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Synthesis and antiviral evaluation of some β-L-2′,3′-dideoxy-5-chloropyrimidine nucleosides and pronucleotides☆

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Abstract

The synthesis and in vitro anti human immunodeficiency virus (HIV) and anti-hepatitis B virus (HBV) activities of some unnatural β-L-nucleoside enantiomers related to the anti-HIV compound 2′,3′-dideoxy-3′-fluoro-5-chlorouridine (β-D-3′Fdd5ClU) are reported. In contrast to β-D-3′Fdd5ClU, β-L-3′Fdd5ClU and the other L-congeners were devoid of significant anti-HIV effects, but β-L-2′,3′-dideoxy-5-chlorocytidine (β-L-dd5ClC) and β-L-2′,3′-dideoxy-3′-fluoro-cytidine (β-L-3′FddC) showed a distinct anti-HBV activity. Three mononucleoside phosphotriester derivatives with *S*-pivaloyl-2-thioethyl (*t*-BuSATE) groups as biolabile phosphate protective groups were also synthesized. The bis(*t*-BuSATE) derivative of β-D-3′Fdd5ClU retained anti-HIV activity in thymidine kinase deficient (TK^-) CEM cells.

Keywords: Human immunodeficiency virus; Hepatitis B virus; β-L-Dideoxynucleoside enantiomers; Pronucleotides

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1. Introduction

Pandemic morbidity and mortality due to the human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are at the origin of intensive efforts in discovering potent selective antiviral agents against these viruses. Several HIV reverse transcriptase inhibitors and protease inhibitors have become available for the treatment of acquired immunodeficiency syndrome (AIDS; Kinchington et al., 1998), but additional anti-HIV agents are still needed to overcome drug resistance and toxicity of the existing drugs. Additionally, although there are over 300 million HBV chronic carriers throughout the world, only one oral agent, namely 2′,3′-dideoxy-3′-thia-β-L-cytidine (lamivudine, 3TC; Fig. 1; Perry and Faulds, 1997) has so far been officially licensed for the treatment of adults with chronic hepatitis B. It is noteworthy that 3TC, which was also approved in November 1995 by the US Food and Drug Administration (FDA) for first-line treatment of HIV infection in adults and children is endowed with the β-L-configuration, which points to the potential of such unnatural enantiomers in antiviral chemotherapy (Wang et al., 1998). Actually, other pyrimidine β-L-dideoxy nucleosides have also shown promising anti-HIV and anti-HBV activity in cell culture experiments, as for instance β-L-2′,3′-dideoxycytidine (β-L-ddC, Fig. 1) and β-L-2′,3′-dideoxy-5-fluorocytidine (β-L-dd5FC, Fig. 1) (Gosselin et al., 1994a; Lin et al., 1994a; Gosselin et al., 1994b; Lin et al., 1994b; Schinazi et al., 1994).

Furthermore, among the various pyrimidine β-D-2′,3′-dideoxynucleoside analogues that have been shown to possess anti-HIV activity (Balzarini et al., 1988; Herdewijn et al., 1991), β-D-2′,3′-dideoxy-3′-fluorothymidine (β-D-3′FddT, Fig. 1) has been described as the most potent inhibitor of HIV-1 replication in various cellular systems, this compound being at least five-times more potent than β-D-2′,3′-dideoxy-3′-azidothymidine (AZT; Balzarini et al., 1988, 1989b; Herdewijn et al., 1991). However, β-D-3′-FddT also has considerable cytotoxic effects (Herdewijn et al., 1991; Daluge et al., 1994). Following the pioneering work of Balzarini et al. (1982), several 3′-fluoro-5-halogeno pyrimidine nucleoside derivatives have been synthesized (Matthes et al., 1989; Van Aerschot et al., 1989; Balzarini et al., 1989b; Herdewijn et al., 1991). Of these compounds, β-D-2′,3′-dideoxy-3′-fluoro-5-chlorouridine (β-D-3′-Fdd5ClU, Fig. 1) emerged as a selective anti-HIV agent. Introduction of a chlorine atom at the C-5 position of uracil resulted in a markedly reduced toxicity compared to that of β-D-3′-FddT and its uracil counterpart (β-D-3′-FddU; Matthes et al., 1989; Van Aerschot et al., 1989; Balzarini et al., 1989a,b; Van Aerschot et al., 1990; Herdewijn et al., 1991). Moreover, β-D-3′-Fdd5ClU showed activity against HIV strains

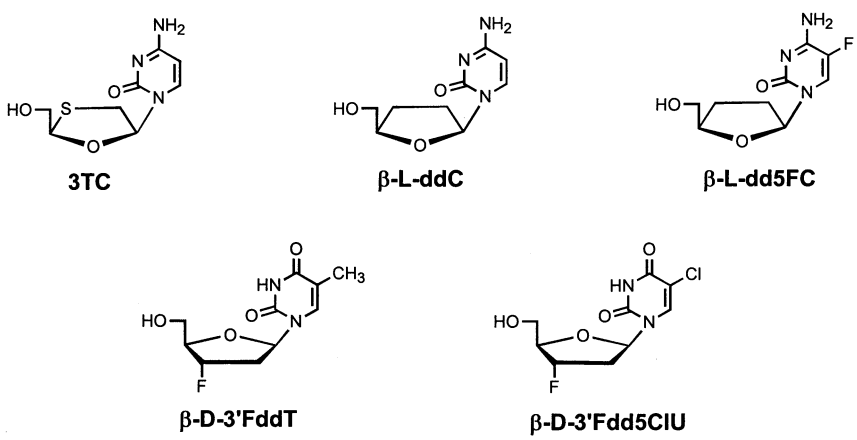


Fig. 1. Anti-HBV and/or anti-HIV pyrimidine β-L and β-D-2′,3′-dideoxynucleosides.

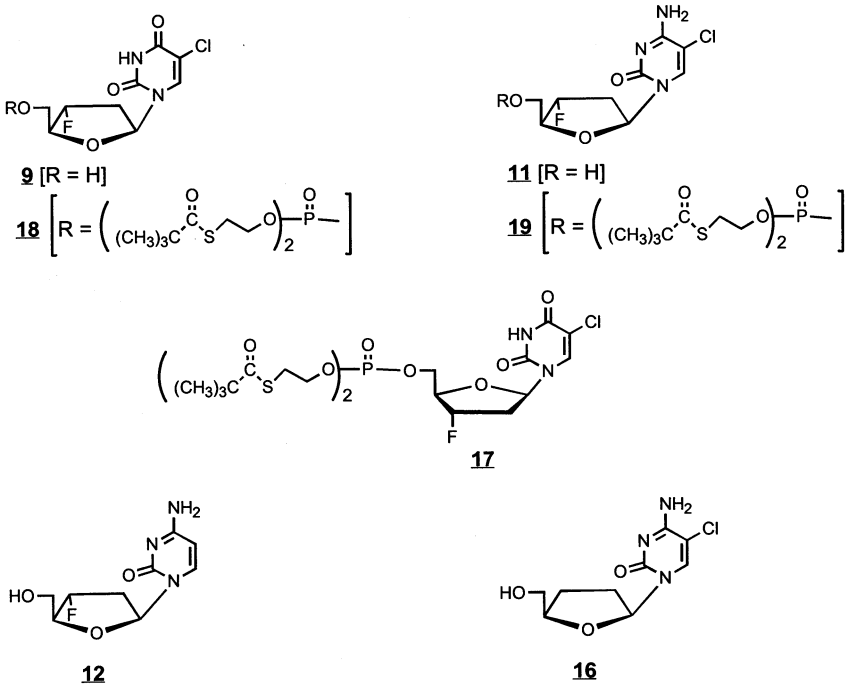


Fig. 2. Structures of the hitherto unknown compounds covered by the present work.

that were resistant to AZT, to β-D-2′,3′-dideoxyinosine (ddI) or to β-D-ddC (Daluge et al., 1994). The promising in vitro anti-HIV activity of β-D-3′-Fdd5ClU alone and in combination with other anti-retroviral agents, along with limited toxicologic effects and desirable pharmacokinetic characteristics has led to the clinical evaluation of this compound (Riddler et al., 1996); unfortunately this compound was dropped from development due to a lack of efficacy in the clinic (Herdewijn, 1997).

