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Synthesis and antiviral activity evaluation of 3′-fluoro-3′-deoxyribonucleosides: broad-spectrum antiviral activity of 3′-fluoro-3′-deoxyadenosine

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Summary

Five 3′-fluorinated ribonucleosides were prepared and evaluated for their inhibitory properties against different viruses. The synthesis of these compounds was achieved by treatment of 2′,5′-di-*O*-tritylated nucleoside analogues possessing a *xylo*-configuration with diethylaminosulfur trifluoride, followed by deprotection. 3′-Fluoro-3′-deoxyadenosine was active against a broad range of viruses, encompassing both DNA viruses [pox (vaccinia)], single-stranded (+) RNA viruses [picorna (polio, Coxsackie B), toga (sindbis, Semliki Forest)] and double-stranded RNA viruses (reo). In its antiviral activity spectrum 3′-fluoro-3′-deoxyadenosine clearly differed from those adenosine analogues that are known as inhibitors of *S*-adenosylhomocysteine hydrolase. 3′-Fluoro-3′-deoxyadenosine also proved effective in vivo, in inhibiting tail lesion formation in mice inoculated intravenously with vaccinia virus.

3′-Fluoro-3′-deoxyribonucleoside; 3′-Fluoro-3′-deoxyadenosine; Adenosylhomocysteine hydrolase, *S*; Vaccinia virus; Picorna virus; Reovirus

Introduction

Examples of broad-spectrum antiviral agents are ribavirin and those compounds that act via inhibition of *S*-adenosylhomocysteine (SAH) hydrolase. Inhibition of SAH hydrolase leads to accumulation of SAH and suppression of *S*-adenosylmethionine-dependent methyltransferase reactions. When such transmethylations are

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required for the maturation of viral mRNA, inhibition of SAH hydrolase may lead to an inhibitory effect on virus replication. SAH hydrolase inhibitors have proved to be active against a variety of DNA viruses [i.e. pox (vaccinia)] and RNA viruses [rhabdo (vesicular stomatitis), paramyxo (parainfluenza), reo (reo, rota)]. Most of these viruses are known to possess their own methyltransferases.

The reaction mechanism of SAH hydrolase has been elucidated (Palmer and Abeles, 1979). This involves an oxidation of the 3′-hydroxyl group of SAH followed by elimination of homocysteine and formation of 3′-*keto*-adenosine by a Michael type addition. Finally the 3′-*keto* group is reduced yielding adenosine. SAH hydrolase inhibitors showing antiviral activity include the acyclic adenosine analogues (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA] and oxidized 2′,3′-*seco*adenosine, and the carbocyclic adenosine analogues carbocyclic 3-deazaadenosine (C-c^{3}Ado), neplanocin A and 3-deazaneplanocin A (De Clercq, 1987, 1988).

In the pursuit of adenosine analogues as SAH hydrolase inhibitors, we reasoned that the presence of a fluorine atom instead of the hydroxyl group in the 3′-*ribo* configuration would give minimal perturbation to the adenosine molecule, and that such type of compounds may interfere with the SAH hydrolase reaction.

Literature search reveals few reports on the synthesis of 3′-fluoro-substituted 3′-deoxynucleosides. In particular, 3′-fluoro-2′,3′-dideoxynucleosides have been synthesized [Herdewijn et al. (1989) and references cited therein]. Among the possible 3′-fluoro-3′-deoxynucleosides, the 1-(3-fluoro-3-deoxy-β-D-*xylo*furanosyl)cytosine (Wright et al., 1970) and its adenine counterpart (Robins et al., 1974; Wright and Taylor, 1968) have been synthesized, as well as the 3′-fluoro*arabino*-nucleosides of uracil (Misra et al., 1984), cytosine (Kowollik and Langen, 1975) and adenine (Miyai et al., 1972). Of the *ribo*furanosyl counterparts, only the 3′-fluoro-3′-deoxyuridine has been reported (Kowollik et al., 1975; Misra et al., 1984).

Numerous attempts have been undertaken to prepare ribonucleosides with a 3′-fluorine substituent. A commonly encountered side reaction during nucleophilic displacement reactions with charged nucleophiles at the 3′-position of *xylo*furanoses is the elimination reaction. The extent at which this elimination reaction occurs is dependent on (a) the electrostatic and steric interactions of the approaching nucleophile and the 2′-*O*-protecting group, (b) the geometry of the furanose, (c) the basicity of the nucleophile and (d) the nature of the leaving group at the 3′-position. Recently we have found that a trityl group at the 2′-*O*-position allowed nucleophilic displacement at the 3′-position with limited side reaction (Herdewijn et al., 1989).

A synthesis of 3′-fluoro-3′-deoxyadenosine (Herdewijn et al., 1989) has been recently described. Tritylation of *xylo*adenosine yielded the tritylated material *13*. Treatment of the latter with diethylaminosulfur trifluoride (DAST) followed by detritylation gave the desired 3′-fluorinated nucleoside *15*. This strategy has now been extended to the synthesis of other 3′-fluorinated 3′-deoxy*ribo*nucleosides.

The cytostatic activity of 3′-fluoro-3′-deoxyadenosine (*15*) in different cell systems has been the subject of a previous report (Van Aerschot et al., 1989). Here we describe the results of the antiviral assays carried out with 3′-fluoro-3′-deoxyadenosine and related 3′-fluorinated 3′-deoxy*ribo*nucleosides (Tables 2–4).

TABLE 1

Electron impact mass spectra of the 3′-fluorinated 3′-deoxyribonucleosides

Compound	M	M-H_2O	M-CH_2O	HBCH-=CH=CHF	HBCH-=CHOH	BCHOH	BCH_2	H_2B	HB	S
4	246(11)	228(23)	216(6)	169(9)	155(2)	141(36)	–	113(100)	112(44)	135(46)
5	245(2)	227(2)	215(5)	–	154(12)	140(29)	–	112(100)	111(31)	135(1)
10	260(13)	242(1)	230(2)	183(4)	–	155(5)	–	127(29)	126(100)	135(11)
15	269(11)	–	239(18)	192(2)	178(42)	164(60)	148(10)	136(70)	135(100)	135(100)
16	270(12)	–	240(6)	193(4)	179(12)	165(15)	149(5)	137(33)	136(100)	135(6)

Main fragment ions in the different spectra are given with relative intensities indicated in parentheses. B = base fragment; S = sugar fragment.

