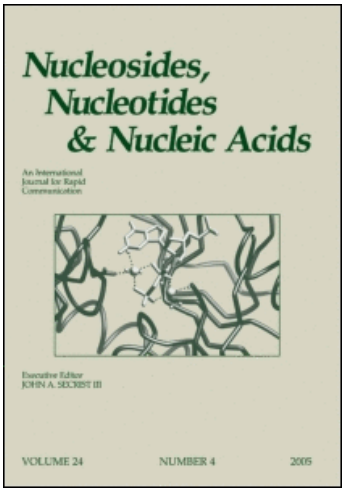

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Synthesis And Antiviral Evaluation Of 2',3'-Secothymidine Analogs of ddT and AZT

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SYNTHESIS AND ANTIVIRAL EVALUATION OF 2',3'-SECOTHYMIDINE ANALOGS OF ddT AND AZT

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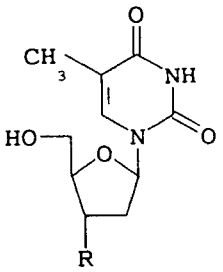
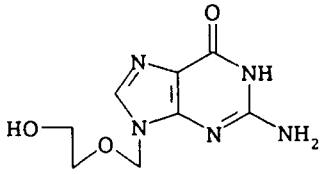
ABSTRACT: 2',3'-Secothymidine derivatives related to AZT and ddT have been synthesized and evaluated for their activity against HIV-1 and various DNA and RNA viruses. These acyclic nucleosides exhibited no antiviral activities.

INTRODUCTION

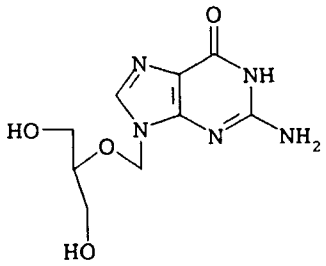
Numerous modified nucleosides have been recently evaluated for activity against human immunodeficiency virus type 1 (HIV-1). Among these may be mentioned, AZT **1** (3'-azido-3'-deoxythymidine) (1-3), 2',3'-dideoxynucleosides such ddT **2** (3'-deoxythymidine) (3-7) and FddT **3** (3'-fluoro-3'-deoxythymidine) (8-11) (Figure 1). Some acyclonucleosides, such as ACV **4** (12-14) (9-(2-hydroxyethoxymethyl)guanine) and DHPG **5** (15-17) (9-(1,3-dihydroxy-2-propoxymethyl)guanine) (Figure 1) also show antiviral activity. 2',3'-Secoribonucleosides



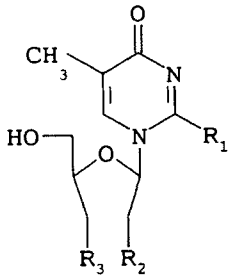
R=N_3 **1** AZT
R=H **2** ddT
R=F **3** FddT



4 ACV



5 DHPG



A

FIG. 1

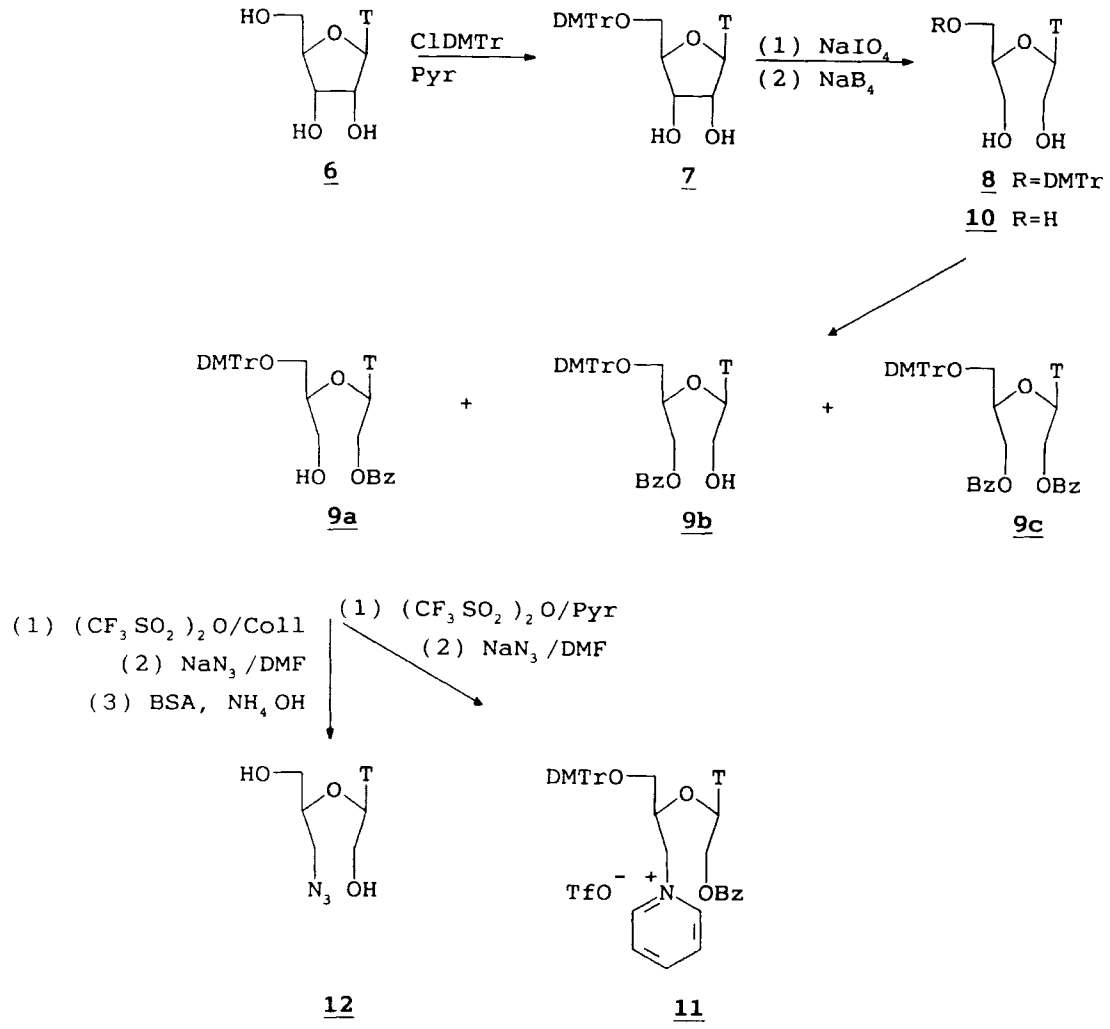
structurally related to the acyclonucleosides **4** and **5** have been described, but lack antiviral activity were reported (18-20). However, it seemed interesting to us to evaluate some 2',3'-seconucleosides structurally related to the anti-HIV derivatives **1**, **2** and **3**.