As a part of our new antiviral drug discovery efforts, and owing to the above considerations, it appeared of interest to us to prepare the β-L-enantiomer **9** (Fig. 2) of 3′Fdd5ClU, hitherto unknown to the best of our knowledge. Here, we report the stereospecific synthesis and antiviral evaluation of this compound as well as several of other pyrimidine β-L-2′,3′-dideoxynucleosides, namely β-L-2′,3′ - dideoxy - 3′ - fluoro - 5 - chlorocytidine (β-L-3′Fdd5ClC, **11**), β-L-2′,3′-dideoxy-3′-fluorocytidine (β-L-3′FddC, **12**) and β-L-2′,3′-dideoxy-5-chlorocytidine (β-L-dd5ClC, **16**) (Fig. 2).

Furthermore, as a result of the potential antiviral activity of pronucleotide derivatives (Alexander and Holy, 1994; Jones and Bischofberger, 1995; Arzumanov and Dyatkina, 1996; Krise and Stella, 1996; Périgaud et al., 1997; Meier, 1998), we also decided to synthesize and to evaluate the antiviral activity of the bis(*S*-pivaloyl-2-thioethyl)phosphotriester derivatives of β-D- and β-L-3′Fdd5ClU and of β-L-3′Fdd5ClC, namely bis(*t*-BuSATE)-β-D-3′Fdd5ClUMP **17**, bis(*t*-BuSATE)-β-L-3′Fdd5ClUMP **18** and bis(*t*-BuSATE)-β-L-3′Fdd5ClCMP **19**, respectively (Fig. 2). As recently demonstrated for acyclovir (Hantz et al., 1999; Périgaud et al., 1999), we hoped that such neutral nucleoside phosphotriesters, which incorporate *S*-acyl-2-thioethyl(-SATE) biolabile phosphate protecting groups, would result in the intracellular delivery of the parent 5′-mononucleotide, thus circumventing the first anabolic phosphorylation step which is usually a rate-limiting step in the mode of action of

antiviral nucleoside analogues (Sommadossi, 1993; Gao et al., 1994).

2. Materials and methods

2.1. Chemistry

2.1.1. General methods

^{1}H, ^{19}F and ^{31}P NMR spectra were recorded at ambient temperature on a Bruker AC 250 (250 MHz) or Bruker AC 400 (400 MHz) spectrometer. ^{1}H NMR chemical shifts (δ) are quoted in parts per million (ppm) referenced to the residual solvent peak (chloroform ($CHCl_3$) set at 7.26 ppm and dimethyl sulfoxide (DMSO-d_5) set at 2.49 ppm) relative to tetramethylsilane (TMS). ^{19}F and ^{31}P chemical shifts are reported in ppm using, respectively, trichlorofluoromethane ($CFCl_3$) and phosphoric acid (H_3PO_4) as external references. The accepted abbreviations are as follows: s, singlet; dd, doublet of doublet; m, multiplet; q, quartet; pt, pseudotriplet; br s, broad signal. FAB mass spectra were recorded in the positive-ion or negative-ion mode on a JEOL DX 300 mass spectrometer operating with a JMA-DA 5000 mass data system and using a mixture of glycerol and thioglycerol (1/1, v/v, G-T) as matrix. Melting points were determined in open capillary tubes with a Gallenkamp MFB-595-010M apparatus and are uncorrected. UV spectra were recorded on an Uvikon 810 (KONTRON) spectrophotometer. Optical rotations were measured in a 1-cm cell on a Perkin-Elmer Model 241 spectropolarimeter. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). Thin-layer chromatography (TLC) was performed on precoated aluminium sheets of silica gel 60 F_{254} (Merck, Art. 5554), visualization of products being accomplished by UV absorbance and by charring with 10% ethanolic sulfuric acid with heating. Column chromatography was carried out on silica gel 60 (Merck, Art. 9385). All moisture-sensitive reactions were carried out under an argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over P_2O_5 under reduced pressure at room temperature.

2.1.2. Chemical synthesis

*2.1.2.1. 1,2-Di-O-acetyl-3,5-di-O-benzoyl-L-xylofuranose (**1**).* This compound was prepared in four steps from L-xylose without purification of the intermediates (Gosselin et al., 1993).

*2.1.2.2. 1-(3,5-Di-O-benzoyl-2-O-acetyl-β-L-xylofuranosyl)-5-chlorouracil (**2**).* A suspension of 5-chlorouracil (5.46 g, 37.3 mmol) in a mixture of hexamethyldisilazane (HMDS, 250 ml) and a catalytic amount of ammonium sulfate was heated under reflux overnight. After cooling, the excess of hexamethyldisilazane was removed in vacuo. Silylated 5-chlorouracil was dissolved in anhydrous 1,2-dichloroethane (245 ml). A solution of protected sugar **1** (11 g, 24.9 mmol) in 1,2-dichloroethane (145 ml) and trimethylsilyltriflate (TMSTf, 9 ml, 49.8 mmol) were added. The reaction mixture was stirred at room temperature for 5 h. After dilution with chloroform (200 ml), the solution was poured into an ice-cold saturated aqueous hydrogen carbonate solution (250 ml). The organic phase was separated, twice washed with water, dried over sodium sulfate and evaporated. Chromatography of the residue on a silica gel column using as eluent a stepwise gradient of methanol (0–5%) in methylene chloride gave pure **2** (10.1 g, 77% yield) as a white foam; mp = 92–97°C; ^{1}H NMR (DMSO-d_6) δ 11.98 (s, 1H, N*H*), 8.10 (s, 1H, *H*-6), 8.0–7.4 (m, 10H, 2 C_6H_5CO), 6.01 (d, 1H, *H*-1′, $J_{1'-2'} = 2.8$ Hz), 5.7 (m, 1H, *H*-3′), 5.57 (t, 1H, *H*-2′, $J_{2'-1'} = J_{2'-3'} = 2.4$ Hz), 4.8 (m, 1H, *H*-4′), 4.7–4.6 (m, 2H, *H*-5′ and *H*-5″), 2.12 (s, 3H, C*H*$_3$); mass spectra FAB > 0: 529 $(M+H)^+$, 469 $(M\text{-}CH_3CO_2)^+$, 407 $(M\text{-}C_6H_5CO_2)^+$, 383 $(S)^+$, 105 $(C_6H_5CO)^+$, 43 $(CH_3CO)^+$; FAB < 0: 527 $(M\text{-}H)^-$, 485 $(M\text{-}CH_3CO)^-$, 423 $(M\text{-}C_6H_5CO)^-$, 145, $(B)^-$, 121 $(C_6H_5CO_2)^-$, 59 $(CH_3CO_2)^-$; UV (ethanol 95) λ_{max} 274 nm (ε 9400), 230 nm (ε 25800); λ_{min} 252 nm (ε 5000); $[\alpha]_D^{20} = -41.7$ ($c = 0.6$, DMSO).

*2.1.2.3. 1-(3,5-Di-O-benzoyl-β-L-xylofuranosyl)-5-chlorouracil (**3**).* A solution of compound **2** (8.76 g, 16.6 mmol) in a mixture of acetic acid (35 ml) and pyridine (140 ml) was treated overnight with hydrazine hydrate ($\cong$ 80% in water, 2.5 ml, 52.0

mmol) with stirring at room temperature. Reaction was quenched by acetone (45 ml) with stirring at room temperature. After 1.5 h, the mixture was partially evaporated in vacuo and extracted from water (200 ml) into ethyl acetate (200 ml). The combined organic phases were washed several times first with an aqueous saturated sodium hydrogen carbonate solution (200 ml), then with water (2×200 ml), dried over sodium sulfate and evaporated to dryness. Chromatography of the residue on a silica gel column using as eluent a stepwise gradient of methanol (0–6%) in methylene chloride led to the isolation of compound **3** (7.9 g, 98% yield) as a white foam; ^{1}H NMR (DMSO-d_6) δ 11.90 (s, 1H, N*H*), 8.02 (s, 1H, *H*-6), 7.9–7.4 (m, 10H, 2 C_6H_5CO), 6.34 (d, 1H, O*H*-2′, $J_{OH\text{-}2'} = 4.5$ Hz), 5.72 (s, 1H, *H*-1′), 5.4 (m, 1H, *H*-3′), 4.8–4.7 (m, 2H, *H*-5′ and *H*-5″), 4.7–4.6 (m, 1H, *H*-4′), 4.4 (m, 1H, *H*-2′); mass spectra FAB > 0: 487 $(M + H)^+$, 341 $(S)^+$, 147 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB < 0: 971 $(2M\text{-}H)^-$, 485 $(M\text{-}H)^-$, 145 $(B)^-$, 121 $(C_6H_5CO_2)^-$.

