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Synthesis and Studies of 3′-*C*-trifluoromethyl Nucleoside Analogues Bearing Adenine or Cytosine as the Base☆

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Abstract—3′-Deoxy-3′-*C*-CF_3, 2′,3′-dideoxy-3′-*C*-CF_3 and 2′,3′-unsaturated-3′-*C*-CF_3 nucleoside derivatives of adenosine and cytidine have been synthesized. All these derivatives were prepared by glycosylation of adenine and uracil with a suitable peracylated 3-trifluoromethyl sugar precursor. The resulting protected nucleosides were subject to appropriate chemical modifications to afford the target nucleoside derivatives. Additionally, the chemical stability in acidic and neutral media of the 2′,3′-dideoxy-3′-*C*-CF_3 and 2′,3′-unsaturated-3′-*C*-CF_3 nucleoside derivatives of adenosine was compared to that of their parent nucleosides 2′,3′-dideoxyadenosine (ddA) and 2′,3′-dideoxy-2′,3′-didehydroadenosine (d_4A). Our results confirm that addition of a trifluoromethyl group at C-3′ on such nucleoside derivatives appears to confer increased chemical stability toward acid-catalyzed cleavage of the glycosidic bond comparatively to their parent counterparts. When evaluated for their antiviral activity in cell culture experiments, two compounds, namely, 2′,3′-dideoxy-3′-*C*-CF_3-adenosine and 2′,3′-dideoxy-2′,3′-didehydro-3′-*C*-CF_3-cytidine exhibited moderate anti-HBV activity with EC_{50} values of 10 and 5 μM, respectively.

Introduction

To date, six nucleoside analogues, namely, 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxycytidine (ddC), 2′,3′-didehydro-3′-deoxythymidine (d_4T), 2′,3′-dideoxy-3′-thia-β-L-cytidine (3TC) and (1*S*,4*R*)-4-[2-amino-6-(cyclopropyl)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol (Abacavir) have been approved by the Food and Drug Administration (FDA) for the treatment of human immunodeficiency virus (HIV) infection. One of them, 3TC was also licensed by FDA for use in hepatitis B virus (HBV) therapy. All these 2′,3′-dideoxynucleoside analogues share a common mechanism of action. They are metabolized by cellular kinases to their 5′-triphosphate forms, which then exert their biological effect as a virus-specific polymerase competitive inhibitors or chain terminators because they lack a hydroxyl group at the C′-3 position.[1] However, inherent drug resistance[2] and toxicity[3] of the currently used antiviral drugs have prompted the development of new agents possessing more potent and broad antiviral activities. In order to discover new nucleoside derivatives with antiviral activity, modifications of the base and/or sugar moiety of natural nucleosides can be attempted. As a part of our ongoing research on this topic, we have synthesized, from a common trifluoromethyl sugar precursor, various 3′-*C*-trifluoromethyl nucleoside analogues bearing adenine and cytosine as the base. Many advantages can

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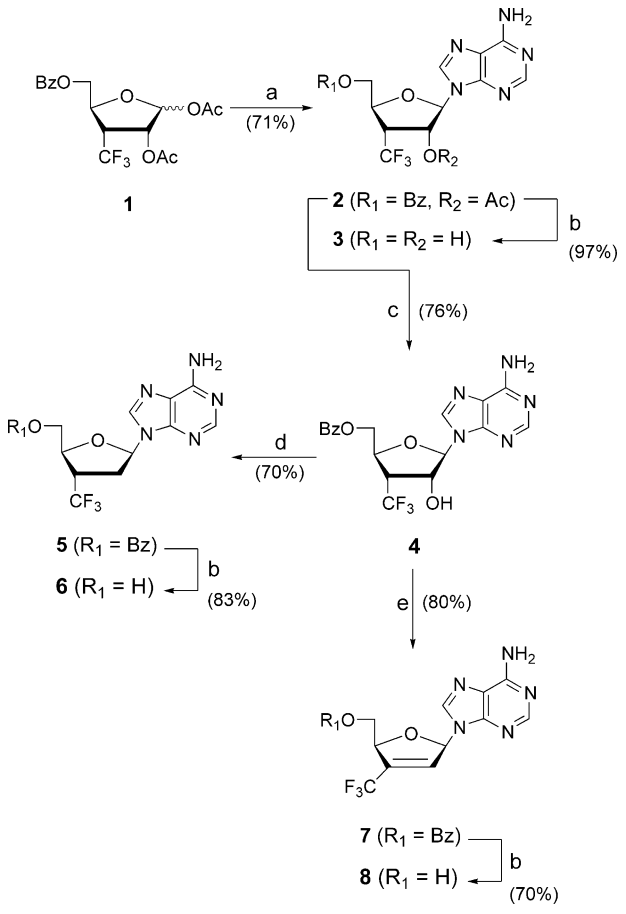
be expected from the presence of a CF_3 group on the sugar moiety of nucleosides, including higher lipophilicity and increased chemical and/or enzymatic stability. Herein, we report on the synthesis of 3′-deoxy-3′-*C*-CF_3, 2′,3′-dideoxy-3′-*C*-CF_3 and 2′,3′-unsaturated-3′-*C*-CF_3 nucleoside derivatives of adenosine (**3**, **6** and **8**) and cytidine (**10**, **13** and **15**), all of them being hitherto unknown except for **6**,[4] **13**[4,5] and **15**.[5] In addition, chemical stability studies within the adenine series are presented for **6** and **8**.