Materials and Methods

Synthesis of the compounds

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Beckman UV 5230 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90A spectrometer with tetramethylsilane as internal standard (s=singlet; d=doublet; t=triplet; m=multiplet; br=broad signal). Electron impact mass spectra (70 eV) were recorded on a AEI-MS12 mass spectrometer. Elemental analyses were carried out by dr. Rozdzinksi at the Institute für Organische Chemie in Stuttgart. Precoated Merck silica gel F254 plates were used for TLC. Column chromatography was performed on Merck silica gel (0.063–0.200 mm). Pyridine was dried by distillation after it had been refluxed on potassium hydroxide for 24 h. Dichloromethane and dichloroethane were dried with calcium chloride and distilled from phosphorus pentoxyde. Diethylaminosulfur trifluoride was bought from Janssen Chimica and used without purification. Acetic acid was distilled on chromium trioxide.

Antiviral assays in vitro

The origin of the viruses was as follows: herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G) (De Clercq et al., 1980); vaccinia virus, vesicular stomatitis virus, Coxsackie virus type B4, sindbis virus and poliovirus type 1 (De Clercq et al., 1975); reovirus type 1 (ATCC VR-230), Semliki Forest virus (ATCC VR-67), and parainfluenza virus type 3 (ATCC VR-93) (American Type Culture Collection, Rockville, MD). The virus stocks were prepared in primary rabbit kidney cells (herpes simplex virus types 1 and 2, vaccinia virus, and vesicular stomatitis virus), VERO cells (parainfluenza virus type 3, sindbis virus, reovirus, Coxsackie virus type B4, and Semliki Forest virus), HeLa cells (poliovirus type 1, Coxsackie virus type B4 and vesicular stomatitis virus) and L929 cells (vaccinia virus). Inhibition of virus-induced cytopathogenicity in vitro and cytotoxicity measurements were performed as described previously (De Clercq, 1985).

Inhibition of SAH hydrolase

SAH hydrolase was purified from murine L929 cells to apparent homogeneity (Cools et al., 1988) and its activity was measured in the synthetic direction using 3 μM [8-^{14}C]adenosine and 2 mM D,L-homocysteine as substrate (Cools and De Clercq, 1989).

Antiviral assay in vivo

The in vivo activity against vaccinia virus was assessed in the pox tail lesion model. Young (25-day-old) NMRI mice, weighing 11–13 g, were inoculated intra-

venously (in a tail vein) with 0.2 ml of a vaccinia virus dilution containing approximately 5×10^4 PFU/ml. The mice were treated intraperitoneally twice daily for five successive days, starting 1 h prior to infection. Tail lesions were counted at 7 days after infection. The vaccinia virus stock used for the animal experiments originated from calf lymph, provided by the Rijksentstofinrichting (Brussels, Belgium). Its titer was 1.5×10^8 PFU/ml; it was stored at 4°C.

Results

Chemistry

N^3-Benzoyl-2',5'-di-O-benzoyl-3'-fluoro-3'-deoxyuridine (2), 2',5'-di-O-benzoyl-3'-fluoro-3'-deoxyuridine (3) After coevaporation with anhydrous benzene, 5.83 g (8 mmol) of 1-(2,5-di-*O*-trityl-β-D-*xylo*furanosyl)uridine (*1*) (Yung and Fox, 1961) was dissolved in 200 ml of dichloromethane and 2.2 ml (16 mmol) of DAST was added. After 1 h at room temperature TLC ($CHCl_3$-acetone 9:1) indicated complete reaction and the mixture was poured into 300 ml of a 10% $NaHCO_3$ solution. After separation of the layers, the organic phase was washed with 300 ml of a 10% $NaHCO_3$ solution and the water phases were extracted once more with 200 ml of $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated. The residue was treated with 200 ml of 80% aqueous acetic acid for 1 h at 90°C. After evaporation, the residue was dissolved in 150 ml of water and washed twice with 200 ml of Et_2O. The water layer was evaporated and the residue was purified on silica gel. A mixture of *4* and uracil was eluted from the column. To this mixture, dissolved in 100 ml of anhydrous pyridine, 6 ml (40 mmol) of benzoyl chloride was added. After 2 h at room temperature, the mixture was concentrated and diluted with 150 ml of $CHCl_3$. The solution was washed twice with 150 ml of water, dried (Na_2SO_4) and evaporated. Purification on 60 g of silica gel [(1 $CHCl_3$; 2) $CHCl_3$-acetone 9:1] yielded 491 mg (0.88 mmol, 11%) of N^3-benzoyl-2',5'-di-*O*-benzoyl-3'-fluoro-3'-deoxyuridine *2* as a foam.

UV (MeOH) λ_{max} 232 and 253 (shoulder) nm; MS m/e 558 (M^+); ^1H NMR ($CDCl_3$)δ: 4.55–4.90 (m, H-4', H-5', H-5''), 5.4–5.6 (m, H-2'), 5.48 (dm, $J_{3',F}$=53 Hz, H-3'), 5.69 (d, J=8.3 Hz, H-5), 6.28 (d, J=6.6 Hz, H-1'), 7.2–8.2 (arom. + H-6)ppm; ^{13}C NMR ($CDCl_3$)δ: 63.2 (d, J=8.6 Hz, C-5'), 73.6 (d, J=15.9 Hz, C-2'), 80.7 (d, J=24.6 Hz, C-4'), 87.5 (C-1'), 89.5 (d, J=191.6 Hz, C-3'), 103.2 (C-5), 139.2 (C-6), 149.0 (C-2), 161.3 (C-4) ppm + arom. signals.

Further elution gave 472 mg (1.04 mmol, 13%) of 2',5'-di-*O*-benzoyl-3'-fluoro-3'-deoxyuridine *3* as a foam.

UV (MeOH)λ_{max} 232 and 257 (broad) nm; MS m/e 454 (M^+); ^1H NMR ($CDCl_3$)δ: 4.55–4.90 (m, H-4', H-5', H-5''), 5.45 (dm, $J_{2',F}$=18 Hz, H-2'), 5.47 (dm, $J_{3',F}$=54 Hz, H-3'), 5.58 (d, J=8 Hz, H-5), 6.30 (d, J=6.5 Hz, H-1'), 7.31 (d, J=8 Hz, H-6), 7.3–7.6 and 7.9–8.15 (arom.), 9.5 (br, NH)ppm; ^{13}C NMR ($CDCl_3$)δ: 63.3 (d, J=8.5 Hz, C-5'), 73.4 (d, J=14.7 Hz, C-2'), 80.6 (d, J=24.4 Hz, C-4'), 87.0 (C-1'), 88.9 (d, J=191.7 Hz, C-3'), 103.4 (C-5), 139.4 (C-6), 150.0 (C-2), 162.7 (C-4) ppm + aromatic signals.