*2.1.2.4. 1-(3,5-Di-O-benzoyl-2-deoxy-β-L-threo-pentofuranosyl)-5-chlorouracil (**5**).* To a solution of compound **3** (7.96 g, 16.3 mmol) in anhydrous acetonitrile (480 ml) were added *O*-phenyl chlorothiocarbonate (3.38 ml, 24.4 mmol) and 4-(dimethylamino)pyridine (DMAP, 5.97 g, 48.9 mmol). The solution was stirred for 1 h at room temperature and then the solvent was removed under reduced pressure. Methylene chloride (300 ml) and water (200 ml) were added. The organic phase was separated and washed successively with ice-cold 0.5 N hydrochloric acid (2×200 ml), water (2×200 ml), then dried over sodium sulfate, filtered and evaporated to dryness. The residue was dissolved in dry toluene, the solution was evaporated under reduced pressure and this process was repeated three times to give the crude thiocarbonate derivative **4**, which was directly dissolved in dry toluene (130 ml) and treated with tris(trimethylsilyl)silane hydride (6.0 ml, 19.6 mmol) and α,α′-azobisisobutyronitrile (AIBN, 870 mg, 5.38 mmol) at 80°C for 1 h 30 min. The solvent was evaporated to leave crude **5**. Purification was accomplished by chromatography on a column of silica gel, using as eluent a stepwise gradient of methanol (0–2%) in methylene chloride. Pooling and evaporation of the appropriate fractions as indicated by TLC gave pure **5** (7.0 g, 91% yield) as a pale-yellow foam; mp = 191–193°C; ^{1}H NMR (DMSO-d_6) δ 11.87 (s, 1H, N*H*), 8.09 (s, 1H, *H*-6), 7.9–7.4 (m, 10H, 2C_6H_5CO), 6.17 (d, 1H, *H*-1′, $J_{1'\text{-}2'} = 7.6$ Hz), 5.71 (t, 1H, *H*-3′, $J_{3'\text{-}2'} = J_{3'\text{-}4'} = 4.2$ Hz), 4.8–4.7 (m, 2H, *H*-5′ and *H*-5″), 4.6 (m, 1H, *H*-4′), 3.0–2.9 (m, 1H, *H*-2′), 2.4 (pd, 1H, *H*-2″, $J_{2'\text{-}2''} = 15.6$ Hz); mass spectra FAB > 0: 941 $(2M + H)^+$, 471 $(M + H)^+$, 325 $(S)^+$, 227 $(M\text{-}2C_6H_5CO_2\text{-}H)^+$, 147 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB < 0: 939 $(2M\text{-}H)^-$, 469 $(M\text{-}H)^-$, 145 $(B)^-$, 121 $(C_6H_5CO_2)^-$; UV (ethanol 95) λ_{max} 275 (ε 9400); λ_{min} 253 (ε 4500); λ_{max} 229 (ε 26600); $[\alpha]_D^{20} = -68.3$ ($c = 1.04$, DMSO).

*2.1.2.5. 1-(2-Deoxy-β-L-threo-pentofuranosyl)-5-chlorouracil (**6**).* A solution of compound **5** (7.08 g, 15.0 mmol) in methanolic ammonia (previously saturated at −10°C and tightly stoppered) (375 ml) was stirred 24 h at room temperature. The solution was evaporated to dryness under reduced pressure, and water (100 ml) and methylene chloride (200 ml) were added. The aqueous phase was separated, twice washed with methylene chloride (2×100 ml) and evaporated to dryness. The residue was chromatographed on column of a silica gel, using as eluent a stepwise gradient of methanol (0–6%) in methylene chloride, to afford pure **6** (3.3 g, 84% yield) which was crystallized from methanol; mp = 180–181°C; ^{1}H NMR (DMSO-d_6) δ 11.8 (br s, 1H, N*H*), 8.23 (s, 1H, *H*-6), 6.04 (dd, 1H, *H*-1′, $J_{1'\text{-}2'} = 8.1$ Hz and $J_{1'\text{-}2''} = 1.6$ Hz), 5.35 (d, 1H, O*H*-3′, $J_{OH\text{-}3'} = 2.0$ Hz), 4.7 (br s, 1H, O*H*-5′,5″), 4.2 (br s, 1H, *H*-3′), 3.8 (m, 1H, *H*-4′), 3.7–3.6 (m, 2H, *H*-5′ and *H*-5″), 2.6–2.5 (m, 1H, *H*-2′), 1.91 (pd, 1H, *H*-2″, $J_{2''\text{-}2'} = 14.7$ Hz); mass spectra FAB > 0: 355 $(M + G + H)^+$, 263 $(M + H)^+$, 147 $(BH_2)^+$; FAB < 0: 261 $(M\text{-}H)^-$, 145 $(B)^-$; UV (ethanol 95) λ_{max} 277 nm (ε 9500); λ_{min} 238 nm (ε 1300); $[\alpha]_D^{20} = +30.0$ ($c = 1.0$, DMSO); Anal. calcd. for $C_9H_{11}ClN_2O_5$: C = 41.15; H = 4.22; Cl = 13.50; N = 10.67; found: C = 41.01; H = 4.13; Cl = 13.58; N = 10.76.

2.1.2.6. 1-(5-O-Benzoyl-2-deoxy-β-L-threo-pentofuranosyl)-5-chlorouracil (7). To a cooled (ice-bath) solution of **5** (1.84 g, 7.0 mmol) in a mixture of anhydrous pyridine (55 ml) and *N*,*N*-dimethylformamide (DMF, 15 ml) was added, dropwise with stirring, a solution of benzoyl chloride (900 μl, 7.7 mmol). The mixture was stirred for 3 h at 0–4°C with the exclusion of moisture. Cooled water (150 ml) was added and the mixture was diluted with methylene chloride (100 ml), washed with a saturated aqueous sodium hydrogen carbonate solution (100 ml) and water (2 × 100 ml), dried over sodium sulfate, filtered and concentrated to dryness. The residue was chromatographed on a column of silica gel, using as eluent a stepwise gradient of methanol (0–3%) in methylene chloride, to afford pure **7** (2.33 g, 91% yield) which was crystallized from methylene chloride; mp = 175–177°C; ^{1}H NMR (DMSO-d_6) δ 11.8 (br s, 1H, N*H*), 8.28 (s, 1H, *H*-6), 8.0–7.5 (m, 5H, C_6H_5CO), 6.12 (d, 1H, *H*-1′, $J_{1'-2'} = 8.1$ Hz), 5.70 (d, 1H, O*H*-3′, $J_{OH-3'} = 3.1$ Hz), 4.6–4.5 (m, 2H, *H*-5′ and *H*-5″), 4.4 (m, 1H, *H*-3′), 4.2 (m, 1H, *H*-4′), 2.7–2.6 (m, 1H, *H*-2′), 2.0 (pd, 1H, *H*-2″, $J_{2''-2'} = 14.8$ Hz); mass spectra FAB > 0: 733 $(2M + H)^+$, 367 $(M + H)^+$, 221 $(S)^+$, 105 $(C_6H_5CO)^+$; FAB < 0: 1097 (3M-H)$^-$, 731 (2M-H)$^-$, 365 (M-H)$^-$, 145 (B)$^-$; UV (ethanol 95) λ_{max} 274 nm (ε 9900); λ_{min} 248 nm (ε 3700); $[\alpha]_D^{20} = -53.4$ ($c = 1.03$, DMSO).

2.1.2.7. 1-(5-O-Benzoyl-3-fluoro-2,3-dideoxy-β-L-erythro-pentofuranosyl)-5-chlorouracil (8). A stirred solution of compound **7** (2.2 g, 6.0 mmol) in a mixture of methylene chloride (53 ml) and pyridine (4 ml) was cooled to 0°C, and diethylaminosulfur trifluoride (F_3SNEt_2 or DAST, 1.96 ml, 15.0 mmol) was added dropwise. The precipitate was slowly warmed to room temperature and was vigorously stirred overnight. The clear solution was cooled to 0°C and a saturated aqueous sodium hydrogen carbonate solution (100 ml) was added to decompose the excess of reagent. After extraction with methylene chloride (100 ml), the organic phase was separated, washed with water (2 × 100 ml), dried over sodium sulfate, filtered and evaporated to dryness. Chromatography on a column of silica gel, using as eluent a stepwise gradient of ethyl acetate (4–20%) in methylene chloride gave **8** (1.55 g, 70% yield), pure enough to be used directly in the next step.