Results and Discussion

The synthesis began with the preparation of an appropriate trifluoromethyl sugar precursor, namely, 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-trifluoromethyl-β-D-ribofuranose[5] (**1**) which was obtained from commercially available diacetone-D-glucose following a modified procedure initially developed by Lavaire et al.[6] The syntheses of the 3′-deoxy-3′-*C*-CF_3-nucleoside derivatives of adenosine (**3**, **6** and **8**) are depicted in Scheme 1. A glycosylation reaction with adenine and **1** using stannic [tin(IV)] chloride as a catalyst[7] afforded 9-(2-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)adenine (**2**) in 71% yield after purification by silica gel column chromatography. The structure of **2** was fully established from ^1H, ^{13}C and UV spectra.

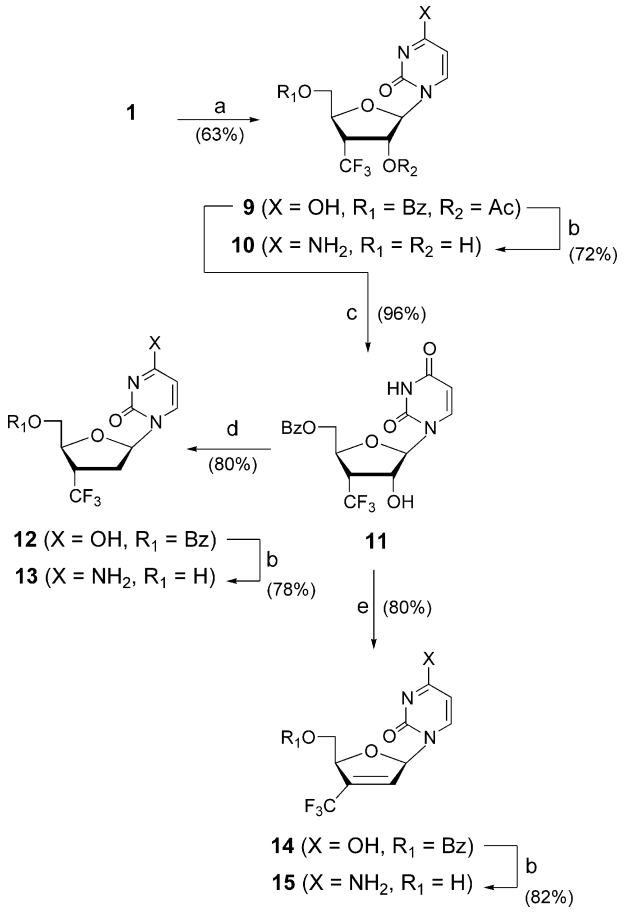


Scheme 1. (a) Adenine, $SnCl_4$, CH_3CN, rt; (b) NH_3/MeOH, rt; (c) H_2NNH_2, AcOH, pyridine, rt; (d) (i) DMAP, $C_6H_5O(S)Cl$, CH_3CN, rt; (ii) $(Me_3Si)_3SiH$, AIBN, toluene, reflux; (e) (i) MsCl, pyridine, rt; (ii) TBAF, THF, 50 °C.

