Nucleosides. 150. Synthesis and Some Biological Properties of 5-Monofluoromethyl, 5-Difluoromethyl, and 5-Trifluoromethyl Derivatives of 2'-Deoxyuridine and 2'-Deoxy-2'-fluoro-β-D-arabinofuranosyluracil†

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A new synthesis of 5-(monofluoromethyl)- and 5-(difluoromethyl)-2'-deoxy-2'-fluoro-β-D-arabinofuranosyluracil (F-FMAU and F_2-FMAU) is reported. 3',5'-Di-O-(*tert*-butyldiphenyl)silylated thymidine or FMAU was photochemically brominated with NBS to the corresponding α-monobromide, which was hydrolyzed to the 5-hydroxymethyl derivative. Further oxidation of the latter with MnO_2 afforded the 5-formyluracil nucleoside. Treatment of these nucleosides with DAST in CH_2Cl_2 gave the protected α-fluorinated nucleosides. Desiylation with TBAF afforded the desired free nucleosides. Also, 5-(trifluoromethyl)-2'-deoxy-2'-fluoro-β-D-arabinofuranosyluracil (F_3-FMAU) was synthesized by copper-catalyzed trifluoromethylation of 5-iodo-2'-fluoro-ara-U (FIAU). These new nucleosides were studied, in comparison with the corresponding 2'-deoxy-*erythro*-pentofuranosyl derivatives, for their inhibitory activity against cellular thymidylate synthase (TS) and [^{3}H]TdR incorporation into DNA, cytotoxicity against HL-60 cells, and antiviral activity against herpes simplex types 1 and 2 (HSV-1 and -2). F_2-TDR and F_3-TDR strongly inhibited TS and were also quite cytotoxic and antiherpetic, whereas FTDR was only active in the antiviral assay. In the 2'-fluoroarabino series, fluorine substitution at the α-methyl function did not alter significantly the antiherpetic activity. Although FMAU and F-FMAU did not inhibit TS to any significant extent, F_2-FMAU and F_3-FMAU were weakly inhibitory. The latter nucleosides did not inhibit [^{3}H]TDR incorporation into DNA, while all the other α-fluorinated thymine nucleosides inhibited the incorporation of radioactivity of [^{3}H]TDR into DNA to various extents. F_2-FMAU and F_3-FMAU were about 2 orders of magnitude less cytotoxic against HL-60 cells than were F_2-TDR and F_3-TDR. The results strongly suggest that in both the 2'-deoxy-2'-fluoroarabino and the 2'-deoxy-*erythro*-pentofurano series the cytotoxic action of the α,α-difluoro and α,α,α-trifluoro derivatives may involve the inhibition of TS. The synthesis of [2-^{14}C]F_2-FMAU, as an experimental imaging agent, is also described. Unfortunately, the highly selective uptake of the labeled compound within infected brain regions previously noted with [2-^{14}C]FMAU was not detected with the derivative [2-^{14}C]F_2-FMAU.

Recently we reported[1,2] the synthesis of α-monofluoro- and α,α-difluorothymine nucleosides from TDR, 5-methyl-UR, and FMAU via partial bromination of the 5-methyl group of these nucleosides followed by nucleophilic displacement of the bromine with fluoride.

Herpes encephalitis in humans is a devastating disease with a 30% mortality rate even after the advent of antiviral therapy. Early diagnosis influences the outcome of therapy, and we have previously reported the use of [2-^{14}C]-FMAU and quantitative autoradiography for imaging of HSV infection in a rat model of HSV encephalitis.[3,4] However, application of this strategy to noninvasive imaging of human HSV encephalitis using PET scanning requires that FMAU be labeled with a positron-emitting isotope such as ^{18}F. Unfortunately, synthesis of FMAU with ^{18}F in the 2'-position is impractical. A derivative of FMAU with an additional fluorine may be of use as an imaging agent, and in order to explore this possibility, we developed a more facile method for preparation of α-fluorinated FMAU which should be applicable to ^{18}F labeling at the α-position of FMAU.

This new procedure was adapted for the synthesis of [2-^{14}C]F_2-FMAU to assess the capacity of F_2-FMAU to image HSV-1 encephalitis in the rat model. We also synthesized the new agent, α,α,α-trifluoro-FMAU (F_3-FMAU) for comparison of all the α-fluorinated FMAU derivatives for their chemical stability, biochemical properties, cytotoxicity, and antiviral activity.

