Fluorinated Pyrimidines. XXVIII. The Synthesis of 5-Trifluoromethyl-6-azauracil and 5-Trifluoromethyl-6-aza-2′-deoxyuridine[1]

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α-Trifluoromethacrylic acid has been converted with hydrogen peroxide to α,β-dihydroxy-α-trifluoromethylpropionic acid, which on treatment with periodate gave the hydrate of perfluoropyruvic acid. The semicarbazone of this keto acid was cyclized with thionyl chloride to 5-trifluoromethyl-6-azauracil. This was converted to the corresponding β-D-deoxyribonucleoside and nucleotide. The nucleoside was bacteriocidal to *Escherichia coli* B in minimal medium but no other significant biological activity could be attributed to any of the compounds described.

The tumor-inhibitory compounds, 5-fluorouracil, 5-fluoro-2′-deoxyuridine, and 5-trifluoromethyl-2′-deoxyuridine[4] have been the subject of a recent review.[5] Tumor growth is inhibited as a result of the metabolic conversion of these compounds to the corresponding deoxyribonucleoside 5′-phosphates which are potent inhibitors of the enzyme, thymidylate synthetase.[6,7] These compounds are detoxified to a lesser extent by tumor cells than by normal cells and thus, some degree of selective action is obtained.[8]

Tumor-inhibitory properties have also been attributed to 6-azauracil and its ribonucleoside, and metabolic conversion to the nucleotide,[9] 6-azauridine 5′-phosphate, which inhibits orotidylate decarboxylase, is a necessary step for tumor inhibition.[10] 6-Azathymine and its deoxyribonucleoside both exhibit antibacterial activity[10] but to date no tumor-inhibitory activity has been ascribed to these analogs.[11]

Although the most active pyrimidine analogs have involved only one minor structural change from the naturally occurring compound, the biological activity of the two groups of analogs described above prompted an investigation of compounds containing two changes, *i.e.*, "fluorinated azapyrimidines." This report describes one such compound, 5-trifluoromethyl-6-azauracil (V), its deoxyribonucleoside (VIII), and deoxyribonucleotide (IX). Concurrent with our work, two other laboratories have prepared V[12,13] and VIII[12,14] by different routes, and recently a second member of this series, 5-fluoromethyl-6-azauracil, has been prepared, by the method described here, both in our laboratory[15] and elsewhere.[16]

Our route to the azapyrimidine (V)[17] was less direct than that of Mertes, *et al.*,[14] and Shen, *et al.*,[12] but involves the preparation of three new compounds. The synthesis was based on that of Bougault and Daniel[18] for azathymine, which was basically the cyclization of the semicarbazone of pyruvic acid. Only brief mention of the silver salt of trifluoropyruvic acid was found in the literature[19] and a new synthesis was thus devised. α-Trifluoromethacrylic acid (I)[20] was treated with hydrogen peroxide and a catalytic amount of osmium tetroxide, and the resulting α,β-dihydroxy-α-trifluoromethylpropionic acid (II) was oxidized with periodate to give the required perfluoropyruvic acid [isolated as the hydrate (III)] in good yield (Scheme I). The cyclization of the semicarbazone (IV) was effected by refluxing in thionyl chloride, rather than with alkali as used by Bougault.[18]

The procedure followed for the preparation of the nucleoside was based on the method of Ryan, Acton, and Goodman[21] to whom we are indebted for a brief description of their work prior to publication. The bis(trimethylsilyl) ether of V was condensed with 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribofuranosyl chloride[22] in the presence of mercuric acetate at room temperature. After filtration and concentration of the filtrate, a solid precipitated, and various fractions thus obtained had melting points in the range 140–170°. This solid was very soluble in cold ethyl acetate and could be precipitated with ethanol, but was not crystalline. If the solid was treated with hot 80% acetic acid, however, higher melting material was obtained. This was no longer soluble in cold ethyl acetate but required heating for solution and also crystallized readily from ethyl acetate–ethanol mixtures. This material was characterized as the protected nucleoside (VII) and it is suggested that the initial product from the condensation perhaps still retained some of the 4-trimethylsiloxy group, which was removed by the