Total deprotection of **2** with methanolic ammonia provided crystalline nucleoside **3**. In order to prepare the target compounds **6** and **8**, regioselective 2′-*O*-deacylation of **2** with hydrazine hydrate[8] was accomplished to give the key derivative **4**. The latter was then treated with *O*-phenyl chloro(thio)formate ($C_6H_5OC(S)Cl$) and 4-dimethylaminopyridine (DMAP) in acetonitrile. The corresponding 2′-*O*-[phenoxy-(thiocarbonyl)] intermediate was subsequently deoxygenated with tris(trimethylsilyl)silane[9] in dry toluene in the presence of α,α′-azoisobutyronitrile (AIBN) to yield the protected 2′,3′-dideoxy-3′-*C*-CF_3-nucleoside derivative **5**. Removal of the benzoyl group with methanolic ammonia afforded the desired dideoxynucleoside **6** as a crystalline solid in 83% after purification on silica gel column. On the other hand, introduction of a double bond between the 2′ and 3′ positions was achieved from **4** via a base-promoted β-elimination. The first step involved the preparation (by treatment of **4** with mesyl chloride) of the corresponding 2′-*O*-mesyl ester, which upon reaction with tetrabutylammonium fluoride[9] (TBAF) in tetrahydrofuran (THF) gave compound **7**. Finally, treatment with methanolic ammonia provided the desired 2′,3′-unsaturated-3′-*C*-CF_3-nucleoside derivative **8** in 70% yield.

The syntheses of the cytidine nucleoside derivatives **10**, **13** and **15** were achieved via the preparation of the 3′-deoxy-3′-*C*-CF_3-nucleosides of uridine[10] (**9**, **12** and **14**), followed by conversion into their corresponding cytidine derivatives as depicted in Scheme 2. Briefly, glycosylation reaction of uracil with sugar **1** was carried out under Vorbrüggen conditions using (trimethylsilyl) trifluoromethane sulfonate (TMSOTf) as a catalyst[11] in anhydrous 1,2-dichloroethane and afforded 1-(2-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)uracil (**9**) in 63% after silica gel column chromatography. Compound **9** was regioselectively deacylated to provide the key intermediate **11**. In order to obtain the 2′,3′-dideoxy-3′-*C*-CF_3 derivative **12**, compound **11** was then treated with [$C_6H_5OC(S)Cl$] in the presence of DMAP (4 equiv). After conventional work-up, the crude material was not purified but directly subjected to a radical reductive process to yield, after column chromatography, an inseparable mixture of compounds **12** and **14** (ratio **12**/**14** = 7/3, as determined by ^1H NMR). Formation of the 2′,3′-unsaturated nucleoside as a side-product is probably due to a β-elimination reaction of the labile transperiplanar H-3′β from the 2′-*O*-[phenoxy(thiocarbonyl)] intermediate promoted by DMAP. To overcome this side reaction, DMAP was replaced by pyridine, and thus, the 2′,3′-dideoxy-3′-*C*-CF_3 derivative **12** was obtained as the sole product in 80% overall yield from **11**. Additionally, the 2′,3′-unsaturated-3′-*C*-CF_3-nucleoside **14** was prepared by a sequence similar to that developed for the synthesis of the didehydro derivative **7** of adenosine. Finally, compounds **9**, **12** and **14** were converted into their corresponding cytidine derivatives **10**, **13** and **15** by using the nitrophenylation-ammonolysis procedure[12] in 72, 78 and 82% overall yield, respectively.

Modifications of the sugar moiety of natural nucleosides have led to the discovery of the 2′,3′-dideoxynucleoside



Scheme 2. (a) Silylated uracil, TMSOTf, 1,2-dichloroethane, rt; (b) (i) 1-methylpyrrolidine, CH_3CN, $(CF_3CO)_2O$, 0 °C; (ii) 4-nitrophenol, 0 °C; (iii) concd aq NH_3/dioxane, 55 °C; (iv) NH_3/MeOH, rt; (c) H_2NNH_2, AcOH, pyridine, rt; (d) (i) pyridine, $C_6H_5O(S)Cl$, CH_2Cl_2, rt; (ii) $(Me_3Si)_3SiH$, AIBN, toluene, reflux; (e) (i) MsCl, pyridine, rt; (ii) TBAF, THF, 50 °C.

