Fluorinated Pyrimidines. 41. Syntheses of 5-Trifluoromethyl-3′-deoxyuridine and 5-Fluoro-3′-deoxyuridine†,[1]

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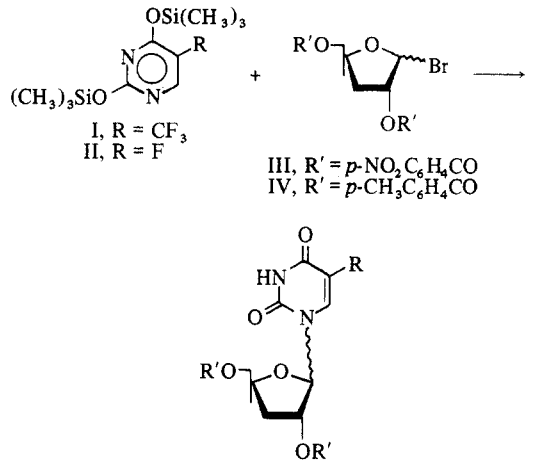
For some time this laboratory has been interested in the structure-activity relationships of two series of clinically active, tumor-inhibitory, fluorinated pyrimidines: 5-trifluoromethyl-2′-deoxyuridine (F_3TdR)[2] and 5-fluoro-2′-deoxyuridine (FUdR).[3,4] We have previously reported both the syntheses and biological activities of a number of analogs of these pyrimidines[5] including some in which the glycosyl moiety had been altered.[6] As a further step in this program we considered it desirable to synthesize the 3′-deoxyribonucleosides of these fluorinated pyrimidines; 5-trifluoromethyl-3′-deoxyuridine [1-(3-deoxy-β-D-*erythro*-pentofuranosyl)-5-trifluoromethyluracil] (3′-F_3TdR) and 5-fluoro-3′-deoxyuridine [1-(3-deoxy-β-D-*erythro*-pentofuranosyl)-5-fluorouracil] (3′-FUdR) and evaluate some of their biological properties.

Although much has been published concerning the biological activity of the nucleoside antibiotic 3′-deoxyadenosine (Cordycepin),[7] the properties of pyrimidine 3′-deoxyribonucleosides have not been as intensely investigated. Walton and coworkers[8] described the syntheses of the 1-(3-deoxy-D-*erythro*-pentofuranosyl) derivatives of uracil, thymine, cytosine, and 5-methylcytosine, but their biological properties were not reported.

3′-F_3TdR (VI) was synthesized according to a modified procedure of Ryan, *et al.*[9] Condensation in acetonitrile of bis-*O*-(trimethylsilyl)-5-trifluoromethyluracil (I)[6c,9] with 3-deoxy-2,5-di-*O*-*p*-nitrobenzoyl-β-D-*erythro*-pentofuranosyl bromide (III)[10] yielded the blocked nucleoside V in 55% yield. V was deblocked with diisopropylamine to give chromatographically homogeneous 3′-F_3TdR (VI) in an overall yield of 41%. Comparison of the uv spectra of VI with those published for 5-trifluoromethyluridine[6c] and 3-methyl-5-trifluoromethyluracil[11] indicated that the position of glycosyl attachment was at N-1.

The anomeric configuration was shown to be β both by nmr and CD. The anomeric proton (τ 4.20) exhibited a singlet in the nmr; in addition, circular dichroism revealed a positive Cotton effect centered at 268 nm. Both observations are in agreement with the work of Walton, *et al.*,[8] for β-pyrimidine 3′-deoxyribonucleosides. Also, the sign of the Cotton effect was in accord with the predictions of Nishimura, *et al.*[12]

3′-FUdR (VIII) was prepared in a similar manner from bis-*O*-(trimethylsilyl)-5-fluorouracil (II)[13] and 3-deoxy-2,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl bromide (IV). This glycosyl halide was synthesized in a two-step procedure starting from methyl 3-deoxy-β-D-*erythro*-pentofuranoside.[10] Nmr indicated that the product obtained from the condensation of II and IV in acetonitrile contained an anomeric mixture of blocked nucleosides with the β anomer predominating in the ratio 6:1. After deblocking with $NaOCH_3$–CH_3OH and preparative tlc, the β anomer was isolated with an overall yield of 21%.



V, R = CF_3; R′ = *p*-$NO_2C_6H_4CO$, β-anomer
VI, R = CF_3; R′ = H, β-anomer
VII, R = F; R′ = *p*-$CH_3C_6H_4CO$, β/α = 6:1
VIII, R = F; R′ = H, β-anomer

Comparison of the uv spectra of VIII with those of the model compounds 3-methyl-5-fluorouracil[6d] and FUdR[14] indicated N-1 to be the site of glycosyl attachment. A β configuration was indicated by a positive Cotton effect from circular dichroism and a singlet in the nmr spectrum representing the absorbance of the anomeric proton (τ 4.15). The coupling constant $J_{H\text{-}1'\text{-}F}$ of 1–2 Hz described by Cushley, *et al.*,[15] in FUdR and 5-fluorouridine was not observed.