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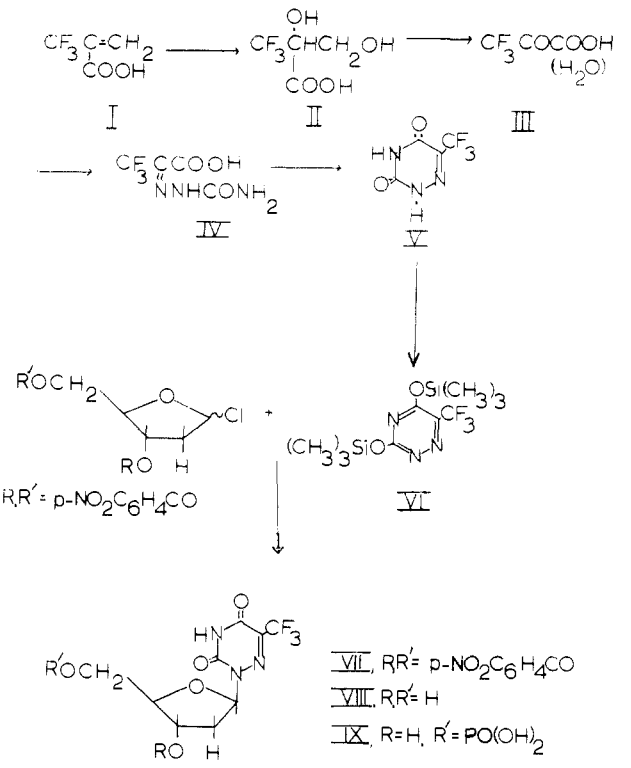
SCHEME I



acid hydrolysis. The *p*-nitrobenzoyl protecting groups were then removed with sodium methoxide yielding the required nucleoside (VIII). Phosphorylation of VIII by the Tener procedure[23] using β-cyanoethyl phosphate[24] gave, in low yield, a nucleotide, presumed from the method of preparation to be the 5′-phosphate.

The pK_{a_1} of V was 5.4 as determined spectrophotometrically,[25] significantly lower than the value 5.9, reported by Mertes,[13] but in general agreement with published data for other 5-substituted azauracils.[26] In contrast to the lability of the trifluoromethyl group in trifluorothymine toward mild alkali,[4] this group in V was found, from examination of the ultraviolet spectrum, to be unaffected by 1 *N* NaOH at room temperature for at least 9 days. The mechanism of hydrolysis of trifluorothymine is presumably similar to that proposed by Cohen and Dinar[27] for 6-trichloromethylpurine; *i.e.*, loss of a proton and then loss of the negative charge left on the ring as fluoride ion. Although they are hydrolyzed at pH 12 at room temperature, the β-D-arabinofuranoside[28] and β-D-deoxyribofuranoside[21] of trifluorothymine are markedly more resistant to alkali than the pyrimidine itself. In the case of the nucleosides, however, the proton must be lost from N-3 instead of N-1 and the negative charge at N-3 is not readily delocalized to the trifluoromethyl group as it is at N-1. As the trifluoromethyl group is hydrolyzed in the case of these nucleosides, some assistance presumably is received from the ring, possibly from the unshared electron pair on N-1. In the case of the azapyrimidine (V), the ultraviolet spectral shifts indicate that the proton on N-3 is the most acidic, as was found by Jonas and Gut[29] for 6-azauracil. Also, the nitrogen at position 6 would effectively block the flow of electrons to the trifluoromethyl group and so only a direct attack by alkali on the trifluoromethyl group carbon is possible, and under the conditions described, this did not occur. 5-Fluoromethyl-6-azauracil has also been found to be unaffected by normal alkali at room temperature,[15] whereas 5-difluoromethyluracil is only stable below pH 4 and cannot exist at physiological pH.[4]

