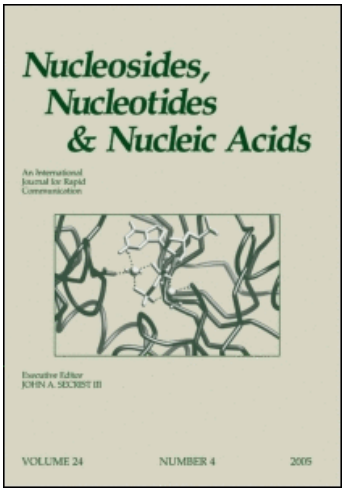

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Synthesis and Anti-Herpetic Activity of A 2'-Fluoroarabinosyl Analog of Trifluridine

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SYNTHESIS AND ANTI-HERPETIC ACTIVITY OF A 2'-FLUOROARABINOSYL ANALOG OF TRIFLURIDINE[1]

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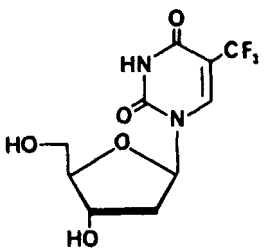
Abstract. The 2'-fluoroarabinosyl analog 2 of trifluridine was found to be a selective inhibitor of herpes simplex virus types 1 and 2 in cell culture. Analog 2 was less active than trifluridine when administered topically against a mouse vaginal infection.

Introduction

One approach to confer antiviral selectivity against herpes viruses utilizes sugar-modified nucleoside analogs which are phosphorylated only in virus-infected cells by the herpes thymidine kinase[5]. Many arabinosyl pyrimidines, such as arabinosylthymine (Ara-T)[6] possess this desired property of selective phosphorylation. Ara-T is active *in vitro* as an anti-herpetic, but its activity in animals is diminished due to rapid urinary clearance[7]. An analog of Ara-T, 2'-fluoro-2'-deoxy-β-D-arabinofuranosylthymine (FMAU, 3), is remarkably potent in treating animals infected with herpes viruses[8,9]. Because of side effects observed with FMAU, however, the compound may not be developed for use in man. Researchers are continuing to synthesize analogs of FMAU which might be less toxic but equally active *in vivo*[10-12].

Trifluridine (TFT, 1) is effective topically in treating herpetic eye infections in man[13], and is approved for that use by the United States Food and Drug Administration. The compound is not selectively antiviral *per se* because it is phosphorylated well in uninfected cells where it inhibits normal cell DNA synthesis.[14] TFT does not appear to be effective when given systemically to combat

herpes encephalitis.[15] The arabinosyl analog of TFT was reported to possess selective antiviral properties against herpes viruses in cell culture, but no effects in animals were reported.[16] We decided to synthesize the 2'-fluoroarabinosyl analog of TFT (2), thinking that it might behave more like FMAU than Ara-T in the treatment of herpes virus infections.



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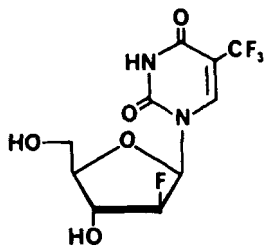

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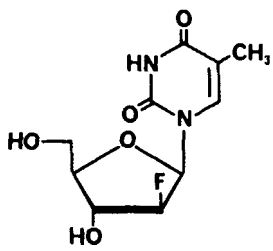