Chemistry

We found that the most convenient method for the preparation of F-FMAU and F_2-FMAU, at this time, was the application of our recently developed procedure[5] for the synthesis of F-TDR and F_2-TDR. FMAU was converted into 3',5'-di-*O*-BDPS-FMAU (**2**, Figure 1), which was photobrominated with NBS[6] to the α-brominated FMAU **3**. Hydrolysis of **3** with $NaHCO_3$ in THF afforded the 5-(hydroxymethyl)uracil nucleoside **4**. Treatment of **4** with DAST[7] afforded the protected F-FMAU (**6**). Oxidation of **4** with active MnO_2 according to the procedure of Mertes et al.[8] afforded the 5-formyluracil derivative **5**,

* Abbreviations: TDR, thymidine; FMAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine; FUDR, 5-fluoro-2'-deoxyuridine; UR, uridine; UDR, 2'-deoxyuridine; FIAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil; NBS, *N*-bromosuccinimide; HSV-1, herpes simplex virus type 1; PET, positron emission tomography; BDPS, *tert*-butyldiphenylsilyl; DAST, (diethylamido)sulfur trifluoride; TS, thymidylate synthase; DMF, dimethylformamide; TBAF, tetra-*n*-butylammonium fluoride; THF, tetrahydrofuran; HMPA, hexamethylphosphoric triamide; HMDS, 1,1,1,3,3,3-hexamethyldisilazane.

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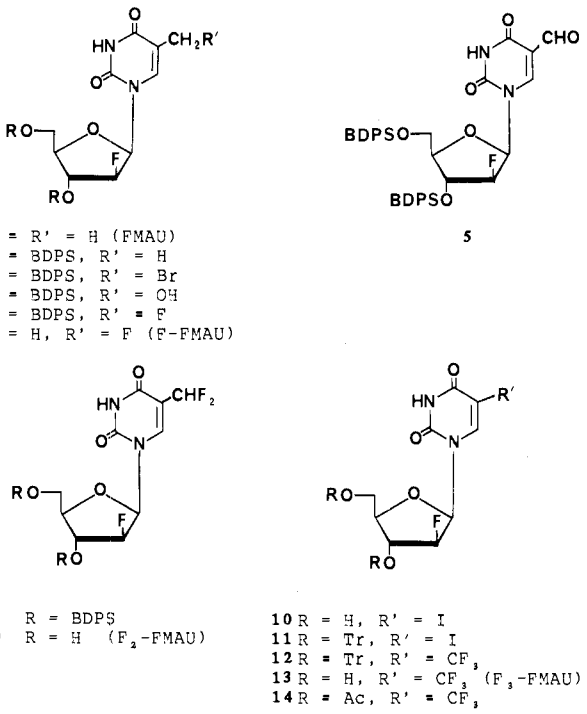


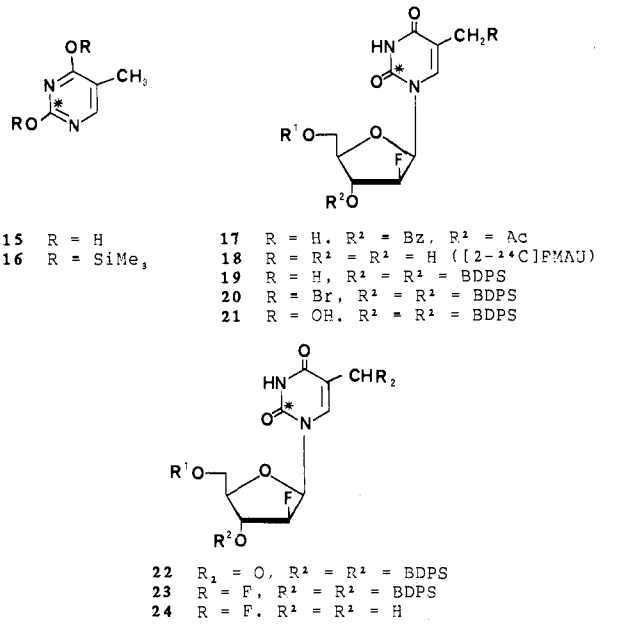
1 R = R' = H (FMAU)
2 R = BDPS, R' = H
3 R = BDPS, R' = Br
4 R = BDPS, R' = OH
6 R = BDPS, R' = F
8 R = H, R' = F (F-FMAU)

5

7 R = BDPS
9 R = H (F_2-FMAU)

10 R = H, R' = I
11 R = Tr, R' = I
12 R = Tr, R' = CF_3
13 R = H, R' = CF_3 (F_3-FMAU)
14 R = Ac, R' = CF_3

Figure 1.



15 R = H
16 R = $SiMe_3$

17 R = H, R^1 = Bz, R^2 = Ac
18 R = R^1 = R^2 = H ([2-^{14}C]FMAU)
19 R = H, R^1 = R^2 = BDPS
20 R = Br, R^1 = R^2 = BDPS
21 R = OH, R^1 = R^2 = BDPS

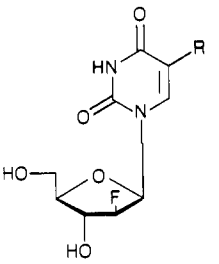
22 R_1 = O, R^1 = R^2 = BDPS
23 R = F, R^1 = R^2 = BDPS
24 R = F, R^1 = R^2 = H

Figure 2.

which, upon reaction with DAST, was converted into the protected F_2-FMAU 7. Compounds 6 and 7 were deprotected with TBAF to give F-FMAU (8) and F_2-FMAU (9), respectively.