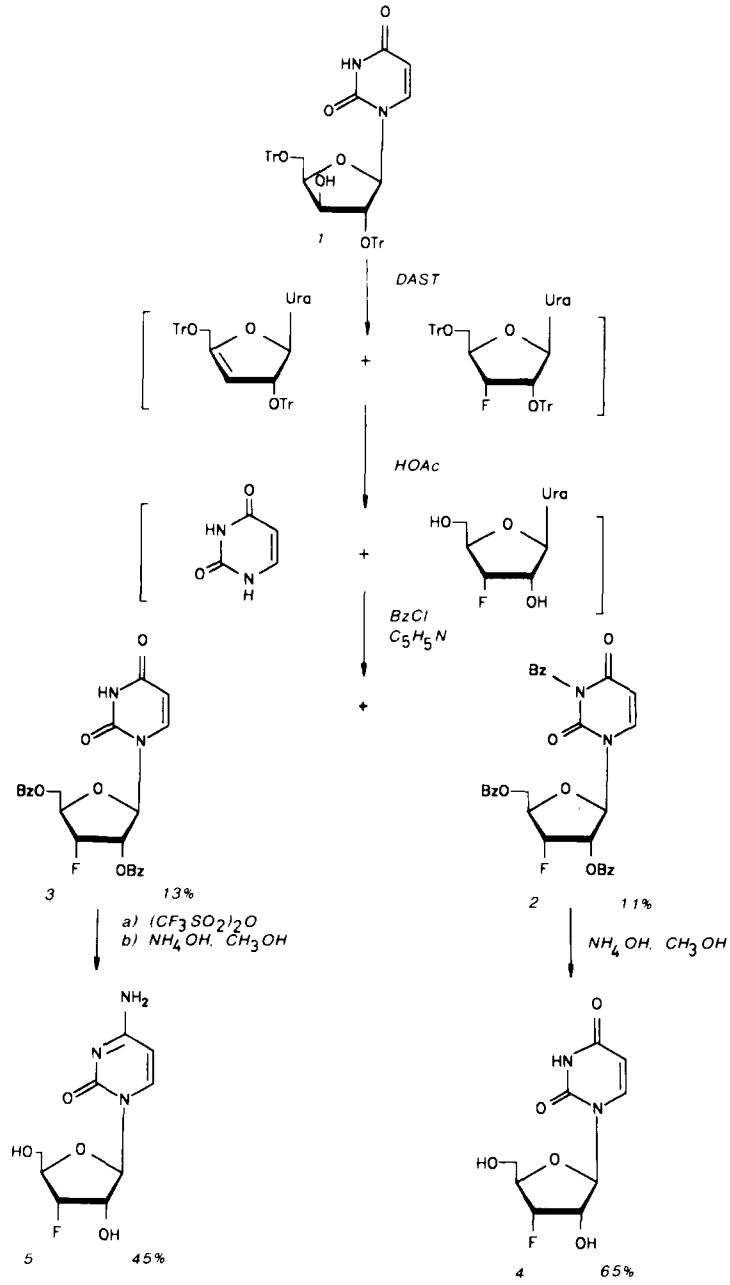

Fig. 1. Synthesis of 3′-fluoro-3′-deoxyuridine (*4*) and of 3′-fluoro-3′-deoxycytidine (*5*).

3'-Fluoro-3'-deoxyuridine (4) The product *2* (491 mg, 0.88 mmol) obtained in the previous preparation was treated overnight with 50 ml of MeOH saturated with ammonia. The mixture was evaporated, adsorbed on 4 g of silica gel and purified on 30 g of silica gel (elution with a gradient from $CHCl_3$-MeOH 95:5 to 9:1). The fractions containing the desired product were pooled and evaporated affording 150 mg (0.57 mmol, 65%) of the title compound which crystallized from MeOH-EtOAc as white needles.

mp 198–199°C [lit. (Misra et al., 1984) 195–196°C and (Kowollik et al., 1975) 196–197°C]; UV (MeOH)λ_{max} 260 nm (ϵ=9100); ^{1}H NMR (DMSO-d_6)δ: 3.62 (d, J=3.5 Hz, H-5', H-5''), 3.95–4.40 (m, H-2', H-4'), 4.96 (dd, J=4 Hz, $J_{3',F}$=55.0 Hz, H-3'), 5.26 (br, 5'-OH), 5.71 (d, J=8 Hz, H-5), 5.85 (br, 2'OH), 5.90 (d, J=7.9 Hz, H-l'), 7.84 (d, J=8 Hz, H-6), 11.3 (br, NH)ppm; ^{13}C NMR (DMSO-d_6)δ: 61.1 (d, J=11 Hz, C-5'), 72.5 (d, J=15.9 Hz, C-2'), 83.6 (d, J=22 Hz, C-4'), 86.5 (C-l'), 93.2 (d, J=181.9 Hz, C-3'), 103.1 (C-5), 141.0 (C-6), 151.4 (C-2), 163.8 (C-4) ppm; *anal.* calcd. for $C_9H_{11}FN_2O_5$: C, 43.91; H, 4.50; N, 11.38. Found: C, 43.77; H, 4.51; N, 11.27.

3'-Fluoro-3'-deoxycytidine (5) A portion of 330 mg (0.72 mmol) of 2',5'-di-*O*-benzoyl-3'-fluoro-3'-deoxyuridine (*3*) was coevaporated with pyridine. To the dried residue was added 15 ml of dichloroethane and 3 ml of pyridine. The solution was cooled on an ice bath and 3 ml of a 10% solution of trifluoromethanesulfonic anhydride in dichloroethane was added dropwise. After 2 h at room temperature the mixture was poured in 100 ml of MeOH saturated with ammonia and the resulting brown solution was left for 15 h at room temperature. Evaporation yielded a dark brown oil which was purified twice on 20 g of silica gel (after adsorption on 4 g of silica gel). Yield: 80 mg (0.32 mmol, 45%) of *5* as a light yellow oil of which 45 mg crystallized from MeOH-EtOAc.

mp: 217–218°C; UV (MeOH)λ_{max} 241 nm (ϵ =7500), 271 nm (ϵ =7800); ^{1}H NMR (DMSO-d_6)δ: 3.59 (d, J=3.3 Hz, H-5', H-5''), 3.95–4.53 (m, H-4', H-2'), 4.95 (dm, $J_{3',F}$=54.5 Hz, H-3'), 5.26 (br, 5'-OH), 5.85 (d, J=7.4 Hz, H-5), 5.96 (d, J=7.5 Hz, H-1'), 5.90 (br, 2'-OH, under H-1' and H-5, exchangeable D_2O), 7.4 (br, NH_2), 7.79 (d, J=7.7 Hz, H-6) ppm; ^{13}C NMR (DMSO-d_6)δ: 61.1 (d, J=12.2 Hz, C-5'), 72.6 (d, J=14.7 Hz, C-2'), 83.2 (d, J=22.0 Hz, C-4'), 87.7 (C-1'), 93.1 (d, J=181.9 Hz, C-3'), 95.6 (C-5), 142.1 (C-6), 156.2 (C-2), 165.6 (C-4) ppm; *anal.* calcd. for $C_9H_{12}FN_3O_4$: C, 44.08; H, 4.93; N, 17.14. Found: C, 44.28; H, 5.06; N, 16.89.