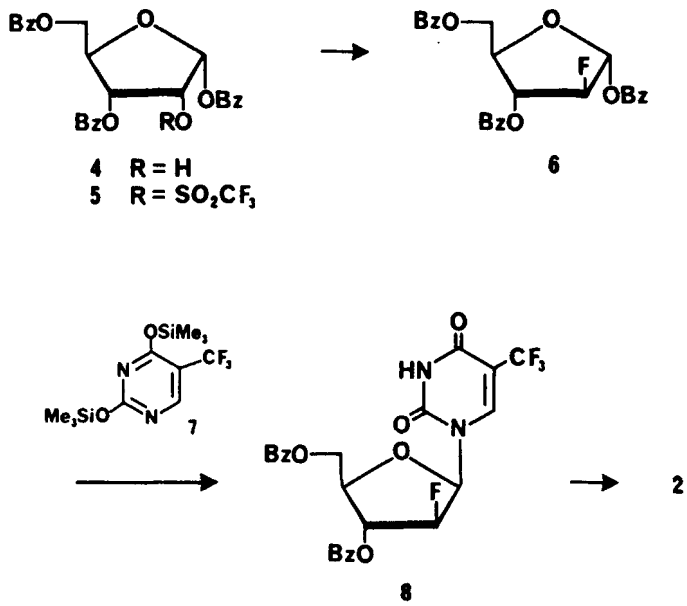
3

Chemistry. Watanabe and co-workers have previously demonstrated the facility with which 1,3-di-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranose couples to pyrimidine bases.[10,17-20] We developed a simplified synthesis (Scheme I) of the related, fully benzoylated fluoro sugar 6 and condensed it with commercially available 5-trifluoromethyluracil.

The easily obtainable 1,3,5-tri-O-benzoyl-α-D-ribofuranose[21] 4 was an attractive precursor for the 2-fluoroarabinose 6. When an attempt to make the fluoro sugar by reacting 4 with diethylaminosulfur trifluoride (DAST) failed, we concentrated our efforts on the displacement of the ribotriflate 5 with fluoride ion. It had been reported[22] that 1,3,5-tri-O-benzyl-2-O-(trifluoromethanesulfonyl)-α-D-ribofuranose reacts with TBAF to form the benzylated 2-fluoroarabinose in 50% yield. However, the presence of the less stable benzoyl protecting groups in the present case led to considerable problems. The preparation of the triflate of the benzoylated ribofuranose 4 proceeded in quantitative yield, but the fluorination proved formidable.

A number of fluoride sources were tried, including potassium fluoride with and without dibenzo-18-crown-6-ether, cesium fluoride, pyridinium hydrofluoride, tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF), tetraethylammonium fluoride (TEAF), and

Scheme I



tetrabutylammonium fluoride (TBAF). Although none of these reagents cleanly effected fluorination, TBAF appeared the most promising and further studies involved TBAF under a variety of conditions. Carrying out the fluorination at room temperature in THF, CCl_4, toluene, or MEK produced the desired fluorosugar in 20-25% yields. Using TBAF in DMF or in a mixture of HMPT and DMSO produced no fluorination, and using anhydrous TBAF, prepared by the method of Cox,[23] resulted in only a 4% yield of fluoro sugar. The highest yields and simplest work-up resulted when the freshly prepared triflate 5 was immediately treated with four equivalents of TBAF in toluene at room temperature. After chromatography, a 19-22% yield (over two steps) of crystalline 6 was isolated.

After the completion of this work, Howell and co-workers reported[24] a similar synthesis of the fluorosugar 6. Using an imidazolylsulfonate instead of a triflate, and fluorination with KHF_2, these investigators converted 4 to 6 in 54% overall yield.

The condensation of the fluoroarabinose with 5-trifluoromethyl-uracil then proceeded uneventfully. A solution of HBr in acetic acid cleanly afforded the bromosugar which was reacted with the silylated

pyrimidine 7 in dichloromethane for 18 hours at room temperature in the presence of mercuric cyanide. After work-up, the β-nucleoside 8 crystallized out in 80% yield. The anomeric configuration was confirmed by the ^{1}H NMR spectrum which contained a large fluorine coupling (21 Hz) to the 1'-proton. Removal of the benzoate protecting groups with ammonium hydroxide provided the final nucleoside 2 in 83% yield.

Results and Discussion

Antiviral Activity of 2. In plaque reduction assays, 2 was approximately as potent as TFT against herpes 1 virus but slightly less active against herpes 2 (Table 1). Both nucleosides were less active than FMAU. In uninfected cells, 2 was the least inhibitory to cell proliferation, with 50% inhibition at 450 μM (Table 1). TFT inhibited cell growth at a concentration lower than that required to inhibit virus plaque formation. These results show that the 2'-fluoroarabinosyl substitution was able to confer selectivity to this thymidine analog.