The chemistry we developed for the side-chain fluorination of TDR and FMAU was not applicable for the preparation of the trifluoromethyl analogue of FMAU, F_3-FMAU, which is a very close analogue of α,α,α-trifluorothymidine (13, F_3-TDR, Figure 2). The latter was reported to be highly antiherpetic but also very toxic.[9] This compound 13 was synthesized by trifluoromethylation of 3',5'-di-*O*-trityl-FIAU 11 with CF_3I–Cu complex[10] to 3',5'-di-*O*-trityl-F_3-FMAU 12, which was then detritylated. Acetylation of 13 with Ac_2O in pyridine afforded 3',5'-di-*O*-acetyl-F_3-FMAU (14). We have thus prepared all the possible fluoromethyl analogues of TDR and FMAU.

Table I. Inhibition of Thymidylate Synthase (TS) in L1210 Cells and [^{3}H]TDR Incorporation into DNA in HL-60 Cells



compd	R	X	ID_{50},[a] μM TS	ID_{50},[a] μM [^{3}H]TDR incorporation
TDR	Me	H	75	0.61
F-TDR	CH_2F	H	11	18.6
F_2-TDR	CHF_2	H	0.063	5.1
F_3-TDR	CF_3	H	0.035	14.0
FMAU	Me	F	>1000	20.0
F-FMAU	CH_2F	F	>1000	189
F_2-FMAU	CHF_2	F	56	>1000
F_3-FMAU	CF_3	F	14	>1000
CHO-UDR	CHO	H	1.0	ND[b]
CHO-FAU	CHO	F	>1000	ND
CH_2OH-UDR	CH_2OH	H	>1000	ND
CH_2OH-FAU	CH_2OH	F	>1000	ND

[a] ID_{50}, 50% inhibitory concentration of drugs. [b] ND, not determined.

All the α-fluorothymine nucleosides (F-, F_2-, and F_3-FMAU) were found to be stable in aqueous solution for at least 72 h, but in a pH 7 phosphate buffer, F-FMAU (8) was completely hydrolyzed within 22 h at room temperature, whereas F_2-FMAU (9) was stable in the buffer solution at least for 28 h. In order to examine whether radioisotopically labeled F_2-FMAU would be metabolized by HSV-infected cells in the central nervous system of the infected rats, and thus be of potential use as an imaging agent in humans, we synthesized [2-^{14}C]F_2-FMAU (Figure 2) starting from [2-^{14}C]thymine (15), which was silylated to 16 and then condensed with 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-α-D-ribosyl bromide[11] to give the protected [2-^{14}C]FMAU 17. Saponification of 17 afforded [2-^{14}C]-FMAU,[12] which was then converted into the radiolabeled F_2-FMAU 24 by the procedure developed for the preparation of F_2-FMAU from FMAU (Figure 1).

Biology

Inhibition of Thymidylate Synthase. The cytotoxicity and antitumor action of FUDR[13,14] and α,α,α-trifluorothymidine (F_3-TDR)[13-15] involve, at least in part, the inhibition of TS. More recently, 5-fluoro-2'-fluoro-ara-U

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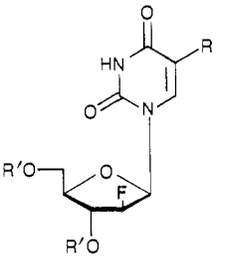
Table II. Antiherpes Activity and Cytotoxicity of α-Fluorinated Thymine Nucleosides



compd	R	R′	X	ED_{50},[a] μM HSV-1 (F)[b]	ED_{50},[a] μM HSV-2 (G)[b]	ID_{50}, μM: HL-60 cell growth 24 h	48 h	72 h
TDR	Me	H	H	>1000	>1000	901	461	336
F-TDR	CH_2F	H	H	>100	>100	178	>1000	>1000
F_2-TDR	CHF_2	H	H	0.80	1.17	4.0	1.25	0.12
F_3-TDR	CF_3	H	H	0.77	1.88	1.5	1.7	0.14
F-FMAU	CH_2F	H	F	0.68	0.80	995	202	119
F_2-FMAU	CHF_2	H	F	0.34	1.04	97	12	6.6
F_3-FMAU	CF_3	H	F	0.83	1.70	304	29	17
Ac-FTDR	CH_2F	Ac	H	>100	>100	3470	976	324
Ac-F_2-TDR	CHF_2	Ac	H	17.0	25.1	83	14	14
Ac-F_3-TDR	CF_3	Ac	H	4.7	16.3	501	19	8.5
Ac-F-FMAU	CH_2F	Ac	F	2.43	5.19	860	500	312
Ac-F_2-FMAU	CHF_2	Ac	F	0.71	2.16	257	83	40
Ac-F_3-FMAU	CF_3	Ac	F	1.3	1.3	54	62	65
FMAU	Me	H	F	0.07	0.07	136	51	25
Ac-FMAU	Me	Ac	F	0.44	1.23	>1000	209	119
CH_2OH-FAU	CH_2OH	H	F	2.0	5.5	>5000	1160	1920

[a] ED_{50} concentration of drugs effective to alleviate 50% of virus. [b] F strain and G strain.