The involvement of N-1 in the glycosidic linkage of VIII, rather than N-3, was inferred from ultraviolet spectral data, a shift in alkali to longer wavelength being expected if N-3 were involved.[29] The β configuration of this linkage was assigned on the basis of the negative Cotton effect in the ORD spectrum[30] and also on the nmr spectrum.[31] In agreement with the findings of Ryan, *et al.*,[21] for trifluorothymidine, the pattern of the anomeric proton was not a simple triplet, but comparison of the spectrum with those of α- and β-fluoro-2′-deoxyuridine enabled the β configuration to be assigned. The specific rotation of VIII reported here ($[\alpha]^{22}D$ −54.9°) differs significantly from that previously reported[12-14] but recently our value has been confirmed.[32]

The nucleotide (IX) prepared here is assumed to be largely the 5′-phosphate, the only evidence for this being the synthetic method used, which Tener[23] has shown to yield predominantly 5′-nucleotides. The phosphate was removed from IX by *Escherichia coli* alkaline phosphatase, a very nonspecific enzyme, but not by the 5′-nucleotidase activity in crude rattlesnake venom (*Crotalus adamanteus*). This latter fact, however, could be because this analog is so different from the enzyme's natural substrates that factors other than the position of the phosphate are involved.

Trifluoropyruvic acid hydrate (III) was found to be a very weak inhibitor of lactate dehydrogenase, and the kinetics indicated that inhibition was competitive with reduced diphosphopyridine nucleotide (DPNH) as hydrogen donor and noncompetitive with reduced acetylpyridine DPN^+ as hydrogen donor.[33]

In bacterial studies[34] it was found that neither the azapyrimidine (V) nor the nucleoside (VIII) would substitute for thymine and support the growth of the thymine-requiring strain of *E. coli* CR34. In tryptone broth neither of the above compounds affected the growth curves of *E. coli* B, but, in minimal medium, although V was still without effect, VIII at a concentration of 25 μg/ml was highly lethal to the cultures. This bacteriocidal effect was not reversed by supplementing the medium with thymidine, even in large doses as is the case for azathymidine,[35] but was reversed when a casein hydrolysate, cystine, cysteine, or glutathione was added to the medium and delayed by the presence of mercaptoethanol. (This has been subsequently shown to be a consequence of contamination of the nucleoside by traces of mercuric ion.)

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At a concentration of 10^{-4} *M*, VIII caused no significant inhibition of DNA synthesis in *Physarum polycephalum* as determined by incorporation experiments with ^{3}H-cytidine.[36] The growth of HeLa cells was not significantly affected by concentrations of V up to 10^{-3} *M* (Figure 1), and only a very slight effect (about 15% inhibition) was found with VIII at the same concentration.[37] The *in vitro* incorporation of ^{14}C-formate into the DNA-thymine of Ehrlich ascites cells was not affected by VIII at the level of 10^{-3} *M*,[38] whereas azathymidine at about the same level causes a 78% inhibition[39] and trifluorothymidine effects a 90% inhibition at only 10^{-6} *M*.[38] Although many nucleosides have been obtained by the enzymic transfer of the deoxyribose moiety from another nucleoside[40,41] to a pyrimidine or purine or to analogs of such bases, no transfer of the deoxyribose moiety from thymidine to V could be detected in either a suspension of *E. coli* B or in a crude extract of Ehrlich ascites cells. A crude extract of nucleoside phosphorylase from Ehrlich ascites cells was without effect on the nucleoside (VIII) at pH 5.9, 6.4, or 7.2,[41] whereas 5-fluoro-2′-deoxyuridine was converted to 5-fluorouracil (52%) at pH 5.9 and 33% at pH 6.4 or 7.2 as determined by the assay procedure of Heidelberger, *et al.*[42] The procedure of Reyes and Heidelberger[7] was used to assay VIII as an inhibitor of thymidylate synthetase in the presence of ATP, but no inhibition could be detected even at levels a thousandfold higher than those necessary for 100% inhibition with 5-fluoro-2′-deoxyuridine.[41] Since the actual inhibitor of this enzyme in the latter case is the nucleoside 5′-phosphate, which is formed enzymically in the incubation mixture, VIII was converted to the corresponding nucleotide (IX) chemically and this was also assayed against thymidylate synthetase. Again, no inhibition was observed. The nucleoside (VIII) was also tested against Adenocarcinoma 775 in female BDF_1 mice at a dose of 400 mg/kg given intraperitoneally on each of 7 days, commencing 1 day after transplantation (Table I). No tumor inhibition was observed.