To explain the basis of selectivity, each compound was evaluated for its ability to inhibit herpes 1 and Vero cell thymidine kinases (TK's). All three analogs were competitive inhibitors of thymidine phosphorylation by herpes 1 TK (Table 2), but 2 was the least competitive of the analogs using cytosol or mitochondrial enzyme sources. Although this assay does not directly demonstrate that each substance is phosphorylated by the respective TK, the cytotoxicity data from Table 1 suggests that this is the case. Certainly there is a correlation between the binding affinity of each compound with cellular TK's to the potency of inhibition of uninfected cell proliferation.

Two animal experiments were conducted to ascertain whether 2 would be effective when administered systemically or applied topically against herpetic diseases. We used 9-[(1,3-dihydroxy-2-propoxy)-methyl]guanine (DHPG) as our positive control in comparing 2 and TFT due to having insufficient quantities of FMAU. The relative potencies of DHPG and FMAU have been shown to be nearly identical in mouse infection models.[9]

TABLE 1. Antiviral and Anticellular Activities of 2, TFT, and FMAU.

	Fifty Percent Inhibitory Concentration (μM)		
Virus[a] or Cell[b]	2	TFT	FMAU
Herpes 1 (F)	3.5	2.2	0.3
Herpes 2 (G)	10	4.0	0.8
Vero Cells	460	1.6	40

[a] Determined by plaque reduction assays in Vero cells. The virus strain is given in parentheses.
[b] Determined by cell proliferation assays.

TABLE 2. Competitive Inhibition of Thymidine Kinase (TK) Activities by 2, TFT and FMAU.

	Inhibition Constant (Ki), μM		
Compound	Herpes 1 TK	Cytosol TK	Mitochondrial TK
2	2.0	>100	35
TFT	0.3	0.4	5
FMAU	0.7	>100	3

In the first experiment mice received ten 50% lethal doses of herpes 2 virus by intraperitoneal injection (16 mice per treatment group), which killed 94% of saline-treated mice. Test compounds were administered once a day for 4 days starting 24 hours after virus inoculation. In the DHPG-treated groups there were 25, 44, and 75% survivors at doses of 3, 10, and 30 mg/kg/day, respectively (the latter two doses causing a statistically significant effect, $p<0.05$). TFT and 2 provided no protection to the mice at $\leq$100 mg/kg/day.

A second experiment compared topical treatments of 2, TFT and DHPG against a herpes 2 vaginal infection in mice (Table 3). In this test, 1 to 5% DHPG in a propylene glycol-based cream reduced the severity of lesions and at the high dose reduced mortality significantly.

TABLE 3. Topical treatment of a herpes 2 vaginal infection in mice with 2, TFT and DHPG.

Compound	Dose[a] (%)	Average Lesion Score[b]	Survivors/ Total (%)
Placebo[c]	-	1.1	14/20 (70)
DHPG	1	0.3[d]	20/20 (100)[e]
DHPG	2.5	0.3[d]	20/20 (100)[e]
DHPG	5	0.3[d]	20/20 (100)[e]
TFT	2.5	1.2	14/20 (70)
TFT	5	0.3[d]	20/20 (100)[e]
2	2.5	1.3	13/20 (65)
2	5	0.9	16/20 (80)

[a]Treatments were given twice a day for 5 days starting 24 hours after virus inoculation.
[b]Determined as a grand average of lesions scored on days 5-9, 11, 13, and 15 post-virus inoculation.
[c]The placebo consisted of drug-free propylene glycol-based cream. Compounds were made up in the same cream.
[d]Statistically significant ($p<.001$).
[e]Statistically significant ($p<.02$).

TFT was equally effective to DHPG at a 5% concentration, but 2 provided no significant benefit at the high dose.

The results of these experiments show that the 2'-fluoroarabinosyl analog of TFT indeed was more selectively antiviral than TFT in cell culture, but was not as effective in topical therapy in animals. Neither compound was active when administered systemically in treating a herpetic infection.

Experimental Section

Chemical Synthesis. General Methods. Nuclear magnetic resonance spectra were recorded on Bruker WM-300 (^{1}H NMR, 300 MHz) and Bruker WH-90 (^{13}C NMR, 22.62 MHz) spectrometers, and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Mass spectra (MS) were recorded on a Finnigan MAT

CH7 spectrometer operating in the direct inlet mode. UV spectra were recorded on a Hewlett-Packard 8450A spectrophotometer. Elemental analysis were obtained by Syntex Analytical Research. Melting points were determined on a hot-stage microscope and are corrected.