(FFAU) was also found to be a potent inhibitor of TS.[16] We therefore examined the inhibitory activity of the α-fluorinated TDR and FMAU derivatives against TS in L-1210 cells in situ by determining the inhibition of the release of tritium from [5-^{3}H]UDR.[17] The results are summarized in Table I.

Stepwise substitution of the hydrogens of the methyl group of thymidine (TDR) with fluorines led to a progressive increase in enzyme inhibitory activity. The largest increment in potency (170-fold) was obtained by the introduction of the second fluorine, giving an ID_{50} of 6.3×10^{-8} M for F_2-TDR, a value which was within 2-fold of that of F_3-TDR. The inhibitory potency of F-TDR ($ID_{50} = 1.1 \times 10^{-6}$ M) was about 7-fold higher than that of TDR. The corresponding 2′-deoxy-2′-fluoroarabinosyl series showed about a 3 orders of magnitude weaker enzyme inhibitory activity. The large potency difference between the mono- and difluoro derivatives in both series suggests different molecular mechanisms of inhibition of TS by these analogues. Details of these mechanisms are currently under investigation.

Since the hydrolysis of the CH_2F and CHF_2 substituents leads to the formation of CH_2OH and CHO groups, respectively, it was important to determine the activity of the corresponding nucleoside derivatives. The results clearly show that formation of the 5-hydroxymethyl and 5-formyl derivatives could not account for the inhibitory activities of the respective mono- and difluoromethyl analogues. This indicates that direct inhibition of TS by the corresponding 5′-monophosphates must occur in the cell as was demonstrated for the structurally related FUDR and F_3-TDR.[14]

Inhibition of Thymidine Incorporation into DNA. The potencies of these nucleosides in inhibiting [^{3}H]TDR incorporation into DNA in the HL-60 cells are also compared in Table I. Incorporation of [*methyl*-^{3}H]TDR (1 μCi, 0.02 μM) into the DNA of HL-60 cells during a 30-min period was determined by the procedures described previously.[18] The ID_{50} values were determined with six concentrations of each nucleoside by using the median effect equation[19] and plot[20] with a microcomputer software.[21] The compounds of the series in decreasing order of their potencies are TDR > F_2-TDR > F_3-TDR > FTDR > FMAU > F-FMAU ≫ F_2-FMAU > F_3-FMAU.

Antiherpes Activity. All of these fluoromethyl nucleosides and some of their 3′,5′-diacetates were screened for their antiviral activity against HSV-1 and HSV-2 (Table II). It is interesting to note that FTDR and its diacetate are not active, although F_2-TDR is rather active but not very toxic against human foreskin fibroblasts. F_3-TDR is reported to be antiherpetic and cytotoxic.[9] On the other hand, in the 2′-fluoroarabino series, fluorine substitution at the α-position appears to weaken slightly the antiviral activity. The acetylation of the nucleosides reduced their activity but not significantly. The acetylated derivatives may act as masked precursors which might be hydrolyzed by esterases to release the corresponding active, free nucleosides.

Cytotoxicity. The cytotoxic effects of these nucleosides as measured by HL-60 cell growth inhibition are compared in Table II. The ID_{50} values were determined from six concentrations of each nucleoside based on the median-effect equation[19] and plot[20] with use of a microcomputer

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Table III. ^{1}H NMR Parameters for the New Compounds[a]

	chemical shifts, δ (in $CDCl_3$)									
compd	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	H-6	5-Me	5-CH_2X	5-CHX_2
2	6.29 dd	4.91 dd	4.49 dd	4.06 m	3.65 dd	3.44 dd	7.70 d	1.67 d		
4	6.28 dd	4.91 dd	4.47 dd	4.10 m	3.54 m		7.71 d		4.10 m	
5	6.24 dd	4.91 dd	4.42 dd	4.17 m	3.56 d		8.28 d			9.85 s
6	6.28 dd	4.92 dd	4.47 dd	4.10 m	3.54 m		7.67 d		4.82 d	
7	6.24 dd	4.89 dd	4.43 dd	4.13 m	3.54 m		7.87 d			6.48 t

	coupling constants, Hz									
compd	$J_{1',2'}$	$J_{1',F}$	$J_{2',3'}$	$J_{2',F}$	$J_{3',4'}$	$J_{3',F}$	$J_{4',5'}$	$J_{4',5''}$	$J_{\alpha H,F}$	$J_{\alpha H,6}$
2	3.02	21.54	0	51.59	3.29	18.43	3.57	4.39		
4	3.02	18.38	0	51.59	3.02	17.29				
5	2.68	20.17	0	51.04	2.47	15.65				1.37
6	3.02	20.85	0	51.46	3.29	17.29			48.30	
7	2.74	20.86	0	51.18	2.47	16.19			54.75	1.65

[a] 6 and 7, X = F; 4, X = OH; 5, X_2 = O.

software.[21] The incubation conditions were as described previously.[22] Times given were the period of exposure to the nucleosides. Usually prolonged exposure (e.g., 72 h) produced more potent inhibition than shorter exposure (e.g., 24 h). For the 72-h exposure, in decreasing order of their potencies, the TDR derivatives are F_2-TDR > F_3-TDR > AcF_3-TDR > AcF_2-TDR ≫ AcFTDR > TDR > F-TDR, and the respective FMAU analogues are F_2-FMAU > F_3-FMAU > FMAU > AcF_2-FMAU > AcF_3-FMAU > AcFMAU > F-FMAU > AcF-FMAU. TDR derivatives were generally more potent than the corresponding FMAU analogues.