analogues family, and some of them have demonstrated potent anti-HIV and anti-HBV activities. However, 2′,3′-dideoxynucleosides and their 2′,3′-unsaturated counterparts are chemically less stable in acidic media than their ribosyl forms due to the lack of the electron-withdrawing inductive effect of the hydroxyl groups. In particular, the 2′,3′-dideoxypurine nucleosides are by far more sensitive to chemical hydrolysis[13] than the dideoxy pyrimidine nucleosides.[14] Owing to the high electron withdrawing power of the CF_3 group[15] and its potential stabilizing effect on the lability of a glycosyl-purine bound, we have examined the chemical stability of the 2′,3′-dideoxy-3′-*C*-CF_3 and 2′,3′-unsaturated-3′-*C*-CF_3 nucleoside derivatives **6** and **8** of adenosine comparatively to 2′,3′-dideoxyadenosine (ddA) and 2′,3′-dideoxy-2′,3′-didehydroadenosine (d_4A). The stabilizing effect of the trifluoromethyl group has been stated in previous publications[5,6] but, until now, it has never been demonstrated on nucleoside structures. The cleavage of the glycosidic bond was studied at pH 2 and 7.2 buffer solutions and 37 °C. Time-dependent degradation of the nucleosides to the free adenine base was analyzed by high performance liquid chromatography (HPLC) of the samples taken at different time intervals. As expected, ddA and d_4A showed chemical instability. This phenomenon was illustrated by the relative high rates of decomposition of ddA and d_4A at pH 2 with a half-life ($t_{1/2}$) of 20 min (Fig. 1, panel A) and less than 1 min (Fig. 2, panel A), respectively. Additionally, d_4A was also been found to present chemical instability at pH 7.2 ($t_{1/2}$ = 2.2 days, Fig. 3, panel A), in accordance to previously reported data.[16] Introduction of the electron withdrawing CF_3 group at the 3′-position increased in a striking way the stability in acidic and neutral media of the nucleoside analogues, as demonstrated for compounds **6** and **8**. In our study, the 2′,3′-dideoxy-3′-*C*-CF_3 nucleoside derivative **6** proved to be over 250-fold more stable ($t_{1/2}$ = 3.7 days, Fig. 1, panel B) at pH 2 than its corresponding 2′,3′-dideoxynucleoside (ddA) counterpart. In a similar manner, the 2′,3′-unsaturated-3′-*C*-CF_3

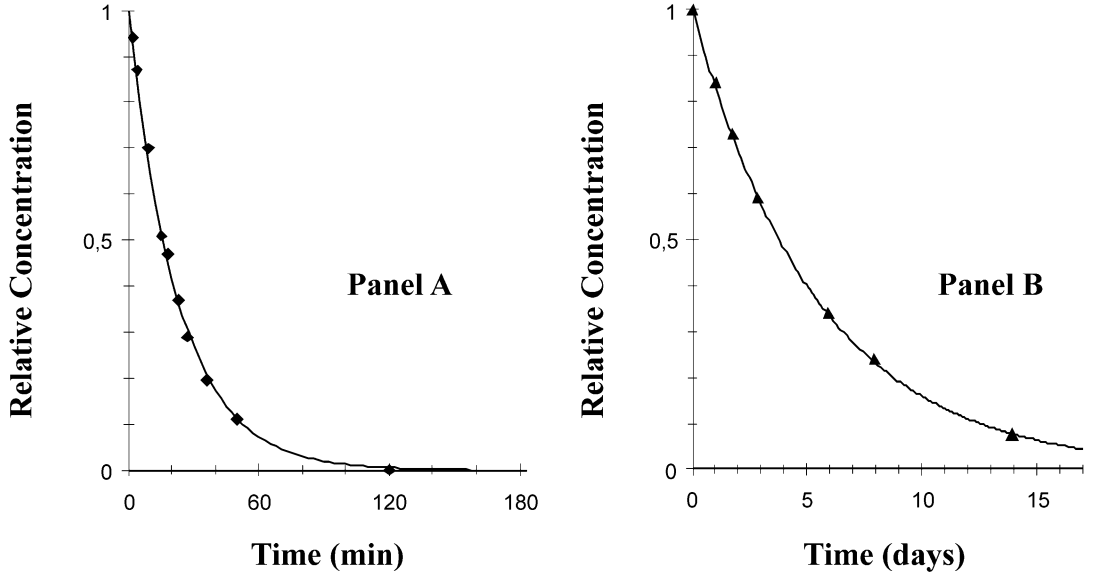


Figure 1. Relative concentration versus time curves for ddA (◆, panel A) and 2′,3′-dideoxy-3′-*C*-CF_3 adenosine (**6**, ▲, panel B) in Glycine/HCl buffer (pH 2) at 37 °C.