1,3,5-Tri-O-benzoyl-2-O-(trifluoromethyl)sulfonyl-α-D-ribofuranose (5). A solution of trifluoromethanesulfonic anhydride (50 g, 177 mmol) in dry CH_2Cl_2 (0.20 L) was added, over 1 h, to a stirred, -10°C solution of 1,3,5-tri-O-benzoyl-α-D-ribofuranose[21a,b] 4 (54.5 g, 118 mmol) in dry CH_2Cl_2 (2.0 L) and dry pyridine (104 mL). When addition was complete the mixture was stirred for 1 h more at -10°C, then allowed to come to room temperature over ca. 0.5 h. The solution was washed with cold, sat. $NaHCO_3$, then with H_2O. The organic phase was dried ($MgSO_4$) and concentrated <u>in vacuo</u> to a syrup, which was co-evaporated with toluene (3 x 50 mL) to remove last traces of pyridine. The final triflate 5 amounted to 70 g (100%) of pale yellow syrup: ^1H NMR ($CDCl_3$) δ 8.01-8.15 (m, 6H, Ar), 7.39-7.69 (m, 9H, Ar), 6.86 (d, J = 4 Hz, 1H, H-1), 5.78 (dd, J = 3, 6 Hz, 1H, H-3), 5.55 (dd, J = 4, 6 Hz, 1H, H-2), 4.86 (ddd, J = 3, 3, 6 Hz, 1H, H-4), 4.76 (dd, J = 3, 12 Hz, 1H, $H\text{-}5_a$), 4.62 (dd, J = 3, 12 Hz, 1H, $H\text{-}5_b$).

2-Deoxy-2-fluoro-1,3,5-tri-O-benzoyl-α-D-arabinofuranose (6). A solution of 5 (70 g, 0.12 mol) and tetrabutylammonium fluoride trihydrate (150 g, 0.48 mmol) in toluene (2.0 L) was stirred for 4 h at room temperature. The dark amber mixture was washed with H_2O (2 x 1 L), filtered, then concentrated in vacuo to a viscous syrup. The syrup was chromatographed on a 3 kg column of silica-gel 60 using a stepwise gradient elution consisting of EtOAc/hexane 1:9 (8 L), 3:17 (6 L), and 1:4 (8 L). This achieved a separation of the desired fluoro sugar from a slightly more polar contaminate. (Identified as tetrabenzoyl-α-D-arabinose). The syrupy product was crystallized from ethyl acetate/hexane to give 11 g (20%) of 6: mp 80-82°C; ^1H NMR ($CDCl_3$) δ 8.04-8.11 (m, 6H, Ar), 7.38-7.64 (m, 9H, Ar), 6.76 (d, J=9 Hz, 1H, H-1), 5.63 (dd, J=3, 20 Hz, 1H, H-3), 5.39 (d, J=48 Hz, 1H, H-2), 4.69-4.83 (m, 3H, H-4 and 5). Anal. Calcd. for $C_{26}H_{21}O_7F$ (464.43); C, 67.24; H, 4.56. Found: C, 67.43; H, 4.62.

2,4-Bis-O-(trimethylsilyl)-5-trifluoromethyluracil (7). A suspension of 5-trifluoromethyluracil (Sigma) (1.55 g, 8.61 mmol) and ammonium sulfate (10 mg) in hexamethyldisilazane (25 mL) was heated at reflux for 2 h. The resulting colorless solution was evaporated in vacuo leaving 7 as a colorless oil.