2.1.2.8. 1-(3-Fluoro-2,3-dideoxy-β-L-erythro-pentofuranosyl)-5-chlorouracil (9). A solution of compound **8** (500 mg, 1.35 mmol) in methanolic ammonia (previously saturated at −10°C and tightly stoppered) (35 ml) was stirred overnight at room temperature. The solution was evaporated to dryness under reduced pressure and the residue was diluted with water (100 ml). The aqueous phase was washed twice with methylene chloride (2 × 100 ml) and then evaporated to dryness. Purification was accomplished by chromatography on a column of silica gel, using as eluent a stepwise gradient of methanol (0–5%) in methylene chloride. Pooling and evaporation of the appropriate fractions gave pure **9** (295 mg, 83% yield) which was crystallized from acetone/hexane; mp = 172–176°C; ^{1}H NMR (DMSO-d_6) δ 11.9 (br s, 1H, N*H*), 8.25 (s, 1H, *H*-6), 6.2 (m, 1H, *H*-1′), 5.29 (dd, 1H, *H*-3′, $J_{3'-F} = 53.7$ Hz and $J_{3'-2'} = 4.2$ Hz), 5.2 (br s, 1H, O*H*-5′,5″), 4.19 (d, 1H, *H*-4′, $J_{4'-F} = 27.4$ Hz), 3.7–3.6 (m, 2H, *H*-5′ and *H*-5″), 2.5–2.2 (m, 2H, *H*-2′ and *H*-2″); ^{19}F NMR (DMSO-d_6) δ-(172.9–173.5) (m, 1F, *F*-3′, $J_{F-3'} = 53.3$ Hz, $J_{F-2'} = 40.5$ Hz, $J_{F-4'} = 27.4$ Hz and $J_{F-2''} = 21.9$ Hz); mass spectra FAB < 0: 263 (M-H)$^-$, 145 (B)$^-$; UV (ethanol 95) λ_{max} 275 nm (ε 8700); λ_{min} 238 nm (ε 1200); $[\alpha]_D^{20} = -4.3$ (c = 0.93, DMSO); Anal. calcd. for $C_9H_{10}ClFN_2O_4$: C = 40.84; H = 3.81; Cl = 13.40; F = 7.18; N = 10.59; found: C = 40.60; H = 3.78; Cl = 14.11; F = 6.72; N = 10.33.

2.1.2.9. 1-(5-O-Benzoyl-3-fluoro-2,3-dideoxy-β-L-erythro-pentofuranosyl)-5-chloro-4-thiouracil (10). To a solution of compound **8** (500 mg, 1.36 mmol) in anhydrous methylene chloride (35 ml) was added Lawesson's reagent (Aldrich; 210 mg, 0.52 mmol). The reaction mixture was refluxed overnight and then the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel, using as eluent a stepwise gradient of methanol (0–2%) in methylene chloride. Pooling and evaporation of the appropriate fractions gave pure **10** (495 mg,

95% yield) as a yellow foam; ^{1}H NMR (DMSO-d_6) δ 13.1 (br s, 1H, NH), 8.00 (s, 1H, *H*-6), 8.0–7.5 (m, 5H, C_6H_5CO), 6.07 (dd, 1H, *H*-1′, $J = 8.0$ Hz and $J = 6.1$ Hz), 5.48 (dd, 1H, *H*-3′, $J_{3'\text{-F}} = 53.4$ Hz and $J_{3'\text{-}2'} = 5.0$ Hz), 4.6–4.5 (m, 3H, *H*-4′, *H*-5′ and *H*-5″), 2.7 (m, 2H, *H*-2′ and *H*-2″); ^{19}F NMR (DMSO-d_6) δ-(170.2–170.8) (m, 1F, *F*-3′, $J_{\text{F-}3'} = 53.4$ Hz, $J_{\text{F-}2'} = 36.1$ Hz and $J_{\text{F-}4'} = J_{\text{F-}2'} = 24.4$ Hz); mass spectra FAB > 0: 769 $(2M + H)^+$, 385 $(M + H)^+$, 163 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB < 0: 383 $(M\text{-}H)^-$, 161 $(B)^-$, 121 $(C_6H_5CO_2)^-$; UV (ethanol 95) λ_{max} 338 nm (ε 15500), 231 nm (ε 14600); λ_{min} 289 nm (ε 2600); $[\alpha]_D^{20} = -43.5$ ($c = 0.46$, DMSO).

*2.1.2.10. 1-(3-Fluoro-2,3-dideoxy-β-L-erythro-pentofuranosyl)-5-chlorocytosine (**11**) and 1-(3-fluoro-2,3-dideoxy-β-L-erythro-pentofuranosyl) cytosine (**12**).* A solution of **10** (495 mg, 1.30 mmol) in methanolic ammonia (previously saturated at −10°C) (30 ml) was heated at 90–100°C for 3 h in a sealed stainless-steel bomb. The mixture was cooled, evaporated to dryness and water (100 ml) and methylene chloride (100 ml) were added. The aqueous phase was separated, twice washed with methylene chloride (2 × 100 ml) and evaporated to dryness. The residue was chromatographed on a column of silica gel, using as eluent a stepwise gradient of methanol (0–15%) in methylene chloride to afford pure **11** (146 mg, 43% yield) and **12** (130 mg, 44% yield).

β-L-3′Fdd5ClC **11**: mp = 203–204°C (crystallized from ethanol); ^{1}H NMR (DMSO-d_6) δ 8.11 (s, 1H, *H*-6), 7.92 and 7.29 (2s, 2H, NH_2), 6.16 (dd, 1H, *H*-1′, $J = 8.9$ Hz and $J = 5.5$ Hz), 5.27 (dd, 1H, *H*-3′, $J_{3'\text{-F}} = 53.8$ Hz and $J_{3'\text{-}2'} = 4.5$ Hz), 5.3 (br s, 1H, O*H*-5′,5″), 4.2 (br d, 1H, *H*-4′, $J_{4'\text{-F}} = 27.4$ Hz), 3.6 (m, 2H, *H*-5′ and *H*-5″), 2.5 (m, 1H, *H*-2′), 2.2 (m, 1H, *H*-2″); ^{19}F NMR (DMSO-d_6) δ-(173.1–173.7) (m, 1F, *F*-3′, $J_{\text{F-}3'} =$ 52.5 Hz, $J_{\text{F-}2'} = 39.8$ Hz, $J_{\text{F-}4'}$ and $J_{\text{F-}2''} = 26.7$ Hz and 21.8 Hz); mass spectra FAB > 0: 790 $(3M + H)^+$, 527 $(2M + H)^+$, 356 $(M + G + H)^+$, 264 $(M + H)^+$, 146 $(BH_2)^+$; FAB < 0: 525 $(2M\text{-}H)^-$, 262 $(M\text{-}H)^-$, 144 $(B)^-$; UV (ethanol 95) λ_{max} 288 nm (ε 6300), 239 nm (sh; ε 5500); λ_{min} 265 nm (ε 4300); $[\alpha]_D^{20} = -2.5$ ($c = 1.0$, DMSO). Anal. calcd. for $C_9H_{11}ClFN_3O_3 \cdot \frac{1}{2} H_2O$: C = 39.64; H = 4.44; F = 6.97; N = 15.41; found: C = 39.65; H = 4.22; F = 6.83; N = 15.41.