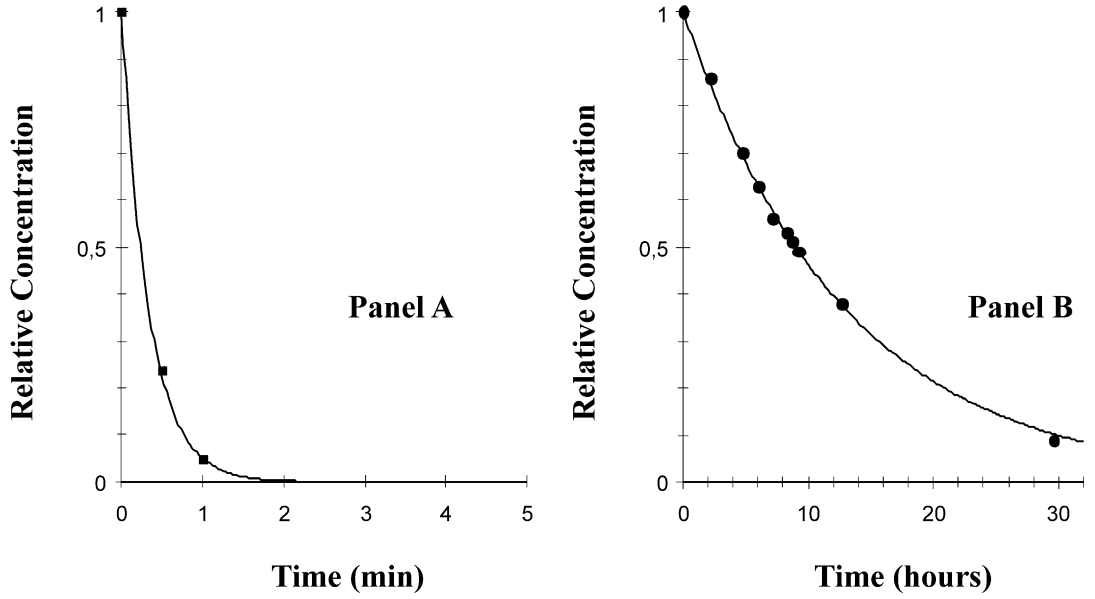


Figure 2. Relative concentration versus time curves for d_4A (■, panel A) and 2′,3′-dideoxy-2′,3′-didehydro-3′-*C*-CF_3 adenosine (**8**,●, panel B) in Glycine/HCl buffer (pH 2) at 37 °C.

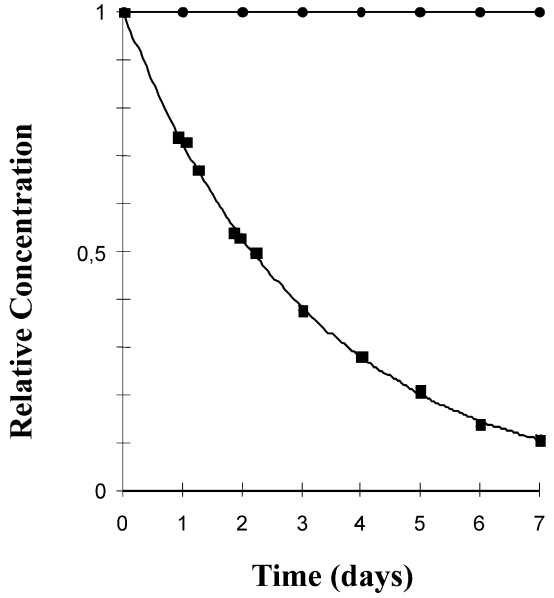


Figure 3. Relative concentration versus time curves for d_4A (■) and 2′,3′-dideoxy-2′,3′-didehydro-3′-*C*-CF_3 adenosine (**8**, ●) in phosphate buffer (pH 7.2) at 37 °C.

nucleoside **8** appeared to be over 500-fold more stable ($t_{1/2}$ = 8.9 h, Fig. 2, panel B) in acidic medium than its 2′,3′-didehydro nucleoside parent (d_4A). At pH 7.2 and in contrast to d_4A, compound **8** was completely stable with no decrease in concentration of **8** or formation of adenine over a 7-day period (Fig. 3, panel B). Also, no decomposition of ddA or nucleoside **6** were detected at pH 7.2 over a 7-day period (data not shown).