1-(2-deoxy-3,5-di-O-benzoyl-2-fluoro-β-D-arabinofuranosyl)-5-trifluoromethyluracil (8). To a solution of 6 (2.00 g, 4.31 mmol) in dry CH_2Cl_2 (20 mL) was added a 30% solution of HBr in AcOH (5.0 mL). After 2 h at room temperature, the solution was concentrated in vacuo and the residue co-evaporated twice with 10 mL of dry toluene. The resulting crystalline bromosugar was dissolved in CH_2Cl_2 (20 mL) and mercuric cyanide (1.1 g, 4.3 mmol) and a solution of 7 (8.6 mmol, from above preparation) in CH_2Cl_2 (20 mL) were added. After stirring for 18 h at room temperature, then diluting with CH_2Cl_2 (50 mL), the mixture was filtered. The filtrate was washed successively with sat. $NaHCO_3$ (50 mL), 30% KI (50 mL), and H_2O (50 mL). The dried ($MgSO_4$) organic phase was evaporated to a crystalline solid which was recrystallized from EtOH to give 1.79 g (80%) of 8: mp 186-187°C; uv λ_{max} (EtOH) 256 nm (ε 13,400), 231 nm (31,300); ^1H NMR ($CDCl_3$) δ 9.41 (br s, 1H, NH), 8.11 (s, 1H, 6H), 8.05-8.08 (m, 4H, Ar), 7.44-7.69 (m, 6H, Ar), 6.32 (dd, J = 3, 21 Hz, 1H, H-1'), 5.64 (dd, J = 2.5, 17 Hz, 1H, H-3'), 5.37 (dd, J = 2.5, 50 Hz, 1H, H-2'), 4.82 (m, 2H, H-5'), 4.57 (dd, J = 2, 3 Hz, 1H, H-4'). Anal. Calcd. for $C_{24}H_{18}N_2O_7F_4$ (522.40): C, 55.18; H, 3.47; N, 5.36. Found: C, 54.97; H, 3.17; N, 5.21.

1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-trifluoromethyluracil (2). A solution of 8 (1.60 g, 3.07 mmol) in MeOH (15 mL) and conc. NH_4OH (15 mL) was kept at room temperature for 16 h before concentrating in vacuo. The aqueous remains were diluted with H_2O (100 mL) and extracted with n-BuOH/CH_2Cl_2 1:9 (3 x 30 mL). The aqueous phase was evaporated to a syrup which was crystallized from EtOH affording 0.80 g (83%) of 2 mp 202-203°C; UV λ_{max} (0.1 N HCl) 259 nm (ε 8,640); ^1H NMR (Me_2SO-d_6) δ 8.51 (s, 1H, H-6), 6.16 (dd, J = 5, 11.5 Hz, 1H, H-1'), 5.16 (ddd, J = 4, 4, 53 Hz, 1H, H-2'), 4.26 (ddd, J = 4, 6, 20 Hz, 1H, H-3'), 3.85 (m, 1H, H-4'),

3.71 (dd, J = 1, 12 Hz, 1H, H-5_a'), 3.59 (dd, J = 4, 12 Hz, 1H, H-5_b'); ^{13}C NMR (Me_2SO-d_6) δ 158.7 (C-4), 149.2 (C-2), 142.1 (C-6), 122.5 (CF_3), 102.8 (C-5), 95.4 (C-2'), 83.3 and 82.8 (C-1' and 3'), 71.6 (C-4'), 58.9 (C-5'); MS 314 (M+), 135 (base). Anal. Calcd. for $C_{10}H_{10}N_2O_5F_4$ (314.20): C, 38.22; H, 3.21; N, 8.92. Found: C, 38.44; H, 3.23; N, 8.80.

Antiviral Standards. Trifluridine was purchased from Sigma Chemical Co., St. Louis, MO. FMAU was a gift from J.J. Fox, Sloan-Kettering Cancer Center, New York, NY. DHPG was synthesized by a published procedure.[25]

Plaque and cytotoxicity assays. Experiments were conducted in Vero cells as described previously.[26] The fifty percent inhibitory concentrations are defined as those causing a 50% reduction of virus plaque numbers, or 50% reduction in the number of uninfected cells after the appropriate incubation period.

Thymidine kinase inhibition assays. Thymidine kinases from herpes 1 infected and uninfected Vero cells were affinity purified by published procedures.[27,28] The Michaelis constant (Km) for thymidine for each enzyme was approximately 0.5 μM. DE-81 paper assays[29] were run using a fixed concentration of 1 μM ^{3}H-thymidine (purchased from ICN Radiochemicals, Irvine, CA) and various inhibitor concentrations. Inhibition constants (Ki values) were estimated from 50% inhibitory concentrations according to the method of Cheng and Prusoff.[30]

Animal experiments. These studies were performed according to methods described in a previous publication.[26] Statistical significance of increases in numbers of survivors was made by the two-tailed Fisher exact test. Lesion score decreases were evaluated statistically by the one-tailed t-test.

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