The cytotoxicities of the α,α-difluoro and α,α,α-trifluoro derivatives F_2-TDR, F_3-TDR, F_2-FMAU, and F_3-FMAU correlate well with their TS inhibitory activities. This strongly suggests that the primary mode of cytotoxic action of these compounds involves the inhibition of DNA biosynthesis at the site of TS.

Imaging Studies. The imaging capability of F_2-FMAU in HSV-1 encephalitis in rat was evaluated as previously described.[3] In short, animals were infected with HSV-1 via the ocular route, and approximately 5 days after infection [2-^{14}C]F_2-FMAU was injected. Animals were sacrificied between 30 min and 6 h later. The brain and various other organs were sectioned, and the radioactivity in sections was determined through quantitative autoradiography. We will report this imaging study in detail elsewhere. However, in brief there was very limited uptake of [2-^{14}C]F_2-FMAU in HSV-infected brain regions in contrast to [2-^{14}C]FMAU and the compound achieved a very brief half-life in blood due to rapid renal excretion. However, even in nephrectomized animals, uptake of compound in infected brain regions was low. We are not fully certain of the reasons for the lack of selective uptake but suspect that it may relate to biological instability which interferes with incorporation of the labeled compound into DNA and hence retention in infected cells. Whatever the reason, our studies unfortunately indicate that the compound is unsuitable as a basis for imaging human HSV encephalitis. We are, therefore, continuing our efforts to assess novel nucleosides as practical imaging probes.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FX90 spectrometer with Me_4Si as the internal standard. Chemical shifts are reported in ppm (δ) and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), dd (double doublets). Values given for coupling constants are first order. Microanalyses were performed by M.H.W. Laboratories and Spang Microanalytical Laboratory. Silica gel TLC were performed on Analtech Uniplates with short-wavelength UV light for visualization. Column chromatography was conducted on flash grade silica gel (Merck 9385, 0.040–0.063 μm).

1-[3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]thymine (2, 3′,5′-Di-*O*-BDPS-FMAU). To a solution of FMAU (0.93 g, 3.57 mmol) and BDPS-Cl (2.79 mL, 10.7 mmol) in DMF (17 mL) was added imidazole (1.07 g, 15.7 mmol), and the mixture was stirred overnight at room temperature. The solvent was removed in vacuo, and the residue partitioned between EtOAc and H_2O (30 mL each). The aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic solutions were dried (Na_2SO_4) and concentrated in vacuo, and the residue was chromatographed on a silica gel column (*n*-hexane–EtOAc, 4:1 and 3:1) to give **2** (2.45 g, 93%) as a colorless foam. The ^{1}H NMR data are given in Table III. Anal. C, H, N.

1-[3,5-Di-O-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]-α-hydroxythymine (4). A mixture of **2** (1.45 g, 1.97 mmol) and NBS (440 mg, 2.47 mmol) in dry CCl_4 (100 mL) was heated under reflux in an N_2 atmosphere and irradiated with 500-W UV lamp for 2 h. The mixture was filtered, and the filtrate was concentrated in vacuo to give crude α-bromide **3** as a foam, which was dissolved in THF (8 mL). A solution of $NaHCO_3$ (198 mg) in H_2O (5 mL) was added, and the mixture was stirred overnight at room temperature and then extracted with $CHCl_3$ (3 × 30 mL). The combined organic extracts were washed (H_2O, 50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed on a silica gel column (*n*-hexane–EtOAc, 5:1 and 3:1) to elute first unreacted **2** (196 mg) followed by **4** (690 mg, 46%), which was obtained as a colorless foam. See Table III for the ^{1}H NMR parameters. Anal. C, H, N.

1-[3,5-Di-O-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-formyluracil (5). To a solution of **4** (0.60 g, 0.80 mmol) in toluene (35 mL) was added active MnO_2 (1.6 g),[8] and the mixture was heated at reflux with stirring for 5 h and then filtered through a Celite pad while hot, and insoluble salts were washed well with $CHCl_3$. The combined filtrate and washings were evaporated, and the residue was chromatographed on a silica gel column (*n*-hexane–EtOAc, 3:1 and 2:1) to give **5** (420 mg, 70%) as a colorless foam. The ^{1}H NMR parameters of **5** are listed in Table III. Anal. C, H, N.