Because of the usual lack of biological activity of α-pyrimidine nucleosides the α anomer obtained in this synthesis was not further characterized.

Biological Activity. The results of studies delineating the effects of 3′-F_3TdR (VI) and 3′-FUdR (VIII) on the growth of HeLa, L-5178Y, Novikoff hepatoma, and Novikoff hepatoma-FUdR resistant cells in culture are summarized in Table I. The methods of testing pyrimidine nucleoside analogs in these cell culture systems have been previously described.[16a] Both VI and VIII appeared to be approximately 0.001 as active as their 2′-deoxyribonucleoside counterparts, F_3TdR and FUdR. Catabolism of the 3′-deoxyribonucleoside to the free base, and then conversion to the active 2′-deoxyribonucleoside could account for the results with 3′-FUdR, as it has been shown that 5-fluorouracil is converted to FUdR.[17] This could not be the case for 3′-F_3TdR, because 5-trifluoromethyluracil is inactive as a growth inhibitor of cells in culture.[18]

The method for determining the effect of 3′-F_3TdR (VI) on the replication of vaccinia virus in HeLa cells has been

Table I. Concentration of Compounds Required for Effective Inhibition of Growth

Cells	FUdR,[a] *M*	3′-FUdR, *M*	F_3TdR,[a] *M*	3′-F_3TdR, *M*
HeLa	5×10^{-7}	10^{-3}	5×10^{-7}	10^{-3}
L-5178Y	5×10^{-10}	10^{-4}	5×10^{-9}	10^{-3}
Novikoff hepatoma	2×10^{-7}	$>10^{-4}$	10^{-5}	
Novikoff hepatoma-FUdR-resistant	2×10^{-3}	$>10^{-3}$	$>10^{-3}$	
Vaccinia viral replication in HeLa cells[b]			5×10^{-6}	$>10^{-4}$

[a] See ref 16a. [b] See ref 16b.

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reported previously.[16b] 3'-F_3TdR is less than 0.01 as active as F_3TdR in this assay system.

In conclusion, neither compound exhibited sufficient biological activity to warrant further study.

Experimental Section§

1-(3-Deoxy-2,5-di-*O*-*p*-nitrobenzoyl-β-D-*erythro*-pentofuranosyl)-5-trifluoromethyluracil (V). Bis-*O*-(trimethylsilyl)-5-trifluoromethyluracil (I)[6c,9] (3.2 g, 10 mmoles) and 3-deoxy-2,5-di-*O*-*p*-nitrobenzoyl-β-D-*erythro*-pentofuranosyl bromide (III)[10] (4.95 g, 10 mmoles) were stirred under anhydrous conditions with 50 ml of dry acetonitrile at room temperature for 72 hr. The reaction mixture was then treated with MeOH and evaporated under reduced pressure to dryness. The residue was dissolved in $CHCl_3$ and chromatographed on 100 g of Woelm neutral alumina (grade II). The column was eluted with 1 l. of $CHCl_3$ and 1 l. of 15% ethyl acetate in $CHCl_3$ to remove the unreacted sugar. The column was then eluted with 500 ml of MeOH, and all fractions containing V (monitored by tlc on silica gel employing the solvent system 20% ethyl acetate in $CHCl_3$) were combined and evaporated to yield 3.29 g of III (55%): mp 127–130° dec; $\lambda_{max}^{H_2O}$ 264 nm; nmr ($CDCl_3$) τ 3.80 (s, 1, H-1'). *Anal.* ($C_{24}H_{17}N_4O_{11}F_3$) C, H, N.

1-(3-Deoxy-β-D-*erythro*-pentofuranosyl)-5-trifluoromethyluracil (VI). A solution of 3.0 g (5 mmoles) of V and 10 ml of diisopropylamine in 50 ml of dry MeOH was refluxed for 15 min. The reaction mixture was evaporated to dryness and partitioned between water and $CHCl_3$. The organic layers were further extracted with water, and aqueous layers were combined and neutralized with Dowex 50-X4 (H+). The resin was filtered and washed with water and MeOH, and the filtrate was evaporated to a yellowish solid, which was recrystallized from ethanol–ether to give 1.2 g (80%) of VI: colorless crystals; mp 195–196°; $\lambda_{max}^{H_2O}$ 264 nm (ϵ 6300), $\lambda_{max}^{0.1\ N\ HCl}$ 264 nm (ϵ 7500); nmr (D_2O) τ 1.13 (s, 1, H-6), τ 4.20 (s, 1, H-1'); $[\theta]_{268}^{25°}$ +13100. *Anal.* ($C_{10}H_{11}N_2O_5F_3$) C, H, N, F.