The 3′-*C*-CF_3 nucleoside derivatives of adenine (**3**, **6** and **8**) and cytosine (**10**, **13** and **15**) were tested for their inhibitory effects on the replication of HIV-1 in MT-4 cells. None of the compounds showed significant anti-HIV activity nor cytotoxicity at the highest concentration tested (generally 100 μM, data not shown). The anti-HBV activity of the synthesized nucleosides was evaluated in 2.2.15 cells (HBV DNA-transfected Hep-G2 cells) and in such experiments 2′,3′-dideoxy-3′-thia-β-L-cytidine (3TC) was included as a reference control. In our cell culture experiments, 2′,3′-dideoxy-3′-*C*-CF_3-adenosine **6** and 2′,3′-dideoxy-2′,3′-didehydro-3′-*C*-CF_3-cytidine **15** exhibited moderate anti-HBV activity, although their EC_{50} (10 μM for **6** and 5 μM for **15**) were higher than 3TC (EC_{50} = 0.05 μM). It is noteworthy that **6** and **15** (as 3TC) did not show cytotoxicity (CC_{50}) in Hep-G2 cells up to a concentration of 200 μM. In the same assays, the others nucleoside analogues were inactive (data not shown) and not cytotoxic.

Conclusion

The syntheses of a series of 3′-deoxy-3′-*C*-CF_3 nucleosides bearing adenine and cytosine as the base were undertaken to discover new nucleoside derivatives as potential anti-HIV and anti-HBV drugs. As demonstrated, we found that introduction of a trifluoromethyl group at C-3′ on acid sensitive purine dideoxy- and didehydronucleosides was able to confer better stability toward chemical hydrolysis compared to their non-trifluoromethylated counterparts. When evaluated against HIV-1 in cell cultures, none of the 3′-deoxy-3′-*C*-CF_3 nucleosides showed any antiretroviral activity. However, when evaluated in anti-HBV assays, two compounds 2′,3′-dideoxy-3′-*C*-CF_3 adenosine **6** and 2′,3′-dideoxy-2′,3′-didehydro-3′-*C*-CF_3 cytidine **15**, showed moderate antiviral activity without concomitant cytotoxicity. Evaluation of the compounds against a broad range of other viruses, as well as, the synthesis of novel trifluoromethylated nucleoside derivatives bearing other purine and pyrimidine bases are currently in progress in our laboratory.

Experimental

General methods

Evaporation of solvents was carried out on a rotary evaporator under reduced pressure. Melting points were determined in open capillary tubes on a Gallenkamp MFB-595-010 M apparatus and are uncorrected. UV spectra were recorded on an Uvikon 931 (Kontron) spectrophotometer. ^{1}H NMR spectra were recorded at 400 MHz, ^{13}C NMR spectra at 100 MHz and ^{19}F NMR at 235 MHz in $(CD_3)_2SO$ at ambient temperature with a Brüker DRX 400. Chemical shifts (δ) are quoted in parts per million (ppm) referenced to the residual solvent peak, $[(CD_3)CD_2H)SO]$ being set at δ-$_H$ 2.49 and δ-$_C$ 39.5 relative to tetramethylsilane (TMS).^{19}F chemical shits are reported using trichlorofluoromethane as external reference. Deuterium exchange and COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in Hertz. 2D ^{1}H–^{13}C heteronuclear COSY were recorded for the attribution of ^{13}C signals. FAB mass spectra were recorded in the positive-ion or negative-ion mode on a JEOL SX 102. The matrix was a mixture (50:50, v/v) of glycerol and thioglycerol (G-T). Specific rotations were measured on a Perkin-Elmer Model 241 spectropolarimeter (path length 1 cm), and are given in units of 10^{-1} ° cm^2 g^{-1}. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). Thin-layer chromatography was performed on precoated aluminium sheets of Silica Gel 60 F_{254} (Merck, Art. 5554), visualization of products being accomplished by UV absorbency followed by charring with 5% ethanolic sulfuric acid and heating. Column chromatography was carried out on Silica Gel 60 (Merck, Art. 9385). Analytical HPLC studies were carried out on a Waters Assoc. unit (600E multisolvent delivery system, 600E system gradient controller, 717 autosampler injector, 996 photodiode array detector and a Millenium data workstation) using a reverse-phase analytical column (Nucleosil, C18, 150 × 4.6 mm, 5 mm) equipped with a prefilter, a precolumn (Nucleosil, C18, 5 mm) and a photodiode array detector (detection at 260 nm). The compound to be analyzed was eluted using a linear gradient of 0–80% acetonitrile in 20 mM triethylammonium acetate buffer (TEAC, pH 7) programmed over a 40-min period with a flow rate of 1 mL/min. All moisture-sensitive reactions were carried out under rigorous anhydrous conditions 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.