1-[3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-(fluoromethyl)uracil (6). A solution of **4** (386 mg, 0.51 mmole) in dry CH_2Cl_2 (2 mL) under argon atmosphere was added dropwise to a cold solution (−15 °C) of DAST (0.062 mL, 0.51 mmol) in CH_2Cl_2 (1 mL), and the mixture was stirred at −15 °C for 30 min and then at room temperature for 1 h. The mixture was poured onto an ice–water mixture (2 mL). The organic layer was separated, washed (H_2O, 1 mL), dried (Na_2SO_4), and concentrated, and the residue was chromatographed (*n*-hexane–EtOAc, 3:1) to give **6** (275 mg, 71%) as a white foam. See Table III for the ^{1}H NMR data. Anal. C, H, F, N.

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1-[3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-fluoro-β-D-arabinofuranosyl]-5-(difluoromethyl)uracil (7). A solution of **5** (400 mg, 0.53 mmol) in dry CH_2Cl_2 (2 mL) was slowly added under argon atmosphere to a solution of DAST (0.077 mL, 0.64 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred overnight at room temperature and then quenched by addition of H_2O (3 mL). The organic layer was separated, washed (H_2O, 2 mL), dried (Na_2SO_4), and concentrated, and the residue was chromatographed (*n*-hexane–EtOAc, 5:1) to give **7** (290 mg, 70%) as a colorless foam. The ^1H NMR parameters of **7** are reported in Table III. Anal. C, H, F, N.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(fluoromethyl)uracil (8, F-FMAU). To a solution of **6** (200 mg, 0.26 mmol) in dry THF (1 mL) was added under argon a 1 M solution of TBAF in THF (0.58 mL), and the mixture was stirred for 40 min at room temperature and then directly chromatographed on a silica gel column (packed with CH_2Cl_2) with CH_2Cl_2–THF (1:2 v/v) as the eluent. After concentration of the combined UV-absorbing fractions, the residue was crystallized from EtOAc to afford **8** (35 mg, 47%), mp 160 °C sintering, slowly decomposed above 200 °C. The ^1H NMR spectrum of this sample was identical with that of F-FMAU.[2]

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(difluoromethyl)uracil (9, F_2-FMAU). Compound **7** (350 mg, 0.45 mmol) was dissolved in dry THF (1.5 mL) and 1 M TBAF in THF (0.83 mL) was added under argon atmosphere. The mixture was stirred at room temperature for 45 min and then placed on a silica gel column (packed with CH_2Cl_2) and eluted with CH_2Cl_2–THF (1:2 v/v). The product was crystallized from $CHCl_3$ to give **9** (77 mg, 57%), mp 175 °C dec. The ^1H NMR spectrum of this sample was identical with that of F_2-FMAU.[2]

1-(2-Deoxy-2-fluoro-3,5-di-*O*-trityl-β-D-arabinofuranosyl)-5-iodouracil (11, 3′,5′-Di-*O*-trityl-FIAU). A mixture of FIAU[24] (1 g, 2.83 mmol) and TrCl (1.74 g, 6.23 mmol) in pyridine (10 mL) was heated at 100 °C for 4 h. An additional charge of TrCl (1.7 g) was added and the mixture was stirred overnight at room temperature and then heated at 100 °C for 4 h. The mixture was poured into ice water, and the gummy precipitate was dissolved in $CHCl_3$ (20 mL), washed with 5% $CdCl_2$ and H_2O, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by silica gel column chromatography (*n*-hexane–EtOAc, 3:1) to give **11** (1.45 g, 60% after crystallization from CH_2Cl_2–MeOH (100:1), mp 137–140 °C. Anal. C, H, N.

1-(2-Deoxy-2-fluoro-3,5-di-*O*-trityl-β-D-arabinofuranosyl)-5-(trifluoromethyl)uracil (12, 3′,5′-Di-*O*-trityl-F_3-FMAU). To a solution of **11** (0.5 g, 0.58 mmol) in dry HMPA (10 mL) were added powdered Cu (1.75 g) and CF_3I (3.36 g). The mixture was stirred in a stainless steel container for 40 h at 110 °C. The mixture was allowed to cool to room temperature, diluted with ice water, and extracted with EtOAc–Et_2O (1:1). The combined extracts were dried (Na_2SO_4) and concentrated in vacuo, and the residue was chromatographed on a silica gel column (*n*-hexane–EtOAc, 4:1) to give **12** (283 mg, 61%, after crystallization from MeOH–H_2O), mp 125–128 °C. Anal. C, H, F, N.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(trifluoromethyl)uracil (13, F_3-FMAU). A solution of **12** (160 mg, 0.70 mmol) in 80% AcOH (12 mL) was heated under reflux for 10 min and then concentrated in vacuo. Traces of AcOH were removed azeotropically by several coevaporations with toluene. The product was separated by TLC (EtOAc as the eluent) and crystallized from Me_2CO–*n*-hexane to give **13** (40 mg, 64%), mp 200–201 °C. Anal. C, H, F, N.