Accordingly, a synthesis of acyclo analogs of general formula **A** (Figure 1) was designed, which allowed enantiomerically pure products to be obtained when $R_3 \neq$ OH. Our synthetic strategy was dictated by the expectation that these nucleosides would have to be converted to triphosphates by cellular and/or viral kinases. Thus, the configuration at C-4' would have to be identical to that of natural nucleosides.

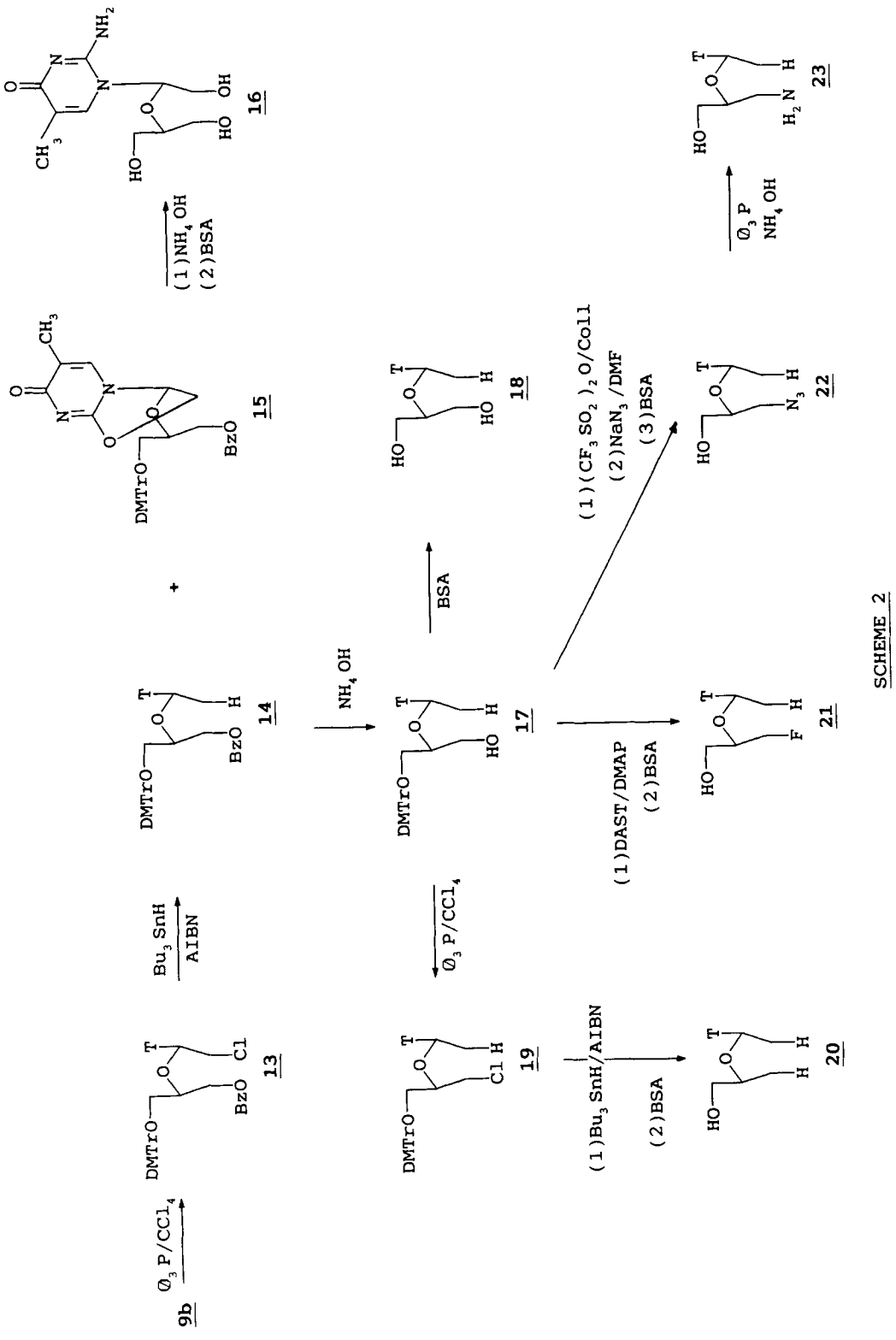
CHEMISTRY

The 2',3'-seconucleosides can be considered as ribonucleosides with the C-2'-C-3' bond missing. Such compounds can be directly derived from pyrimidine and purine ribonucleosides (20-24). As we focused on 2',3'-seco-2'-hydroxythymidine, we synthesized first the ribothymidine (**6**) by a coupling reaction between 1-O-acetyl-2,3,5-tri-O-benzoylribose and silylated thymine (25). The benzoate protecting groups were easily removed with sodium methoxide in methanol to give **6** as a crystalline solid in 80% yield (26) (Scheme 1). The 5'-hydroxyl group was selectively protected with a 4,4'-dimethoxytrityl group (DMTr) (27) before subsequent oxidation and reduction with retention of chirality at C-4' (28-29). Oxidation of 5'-O-(4,4'-dimethoxytrityl)-ribothymidine (**7**) with $NaIO_4$ in water-dioxane mixture followed by reduction with $NaBH_4$ (28) afforded compound **8** in good yield (Scheme 1). Treatment of **8** in acidic medium afforded the parent 2',3'-secoribothymidine (**10**). Functionalization of the 2' and 3' primary hydroxyl group was performed by treatment of **8** with benzoyl chloride at -45°C (30) and gave a mixture of the corresponding 2' or 3'-mono **9a,b** and di-O-benzoyl derivatives **9c** in 41%, 18% and 14% yields respectively (Scheme 1). After chromatographic separation the structure of these key intermediates was proven by ^1H NMR spectroscopic decoupling experiments. Reaction of **9a** with trifluoromethanesulfonic anhydride in dichloromethane-pyridine followed by addition of an excess of sodium azide in DMF (31) gave only the pyridinium derivative **11** (Scheme 1). That was not completely unexpected, as pyridine is nucleophilic enough to displace the highly reactive triflate group (32-33). Replacement of pyridine by the less nucleophilic base collidine (34-35) afforded the expected 3'-azido-3'-deoxy-2',3'-seco-2'-hydroxythymidine (**12**) after consecutive treatment with 2% benzenesulfonic acid (BSA) and ammonium hydroxide (Scheme 1).

Replacement of the 2'-OH group in **9b** by chlorine was performed with triphenylphosphine in CCl_4-pyridine (36) yielding the intermediate **13** (Scheme 2). Reduction of this



SCHEME 1



SCHEME 2

2'-chloro derivative 13 with n-Bu_3SnH and azobisisobutyronitrile (AIBN) in absolute EtOH resulted in the formation of 3'-O-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2',3'-secothymidine (14) and 2,2'-anhydro-3'-O-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2',3'-secothymidine 15 in 68% and 16% yields, respectively (Scheme 2). Direct radical deoxygenation of thiobenzoate derivative of 9b by the Barton and McCombie procedure (38) gave compounds 14 and 15 in 8% and 15% yields respectively, and thus was less satisfactory as a route to 14.