1-(3,5-Di-O-benzoyl-β-D-xylofuranosyl)thymine (6) An amount of 13.12 g (25.8 mmol) of 1-(2-*O*-acetyl-3,5-di-*O*-benzoyl-β-D-*xylo*furanosyl)thymine was dissolved in 250 ml of pyridine-acetic acid (4:1) and 3.75 ml (77 mmol) of hydrazine hydrate was added. The mixture was stirred overnight at room temperature after which TLC ($CHCl_3$-acetone 8:2) indicated complete deacetylation. An amount of 75 ml of acetone was added and the mixture was stirred for another 2 h. After evaporation the residue was dissolved in EtOAc and washed twice with a saturated $NaHCO_3$ solution and with brine. The water phases were extracted twice with

EtOAc. Chromatographic purification of the combined organic phases on silica gel ($CHCl_3$ to $CHCl_3$-MeOH 98:2) afforded 10.3 g (22.08 mmol, 85%) of the title compound as a foam [during the course of this work this compound was described (Robins et al., 1988)].

UV (MeOH)λ_{max} 230 and 269 nm, λ_{min} 252 nm; MS m/e 466 (M^+), 341 (s), 127 (B+2H), 126 (B+H); ^{1}H NMR ($CDCl_3$) δ: 1.82 (s, CH_3), 4.52 (s, H-4′), 4.76 (m, H-5′, H-5″), 4.92 (m, H-2′), 5.59 (d, H-3′), 5.72 (br, 2′-OH), 5.89 (s, H-1′), 7.2–8.0 (m, aromatic H, H-6), 10.55 (br, NH) ppm; ^{13}C NMR ($CDCl_3$) δ: 12.1 (CH_3), 61.5 (C-5′), 76.9 (C-2′), 79.6 and 80.1 (C-3′, C-4′), 92.4 (C-1′) ppm + aromatic signals.

1-(2-0-Trityl-β-D-xylofuranosyl)thymine (7) After coevaporation with anhydrous pyridine, 10.3 g (22.08 mmol) 1-(3,5-di-*O*-benzoyl-β-D-*xylo*furanosyl)thymine (*6*) was dissolved in 200 ml anhydrous pyridine and 12.5 g (45 mmol) trityl chloride was added. The mixture was stirred for 48 h at 90°C and concentrated. After addition of 500 ml of $CHCl_3$ the organic layer was washed twice with 500 ml of a 10% $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated. The residue was dissolved in 300 ml anhydrous toluene-MeOH (2:1) and approximately 0.5 g of sodium was added. After 30 min at room temperature the solution was neutralized by addition of Dowex AG50W-X8 resin and filtered. The filtrate and washings (acetone) were evaporated and the residue was purified on 200 g of silica gel [elution 1) $CHCl_3$; 2) $CHCl_3$-acetone 9:1; 3) $CHCl_3$-acetone 8:2] affording 10.33 g (20.75 mmol, 94%) of *7* as a foam.

UV (MeOH)λ_{max} 230 and 267 nm, λ_{min} 244 nm; ^{1}H NMR ($CDCl_3$) δ: 1.73 (d, J=1.3 Hz, CH_3), 3.72 (d, J=3 Hz, H-3′), 3.95 (m, H-5′, H-5″), 4.06 (m, H-4′), 4.32 (d, J=2 Hz, H-2′), 5.77 (d, J=2 Hz, H-1′), 7.05 (d, J=1.3 Hz, H-6), 7.1–7.5 (m, trityl), 9.1 (br, NH)ppm; ^{13}C NMR ($CDCl_3$) δ: 12.0 (CH_3), 60.5 (C-5′), 76.9 (C-3′), 80.3 (C-2′), 83.2 (C-4′), 88.6 (Ph_3C), 93.1 (C-1′), 110.7 (C-5), 138.6 (C-6), 150.0 (C-2), 163.7 (C-4) ppm.

1-(2,5-Di-O-trityl-β-D-xylofuranosyl)thymine (8) A portion of 10.2 g (20.37 mmol) of *7* was coevaporated twice with pyridine and to the oil was added 200 ml of anhydrous pyridine and 11.1 g (40 mmol) of trityl chloride. The mixture was heated for 15 h at 60°C and concentrated. After addition of 300 ml of $CHCl_3$, the organic

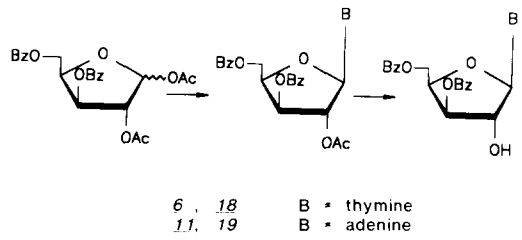

Fig. 2. Sugar-base condensation of the protected xylofuranosyl derivatives of adenine and thymine, followed by selective 2′-deprotection.

layer was washed with a 10% $NaHCO_3$ solution and with water (300 ml each). The organic layer was dried (Na_2SO_4), evaporated and purified on 200 g of silica gel [elution: 1) cyclohexane-$CHCl_3$ 1:1, 2) $CHCl_3$-acetone 9:1]. The title product *8* was isolated as a foam (13.77 g, 18.53 mmol, 91%).

UV ($CHCl_3$)λ_{max} 230 and 265 nm, λ_{min} 246 nm; ^{1}H NMR ($CDCl_3$) δ: 1.78 (d, J=1.5 Hz, CH_3), 3.02 (d, J=5 Hz, H-5′), 3.33 (m, H-3′), 3.43 (d, J=5 Hz, H-5″), 4.07 (m, H-4′), 4.20 (d, J=1.7 Hz, H-2′), 5.94 (d, J=1.7 Hz, H-1′), 7.10 (d, J=1.5 Hz, H-6), 7.1–7.5 (m, trityl), 8.34 (br, NH)ppm; ^{13}C NMR ($CDCl_3$) δ: 12.2 (CH_3), 61.8 (C-5′), 76.2 (C-3′), 80.0 (C-2′), 83.5 (C-4′), 87.3 and 88.6 (2 Ph_3C), 92.7 (C-1′), 110.8 (C-5), 138.2 (C-6), 150.0 (C-2), 163.4 (C-4)ppm + trityl signals.