1-(3,5-Di-*O*-acetyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-(trifluoromethyl)uracil (14, 3′,5′-Di-*O*-acetyl-F_3-FMAU). A mixture of **13** (30 mg, 0.10 mmol) and Ac_2O in pyridine (1 mL) was kept at room temperature for 4 h and then concentrated in vacuo, and the residue was crystallized from CH_2Cl_2–*n*-hexane to give **14** (30 mg, 79%), mp 117–119 °C. Anal. C, H, F, N.

[2-^{14}C]F_2-FMAU. To a mixture of [2-^{14}C]thymine[25] (**15**, 100 mCi, 227 mg, 1.80 mmol), cold thymine (571 mg, 4.53 mmol), $(NH_4)_2SO_4$ (74 mg), and $Cl(CH_2)_2Cl$ (25 mL) was added HMDS (1.46 mL, 6.94 mmol). The mixture was heated at reflux for 1 h and then cooled to room temperature followed by removal of NH_3 in vacuo (30 mmHg, 20 min). To this mixture was added a solution of 3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide[11] (2.28 g, 6.32 mmol) in $Cl(CH_2)_2Cl$ (9 mL). The mixture was heated at reflux for 2.5 h. The reaction was quenched by addition of ice water (50 mL) and then extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with H_2O (30 mL), dried (Na_2SO_4), and concentrated to give crude, protected [2-^{14}C]FMAU (**17**, 2.4 g), which was dissolved in saturated NH_3–MeOH (200 mL). After 3 days at room temperature, the mixture was concentrated in vacuo. The residue was partitioned between H_2O and CCl_4 (50 mL each). The aqueous layer was washed (CCl_4) and concentrated in vacuo, and the residue was triturated with MeOH. [2-^{14}C]FMAU (**18**, 447 mg, 27%) crystallized was collected by filtration. An additional crop (514 mg) of **18** was obtained from the mother liquor (combined yield, 58% from thymine).

To a solution of **18** (553 mg, 2.13 mmol) and BDPSCl (1.66 mL, 6.38 mmol) in dry DMF (10 mL) was added imidazole (638 mg, 9.37 mmol), and the mixture was stirred for 24 h at room temperature. The solvent was removed in vacuo, and the residue partitioned between CH_2Cl_2 and H_2O (10 mL each). The aqueous layer was washed with CH_2Cl_2 (2 × 10 mL), the combined organic solutions were dried (Na_2SO_4) and concentrated, and the residue was chromatographed on a column of silica gel (*n*-hexane–EtOAc, 5:1) to give 3′,5′-di-*O*-BDPS-[2-^{14}C]FMAU (**19**, 1.46 g, 93%), which was dissolved in CCl_4 (150 mL). NBS (440 mg, 2.47 mmol) was added, and the mixture was heated at reflux for 2 h under N_2 while being irradiated with a 500-W Hg lamp and then concentrated in vacuo. To the residue (mainly the 3′,5′-di-*O*-BDPS-5-bromomethyl-[2-^{14}C]FAU (**20**) and imidazole) was added THF (8 mL) and $NaHCO_3$ (198 mg in 5 mL of H_2O), and the mixture was stirred overnight at room temperature and then extracted with $CHCl_3$ (3 × 30 mL). The combined extracts were dried (Na_2SO_4) and concentrated in vacuo, and the residue was chromatographed (*n*-hexane–EtOAc, 5:1) to give 306 mg of **18** and 690 mg of 3′,5′-di-*O*-BDPS-5-hydroxymethyl-[2-^{14}C]FAU (**21**). The recovered **18** was treated with NBS (93 mg) in 32 mL of CCl_4 as described above, and an additional amount (60 mg) of **21** was obtained.

A mixture of **21** (750 mg, 1.00 mmol) and active MnO_2 (2.0 g) in toluene (40 mL) was heated under reflux with stirring for 5 h and then filtered through a Celite pad while hot. The MnO_2 was washed well with $CHCl_3$. The combined filtrate and washings were concentrated, and the residue was chromatographed (*n*-hexane–EtOAc, 5:1) to give 3′,5′-di-*O*-BDPS-5-formyl-[2-^{14}C]FAU (**22**, 490 mg), which was dissolved in dry CH_2Cl_2 (2 mL). To the solution was added under argon atmosphere a solution of DAST (0.094 mL) in dry CH_2Cl_2 (1 mL), and the mixture was stirred overnight at room temperature. Ice water (3 mL) was added, and the organic layer was separated, washed (H_2O, 3 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed (*n*-hexane–EtOAc, 5:1) to give 350 mg of 3′,5′-di-*O*-BDPS-F_2-[2-^{14}C]FMAU (**23**).

To a solution of **23** (350 mg) in THF (1 mL) was added 1 M TBAF in THF (1.8 mL), and the mixture was stirred at room temperature for 30 min. After removal of the solvent in vacuo, the residue was chromatographed (on a silica gel column packed with CH_2Cl_2) with CH_2Cl_2–THF (1:2 v/v) to give [2-^{14}C]F_2-FMAU (**24**) (77 mg, after crystallization from $CHCl_3$), mp 175 °C dec.