Deprotection of compounds 14 and 15 afforded 2',3'-secothymidine (18) and 2',3'-secoisocytidine (16), respectively (Scheme 2). The 2-amino derivative 16 was obtained by hydrolysis of the 2,2'-anhydro compound 15 with ammonium hydroxide. Debenzoylation of 14 followed by chlorination of 3'-hydroxyl of 17 provided 3'-chloro- 3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2',3'-secothymidine (19) (Scheme 2). Radical deoxygenation and detritylation of 19 furnished 3'-deoxy-2',3'-secothymidine (2',3'-seco-ddT) (20) (Scheme 2). Fluorination of 17 with diethylaminosulfur trifluoride (DAST) in dichloromethane (41-43) followed by treatment with 2% BSA gave 3'-fluoro-3'-deoxy-2',3'-secothymidine (2',3'-seco-FddT) (21) (Scheme 2). The seco analog of AZT, 3'-azido-3'-deoxy-2',3'-secothymidine (2',3'-seco-AZT) (22) was synthesized by the same procedure as compound 12 (Scheme 2). Treatment of 2',3'-seco-AZT (22) with triphenylphosphine in pyridine and ammonium hydroxide (44) afforded 3'-amino-3'-deoxy-2',3'-secothymidine (23) (Scheme 2).

BIOLOGICAL RESULTS

All of the new 2',3'-secothymidine derivatives prepared in this study (10, 12, 16, 18, 20-23) were evaluated for their antiviral activities against representative RNA and DNA virus in cell cultures. At concentrations up to 10^{-4} M, none of them inhibited the replication of HIV-1 (CME and MT4 cells); of DNA viruses (CMV, HSV-1, HSV-2, vaccinia) and of RNA viruses (parainfluenza-3, Rous Sarcoma, Sindbis, coxsackie-B3, polio-1). None of the compounds (10, 12, 16, 18,

20-23) showed any cytostatic effect at the highest concentration tested (10^{-4} M).

EXPERIMENTAL

Chemical Synthesis. General Procedures

Ultraviolet (UV) spectra were recorded in 95% EtOH or 0.1M ammonium acetate buffer (pH 5.9) on a Uvikon-810 spectrometer from 220 to 350 nm. ^{1}H and ^{19}F nuclear magnetic resonance spectra was obtained at ambient temperature in DMSO-d_6 on a Brüker AC 250 spectrometer. ^{1}H NMR chemical shifts are expressed in parts per million with DMSO set at 2.49 ppm as the reference. ^{19}F NMR shifts in ppm were reported relative to external $CFCl_3$. Fast-atom-bombardment mass spectra (FAB-MS) were recorded in the positive (FAB > 0) and negative-ion (FAB < 0) mode on a JEOL DX 300 mass spectrometer, with a JMA-DA 5000 mass data system; Xenon was used for the atom gun at 3 KeV with a total discharge current of 20 μA and the matrix was glycerol (G), thioglycerol (TG), or 3-nitrobenzyl alcohol (NOBA). Thin-layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel $60F_{254}$ (Merck No.5554), visualization of products being a accomplished by UV absorbance followed by spraying with 10% ethanolic sulfuric acid and heating. The solvent systems used were: dichloromethane: methanol (90:10) (A); dichloromethane: methanol (95:5) (B). Column chromatography was performed on silica gel 60A (Merck No. 7736) or RP-2 silanized silica gel (Merck No. 7719). High-pressure liquid chromatographic (HPLC) studies were carried out on a Radial-Pak C_{18} (10 μm) cartrige in a Waters RCM-8×10 module or on a Beckman C_{18} XLODS (3 μm) column. A Waters 990 photodiode array detector and NEC APC IV computer controlling the data acquisition were used .Condition A for analytical HPLC: Beckman C_{18} column; solvent A: 0.1 M ammonium acetate buffer (pH 5.9); solvent B: acetonitrile; gradient 0-30% solvent B in 30 min, flow rate 1 ml/min. Condition B for purification by HPLC: Radial-Pak C_{18} column; solvent: H_2O; flow rate 2 ml/min.