1-(3-Fluoro-3-deoxy-β-D-ribofuranosyl)thymine (10) A portion of 5.96 g (8.02 mmol) of *8* was coevaporated twice with anhydrous benzene and dissolved in 250 ml of dichloromethane. To this solution 2.2 ml (16 mmol) of DAST was added at room temperature and the solution was stirred for 20 min. TLC ($CHCl_3$-acetone 95:5) revealed two major products of which the lower one had approximately the same mobility as the starting product. Prolonged reaction did not alter the ratio of products and after 3 h the mixture was poured into 250 ml of a 10% $NaHCO_3$ solution and stirred for 15 min. Both layers were separated, the water layer was extracted with 150 ml of dichloromethane and the combined organic layers were dried and evaporated. After two chromatographic purifications 2.68 g (3.69 mmol, 46%) of what we believe to be the 3′,4′-elimination product and 2.03 g (2.72 mmol, 34%) of 1-(2,5-di-*O*-trityl-3-fluoro-3-deoxy-β-D-*ribo*furanosyl)thymine (*9*) was obtained as a foam.

UV ($CHCl_3$) λ_{max} 230 and 266 nm; ^{1}H NMR ($CDCl_3$) δ: 1.30 (s, CH_3), 3.33 (dd, J=4 Hz, $J_{3',F}$=55 Hz, H-3′), 3.93 (m, H-5′), 4.26 (m, H-5″), 4.44 (ddd, J=8.2 Hz and 4 Hz, $J_{2',F}$=20 Hz, H-2′), 6.53 (d, J=8.2 Hz, H-1′), 7.05–7.65 (m, trityl, and H-6), 8.92 (s, NH)ppm; ^{13}C NMR ($CDCl_3$)δ: 11.2 (CH_3), 63.6 (d, J=11 Hz, C-5′), 75.6 (d, J=16 Hz, C-2′), 82.4 (d, J=25.6 Hz, C-4′), 85.7 (C-1′), 87.7 and 87.9 (2 Ph_3C), 90.4 (d, J=180 Hz, C-3′), 111.7 (C-5), 135.6 (C-6), 150.5 (C-2), 163.3 (C-4) ppm + trityl signals.

The foam obtained in the previous preparation was treated with 150 ml of 80% acetic acid for 1 h at 95°C. The mixture was evaporated to dryness and the residue was dissolved in 100 ml of water. The water phase was washed three times with 100 ml of diethylether and the combined organic layers were extracted with 50 ml of water. After evaporation in vacuo of the water layer, the residue was dissolved in MeOH and adsorbed on 5 g of silica gel. Purification on 40 g of silica gel ($CHCl_3$-MeOH 95:5) yielded 424 mg (1.63 mmol, 60%) of the title compound *10*. After standing for 2 weeks in MeOH-benzene 166 mg of crystalline material was isolated.

mp 163–164°C; UV (MeOH)λ_{max} 266 (ε=9000); ^{1}H NMR (DMSO-d_6) δ: 1.79 (d, J=1.3 Hz, CH_3), 3.61 (d, J=3.2 Hz, H-5′, H-5″), 4.15 (dt, $J_{4',F}$=27.8 Hz, H-4′), 4.28 (dm, $J_{2',F}$=25 Hz, H-2′), 4.96 (dd, J=4 Hz, $J_{3',F}$=55 Hz, H-3′), 5.28 (br, 5′-OH), 5.77 (d, J=6.1 Hz, 2′-OH), 5.89 (d, J=7.5 Hz, H-1′), 7.66 (d, J=1.3 Hz, H-6), 11.35 (br, NH)ppm; ^{13}C NMR (DMSO-d_6)δ: 12.2 (CH_3), 60.7 (d, J=11 Hz,

C-5′), 71.7 (d, J=14.9 Hz, C-2′), 82.9 (d, J=22 Hz, C-4′), 85.8 (C-1′), 92.7 (d, 181.9 Hz, C-3′), 110.0 (C-5), 135.9 (C-6), 151.0 (C-2), 163.6 (C-4)ppm; *anal.* calcd. for $C_{10}H_{13}FN_2O_5$: C, 46.16; H, 5.04; N, 10.77. Found: C, 46.21; H, 5.09; N, 10.59.

N^6-Trityl-9-(2-O-trityl-β-D-xylofuranosyl)adenine (12) After coevaporation with 150 ml of anhydrous pyridine, 23.6 g (49.6 mmol) of 9-(3,5-di-O-benzoyl-β-D-*xylo*furanosyl)adenine (*11*) (Gosselin and Imbach, 1982) was dissolved in 750 ml of anhydrous pyridine and 55.6 g (200 mmol) of trityl chloride was added. The mixture was left at 90°C for 72 h and concentrated. The oily residue was dissolved in 1 l of $CHCl_3$ and washed twice with 1 l of a saturated solution of $NaHCO_3$. The water layer was extracted once with 1 l of $CHCl_3$. The combined organic layers were dried (Na_2SO_4), evaporated and coevaporated twice with toluene. The residue was dissolved in 700 ml of a mixture of anhydrous toluene-MeOH (5:2) and approximately 0.5 g of sodium was added. After 30 min at room temperature TLC ($CHCl_3$) indicated complete debenzoylation. The mixture was neutralized by addition of Dowex AG50W-X8 resin, filtered and the ion exchange resin was washed with MeOH and acetone. The filtrate and washings were evaporated and the residue was purified on 600 g of silica gel (elution with a gradient from $CHCl_3$-cyclohexane 1:1 to pure $CHCl_3$). Evaporation of the desired fractions yielded 31 g (41.17 mmol, 83%) of the title compound as a foam.

UV ($CHCl_3$)λ_{max} 230 and 275 nm; ^{1}H NMR ($CDCl_3$)δ: 3.92 (d, J=5 Hz, H-5′, H-5″), 4.26 (m, H-3′, H-4′), 4.69 (d, J=2 Hz, H-2′), 5.47 (d, J=2 Hz, H-1′), 7.10–7.50 (m, trityl), 7.04 and 7.83 (2 s, H-2, H-8)ppm; ^{13}C NMR ($CDCl_3$) δ: 61.0 (C-5′), 71.4 (Ph_3C-N), 77.3 (C-3′), 82.1 (C-2′), 83.5 (C-4′), 88.7 (Ph_3C-O), 91.5 (C-1′), 121.4 (C-5), 140.2 (C-8), 146.3 (C-4), 150.9 (C-2), 154.2 (C-6)ppm + trityl signals.