TABLE I

Days after transplantation	Controls (6 mice) Wt change, g	Controls (6 mice) Tumor vol, mm³	Treated (2 mice) Wt change, g	Treated (2 mice) Tumor vol, mm³	T/C
11	+0.9	4709	+1.8	4150	0.88
15	+4.3	9350	+5.4	9240	0.98

In general, V and its derivatives exhibit a distinct lack of biolcgical effects. The differences between V and its naturally occurring analog, thymine, are probably so great that enzymes normally involved in thymine metabolism no longer recognize this grossly altered base (V). Possibly one of the most drastic differences lies in the pK_{a_1} values of the two compounds,

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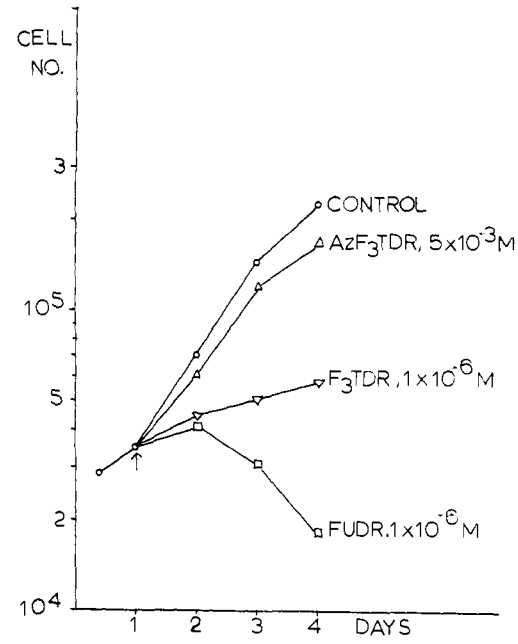

Figure 1. The effect of the deoxyribonucleosides of 5-fluorouracil (FUDR), 5-trifluoromethyluracil (F_3TDR), and 5-trifluoromethyl-6-azauracil (AzF_3TDR) on the growth curves of HeLa cells.

9.5 for thymine[14] and 5.4 for V. Thus, at physiological pH, V exists almost completely as an anion, whereas thymine exists as the neutral molecule.

Experimental Section

Melting points are corrected and analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich.

α,β-Dihydroxy-α-trifluoromethylpropionic Acid (II).—α-Trifluoromethacrylic acid (I, 6.30 g)[20] in water (5 ml) was treated with a 2% aqueous solution of OsO_4 (1 ml), and 30% H_2O_2 solution (9 ml) was added in portions over a period of 24 hr at room temperature. A current of air was passed over the solution to remove the OsO_4 and the solution was then diluted with water (5 ml) and extracted with ether. After drying and evaporation of the ethereal solution an oil was obtained which crystallized to a white solid (5.54 g, 71%, mp 85–92°) on trituration with dichloromethane. This solid could be recrystallized from ethyl acetate–chloroform and an analytical sample so obtained had mp 97–98.5°.

Anal. Calcd for $C_4H_5F_3O_4$: C, 27.59; H, 2.90. Found: C, 27.49, H, 2.91.

Trifluoropyruvic Acid Hydrate (III).—Crude II (8.7 g) in water (50 ml) was treated with sodium metaperiodate (10.2 g) in water (90 ml) at room temperature for 40 min. The solution was then saturated with NaCl and extracted with ether. After drying and evaporation of the ethereal solution, the resulting oil was crystallized by trituration with dichloromethane. Crude product (6.15 g, mp 103–111°) corresponding to 77% of theory was thus obtained. Recrystallization from ethyl acetate–chloroform gave very hygroscopic crystals, mp 119.5–122.5°.

Anal. Calcd for $C_3H_3F_3O_4$: C, 22.51; H, 1.89. Found: C, 22.65; H, 2.06.