β-L-3′FddC **12**: mp = 180–220°C (decomposition) (crystallized from methanol); ^{1}H NMR (DMSO-d_6) δ 7.76 (d, 1H, *H*-6, $J_{6\text{-}5} = 7.5$ Hz), 7.21 and 7.17 (2s, 2H, NH_2), 6.20 (dd, 1H, *H*-1′, $J = 9.2$ Hz and $J = 5.4$ Hz), 5.73 (d, 1H, *H*-5, $J_{5\text{-}6} = 7.5$ Hz), 5.26 (dd, 1H, *H*-3′, $J_{3'\text{-F}} = 53.9$ Hz and $J_{3'\text{-}2'} = 4.5$ Hz), 5.1 (br s, 1H, O*H*-5′,5″), 4.13 (td, 1H, *H*-4′, $J_{4'\text{-F}} = 27.7$ Hz and $J_{4'\text{-}5',5''} = 4.1$ Hz), 3.6–3.5 (m, 2H, *H*-5′ and *H*-5″), 2.5–2.4 (m, 1H, *H*-2′), 2.3–2.2 (2m, 1H, *H*-2″, $J_{2''\text{-F}} = 40.8$ Hz); ^{19}F NMR (DMSO-d_6) δ-(173.0–173.7) (m, 1F, *F*-3′, $J_{\text{F-}3'} = 54.1$ Hz, $J_{\text{F-}2'} = 40.4$ Hz, $J_{\text{F-}4'}$ and $J_{\text{F-}2''} = 27.6$ Hz and 21.0 Hz); mass spectra FAB > 0: 688 $(3M + H)^+$, 459 $(2M + H)^+$, 322 $(M + G + H)^+$, 230 $(M + H)^+$, 112 $(BH_2)^+$; FAB < 0: 228 $(M\text{-}H)^-$, UV (ethanol 95) λ_{max} 270 nm (ε 8000), 237 nm (sh; ε 7400); λ_{min} 254 nm (ε 6800); $[\alpha]_D^{20} = -28.9$ ($c = 0.38$, DMSO). Anal. calcd. for $C_9H_{12}FN_3O_3$.: C = 47.16; H = 5.28; F = 8.29; N = 18.33; found: C = 47.09; H = 5.41; F = 7.93; N = 18.31.

*2.1.2.11. 1-(5-O-Benzoyl-2,3-dideoxy-β-L-pento-furanosyl)-5-chlorouracil (**14**).* To a solution of compound **7** (500 mg, 1.36 mmol) in anhydrous acetonitrile (85 ml) were added *O*-phenyl chlorothiocarbonate (470 μl, 3.40 mmol) and DMAP (1.66 g, 13.6 mmol). The suspension was stirred overnight at room temperature, then *O*-phenyl chlorothiocarbonate was added again (470 μl, 3.40 mmol) and stirring was maintained for 2 h. The mixture was concentrated in vacuo, diluted with methylene chloride (100 ml) and water (100 ml). The organic layer was washed successively with ice-cold 0.5 N hydrochloric acid (3 × 100 ml) and water (2 × 100 ml), dried over sodium sulfate, filtered and concentrated to dryness. The crude thiocarbonate derivative **13** was dissolved in dry dioxane (11 ml) and treated with tris(trimethylsilyl)silane hydride (500 μl, 1.63 mmol) and AIBN (72 mg, 0.45 mmol). The mixture was refluxed for 4 h, then cooled to room temperature and concentrated to dryness. Column chromatography of the residue, using a stepwise gradient of methanol (0–10%) in methylene chloride afforded **14** (300

mg, 63% yield) as a foam; mp = 72–76°C; ^{1}H NMR (DMSO-d_6) δ 11.9 (br s, 1H, N*H*), 8.0–7.5 (m, 5H, C_6H_5CO), 7.89 (s, 1H, *H*-6), 5.9 (m, 1H, *H*-1′), 4.6–4.5 (m, 2H, *H*-5′ and *H*-5″), 4.4–4.3 (m, 1H, *H*-4′), 2.4–2.3 (m, 1H, *H*-2′), 2.1–2.0 (m, 3H, *H*-2″, *H*-3′ and *H*-3″); mass spectra FAB > 0: 351 $(M+H)^+$, 205 $(S)^+$, 147 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB < 0: 349 $(M\text{-}H)^-$, 145 $(B)^-$, 121 $(C_6H_5CO_2)^-$; UV (ethanol 95) λ_{max} 275 nm (ε 8800), 226 nm (ε 15500); λ_{min} 249 nm (ε 3400); $[\alpha]_D^{20} = -8.05$ ($c = 0.87$, DMSO).

*2.1.2.12. 1-(5-O-Benzoyl-2,3-dideoxy-β-L-pentofuranosyl)-5-chloro-4-thiouracil (**15**).* To a solution of compound **14** (569 mg, 1.62 mmol) in anhydrous methylene chloride (40 ml) was added Lawesson's reagent (460 mg, 1.13 mmol). The reaction mixture was refluxed overnight and then the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel, using as eluent a stepwise gradient of methanol (0–2%) in methylene chloride. Pooling and evaporation of the appropriate fractions gave pure **15** (405 mg, 68% yield) as a yellow foam; mp = 65–70°C; ^{1}H NMR (DMSO-d_6) δ 13.0 (br s, 1H, NH), 8.0–7.5 (m, 6H, C_6H_5CO and *H*-6), 5.88 (dd, 1H, *H*-1′, $J_{1'-2'} = 6.9$ Hz and $J_{1'-2''} = 3.4$ Hz), 4.6–4.5 (m, 2H, *H*-5′ and *H*-5″), 4,4 (m, 1H, *H*-4′), 2.4–2.3 (m, 1H, *H*-2′), 2.2–2.1 (m, 1H, *H*-2″), 2.1–2.0 (m, 2H, *H*-3′ et *H*-3″); mass spectra FAB > 0: 367 $(M+H)^+$, 205 $(S)^+$, 163 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; FAB < 0: 365 $(M\text{-}H)^-$, 161 $(B)^-$, 121 $(C_6H_5CO_2)^-$; UV (ethanol 95) λ_{max} 339 nm (ε 13900), 229 nm (ε 13400); λ_{min} 288 nm (ε 2000), 219 nm (ε 10300); $[\alpha]_D^{20} = -69.0$ ($c = 1.0$, DMSO).

*2.1.2.13. 1-(2,3-Dideoxy-β-L-pentofuranosyl)-5-chlorocytosine (**16**).* A solution of **15** (374 mg, 1.02 mmol) in methanolic ammonia (previously saturated at −10°C) (25 ml) was heated at 90–100°C for 3 h in a sealed stainless-steel bomb. The mixture was cooled, evaporated to dryness and water (100 ml) and methylene chloride (100 ml) were added. The aqueous phase was separated, twice washed with methylene chloride (2 × 100 ml) and evaporated to dryness. The residue was chromatographed on a column of silica gel, using as eluent a stepwise gradient of methanol (0–15%) in methylene chloride to afford pure **16** (127 mg, 51% yield) which was crystallized from methanol; mp = 194–197°C; ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H, *H*-6), 7.74 et 7.11 (2s, 2H, NH_2), 5.84 (dd, 1H, *H*-1′, $J_{1'-2'} = 6.5$ Hz et $J_{1'-2''} = 2.5$ Hz), 5,2 (br s, 1H, O*H*-5′,5″), 4.1–4.0 (m, 1H, *H*-4′), 3.7 (pd, 1H, *H*-5′, $J_{5'-5''} = 12.0$ Hz), 3.5 (pd, 1H, *H*-5″, $J_{5''-5'} = 12.0$ Hz), 2.3–2.2 (m, 1H, *H*-2′), 1.9–1.8 (m, 1H, *H*-2″), 1.8–1.7 (m, 2H, *H*-3′ and *H*-3″); mass spectra FAB > 0: 736 $(3M+H)^+$, 491 $(2M+H)^+$, 338 $(M+G+H)^+$, 246 $(M+H)^+$, 146 $(BH_2)^+$, 101 $(S)^+$; FAB < 0: 244 $(M\text{-}H)^-$, 144 $(B)^-$; UV (ethanol 95) λ_{max} 288 nm (ε 6900); λ_{min} 263 nm (ε 4300), λ_e 218 nm (ε 11800); $[\alpha]_D^{20} = -53.1$ ($c = 0.96$, DMSO); Anal. calcd. for $C_9H_{12}ClN_3O_3$: C = 44.00; H = 4.93; Cl = 14.43; found: C = 44.30; H = 5.01; Cl = 14.57.