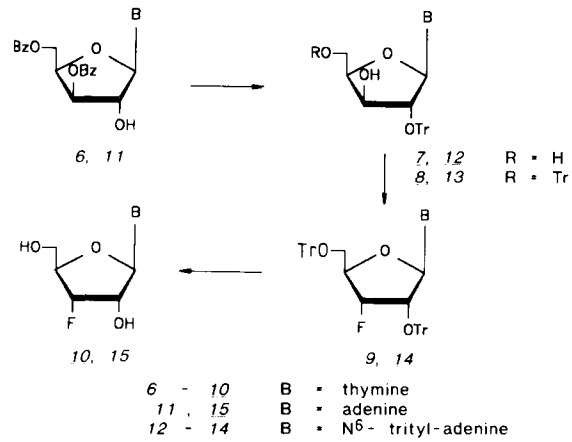

Fig. 3. Synthesis of 1-(3-fluoro-3-deoxy-β-D-ribofuranosyl)thymine (*10*) and 3′-fluoro-3′-deoxyadenosine (*15*).

N^6-Trityl-9-(2,5-di-O-trityl-β-D-xylofuranosyl)adenine (13) After coevaporation of 31.0 g (41.17 mmol) of *12* with pyridine, 750 ml of anhydrous pyridine and 23 g (82.5 mmol, 2 eq.) of trityl chloride were added. The reaction mixture was stirred for 48 h at room temperature and concentrated. The oily residue was diluted with 1 l of $CHCl_3$ and washed twice with 1 l of a saturated solution of $NaHCO_3$. The water phase was extracted with 1 l of $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated. Crystallization from cyclohexane-$CHCl_3$ yielded a first crop of 10.4 g (10.45 mmol) of *13*. The filtrate and washings were evaporated and purified on 400 g of silica gel (cyclohexane-$CHCl_3$ 1:1 to pure $CHCl_3$). Crystallization of the pure fractions from cyclohexane-$CHCl_3$ yielded in three crops 24.55 g more of the title compound (a total of 34.95 g, 35.12 mmol, 85%).

UV ($CHCl_3$)λ_{max} 230 and 275 nm (ϵ=23000); ^{1}H NMR ($CDCl_3$)δ: 3.51 (m, H-5′, H-5″). 3.96 (dd, J=3.3 Hz, $J_{3',OH}$=11.5 Hz, H-3′), 4.29 (m, H-4′), 4.57 (d, J=1.5 Hz, H-2′), 5.45 (d, J=1.5 Hz, H-1′), 7.1–7.5 (m, trityl), 6.96 and 7.76 (2 s, H-2, H-8)ppm; ^{13}C NMR ($CDCl_3$)δ: 62.4 (C-5′), 71.4 (Ph_3C-N), 76.9 (C-3′), 82.4 (C-2′), 84.5 (C-4′), 87.1 and 88.6 (2 Ph_3C-O), 92.0 (C-1′), 121.3 (C-5), 140.4 (C-8), 146.4 (C-4), 150.9 (C-2), 154.1 (C-6)ppm + trityl signals.

3′-Fluoro-3′-deoxyadenosine (15) The tritylated material *13* (26.32 g, 26.45 mmol) was dissolved in 750 ml of dichloroethane to which 8.6 ml (65 mmol, 2.5 eq.) of DAST was added. The reaction mixture was left for 2 h at room temperature, poured into 1 l of a saturated $NaHCO_3$ solution and stirred for 15 min. Both layers were separated and the water layer was extracted once more with 500 ml of $CHCl_3$. The organic phase was dried (Na_2SO_4), evaporated and treated with 750 ml of 80% acetic acid for 1 h at 100°C. After evaporation the residue was dissolved in 200 ml of water and washed with 300 ml of diethyl ether (3 ×). The organic washings were extracted once more with 100 ml of water. After evaporation of the water phase the residue was purified on 80 g of silica gel and crystallized from MeOH-Et_2O yielding 2.51 g (9.34 mmol). A second batch of *13* (8.63 g, 8.67 mmol) yielded 650 mg (2.42 mmol) of *15* under the same reaction conditions. The brown filtrates of both reactions were combined, evaporated and acylated with 50 ml of acetic anhydride-pyridine (1:4) for 2 h at room temperature. Evaporation and purification on silica gel afforded the tri- and tetra-acetylated products, which were combined and deacylated with methanol saturated with ammonia. Purification on silica gel yielded 1.75 g of *15* (affording a total of 4.91 g, 18.26 mmol, 52%): mp 205°C; MS (m/e) 269 (M^+) [lit. (Herdewijn et al., 1989): the indicated mp of 164°C and MS (m/e) 260 (M^+) were incorrect].

3′-Fluoro-3′-deoxyinosine (16) A mixture of 320 mg (1.19 mmol) of 3′-fluoro-3′-deoxyadenosine (*15*) and 0.5 ml of a suspension of adenosine deaminase in 100 ml 0.05 *M* phosphate buffer, pH 7.5, was incubated for 30 min at 37°C. The reaction mixture was evaporated, dissolved in methanol and adsorbed on 6 g of silica gel. Purification on 25 g of silica gel (gradient from $CHCl_3$-MeOH 95:5 to $CHCl_3$-MeOH 88:12) yielded 260 mg (0.96 mmol, 81%) of *16* which crystallized from MeOH.

mp 228–230°C; UV (MeOH)λ_{max} 250 nm (ϵ=11100); ^{1}H NMR (DMSO-d_6) δ:

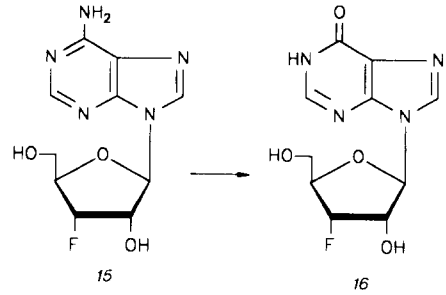

Fig. 4. Deamination of *15* to 3′-fluoro-3′-deoxyinosine (*16*).

3.64 (d, J=4 Hz, H-5′, H-5″), 4.28 (dt, J=4 Hz, $J_{4',F}$=26.8 Hz, H-4′), 4.83 (ddd, J=4 and 7.5 Hz, $J_{2',F}$=25.0 Hz, H-2′), 5.10 (dd, J=4 Hz, $J_{3',F}$=54.2 Hz, H-3′), 5.94 (d, J=7.5 Hz, H-1′), 8.10 (s) and 8.36 (s), (H-2, H-8); ^{13}C NMR δ: 60.8 (d, J=11.0 Hz, C-5′), 72.7 (d, J=15.8 Hz, C-2′), 83.8 (d, J=22.0 Hz, C-4′), 86.3 (s, C-1′), 92.17 (d, J=181.9 Hz, C-3′), 124.6 (C-5), 138.18 (C-8), 146.0 (C-2), 148.5 (C-4), 156.5 (C-6); *anal.* calcd. for $C_{10}H_{11}FN_4O_4$: C, 44.45; H, 4.10; N, 20.73. Found: C, 44.49; H, 4.23; N, 20.55.