5'-O-(4,4'-Dimethoxytrityl)-2'-hydroxythymidine (7).

4,4'-Dimethoxytrityl chloride (8.98 g, 26.5 mmol) was added to a solution of 2'-hydroxythymidine (6) (5.70 g, 22.1 mmol) in 260 ml of dry pyridine. The reaction mixture was stirred at room temperature for 4 h. After the usual work up and purification on a silica gel column eluted with dichloromethane: methanol (from 100:0 to 95:5), the compound 7 was isolated as a colorless foam in 80% yield (9.90 g). TLC (solvent A) one spot with R_f = 0.40; UV (EtOH) λ_{max} 268 nm (ϵ 8550), λ_{max} 232 nm (ϵ 17700), λ_{min} 246 nm (ϵ 7200), λ_{min} 225 nm (ϵ 17800); FAB > 0 m/z: 653 $(M+G+H)^+$, 561 $(M+H)^+$, 127 $(B+H_2)^+$, FAB < 0 m/z: 559 $(M-H)^-$, 257 $(M-DMTr)^-$, 125 $(B)^-$; ^1H NMR δ 11.33 (s, 1, NH), 7.50 (s, 1, H_6), 7.40-6.86 (m, 13, H-DMTr), 5.80 (d, $J_{1'-2'}$ = 5.2 Hz, 1, $H_{1'}$), 5.18 (br m, 1, OH), 5.48 (br m, 1, OH), 4.18 (t, 1, $H_{2'}$), 4.10 (t, 1, $H_{3'}$), 3.96 (m, 1, $H_{4'}$), 3.74 (s, 6, OCH_3), 3.21 (m, 2, $H_{5'-5''}$), 1.42 (s, 3, CH_3).

5'-O-(4,4'-Dimethoxytrityl)-2',3'-seco-2'-hydroxythymidine (8).

To a solution of 5'-O-(4,4'-dimethoxytrityl)-2'-hydroxythymidine (7) (9.4 g, 16.8 mmol) in dioxane (200 ml) and water (40 ml) was added a solution of sodium periodate (3.95 g, 18.5 mmol) in water (40 ml). The reaction mixture was stirred at room temperature for 1 h. A precipitate of $NaIO_3$ was formed after the addition of $NaIO_4$. Dioxane (200 ml) was then added, the suspension was stirred for 15 min and filtered, and the filter cake was washed with dioxane (150 ml). To the combined filtrates was added $NaBH_4$ (0.7 g, 18.5 mmol), and the mixture was stirred for 30 min at 20°C. After addition of acetone (10 ml) and stirring for 10 min, the solution was neutralized with acetic acid: pyridine (1:1), and then evaporated to a volume of approximately 100-150 ml. This solution was diluted with dichloromethane, extracted with $NaHCO_3$ solution, washed with water, dried with Na_2SO_4 and evaporated. The residue was purified by flash-chromatography on silica gel with dichloromethane: methanol (from 100:0 to 90:10) to obtain compound 8 (8.90 g,

94%) as a white foam upon evaporation. TLC (solvent A) one spot with R_f = 0.38; UV (EtOH) λ_{max} 268 nm (ϵ 9570), λ_{max} 234 nm (ϵ 19900), λ_{min} 254 nm (ϵ 8020), λ_{min} 225 nm (ϵ 18630); FAB > 0 m/z: 563 $(M+H)^+$, 127 $(B+H_2)^+$, FAB < 0 m/z: 561 $(M-H)^-$, 259 $(M-DMTr)^-$, 125 $(B)^-$; ^{1}H NMR δ 11.36 (s, 1, NH), 7.53 (s, 1, H_6), 7.31-6.86 (m, 13, H-DMTr), 5.83 (t, 1, $H_{1'}$), 5.15 (t, 1, $OH_{2'}$), 4.79 (t, 1, $OH_{3'}$), 3.75 (s, 6, OCH_3), 3.66 (m, 3, $H_{2',2'',4'}$), 3.40 (m, 2, $H_{3',3''}$), 2.97 (m, 2, $H_{5',5''}$), 1.61 (s, 3, CH_3).

2'-O-Benzoyl, 3'-O-benzoyl, and 2',3'-di-O-benzoyl-5'-O-(4, 4'-dimethoxy- trityl)-2',3'-seco-2'-hydroxythymidine (9a-c).

Compound **8** (8.8 g, 15.6 mmol) was coevaporated with dry pyridine (3×100 ml). The resulting solid suspended in dry pyridine (150 ml) and stirred at -45°C in a dry ice-acetone bath. A solution of benzoyl chloride (1.55 ml, 17.16 mmol) in anhydrous dichloromethane (6 ml) was slowly added to the stirred mixture. After 30 min the reaction was warmed to -20°C for 2 h, and then was quenched with water (5 ml) and evaporated to dryness. The gum was taken up in dichloromethane, and the solution was extracted with $NaHCO_3$ solution, washed with water, dried over Na_2SO_4 and then coevaporated with toluene and dichloromethane. The syrup was chromatographed on a silica gel column. Products were eluted with CH_2Cl_2: MeOH (from 100:0 to 90:10). Fractions containing pure product were combined and evaporated.