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Substrate Analogue Inhibitors of the IgA1 Proteinases from *Neisseria gonorrhoeae*

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Substrate analogues based on the amino acid sequence of the hinge region of human IgA1 around the cleavage site of the IgA1 proteinases secreted by *Neisseria gonorrhoeae* are competitive inhibitors of these enzymes. The octapeptide Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser, which occurs between residues 233 and 240, has an IC_{50} value of 0.26 mM for the type 1 proteinase and 0.50 mM for the type 2 enzyme. Acetylation of the octapeptide N-terminal amino group lowers affinity for the type 1 proteinase sixfold but does not change binding to the type 2 enzyme. Amidation of the C-terminal carboxyl group does not change binding to the type 1 proteinase but improves IC_{50} for the type 2 enzyme. Simultaneous blockade of both the N- and C-termini drastically lowers affinity of the octapeptide for both proteinases. Sequential replacement of the hydroxy amino acids in the blocked octapeptide with cysteine yields a series of inhibitors that generally bind to the neisserial IgA1 proteinases as well as or better than the unblocked octapeptide. The most effective inhibitor contains a cysteine residue at position 6 (P_3') and has an IC_{50} value for the type 2 IgA1 proteinase of 50 μM. Dimerization of the cysteine-containing octapeptides significantly diminishes inhibitory properties. The substrate analogues described here are the first synthetic inhibitors of the neisserial IgA1 proteinases to be reported.

Pathogenic members of the genera *Neisseria*, *Hemophilus*, and *Streptococci* secrete highly specific proteolytic enzymes (EC 3.4.24.13), which inactivate human IgA1 by cleavage of hinge-region peptide bonds on the C-terminal side of specific prolyl residues. Cleavage occurs within a 16-residue sequence formed by duplication of the octapeptide Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser.[1] The only known substrate for these proteinases is IgA1 from humans and great apes,[2] although isolated human IgA1 heavy chain (α-chain) is reported to be slowly cleaved by an IgA1 proteinase from *Hemophilus*.[3]

Strains of *N. gonorrhoeae* produce one of two related proteinases (type 1 or type 2) that cleave IgA1 at slightly different positions (Figure 1). The IgA1 proteinase secreted by *S. sanguis* is inhibited by EDTA;[4] while an IgA1 proteinase isolated from *B. melaninogenicus* is blocked by reagents that inhibit cysteine proteases.[5]

Previous research[6,7] indicated that synthetic peptides homologous with the amino acid sequence of IgA1 between residues 225 and 240 could inhibit the type 2 proteinase from *N. gonorrhoeae*. IC_{50} values of a more complete set of substrate analogue inhibitors for both the type 2 and type 1 proteinases are reported here.

Results

The amino acid sequence and IC_{50} values of the substrate analogue inhibitors for the neisserial proteinases are given in Table I. These values are different from those given in a preliminary report[7] and reflect development of a more consistent assay. Previously reported IC_{50} values from this laboratory should be viewed with this in mind.

Both the hexadecapeptide (HRP-1) and the octapeptide (HRP-2) inhibit the IgA1 proteinases from *N. gonorrhoeae* in the high micromolar range. Amidation of the C-terminal carboxyl group in the octapeptide (HRP-75) slightly decreases binding to the type 1 enzyme while increasing affinity for the type 2 proteinase. Acetylation of the N-terminus does not markedly change affinity for the type 2 proteinase but increases the IC_{50} value for the type 1 enzyme sixfold. Simultaneous blockade of both the N- and C-terminal residues (HRP-25) increases the IC_{50} value

Table I. Inhibition of the Neisserial IgA1 Proteinases

		IC_{50}, mM	
		type 1	type 2
HRP-1	Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser	0.31	[a]
HRP-2	Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser	0.26	0.50
HRP-25	Ac———NH_2	9.35	6.68
HRP-59	Ac———	1.57	0.51
HRP-75	———NH_2	0.40	0.18
HRP-18	(Ac———Cys-NH_2)$_2$	[b]	3.30
HRP-19	(Ac———Cys——NH_2)$_2$	5.15	[b]
HRP-20	(Ac———Cys———NH_2)$_2$	0.20[c]	0.17[c]
HRP-21	(Ac-Cys———NH_2)$_2$	1.81	3.45
HRP-61	Ac———Cys-NH_2	1.03	0.49
HRP-62	Ac———Cys——NH_2	0.20	0.05
HRP-63	Ac———Cys———NH_2	ND[d]	ND[d]
HRP-64	Ac-Cys———NH_2	0.31	0.12

[a] 19% inhibition at 0.30 mM. [b] 0% inhibition at >2 mM. [c] Assayed in 40% TFE. [d] ND: not determined because of poor solubility. Addition of adequate TFE to solubilize HRP-63 denatures the IgA1 proteinases.

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