Trifluoropyruvic Acid Semicarbazone (IV).—A solution of III (5 g) and semicarbazide hydrochloride (3.7 g) in water (10 ml) was heated under reflux for 25 min. After cooling the crystalline product was collected by filtration and further product was obtained by evaporation of the filtrate. The total yield was 4.56 g (73%, mp 216–218°). An analytical sample recrystallized from water had mp 217–219°; ultraviolet absorption spectra, in 2 *N* HCl λ_{max} 260 mμ, in 1 *N* NaOH λ_{max} 261 mμ, in water λ_{max} 250 mμ (ϵ 16,500).

Anal. Calcd for $C_4H_4F_3N_3O_3$: C, 24.13; H, 2.03; N, 21.11. Found: C, 24.20; H, 2.13; N, 21.15.

5-Trifluoromethyl-6-azauracil (V).—Compound IV (3.76 g) in $SOCl_2$ (100 ml) was heated under reflux for 24 hr. The $SOCl_2$ was then distilled and the semisolid residue was dissolved in water

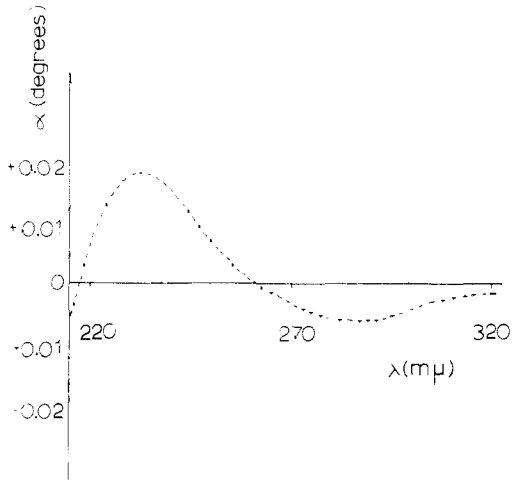

Figure 2.—The ORD spectrum of VIII in 0.1 *N* HCl.

and evaporated to dryness. The residue was sublimed *in vacuo* at 100–120° and the white sublimate was crystallized from ethyl acetate–Skelly B and/or ethyl acetate–chloroform, yielding 2.82 g (82%) of crystalline product, mp 156–158°. An analytical sample had mp 157.5–158.5°; the infrared spectrum in dioxane showed a twin peak in the carbonyl region at 1750 and 1720 cm^{-1}; ultraviolet absorption spectra, in 0.1 *N* HCl λ_{max} 262 mμ (ε 6000), in 1 *N* NaOH λ_{max} 292 mμ (ε 7100); the pK_{a_1} as determined by the spectrophotometric method of Shugar and Fox[25] was 5.4.

Anal. Calcd for $C_4H_2F_3N_3O_2$: C, 26.53; H, 1.11; N, 23.20. Found: C, 26.48; H, 1.27; N, 23.12.

1-(2′-Deoxy-3′,5′-di-*O*-*p*-nitrobenzoyl-β-D-ribofuranosyl)-5-trifluoromethyl-6-azauracil (VII).—Compound V (2.31 g) was dried and then heated under reflux in hexamethyldisilazane (30 ml) under nitrogen for 3.5 hr. The hexamethyldisilazane was removed by vacuum distillation in an atmosphere of nitrogen leaving a light yellow oil of the bistrimethylsiloxy derivative (VI). This was added to 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribofuranosyl chloride (6.6 g)[22] in benzene (325 ml). Mercuric acetate (4.15 g) was added, and the flask was closed and stirred at room temperature for 3 days. The suspension was filtered and the pad was washed with benzene. The filtrate and washings were evaporated to a pale yellow foamed glass (8.8 g). This was heated under reflux in 80% acetic acid (75 ml) for 30 min to remove the 4-trimethylsiloxy group and filtered, and water was added to the cloud point. Three crops of crystals were obtained and each crop was crystallized again from aqueous acetic acid and then from ethyl acetate–ethanol. A total of 2.76 g of product (36.4%), mp 202–206°, was obtained in this experiment although yields as high as 52% have been obtained on smaller scale runs. An analytical specimen crystallized from ethyl acetate–ethanol had mp 206.5–207.5°, $[\alpha]^{22}D$ −32.6° (*c* 0.2, ethyl acetate).