Methyl 3-Deoxy-2,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranoside. A solution of 2.2 g (15 mmoles) of methyl 3-deoxy-β-D-*erythro*-pentofuranoside[10] in 50 ml of dry pyridine was cooled in an ice bath and treated with 6.9 g (45 mmoles) of *p*-toluoyl chloride. After stirring at room temperature overnight, the mixture was added with stirring to 100 g of ice and the resulting mixture extracted with ether. The ethereal extracts were combined, washed successively with water, dil H_2SO_4, and satd $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated. The yellowish syrup was purified on 100 g of Woelm neutral alumina (activity grade II) with $CHCl_3$ as the eluant. Evaporation of the solvent yielded 4.1 g (80%) of colorless crystalline methyl 3-deoxy-2,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranoside: mp 80° dec. *Anal.* ($C_{22}H_{24}O_6$) C, H.

3-Deoxy-2,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl Bromide (IV). To an ice-cold solution of 3.8 g (10 mmoles) of methyl 3-deoxy-2,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranoside in 15 ml of glacial AcOH was added 1 ml of acetyl bromide and 15 ml of 42% HBr–AcOH. The solution was kept ice cold for 45 min, whereupon it was evaporated to a viscous oil. Several portions of toluene were successively added and evaporated *in vacuo* to yield IV, 3.46 g (80%), as a faintly colored syrup, which was used without any further characterization.

1-(3-Deoxy-2,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl)-5-fluorouracil and 1-(3-Deoxy-2,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (VII). Bis-*O*-(trimethylsilyl)-5-fluorouracil (II)[13] (2.74 g, 10 mmoles) and IV (3.46 g, 8 mmoles) were stirred in 70 ml of dry acetonitrile for 2 days. Methanol was added and the reaction mixture filtered and evaporated to give a brown glass. This glass was dissolved in $CHCl_3$ and chromatographed on 100 g of silica gel using $CHCl_3$ as eluent. The fractions containing the blocked nucleosides were combined and evaporated to yield 2.56 g (66%) of a colorless glassy solid containing a 6:1 mixture of β and α anomers of VII.

1-(3-Deoxy-β-D-*erythro*-pentofuranosyl)-5-fluorouracil (VIII). The mixture of blocked nucleosides (VII) (2.2 g, 4.5 mmoles) was dissolved in 100 ml of dry MeOH and treated with 1.00 g of NaOMe. The mixture was stirred for 2 hr at room temperature, after which time Dowex 50-X4 (H^+) was added to adjust the pH to 3–4. The resin was filtered and washed several times with MeOH. The filtrate and washings were combined and evaporated to an oil. This oil was extracted repeatedly with ether to remove methyl *p*-toluate, and the resulting solid was chromatographed on 100 g of silica gel with $CHCl_3$ as the eluant. After the residual methyl *p*-toluate was eluted, the column was washed with MeOH to give 850 mg (75%) of the anomeric mixture as a colorless solid. Preparative tlc on 1.75-mm silica gel plates employing a solvent system of ethyl acetate–MeOH–H_2O–heptane, 10:6:5:3 (upper phase),[9] resolved both anomers (R_f β 0.13; α 0.10). After recrystallization from amyl acetate–MeOH, 370 mg (21% based on IV) of VIII was obtained as microcrystals: mp 148° sint, 150–152° dec; $\lambda_{max}^{H_2O}$ 269 nm (ϵ 7210), $\lambda_{max}^{0.1\ N\ NaOH}$ 269 nm (ϵ 6900); nmr (D_2O) τ 1.85 (d, 1, J = 6.0 Hz, H-6), τ 4.15 (s, 1, H-1'), $[\theta]_{270}^{25°}$ + 7700. *Anal.* ($C_9H_{11}N_2O_5F$) C, H, N, F.

§ Melting points were determined on a Thomas-Hoover Apparatus and are uncorrected. Uv spectra were recorded on a Cary spectrophotometer Model 15 and nmr spectra on a Perkin-Elmer Model R12 using tetramethylsilane as internal reference. Circular dichroism spectra were obtained on a Cary spectropolarimeter Model 60. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. All analytical results were within ±0.4% of the theoretical values. Analytical tlc was performed on Eastman Chromatogram Sheets silica gel with fluorescent indicator. Preparative tlc plates were prepared with EM silica gel PF-254 and column chromatography performed with Baker silica gel 60–200 mesh.

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3,5-Disubstituted-6-methyl-2-pyridone. Dihydrazide, Dicarbamate, and Monoaminoalkyl Ester

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Many pharmacologically active 2-pyridones have been prepared[1] and it was of interest to synthesize simple 2-pyridones bearing known pharmacophores to investigate the efficacy of the 2-pyridone moiety as a carrier and Ph replacement.