1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-trifluoromethyl-D-ribofuranose (1). Compound (**1**) was prepared from commercially available diacetone-D-glucose following a modified procedure initially developed by Lavaire et al.[6] for the synthesis of 1,2,5-tri-*O*-acetyl-3-deoxy-3-*C*-trifluoromethyl-D-ribofuranose. During the course of our studies, the synthesis of **1** has been described with a different strategy starting from D-xylose.[5] An analytical sample of **1** was obtained after crystallization from petroleum ether providing the β-anomer. Its physico chemical properties were similar to those previously described:[5] mp 127 °C (lit.[5] 126.4–126.6 °C); $[\alpha]_D^{20} = -20$ (*c* 0.95 in DMSO). Anal. calcd for $C_{17}H_{17}O_7F_3$: C, 52.31; H, 4.39; F, 14.60. Found: C, 52.15; H, 4.52; F, 14.52.

9-(2-*O*-Acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)adenine (2). Stannic chloride (1.32 mL, 11.3 mmol) was added cautiously to a stirred suspension of adenine (0.83 g, 6.15 mmol) and 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-trifluoromethyl-D-ribofuranose **1** (2 g, 5.1 mmol) in dry acetonitrile (46 mL) at room temperature. After 14 h, pyridine (9 mL) was added to the resultant solution. The white precipitate was filtered and washed with chloroform (2 × 50 mL). The combined filtrates were washed with a solution of saturated sodium hydrogen carbonate (2 × 50 mL), water (2 × 50 mL), dried over sodium sulfate and evaporated. Silica gel column chromatography of the residue using a stepwise gradient of methanol (0–5%) in dichloromethane afforded the title compound **2** as a white foam (1.7 g, 71%): $[\alpha]_D^{20} = -34$ (*c* 1.06 in DMSO); UV λ_{max} (EtOH)/nm 260 nm (ε 14,500), 232 nm (ε 15,600), λ_{min} (EtOH)/nm 245 (ε 11,100); ^{1}H NMR (DMSO-d_6) δ 2.10 (3H, s, CH_3CO), 4.41–4.46 (2H, m, H-5′ and H-3′), 4.68 (1H, dd, H-5″, $J_{5'',4'} = 2.6$, $J_{5'',5'} = 12.6$), 4.80 (1H, m, H-4′), 6.24 (1H, d, H-1′, $J_{1',2'} = 3.0$), 6.28 (1H, dd, H-2′, $J_{2',3'} = 6.7$), 7.39 (2H, br s, NH_2), 7.45–7.86 (5H, m, C_6H_5CO), 8.08 (1H, s, H-2), 8.29 (1H, s, H-8); ^{13}C NMR (DMSO-d_6) δ 21.3 (*C*H$_3$CO), 45.2 (C-3′, q, $^2J_{C,F} = 27.4$), 64.1 (C-5′), 73.9 (C-2′), 76.8 (C-4′), 88.4 (C-1′), 119.9 (C-5), 126.0 (q, CF_3, $^1J_{C,F} = 277.0$), 129.6–134.4 (C-Arom), 141.0 (C-8), 149.6 (C-4), 153.8 (C-2), 157.1 (C-6), 166.1 (CO), 170.1 (CO); ^{19}F NMR (DMSO-d_6) δ−62.0 (d, CF_3, $J_{F,H} = 9.4$) ; *m*/*z* (FAB > 0) 466 $(M+H)^+$, 331 $(S)^+$, 136 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; *m*/*z* (FAB < 0) 464 $(M-H)^-$, 134 $(B)^-$, 121 $(C_6H_5CO_2)^-$. Anal. calcd for $C_{20}H_{18}F_3N_5O_5$: C, 51.62; H, 3.90; N, 15.05; F, 12.25. Found: C, 51.42; H, 3.82; N, 14.85; F, 12.01.