*2.1.3. General procedure for the preparation of the phosphotriesters **17–19***

1*H*-Tetrazole (3 eq.) was added to a stirred solution of β-D-3′Fdd5ClU (100 mg, 0.38 mmol), β-L-3′Fdd5ClU **9** (47 mg, 0.18 mmol) or β-L-3′Fdd5ClC **11** (66 mg, 0.25 mmol) and bis(*S*-pivaloyl-2-thioethyl)*N*,*N*-diisopropylphosphoramidite (1.2 eq.; synthesized as already described (Lefebvre et al., 1995)) in a mixture of tetrahydrofuran and *N*,*N*-dimethylformamide (1:2, v/v; 5 ml/mmol of nucleoside). After 30 min at room temperature, the reaction mixture was cooled to −40°C and a 3-M solution of *tert*-butyl hydroperoxide (795 μl or 2.37 mmol/mmol of nucleoside) was added. Then, the reaction mixture was allowed to warm to room temperature over 1 h and diluted with methylene chloride (2 ml/mmol of nucleoside). Sodium hydrogen sulfite (10% aqueous solution, 3 ml/mmol of nucleoside) was added to reduce the excess of *tert*-butyl hydroperoxide. The organic layer was separated, diluted with methylene chloride, washed with a saturated aqueous sodium hydrogen carbonate solution and then with water, dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was taken up in the minimum amount of methylene chloride and chromatographed on a silica gel

column using as eluent a stepwise gradient of methanol (0–6%) in methylene chloride. The title compound **17** (188 mg, 79% yield), **18** (87 mg, 78% yield) and **19** (92 mg, 46% yield) were obtained as pale yellow oil.

*2.1.3.1. 1-[5-O-[O,O′-Bis(S-pivaloyl-2-thioethyl) phosphoryl]- 2,3- dideoxy - 3 - fluoro - β - D - erythro-pentofuranosyl]-5-chlorouracil (**17**).* ^{1}H NMR (DMSO-d_6) δ 12.0 (s, 1H, N*H*), 8.00 (s, 1H, *H*-6), 6.18 (dd, 1H, *H*-1′, $J = 8.8$ Hz and $J = 5.7$ Hz), 5.34 (dd, 1H, *H*-3′, $J_{3'\text{-F}} = 53.4$ Hz and $J_{3'\text{-}2'} = 4.8$ Hz), 4.37 (td, 1H, *H*-4′, $J_{4'\text{-F}} = 26.5$ Hz, $J_{4'\text{-}5',5''} = 4.6$ Hz), 4.2 (m, 2H, *H*-5′ and *H*-5″), 4.1 (m, 4H, 2CH_2O), 3.1 (m, 4H, 2CH_2S), 2.5–2.3 (m, 2H, *H*-2′ and *H*-2″), 1.19 (d, 18H, 2(CH_3)$_3$C, J = 1.8 Hz); ^{19}F NMR (DMSO-d_6) δ-(174.0–174.7) (m, 1F, *F*-3′, $J_{\text{F-}3'} = 52.9$ Hz, $J_{\text{F-}2'} = 36.2$ Hz et $J_{\text{F-}2''} = J_{\text{F-}4'} = 25.7$ Hz); ^{31}P NMR (DMSO-d_6) δ -0.70 (s); mass spectra FAB > 0: 633 $(M + H)^+$, 573 $(M\text{-}(CH_3)_3C + 2H)^+$, 345 $(M\text{-}2((CH_3)_3CC(O)SCH_2CH_2) + 3H)^+$, 147 $(BH_2)^+$, 145 $((CH_3)_3CC(O)SCH_2CH_2)^+$, 57 $((CH_3)C)^+$; FAB < 0: 631 $(M\text{-}H)^-$, 487 $(M\text{-}(CH_3)_3CC(O)SCH_2CH_2)^-$, 385 $(((CH_3)_3CC(O)SCH_2CH_2O)_2PO_2)^-$, 145 $(B)^-$; UV (ethanol 95) λ_{max} 275 nm (ε 8700); λ_{min} 248 nm (ε 3700); $[\alpha]_D^{20} = +5.4$ ($c = 1.29$, DMSO).

*2.1.3.2. 1-[5-O-[O,O-Bis(S-pivaloyl-2-thioethyl) phosphoryl]- 2,3- dideoxy - 3 - fluoro - β - L - erythro-pentofuranosyl]-5-chlorouracil (**18**).* ^{1}H NMR (DMSO-d_6) δ 11.9 (sl, 1H, N*H*), 7.99 (s, 1H, *H*-6), 6.16 (dd, 1H, *H*-1′, $J = 8.7$ Hz and $J = 5.8$ Hz), 5.32 (dd, 1H, *H*-3′, $J_{3'\text{-F}} = 53.4$ Hz and $J_{3'\text{-}2'} = 4.8$ Hz), 4.3 (dl, 1H, *H*-4′, $J_{4'\text{-F}} = 26.5$ Hz), 4.2 (m, 2H, *H*-5′ and *H*-5″), 4.05 (q, 4H, 2CH_2O, $J = 6.2$ Hz), 3.1 (m, 4H, 2CH_2S), 2.5–2.4 (m, 2H, *H*-2′ and *H*-2″), 1.16 (d, 18H, 2(CH_3)$_3$C, $J = 1.5$ Hz); ^{19}F NMR (DMSO-d_6) δ-(174.0–174.5) (m, 1F, *F*-3′, $J_{\text{F-}3'} = 51.8$ Hz, $J_{\text{F-}2'} = 37.4$ Hz and $J_{\text{F-}2''} = J_{\text{F-}4'} = 24.1$ Hz); ^{31}P NMR (DMSO-d_6) δ -0.77 (s); mass spectra FAB > 0: 633 $(M + H)^+$, 489 $(M\text{-}(CH_3)_3CC(O)SCH_2CH_2 + 2H)^+$, 345 $(M\text{-}2(CH_3)_3CC(O)SCH_2CH_2 + 3H)^+$, 147 $(BH_2)^+$, 145 $((CH_3)_3CC(O)SCH_2CH_2)^+$; FAB < 0: 631 $(M\text{-}H)^-$, 487 $(M\text{-}(CH_3)_3CC(O)SCH_2CH_2)^-$, 385 $(((CH_3)_3CC(O)SCH_2CH_2O)_2PO_2)^-$; 145 $(B)^-$; UV (ethanol 95) λ_{max} 275 nm (ε 7000); λ_{min} 248 nm (ε 3100); $[\alpha]_D^{20} = -7.4$ ($c = 1.22$, DMSO).

*2.1.3.3. 1-[5-O-[O,O-Bis(S-pivaloyl-2-thioethyl) phosphoryl]- 2,3-dideoxy - 3 - fluoro - β - L - erythro-pentofuranosyl]-5-chlorocytosine (**19**).* ^{1}H NMR (DMSO-d_6) δ 8.0 and 7.4 (2sl, 2H, NH_2), 7.87 (s, 1H, *H*-6), 6.15 (dd, 1H, *H*-1′, $J = 8.9$ Hz and $J = 5.5$ Hz), 5.32 (dd, 1H, *H*-3′, $J_{3'\text{-F}} = 53.4$ Hz, and $J_{3'\text{-}2'} = 4.5$ Hz), 4.4 (dl, 1H, H-4′, $J_{4'\text{-F}} = 26.6$ Hz), 4.2 (m, 2H, *H*-5′ and *H*-5″), 4.1–4.0 (m, 4H, 2CH_2O), 3.1 (m, 4H, 2CH_2S), 2.5–2.2 (m, 2H, *H*-2′ and *H*-2″), 1.15 (d, 18H, 2(CH_3)$_3$C, $J = 1.8$ Hz); ^{31}P NMR (DMSO-d_6) δ -0.98 (s); mass spectra FAB > 0: 632 $(M + H)^+$, 246 $(M\text{-}(CH_3)_3CC(O)SCH_2CH_2O)_2PO_2)^+$, 146 $(BH_2)^+$, 145 $((CH_3)_3CC(O)SCH_2CH_2)^+$, 57 $((CH_3)_3C)^+$; FAB < 0: 1261(2M-H)$^-$, 630 $(M\text{-}H)^-$, 486 $(M\text{-}(CH_3)_3CC(O)SCH_2CH_2)^-$, 385 $(((CH_3)_3CC(O)SCH_2CH_2O)_2PO_2)^-$; 144 $(B)^-$; UV (ethanol 95) λ_{max} 288 nm (ε 6100), 222 nm (ε 14300); λ_{min} 266 nm (ε 4600).

2.2. Biological evaluations in cell culture experiments

2.2.1. Anti-HIV assays

Human immunodeficiency virus type 1 (HIV-1/IIIB) was obtained from Dr R.C. Gallo (at that time at the National institutes of Health, Bethesda, MD). HIV-2/ROD was provided by Dr L. Montagnier (at that time at the Pasteur Institute, Paris, France). CEM/0 cells were obtained from the American Tissue Culture Collection (Rockville, MD) and CEM/TK$^-$ cells were a kind gift from Drs S. Eriksson and A. Karlsson (Karolinska Institute, Stockholm, Sweden). CEM cells were infected with HIV-1 as previously described (Balzarini et al., 1993). Briefly, 4×10^5 CEM cells/ml were infected with HIV-1 or HIV-2 at 100 $CCID_{50}$ (50% cell culture infective dose) per ml of cell suspension. Then, 100 μl of the infected cell suspension were transferred to microtiter plate wells and mixed with 100 μl of the appropriate dilutions of the test compounds. After 4 days, giant cell formation was recorded microscopically in the HIV infected cell cultures.