Antiviral activity

The five 3′-fluorinated 3′-deoxyribonucleosides were examined for their antiviral activity against herpes simplex virus type 1 and type 2, vaccinia virus and vesicular stomatitis virus in primary rabbit kidney cells (Table 2). None of the com-

TABLE 2

Antiviral and cytotoxic activity of 3′-fluorinated 3′-deoxyribonucleosides in primary rabbit kidney cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)			
		Herpes simplex virus type 1 (KOS)	Herpes simplex virus type 2 (G)	Vaccinia virus	Vesicular stomatitis virus
3′F3′dUrd (*4*)	>400	>400	>400	>200	>200
3′F3′dCyd (*5*)	>400	>400	>200	>200	>400
3′F3′dThd (*10*)	>400	>400	>200	>400	>200
3′F3′dAdo (*15*)	40	>10	>10	1	>40
3′F3′dIno (*16*)	>400	>400	>400	70	>400
Tubercidin	0.4	0.4	>0.1	>0.1	0.07
(*S*)-DHPA	>400	>400	>400	70	7
Ribavirin	>400	>400	>400	20	150
C-c^{3}Ado	>400	300	>400	2	0.7

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

TABLE 3

Antiviral and cytotoxic activity of 3′-fluorinated 3′-deoxyribonucleosides in VERO cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)				
		Parainfluenza virus type 3	Reovirus type 1	Sindbis virus	Coxsackie virus type B4	Semliki Forest virus
3′F3′dUrd (*4*)	400	>100	20	>200	300	300
3′F3′dCyd (*5*)	400	>200	>100	150	>400	300
3′F3′dThd (*10*)	>400	>400	>400	>400	>400	>400
3′F3′dAdo (*15*)	40	>10	1	2	4	4
3′F3′dIno (*16*)	>400	>200	70	150	300	>400
Tubercidin	0.4	>0.04	>0.04	0.07	0.07	>0.1
(*S*)-DHPA	200	70	>200	>100	>200	>200
Ribavirin	>400	70	70	70	70	70
C-c^{3}Ado	>200	1	7	150	10	>200

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

pounds inhibited herpes simplex virus or vesicular stomatitis virus replication. Yet, 3′-fluoro-3′-deoxyadenosine (3′F3′dAdo) (*15*) proved markedly inhibitory to vaccinia virus at a concentration well below the cytotoxic concentration. The activity shown by compound *15* against vaccinia virus was comparable to that of the reference compound C-c^{3}Ado. In contrast to the SAH hydrolase inhibitors (*S*)-DHPA and C-c^{3}Ado, however, 3′F3′dAdo did not show activity against vesicular stomatitis virus.

In VERO cells (Table 3), 3′-fluoro-3′-deoxyuridine had some activity against reovirus but not parainfluenza, Sindbis, Coxsackie or Semliki Forest virus. The two

TABLE 4

Antiviral and cytotoxic activity of 3′-fluorinated 3′-deoxyribonucleosides in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus type B4	Polio virus type 1
3′F3′dUrd (*4*)	>400	>400	150	150
3′F3′dCyd (*5*)	>200	>200	150	150
3′F3′dThd (*10*)	>400	>400	>400	>400
3′F3′dAdo (*15*)	40	>10	2	4
3′F3′dIno (*16*)	>400	>400	>400	>400
Tubercidin	1	0.07	0.07	0.2
(*S*)-DHPA	>400	20	>400	>400
Ribavirin	100	7	20	70
C-c^{3}Ado	>200	2	>200	>200

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%.

TABLE 5

Effects of L-homocysteine (L-Hcy) and adenosine (Ado) on the anti-vaccinia activity of 3′-fluoro-3′-deoxyadenosine (*15*) in L929 cells

Compound	Additional treatment	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)
15	None	40	0.25
15	0.5 mM L-Hcy	40	0.25
15	1 mM L-Hcy	40	0.25
15	0.1 mM Ado	100	1
15	0.5 mM Ado	100	2.5
15	1 mM Ado	≥100	2.5

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce vaccinia virus-induced cytopathogenicity by 50%.

other 3′-fluorinated pyrimidine nucleosides did not show significant antiviral activity. 3′F3′dAdo was markedly inhibitory to reovirus, Sindbis, Coxsackie and Semliki Forest virus at concentrations which were 10- to 40-fold lower than those that disturbed normal cell morphology. 3′F3′dIno, the deaminated product of 3′F3′dAdo, had virtually no inhibitory effect on any of the viruses tested, except for a slight inhibitory effect on reovirus.

In Hela cells (Table 4), only 3′F3′dAdo showed an appreciable activity against Coxsackie and poliovirus. Again, vesicular stomatitis virus was not sensitive to the inhibitory effect of 3′F3′dAdo.

As described previously (Van Aerschot et al., 1989) none of the test compounds (*4, 5, 10, 15, 16*) exhibited any activity against the replication of human immunodeficiency virus type 1 (HIV-1) in MT4 cells.

Since 3′F3′dAdo proved effective against a broad spectrum of viruses, further studies were undertaken to assess whether the compound may achieve such broad-spectrum antiviral activity through inhibition of SAH hydrolase. However, 3′F3′dAdo was not inhibitory to SAH hydrolase (purified from murine L929 cells) unless it was used at a rather high concentration (50% inhibitory concentration: 90 μM). Genuine inhibitors of SAH hydrolase such as C-c^{3}Ado, neplanocin A and

TABLE 6

Effect of 3′-fluoro-3′-deoxyadenosine on the formation of vaccinia tail lesions in mice

Dose (mg/kg/day)	Number of lesions		*P* (compared with control group)[a]
	Per individual mouse	Average ±SD	
100	0,2,1,2,3,12,12,11,9,7	5.9±4.8	<0.005
50	43,24,36,30,11,35,32,26,12,29	24.9±13.4	<0.1
10	16,36,37,32,18,42,55,41,43,19	33.9±12.7	NS[b]
Control	58,39,17,43,38,33,37,28,36,27	33.9±14.7	–

[a]Statistical significance assessed by Student's *t*-test.
[b]Not significant.

3-deazaneplanocin A inhibit SAH hydrolase in the 1-10 nM range (Cools and De Clercq, 1989).

Also, treatment of L929 cells with 3'F3'dAdo at 50 μM for up to 4 hours did not influence cellular SAH pool levels (data not shown). Furthermore, addition of exogenous homocysteine did not enhance the antiviral activity of 3'F3'dAdo (Table 5). Yet, homocysteine is known to potentiate the antiviral effects of those nucleoside analogues that are targeted at SAH hydrolase (De Clercq et al., 1989). On the other hand, adenosine partially counteracted the activity of 3'F3'dAdo, in that its minimum inhibitory concentration for vaccinia virus was increased 10-fold in the presence of adenosine (Table 5).