Anal. Calcd for $C_{23}N_{16}F_3N_5O_{11}$: C, 46.39; H, 2.71; N, 11.77. Found: C, 46.48; H, 2.90; N, 11.62.

5-Trifluoromethyl-6-aza-2′-deoxyuridine (VIII).—Compound VII (1 g) in methanol (50 ml) was treated with 0.3 *N* sodium methoxide in methanol (15 ml) and heated to reflux for 10 min and then left overnight at room temperature. The solution was neutralized with Dowex 50 (H^+), evaporated to dryness, and partitioned between chloroform and water. The aqueous phase was evaporated to dryness, redissolved in water, filtered, and again evaporated. The residue was triturated with CH_2Cl_2 and left at room temperature. The resulting crystals were collected and recrystallized from ethyl acetate–dichloromethane yielding 300 mg (60%) of product, mp 152–153°, $[\alpha]^{22}D$ −54.9° (*c* 0.3, H_2O). The melting point was not improved by further crystallization.

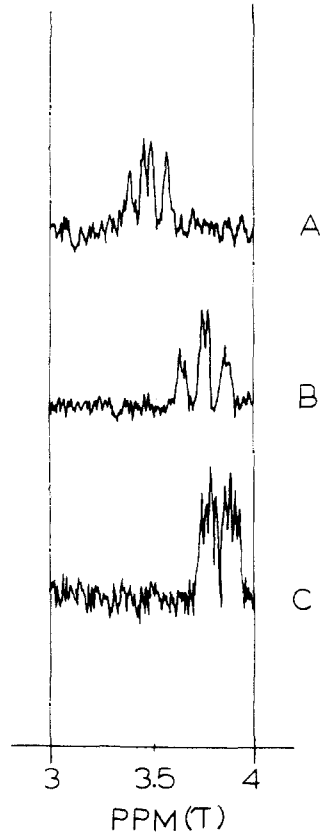

Figure 3.—The nmr pattern of the anomeric proton in VIII (A), β-fluorodeoxyuridine (B), and α-fluorodeoxyuridine (C).

Anal. Calcd for $C_9H_{10}F_3N_3O_5$: C, 36.37; H, 3.39; N, 14.14. Found: C, 36.31; H, 3.41; N, 14.11.

The ORD curve of VIII in 0.1 *N* HCl showed a negative Cotton effect with a trough at 286 and peak at 233 mμ consistent with a β configuration of the glycosidic link (Figure 2). The nmr spectrum of VIII in D_2O was also consistent with a β configuration of the glycosidic link (Figure 3); ultraviolet absorption spectra, in 0.1 *N* HCl λ_{max} 267–268 mμ (ε 6400), in 1 *N* NaOH λ_{max} 264 mμ (ε 5850).

5-Trifluoromethyl-6-aza-2′-deoxyuridine 5′-Phosphate (IX). Compound VIII (29.7 mg, 0.1 mmole) was phosphorylated according to the procedure used by Tener[23] for the phosphorylation of thymidine. After the removal of N,N′-dicyclohexylurea the filtrate contained only 61.5 OD units, *i.e.*, about 10% of the original ultraviolet absorption, and no ultraviolet absorption could be found in the solid removed in the filtration. The filtrate was fractionated on a column of Dowex 1 (formate) using a linear gradient of formic acid (1 l. of 4 *N* formic acid into 1 l. of 1 *N* formic acid) and 15-ml fractions were collected. A small peak of unchanged VIII came off in tubes 4–7 and the major peak (IX) in tubes 81–99, followed by a very small peak, 109–129 possibly the 3′-nucleotide. The major peak was concentrated and finally freeze dried. No further purification was attempted and from the ultraviolet absorption the yield was calculated to be 1.8 mg. On paper chromatography in 1-propanol–H_2O (7:3) one ultraviolet-absorbing component was detected and this also gave a positive color with the Hanes–Isherwood phosphate spray[43] ultraviolet absorption spectra, in 0.1 *N* HCl λ_{max} 266–267 mμ, in water λ_{max} 264 mμ.

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