Compound 9a: 4.24 g (41%); TLC (solvent A) one spot with R_f = 0.68; UV (EtOH) λ_{max} 268 nm (ϵ 10760), λ_{max} 229 nm (ϵ 33400), λ_{min} 255 nm (ϵ 9400); FAB > 0 m/z: 667 $(M+H)^+$, 127 $(B+H_2)^+$, FAB < 0 m/z: 665 $(M-H)^-$, 363 $(M-DMTr)^-$, 125 $(B)^-$; ^{1}H NMR δ 11.47 (s, 1, NH), 8.07-6.84 (m, 19, H-Bz, H-DMTr, H_6), 6.20 (t, 1, $H_{1'}$), 4.85 (t, 1, $OH_{3'}$), 4.72-4.47 (m, 2, $H_{2',2''}$),3.74 (s, 6, OCH_3), 3.67 (m, 1, $H_{4'}$), 3.45 (m, 2, $H_{3',3''}$), 3.02 (m, 2, $H_{5',5''}$), 1.56 (s, 3, CH_3).

Compound 9b: 1.87 g (18%); TLC (solvent A) one spot with R_f = 0.60; UV (EtOH) λ_{max} 268 nm (ϵ 11270), λ_{max} 229 nm (ϵ 32950), λ_{min} 256 nm (ϵ 10200); FAB > 0 m/z: 667 $(M+H)^+$, 127 $(B+H_2)^+$, FAB < 0 m/z: 665 $(M-H)^-$, 363 $(M-DMTr)^-$, 125

$(B)^-$; ^1H NMR δ 11.30 (s, 1, NH), 7.87-6.82 (m, 19, H-Bz, H-DMTr, H_6), 5.88 (t, 1, $H_{1'}$), 5.15 (t, 1, $OH_{2'}$), 4.47-4.31 (m, 2, $H_{3',3''}$), 3.95 (m, 1, $H_{4'}$), 3.72 (s, 6, OCH_3), 3.61 (m, 2, $H_{2',2''}$), 3.11 (m, 2, $H_{5',5''}$), 1.63 (s, 3, CH_3).

Compound 9c: 1.68 g (14%); TLC (solvent A) one spot with R_f = 0.78; UV (EtOH) λ_{max} 268 nm (ε 10100), λ_{max} 230 nm (ε 43600), λ_{min} 258 (ε 9600); FAB > 0 m/z: 771 $(M+H)^+$, 645 $(M-B)^+$, 127 $(B+H_2)^+$, FAB > 0 m/z: 769 $(M-H)^-$, 125 $(B)^-$; ^1H NMR δ 11.49 (s, 1, NH), 7.89-6.78 (m, 24, H-Bz, H-DMTr, H_6), 6.30 (t, 1, $H_{1'}$), 4.67-4.36 (m, 4, $H_{2',2'',3',3''}$), 4.06 (m, 1, $H_{4'}$), 3.72 (s, 6, OCH_3), 3.15 (m, 2, $H_{5',5''}$), 1.62 (s, 3, CH_3).

2',3'-Seco-2'-hydroxythymidine (10).

Compound 8 (704 mg, 1.25 mmol) in dichloromethane: methanol (25 ml) (7:3) was stirred at 0°C. A solution of 10% BSA in CH_2Cl_2: MeOH (6.25 ml) (7:3) was slowly added to the stirred mixture. After 30 min, the solution was neutralized with 32% aqueous ammonia, and evaporated to dryness, and the residue was partitioned between H_2O and CH_2Cl_2. The aqueous phases were evaporated and chromatographed on RP-2 silanized silica gel column (eluent: H_2O). Fractions containing pure product were combined, evaporated and lyophilized in dioxane to obtain a white powder (265 mg, 82%). HPLC analysis on C_{18} column (condition A) R_t = 4.78 min, purity = 100%; UV (EtOH) λ_{max} 264 nm (ε 7730), λ_{min} 233 nm (ε 1770); FAB > 0 m/z: 261 $(M+H)^+$, 127 $(B+H_2)^+$; ^1H NMR δ 11.20 (s, 1, NH), 7.52 (s, 1, H_6), 5.80 (t, 1, $H_{1'}$), 5.07 (t, 1, $OH_{2'}$), 4.70 (t, 1, $OH_{3'}$), 4.58 (t, 1, $OH_{5'}$), 3.63-3.43 (m, 5, $H_{2',2'',3',3'',4'}$), 3.34 (m, 2, $H_{5',5''}$), 1.75 (s, 3, CH_3).

3'-Azido-3'-deoxy-2',3'-seco-2'-hydroxythymidine (12) and pyridinium derivative 11.

Compound 12: A solution of compound 9a (667 mg, 1 mmol) in anhydrous dichloromethane (10 ml) was stirred at -30°C in a dry ice-acetone bath and treated with collidine (1 ml, 7.5 mmol) and subsequently with a solution of trifluoromethanesulfonic anhydride (250 μl, 1.5 mmol) in dry CH_2Cl_2