3'F3'dAdo was further investigated for its in vivo antiviral efficacy. It proved effective in suppressing the formation of tail lesions in mice infected intravenously with vaccinia virus (Table 6). Following a 5-day treatment with 3'F3'dAdo at 50 mg/kg/day, the number of pox tail lesions was reduced by approximately 25%, and when 3'F3'dAdo was administered at 100 mg/kg/day, the pox tail lesion number was reduced by 80%. At 200 mg/kg/day 3'F3'dAdo was lethal to the mice.

Discussion

Chemistry

Treatment of 1-(β-D-*xylo*furanosyl)uracil with 5 eq. of triphenylmethyl chloride in pyridine for 24 h at 80°C yielded a complex mixture of the mono- and di-tritylated nucleosides. After extensive chromatographic purification 55% of the 2',5'-ditrityl derivative *1* (Yung and Fox, 1961), 32% of the 3',5'-ditrityl isomer and 11% of the 5'-monotritylated derivative were isolated. The 2',5'-ditrityl derivative *1* was treated with 2 eq. of DAST in dichloromethane for 1 h at room temperature. After work-up the residue was treated with 80% acetic acid for 1 h at 90°C. Chromatographic purification yielded a mixture of 3'-fluoro-3'-deoxyuridine and uracil, which could not be separated. Therefore the mixture was treated with benzoyl chloride in pyridine, yielding 11% of the tri-*O*-benzoyl derivative *2* and 13% of the di-*O*-benzoyl analogue *3*. Debenzoylation of *2* with methanol saturated with ammonia gave 65% of 3'-fluoro-3'-deoxyuridine (*4*). The di-*O*-benzoyl analogue *3* was treated with trifluoromethanesulfonic anhydride, followed by methanol saturated with ammonia. This gave after purification 45% of 3'-fluoro-3'-deoxycytidine (*5*).

Because of the difficulties encountered in the 2',5'-selective protection of 1-(β-D-*xylo*furanosyl)uracil, an alternative strategy was used for the thymine analogue. The *xylo*-nucleoside was prepared by sugar base condensation of thymine with 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-D-*xylo*furanose (*17*) (Gosselin and Imbach, 1982). After refluxing the silylated thymine and the protected sugar for 5 h in 1,2-dichloroethane in the presence of 1.2 eq. of trimethylsilyl trifluoromethanesulfonate, 57% of 1-(2-*O*-acetyl-3,5-di-*O*-benzoyl-β-D-*xylo*furanosyl)thymine (*18*) was isolated. Alternatively, condensation with 1.5 eq. of stannic chloride as a catalyst in acetonitrile yielded only 32% of *18*. Emulsions, which were formed during the

extractions, substantially lowered the yield (Vorbrüggen et al., 1981).

Selective deacetylation with hydrazine hydrate in a mixture of pyridine and acetic acid (4:1) yielded the 1-(3,5-di-*O*-benzoyl-β-D-*xylo*furanosyl)thymine *6* [during the course of this work, the products *6* and *18* were described (Robins et al., 1988]. Tritylation of this 2′-deacetylated material followed by deesterification gave 1-(2-*O*-trityl-β-D-*xylo*-furanosyl)thymine (*7*), which upon further tritylation yielded the 2′,5′-ditritylated analogue *8* in 78% overall yield from the protected derivative *18*. Treatment of *8* with 2 eq. of DAST for 3 h at room temperature gave 34% of the fluorinated analogue *9*, which was detritylated to yield 60% of 1-(3-deoxy-3-fluoro-β-D-*ribo*furanosyl)thymine (*10*).

A large scale preparation of *15* was started, following the same strategy for selective protection as for the synthesis of *10*. Sugar-base condensation on a 75 mmol scale with 1.5 eq. trimethylsilyl trifluoromethanesulfonate yielded 70% of the protected *xylo*adenosine *19*. Selective deprotection at the 2′-position with hydrazine yielded 84% of *11* (Gosselin and Imbach, 1982). Protection of the 2′-position with trityl chloride was accompanied by tritylation of the amino function yielding 89% of N^6-trityl-9-(2-*O*-trityl-β-D-*xylo*furanosyl)adenine (*12*) after debenzoylation. Tritylation of the 5′-hydroxyl group afforded 82% of the tritylated derivative *13*. Fluorination was carried out with 2 eq. of DAST for 20 min at room temperature, followed by detritylation, yielding 52% of 3′-fluoro-3′-deoxyadenosine (*15*) after extensive work-up. This means an overall yield of 22% starting from adenine. Finally, enzymatic deamination of *15* gave 81% of 3′-fluoro-3′-deoxyinosine. All fluorinated nucleosides were completely characterized by UV, MS, ^{1}H and ^{13}C NMR and elemental analysis. Main fragment ions are given in Table 1.

Antiviral activity

The 3′-fluorinated 3′-deoxyribonucleosides, except for the adenosine analogue 3′Fd3′dAdo, were virtually inactive in our broad-spectrum antiviral assay systems. 3′F3′dAdo was effective against a broad range of viruses including DNA viruses [pox (vaccinia)], double-stranded RNA viruses (reo), single-stranded (+) RNA viruses [picorna (polio, Coxsackie B), toga (Sindbis, Semliki Forest)]. The antiviral activity spectrum of 3′F3′dAdo is clearly different from that of the adenosine analogues neplanocin A, C-c^{3}Ado and (*S*)-DHPA. These adenosine analogues, which are known as SAH hydrolase inhibitors, are particularly active against (−)RNA viruses (De Clercq, 1987).

The mechanism by which 3′F3′dAdo exerts its antiviral effect remains to be elucidated. Clearly, it cannot be attributed to inhibition of SAH hydrolase. The compound is a very poor SAH hydrolase inhibitor, and, at concentrations well above the antivirally active concentrations, it does not influence SAH pool levels in the cells. Also, exogenous addition of homocysteine did not enhance the antiviral activity of 3′F3′dAdo, although homocysteine is supposed to do so with SAH hydrolase inhibitors (De Clercq et al., 1989).

3′F3′dAdo was effective in vivo in suppressing vaccinia virus-induced tail lesions in mice. In its in vitro and in vivo activity against vaccinia virus, 3′F3′dAdo was

comparable to C-c^{3}Ado, which has been previously shown to suppress the formation of vaccinia tail lesions in mice when administered at the relatively high dose of 5 mg/mouse (approximately 500 mg/kg) (De Clercq et al., 1984).

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