The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were defined as the compound concentrations required to reduce by 50% the number of giant cells in the virus-infected cell cultures and the number of viable cells in the mock-infected cell cultures, respectively.

2.2.2. Anti-HBV assays

The 2.2.15 cells (HBV DNA-transfected human hepatoblastoma-derived HepG2 cells) were cultured as described previously (Korba and Guerin, 1992) and inhibition of HBV intracellular DNA (HBV replicative intermediate) was determined with minor modifications (El Alaoui et al., 1996). Briefly, cells cultured in Dubelcco's modified Eagle's medium (MEM) supplemented with 4% fetal bovine serum and 0.5 mM glutamine were treated with compounds for 9 days, and culture medium was changed every 3 days. Hep-G2 cells and untreated 2.2.15 cells were used as negative and positive controls. At harvest, the medium was removed and cells were lysed. Total intracellular DNA was recovered and subjected to Southern blot analysis using a ^{32}P-labeled HBV specific probe (pTHBV plamid which contains the full length HBV genome) kindly provided by Dr Raymond F. Schinazi (Emory University, Atlanta, GA). Inhibition of the viral replicative intermediate DNA in compound-treated cells versus control untreated cells was determined.

Cytotoxicity assays were conducted in HepG2 cells, which were maintained in MEM supplemented with 10% heat-inactived fetal bovine serum, 1% sodium pyruvate and 1% penicillin/streptomycin. Each compound was tested in four concentrations in triplicate cultures and the median cytotoxic concentration (CC_{50}) was determined as previously described (Schinazi et al., 1994), by measuring the uptake of neutral red dye in a 96-well cell culture plate.

2.2.3. Broad-spectrum antiviral activity assays

For herpes simplex viruses (HSV), vaccinia virus (VV), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), coxsackie virus type B4 (CBV), vesicular stomatitis virus (VSV) parainfluenza virus type 3 (PIV-3), respiratory syncytial virus (RSV), Sindbis virus, Punta Toto virus and reovirus type 1, the origin of the virus stocks (Witvrouw et al., 1998) and the assay procedures (De Clercq and Merigan, 1971; De Clercq et al., 1975, 1980; Andrei and De Clercq, 1990) have been described previously. For instance, VZV assays were carried out in human embryonic lung (HEL) cells using TK^+ (OKA and YS) and TK^- (07/1 and YS/R) VZV strains. Virus input was 20 plaque forming units (PFU), and acyclovir (ACV) was used as the reference compound. CMV assays were carried out in HEL cells using AD-169 and Davis CMV strains. Virus input was 100 PFU. Ganciclovir (GCV) was used as the reference compound. HSV assays were carried out against HSV-1 TK^+ (KOS, F and McIntyre) and HSV-2 (G and Lyons) and against HSV-1 TK^- (B2006) and HSV-1 TK^-/TK^+ (VMW1837) in embryonic skin muscle (E_6SM) and human embryonic lung (HEL) cell cultures. Ribavirin, GCV and ACV were used as reference compounds.

3. Results and discussion

3.1. Chemical results

3.1.1. Stereospecific synthesis of 2′,3′-dideoxynucleoside β-L-enantiomers (Scheme 1)

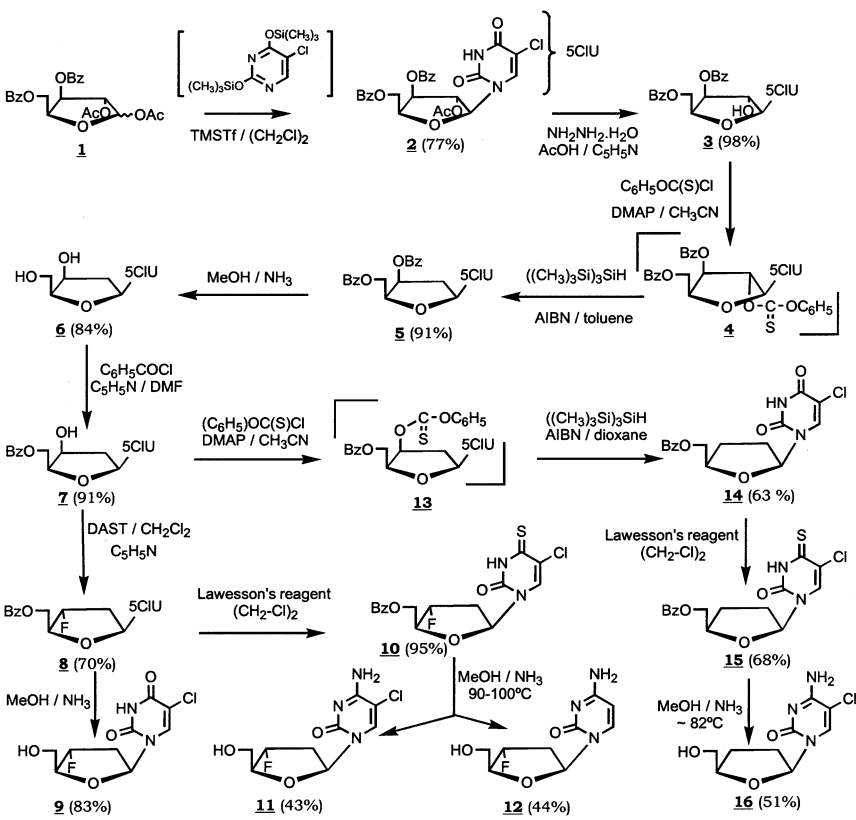
Starting from L-xylose, we first prepared in several steps and in good yield the fully acylated L-sugar **1** (Gosselin et al., 1993). This sugar was condensed with silylated 5-chlorouracil (Jeong et al., 1993) to give exclusively the corresponding fully protected β-L-nucleoside anomer **2**. This compound was selectively deacetylated at its 2′-position (Ishido et al., 1979), and then subjected to a Barton-type reductive deoxygenation (Barton and McCombie, 1975). Thus, treatment of **3** with phenyl chlorothionoformate and diethylaminopyridine, followed by subsequent radical deoxygenation afforded **5**. Debenzoylation with saturated methanolic ammonia afforded **6**, which was selectively benzoylated at the 5′-position to give the key intermediate **7**. This intermediate **7** was con-

verted to β-L-3′-Fdd5ClU **9** via subsequent fluorination with DAST (Herdewijn et al., 1989; Pankiewicz et al., 1992) and debenzoylation (Scheme 1).

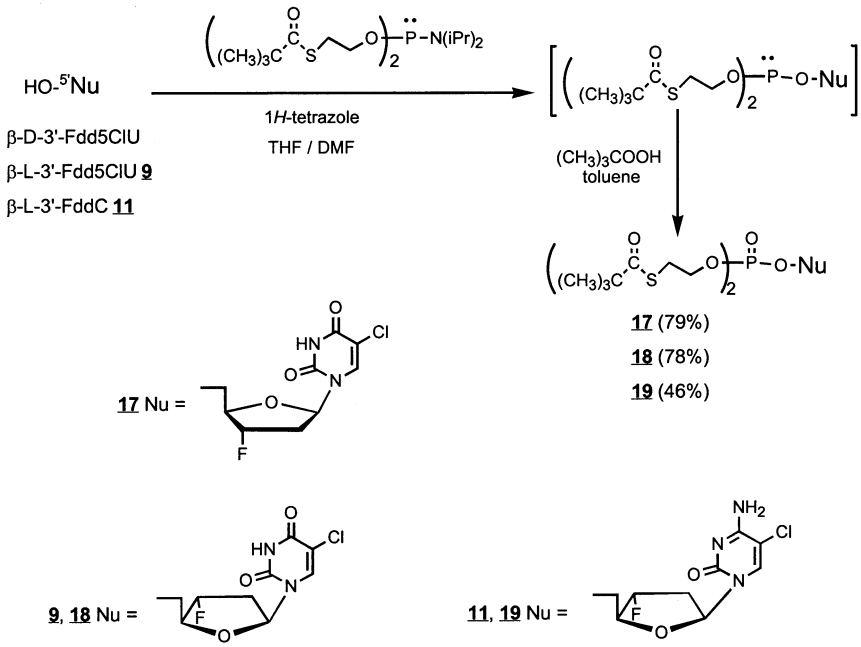
With regards to the β-L-5-chlorocytidine derivatives, β-L-3′-Fdd5ClC **11** and β-L-dd5ClC **16** were obtained by amination of their properly protected β-L-5-chlorouridine counterparts. Thus, compounds **8** and **14** were first converted into their corresponding thioamide derivatives (**10** and **15**, respectively) by treatment with Lawesson's reagent (Cava and Levinson, 1985) in refluxing methylene chloride. Subsequent treatment with methanolic ammonia at 90–100°C afforded the desired β-L-3′-Fdd5ClC **11** and β-L-dd5ClC **16**, respectively. It is noteworthy that during the column chromatography of **11**, β-L-3′-FddC **12** was also isolated in 44% yield. Such a dechlorination side reaction during methanolic ammonia treatment could be minimized by lowering the temperature as exemplified for the amination of **15**.