(2.5 ml). The cooling bath was removed and the reaction was stirred for another 15 min. A solution of sodium azide (650 mg, 10 mmol) in dimethylformamide (10 ml) was added and the reaction was stirred overnight at room temperature. CH_2Cl_2 (70 ml) was added, the organic layer was washed with H_2O (3×50 ml), dried over Na_2SO_4 and evaporated to dryness. The oily residue was treated with 2% BSA (25 ml) and was stirred for 30 min at 0°C. The mixture reaction was neutralized with $NaHCO_3$ solution and the organic layer was separated, washed with H_2O, dried and evaporated. The syrup was dissolved in methanol saturated with ammonia, and kept overnight at room temperature. After evaporation, the residue was chromatographed on RP-2 silanized silica gel column (eluent: H_2O). Fractions containing pure product were combined, evaporated, and lyophilized in dioxane: H_2O (1:1) to obtain a white powder (95 mg, 33%). HPLC analysis on C_{18} column (condition A) R_t = 12.01 min, purity = 100%; UV (EtOH) λ_{max} 264 nm (ϵ 8350), λ_{min} 232 nm (ϵ 1550); FAB > 0 m/z: 286 $(M+H)^+$, 127 $(B+H_2)^+$; ^1H NMR δ 11.29 (s, 1, NH), 5.82 (t, 1, $H_{1'}$), 5.13 (br m, 1, $OH_{2'}$), 4.81 (br m, 1, $OH_{5'}$), 3.67-3.53 (m, 4, $H_{2',2'',3',3''}$), 3.32 (m, 3, $H_{4',5',5''}$), 1.79 (s, 3, CH_3).

Compound 11: This product was obtained in 56% yield by the same method described above. In this reaction collidine was replaced by pyridine and treatment with BSA and ammonium hydroxide was omitted. TLC (solvent A) one spot with R_f = 0.19; UV (EtOH) λ_{max} 269 nm, λ_{max} 230 nm, λ_{min} 255 nm; FAB > 0 m/z: 728 $(M)^+$, FAB < 0 m/z: 149 $(CF_3SO_3)^-$; ^1H NMR δ 11.45 (s, 1, NH), 9.03 (d, J_{o-m} = 5.9 Hz, 2,H-*o*-pyridine), 8.49 (t, J_{p-m} = 7.8 Hz, 1, H-*p*-pyridine), 8.02-6.82 (m, 21, H-DMTr, H-*m*-pyridine, H-Bz, H_6), 6.02 (t, 1, $H_{1'}$), 4.85 (m, 2, $H_{3',3''}$), 4.53 (m, 1, $H_{4'}$), 4.25 (m, 2, $H_{2',2''}$), 3.78 (s, 6, OCH_3), 3.08 (m, 2, $H_{5',5''}$), 1.60 (s, 3, CH_3).

2'-Chloro-3'-O-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2',3'-secothymidine (13).

Triphenylphosphine (2.5 g, 9.5 mmol) was added to a solution of **9b** (3.15 g, 4.75 mmol) in DMF (25 ml). After

the mixture was stirred for 10 min, CCl_4 (0.8 ml, 7.12 mmol) in pyridine (1.4 ml) was added, and the solution was stirred at room temperature overnight. Then 1-butanol (2.5 ml) was added, followed after 10 min by CH_2Cl_2 (60 ml) and of $NaHCO_3$ solution (30 ml). The organic layer was washed with H_2O (2×50 ml), dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash-chromatography on silica gel with CH_2Cl_2: MeOH (from 100:0 to 90:10) to obtain 2'-chloro-3'-O-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2',3'- secothymidine (13) (2.74 g, 84%) as a foam upon evaporation. TLC (solvent B) one spot with R_f = 0.57; UV (EtOH) λ_{max} 268 nm (ϵ 11220), λ_{max} 233 nm (ϵ 31850), λ_{min} 254 nm (ϵ 9770); FAB > 0 m/z: 685 $(M+H)^+$, 127 $(B+H_2)^+$; ^{1}H NMR δ 11.48 (s, 1, NH), 7.87-6.81 (m, 19, H-DMTr, H-Bz, H_6), 6.06 (t, 1, $H_{1'}$), 4.40 (m, 2, $H_{3',3''}$), 4.00 (m, 1, $H_{4'}$), 3.95 (m, 2, $H_{2',2''}$), 3.70 (s, 6, OCH_3), 3.10 (m, 2, $H_{5',5''}$), 1.62 (s, 3, CH_3).

3'-O-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2',3'-secothymidine (14) and 2,2'-anydro-3'-O-benzoyl-5'-O-(4,4'-dimethoxytrityl)-2',3'-secothymidine (15).

A solution of compound 13 (2.7 g, 3.95 mmol), n-Bu_3SnH (11.7 ml, 43.5 mmol) and AIBN (165 mg, 1 mmol) in absolute EtOH (90 ml) was refluxed for 48 h under argon. After evaporation of the solvent the residue was purified by chromatography on silica gel column to obtain two compounds.

Compound 14: 1.75 g (68%); TLC (solvent B) one spot with R_f = 0.49; UV (EtOH) λ_{max} 268 nm (ϵ 11000), λ_{max} 231 nm (ϵ 31000), λ_{min} 256 nm (ϵ 9750); FAB > 0 m/z: 651 $(M+H)^+$, 127 $(B+H_2)^+$; ^{1}H NMR δ 11.40 (s, 1, NH), 7.88-6.83 (m, 19, H-DMTr, H-Bz, H_6), 6.06 (q, 1, $H_{1'}$), 4.44 (m, 2, $H_{3',3''}$), 3.90 (m, 1, $H_{4'}$), 3.73 (s, 6, OCH_3), 3.06 (m, 2, $H_{5',5''}$), 1.64 (s, 3, CH_3), 1.40 (d, $J_{1',2'}$ = 6 Hz, 3, CH_3).

Compound 15: 410 mg (16%); TLC (solvent B) one spot with R_f = 0.20; UV (EtOH) λ_{max} = 228 nm (ϵ 32000), λ_{sh} = 277 nm (ϵ 4300), λ_{sh} = 267 nm (ϵ 6700); FAB > 0 m/z: 649 $(M+H)^+$; ^{1}H NMR δ 7.86-6.82 (m, 19, H-DMTr, H-Bz, H_6), 6.16 (br d, 1, $H_{1'}$), 4.75 (m, 1, $H_{2'}$), 4.58 (m, 1, $H_{4'}$), 4.48 (m, 2, $H_{2'',3'}$), 4.35 (m, 1, $H_{3'}$), 3.72 (s, 6, OCH_3), 3.17 (m, 2, $H_{5',5''}$), 1.68 (s, 3, CH_3).

5-Methyl-2'-deoxy-2',3'-secoisocytidine (16).

Compound **15** (105 mg, 0.16 mmol) was treated successively with 32% aqueous ammonia and 2% BSA in CH_2Cl_2:MeOH (7:3). Purification of **16** was performed on RP-2 silanized silica gel column (eluent: H_2O) followed by HPLC on C_{18} column (condition B). Fractions containing pure product were combined and evaporated, and a aqueous solution of the residue was lyophilized to obtain a white powder (15 mg, 37%). HPLC analysis on C_{18} column (condition A) R_t = 2.48 min, purity = 100%; UV (pH 5.9) λ_{max} 260 nm, λ_{min} 248 nm; FAB > 0 m/z: 282 $(M+Na)^+$, 260 $(M+H)^+$, 126 $(B+H_2)^+$; ^1H NMR δ 7.33 (s, 1, H_6), 6.63 (s, 2, NH_2), 5.48 (t, 1, $H_{1'}$), 5.20 (t, 1, $OH_{2'}$), 4.80 (t, 1, $OH_{3'}$), 4.72 (t, 1, $OH_{5''}$), 3.64 (m, 3, $H_{2',2'',4'}$), 3.50 (m, 2, $H_{3',3''}$), 3.37 (m, 2, $H_{5',5''}$), 1.73 (s, 3, CH_3).

5'-O-(4,4'-Dimethoxytrityl)-2',3'-secothymidine (17).

A solution of **14** (1.60 g, 2.45 mmol) of MeOH (10 ml) and aqueous ammonia (30 ml) was stirred at room temperature overnight and evaporated to dryness. The residue was chromatographed on silica gel column to obtain a white foam (1.07 g, 80%). TLC (solvent B) one spot with R_f = 0.23; UV (EtOH) λ_{max} 269 nm (ϵ 8200), λ_{max} 235 nm (ϵ 17100), λ_{min} 256 nm (ϵ 7000), λ_{min} 226 nm (ϵ 15700); FAB < 0 m/z: 545 $(M-H)^-$, 243 $(M-DMTr)^-$, 125 $(B)^-$; ^1H NMR δ 11.38 (s, 1, NH)), 8.00-6.86 (m, 14, H-DMTr, H_6), 6.00 (q, 1, $H_{1'}$), 4.78 (t, 1, $OH_{3'}$), 3.75 (s, 6, OCH_3), 3.54 (m, 1, $H_{4'}$), 3.41 (m, 2, $H_{3',3''}$), 2.93 (m, 2, $H_{5',5''}$), 1.62 (s, 3, CH_3), 1.45 (d, $J_{1',2'}$ = 5.9 Hz, 3, CH_3).

2',3'-Secothymidine (18).

This compound was obtained as a white powder from **17** (110 mg, 0.2 mmol) in 78% yield (38 mg) by the method described for compound **10**. HPLC analysis on C_{18} column (condition A) R_t = 11.96 min, purity = 100%; UV (EtOH) λ_{max} 266 nm (ϵ 8620), λ_{min} 232 nm (ϵ 3200); FAB > 0 m/z: 267 $(M+Na)^+$, 245 $(M+H)^+$, 127 $(B+H_2)^+$, 119 $(M-B)^+$; ^1H NMR δ 11.26 (s, 1, NH), 7.60 (s, 1, H_6), 4.74 (t, 1, $OH_{3'}$), 4.60 (t, 1,

$OH_{5'}$), 3.45 (m, 3, $H_{3'-4'}$), 3.32 (m, 2, $H_{5'-5''}$), 1.80 (s, 3, CH_3), 1.38 (d, $J_{1'-2'}$ = 5.9 Hz, 3, CH_3).

3'-Chloro-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2',3'-seco-thymidine (19).

This compound was obtained as a white foam from **17** (290 mg, 0.53 mmol) in 77% yield (230 mg) by the method described for compound **13**. TLC (solvent B) one spot with R_f = 0.45; UV (EtOH) λ_{max} 266 nm (ϵ 9000), λ_{max} 233 nm (ϵ 19600), λ_{min} 255 nm (ϵ 8150), λ_{min} 226 nm (ϵ 19000); FAB > 0 m/z: 657 $(M+G+H)^+$, 565 $(M+H)^+$, 127 $(B+H_2)^+$, FAB < 0 m/z: 563 $(M-H)^-$, 261 $(M-B)^-$, 125 $(B)^-$; ^1H NMR δ 11.38 (s, 1, NH), 7.54 (s, 1, H_6), 7.30-6.85 (m, 13, H-DMTr), 5.99 (q, 1, $H_{1'}$), 3.74 (m, 9, OCH_3, $H_{3'-4'}$), 2.98 (m, 2, $H_{5'-5''}$), 1.62 (s, 3, CH_3), 1.45 (d, $J_{1'-2'}$ = 6 Hz, 3, CH_3).

3'-Deoxy-2',3'-secothymidine (20).