3.1.2. Synthesis of the bis(t-BuSATE) pronucleotides of β-D and β-L-3′-Fdd5ClU and β-L-3′-Fdd5ClC (Scheme 2)

The three bis(*t*-BuSATE)phosphotriester derivatives **17**–**19** were synthesized according to a published general procedure (Lefebvre et al., 1995). Thus, coupling β-D-3′-Fdd5ClU, its corresponding β-L enantiomer **9** and β-L-3′-Fdd5ClC **11** with an appropriate phosphoramidite reagent in the presence of 1-*H*-tetrazole, followed by in situ oxidation with *tert*-butyl hydroperoxide gave the desired bis(*t*-BuSATE) pronucleotides **17**–**19** which were isolated as pale yellow oils after purification on silica gel column chromatography.



Scheme 1. Chemical syntheses of pyrimidine β-L-5-dideoxynucleoside derivatives.



Scheme 2. Chemical syntheses of bis(*t*-BuSATE) pronucleotides.

Table 1
Anti-HIV-1, anti-HIV-2 activity and cellular toxicity of the title compounds in human CEM and hepatocyte cells

Compounds	EC_{50}[a] (μM)				CC_{50}[d] (μM)	
	HIV-1[b] CEM/O	HIV-2[b]		HBV R.I.[c] 2.2.15 cells	CEM/O	Hep-G2
		CEM/O	CEM/TK$^-$[e]			
3TC	0.039	0.1	0.17	0.01	≥250	>200
β-D-3′Fdd5ClU	0.50	2.5	>250	>10	>250	>200
β-L-3′Fdd5ClU **9**	>250	>250	ND	>10	>500	>200
β-L-3′Fdd5ClC **11**	>50	>50	ND	>10	≥500	>200
β-L-3′FddC **12**[f]	ND	ND	ND	1	ND	>200
β-L-dd5ClC **16**	>250	>250	ND	8	>250	>200
Bis(*t*BuSATE)-β-D-3′Fdd5ClUMP **17**	2.1	5.0	27.5	>10	58.0	>200
Bis(*t*BuSATE)-β-L-3′Fdd5ClUMP **18**	>50	>50	ND	>10	41	75
Bis(*t*BuSATE)-β-L-3′Fdd5ClCMP **19**	>50	>50	ND	>10	41	ND

[a] Fifty percent effective concentration, or compound concentration required to protect CEM cells against HIV-induced giant cell formation by 50% or to inhibit 50% of HBV DNA R.I. as compared to control.

[b] Anti-HIV experiments were performed with HIV-1 (IIIB) and HIV-2 (ROD) in CEM cells.

[c] R.I. represents the replicative intermediate (intracellular) HBV DNA.

[d] Fifty percent cytotoxic concentration, or compound concentration required to reduce CEM cell viability by 50% or to inhibit 50% of Hep-G2 cell growth as compared to control.

[e] CEM/TK$^-$ = thymidine kinase deficient CEM cells.

[f] β-L-3′FddC **12** was independently found virtually inactive (EC_{50} = 82 μM) against HIV-1 Lai in CEM-SS cells and fully inactive (EC_{50} >100 μM) against HIV-1 IIIB in MT-4 cells, without cytotoxicity (CC_{50} >100 μM) in both cases (Dr A.-M. Aubertin, Strasbourg, France; unpublished results).

3.2. Biological results (Table 1)

3.2.1. Antiviral activity of 2′,3′-dideoxynucleoside β-L-enantiomers

The antiretroviral activities of β-L-3′-Fdd5ClU **9**, β-L-3′-Fdd5ClC **11**, β-L-3′-FddC **12** and β-L-dd5ClC **16** were evaluated in CEM cells against HIV-1 and HIV-2 (data presented in Table 1). Results obtained with (−)-β-L-2′,3′-dideoxy-3′-thiacytidine (3TC, Lamivudine) and with β-D-3′-Fdd5ClU were included for comparison. The reference β-D-enantiomer of 3′-Fdd5ClU was noted to be a little less active than 3TC against HIV-1 and HIV-2 in CEM/0 cells, and fully inactive in a thymidine kinase-deficient cell line (CEM/TK$^-$) owing to its marked dependence on cytosolic thymidine kinase for activation. The β-L-enantiomer **9** of 3′-Fdd5ClU, as well as β-L-3′-Fdd5ClC **11**, β-L-3′-FddC **12** and β-L-dd5ClC **16**, were inactive against HIV (Table 1). When tested against HBV replication in 2.2.15 cells, β-D-3′-Fdd5ClU, β-L-3′-Fdd5ClU **9** and β-L-3′-Fdd5ClC **11** proved to be completely inactive, while β-L-3′-FddC **12** and β-L-dd5ClC **16** had an EC_{50} of 1 and 8 μM, respectively, **12** being far less active than the reference compound 3TC (EC_{50} 0.01 μM; Table 1).

The compounds were also evaluated against a panel of DNA and RNA viruses (Section 2), but none of them showed marked antiviral effects or detectable alteration of host cell morphology at the highest concentration tested (generally 200 μg/ml; data not shown).

3.2.2. Antiviral activity of the bis(t-BuSATE) pronucleotides

Bis(*t*-BuSATE)-β-D- and β-L-3′-Fdd5ClUMP (**17** and **18**, respectively), as well as bis(*t*-BuSATE)-β-L-3′-Fdd5ClCMP **19** were tested against HIV and HBV replications in the same conditions as their nucleoside parents. None of them showed anti-HBV activity (Table 1). On the other hand, bis(*t*BuSATE)-β-D-3′-Fdd5ClUMP **17** had comparable activity against HIV-1 and HIV-2 as its nucleoside parent β-D-3′-Fdd5ClU. And, interestingly, this pronucleotide **17** remained significantly active in CEM/TK$^-$ cells in contrast to its nucleoside parent. This may point to some intracellular release of the 5′-monophosphate derivative from **17**. If one assumes that such an intracellular delivery of β-L-3′-Fdd5ClUMP and β-L-3′-Fdd5ClCMP from the β-L-pronucleotides **18** and **19** also occurs, the lack of anti-HIV and anti-HBV activity of these unnatural enantiomers may be related to inadequate second and/or third phosphorylation steps, or to inefficient inhibition of viral DNA polymerases by their triphosphate forms.

4. Conclusion

In conclusion, we found that the unnatural β-L-enantiomers of uracil and cytosine 2′,3′-dideoxypentofuranosyl nucleosides **9** and **11** bearing both a fluorine atom at the C-3′ position of the sugar moiety and a chlorine atom at the C-5 position of the base do not inhibit HIV and HBV replication in cell culture. In addition, their lack of activity cannot be overcome by using their pronucleotide derivatives **18** and **19**, respectively. Furthermore, in the cytosine series and compared to previous results (Schinazi et al., 1994; Gosselin et al., 1994a; Lin et al., 1994a; Gosselin et al., 1994b; Lin et al., 1994b) the introduction of either a fluorine atom at the C-3′ position or a chlorine atom at the C-5 position of β-L-ddC resulted in nucleoside analogues (β-L-3′-FddC **12** and β-L-dd5ClC **16**, respectively) that had still retained considerable (although not as pronounced as for 3TC) activity against HBV. The search for more promising β-L-nucleoside analogues is currently the subject of active investigations in our laboratories.

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