This compound was obtained from **19** (210 mg, 0.37 mmol) in 65% yield (55 mg) after lyophilization in dioxane by the method described for compound **14** followed by treatment with 2% BSA. Purification of **20** was performed on silica gel column with CH_2Cl_2: MeOH (from 100:0 to 70:30). HPLC analysis on C_{18} column (condition A) R_t = 10.76 min, purity 100%; UV (EtOH) λ_{max} 266 nm (ϵ 7460), λ_{min} 232 nm (ϵ 1830); FAB > 0 m/z: 457 $(M_2+H)^+$, 251 $(M+Na)^+$, 229 $(M+H)^+$, 127 $(B+H_2)^+$, 103 $(M-B)^+$, FAB < 0 m/z: 319 $(M+G-H)^-$, 227 $(M-H)^-$, 125 $(B)^-$; ^1H NMR δ 11.26 (s, 1, NH), 7.58 (s, 1, H_6), 5.84 (q, 1, $H_{1'}$), 4.65 (t, 1, $OH_{5'}$), 3.34 (m, 1, $H_{4'}$), 3.23 (m, 2, $H_{5'-5''}$), 1.79 (s, 3, CH_3), 1.36 (d, $J_{1'-2'}$ = 6 Hz, 3, CH_3), 1.07 (d, $J_{3'-4'}$ = 6 Hz, 3, CH_3).

3'-Fluoro-3'-deoxy-2',3'-secothymidine (21).

A solution of compound **17** (250 mg, 0.46 mmol) in dry dichloromethane (1.85 ml) and collidine (185 µl) was stirred at 0°C. DAST (92 µl, 0.69 mmol) in CH_2Cl_2 (1.85 ml) was slowly added to the stirred mixture and the solution warmed to room temperature. After 5 h the reaction was quenched with MeOH (2.5 ml), diluted with dichloromethane (10 ml),

extracted with $NaHCO_3$ solution and washed with water. The organic layer was dried over Na_2SO_4, evaporated to dryness and chromatographed on silica gel column with CH_2Cl_2 : MeOH (from 100:0 to 90:10). Fractions containing pure compound were combined, evaporated and treated by the method described for compound **10**. Purification of **21** was performed on RP-2 silanized silica gel column with H_2O: MeOH (from 100:0 to 50:50). Fractions containing pure product **21** were evaporated and lyophilized in H_2O: dioxane (1:1) to obtain a white powder (68 mg, 60%). HPLC analysis on C_{18} column (condition A) R_t = 15.37 min, purity 100%; UV (EtOH) λ_{max} 264 nm (ϵ 7240), λ_{min} 232 nm (ϵ 1600); FAB > 0 m/z: 247 $(M+H)^+$, 127 $(B+H_2)^+$, 121 $(M-B)^+$; ^1H NMR δ 11.30 (s, 1, NH), 7.60 (s, 1, H_6), 5.94 (q, 1, $H_{1'}$), 4.84 (t, 1, $OH_{5'}$), 4.73-4.34 (m, 2, $H_{3',3''}$), 3.55 (m, 1, $H_{4'}$), 3.34 (m, 2, $H_{5',5''}$), 1.81 (s, 3, CH_3), 2.83 (d, $J_{1',-2'}$ = 6 Hz, 3, CH_3); ^{19}F NMR δ -231.85 (dt, $J_{F-H_{3'}} = J_{F-H_{3''}}$ = 47.5 Hz, $J_{F-H_{4'}}$ = 21.7 Hz).

3'-Azido-3'-deoxy-2',3'-secothymidine (22).

This product was obtained from **17** (274 mg, 0.5 mmol) by the same procedure as described for compound **12** except for the treatment with ammonium hydroxide. Purification of **22** was performed on RP-2 silanized silica gel column with H_2O: MeOH (from 100:0 to 50:50). Fractions containing pure product were evaporated and lyophilized from dioxane to obtain 88 mg (65%). HPLC analysis on C_{18} column (condition A) R_t = 20.68 min, purity 100%; UV (EtOH) λ_{max} 264 nm (ϵ 8000), λ_{min} 232 nm (ϵ 1780); FAB > 0 m/z: 292 $(M+Na)^+$, 270 $(M+H)^+$, 244 $(M-N_2+H_3)^+$, 127 $(B+H_2)^+$; ^1H NMR δ 11.32 (s, 1, NH), 7.60 (s, 1, H_6), 5.98 (q, 1, $H_{1'}$), 4.80 (t, 1, $OH_{5'}$), 361 (m, 1, $H_{3'}$), 3.52 (m, 1, $H_{4'}$), 3.30 (m, 3, $H_{3'',5',5''}$), 1.80 (s, 3, CH_3), 1.42 (d, $J_{2',-1'}$ = 6 Hz, 3, CH_3).

3'-Amino-3'-deoxy-2',3'-secothymidine (23).

3'-Azido-3'-deoxy-2',3'-secothymidine (**22**) (74 mg, 0.27 mmol) and triphenylphosphine (113 mg, 0.43 mmol) were dissolved in pyridine (0.5 ml) and kept at room temperature for 2 h. Concentrated ammonium hydroxide (0.8 ml) was then

added and the solution was left at room temperature with stirring overnight. Pyridine and ammonium hydroxide were removed by evaporation, water was added, and triphenylphosphine and triphenylphosphine oxyde were extracted with dichloromethane. The aqueous layer was purified on RP-2 silanized silica gel column with H_2O: MeOH (from 100:0 to 0:100) to obtain compound **23** (40 mg, 62%) after lyophilization in H_2O: dioxane (1:1). HPLC analysis on C_{18} column (condition A) R_t = 9.36 min, purity 100%; UV (EtOH) λ_{max} 265 nm (ϵ 6880), λ_{min} 234 nm (ϵ 2100); FAB > 0 m/z: 266 $(M+Na)^+$, 244 $(M+H)^+$, 127 $(B+H_2)^+$, 118 $(M-B)^+$; ^1H NMR δ 7.54 (s, 1, H_6), 5.85 (q, 1, $H_{1'}$), 3.30 (m, 7, $H_{3',3'',4'}$, NH, NH_2, $OH_{5'}$), 2.66 (m, 2, $H_{5',5''}$).

BIOLOGICAL METHODS

The antiviral assays were performed as previously reported (45).

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