9-(3-Deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)adenine (3). A solution of **2** (0.350 g, 0.75 mmol) in methanolic ammonia (previously saturated at −10 °C and tightly stoppered) (20 mL) was stirred for 14 h at room temperature, then evaporated to dryness. The residue was subjected to silica gel column chromatography with a stepwise gradient of methanol (0–10%) in dichloromethane to afford the title compound **3** (0.235 g, 97%) which was crystallized from methanol: mp 256 °C; $[\alpha]_D^{20} = -42$ (*c* 1.01 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 12,600); ^{1}H NMR (DMSO-d_6) δ 3.41–3.54 (2H, m, H-5′ and H-3′), 3.74–3.78 (1H, m, H-5″), 4.40 (1H, m, H-4′), 4.97 (1H, m, H-2′), 5.49 (1H, t, OH-5′, $J = 4.7$), 5.91 (1H, d, H-1′, $J_{1',2'} = 4.3$), 6.27 (1H, d, OH-2′, $J = 5.7$) 7.35 (2H, br s, NH_2), 8.14 (1H, s, H-2), 8.38 (1H, s, H-8); ^{13}C NMR (DMSO-d_6) δ 45.9 (q, C-3′, $^2J_{C,F} = 25.2$), 62.6 (C-5′), 73.5 (C-2′), 79.3 (C-4′), 90.4 (C-1′), 120.0 (C-5), 126.8 (q, CF_3, $^1J_{C,F} = 278.7$), 140.4 (C-8), 149.7 (C-4), 153.4 (C-2), 157.0 (C-6); ^{19}F NMR (DMSO-d_6) δ−61.8 (d, CF_3, $J_{F,H} = 10.1$); *m*/*z* (FAB > 0) 320 $(M+H)^+$, 136 $(BH_2)^+$. Anal. calcd for $C_{11}H_{12}F_3N_5O_3 \cdot 0.5\ CH_3OH$: C, 41.20; H, 4.21; N,

20.89; F, 17.00. Found: C, 41.05, H, 3.80; N, 21.24; F, 16.98.

9-(5-*O*-Benzoyl-3-deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)adenine (4). Hydrazine hydrate (0.51 mL, 10.5 mmol) was added to a stirred solution of **2** (1.63 g, 3.5 mmol) in an acetic acid–pyridine (v/v, 1:4, 33.3 mL) mixture. After 60 h, acetone (10 mL) was added and the solution was stirred at room temperature for 30 min. A solution of saturated sodium hydrogen carbonate (50 mL) was then added, and the aqueous phase was extracted with dichloromethane (2 × 50 mL). The organic phase was washed with water (2 × 50 mL), dried over sodium sulfate and evaporated to dryness. Silica gel column chromatography of the residue using a stepwise gradient of methanol (0–6%) in dichloromethane afforded the title compound **4** as a white foam (1.13 g, 76%) which was crystallized from acetonitrile: mp 190 °C; $[\alpha]_D^{20} = -39$ (*c* 1.27 in DMSO); UV λ_{max} (EtOH)/nm 260 nm (ε 14,000), 232 nm (ε 14700), λ_{min} (EtOH)/nm 245 (ε 10,500); ^{1}H NMR (DMSO-d_6) δ 3.98 (1H, m, H-3′), 4.42 (1H, dd, H-5′, $J_{5',4'} = 4.7$, $J_{5',5''} = 12.4$), 4.63 (1H, dd, H-5″, $J_{5'',4'} = 2.8$), 4.73 (1H, m, H-4′), 5.21 (1H, m, H-2′), 5.98 (1H, d, H-1′, $J_{1',2'} = 4.3$), 6.40 (1H, d, 2′-OH, $J = 5.8$), 7.33 (2H, br s, NH_2), 7.46–7.89 (5H, m, C_6H_5CO), 8.09 (1H, s, H-2), 8.31 (1H, s, H-8); ^{13}C NMR (DMSO-d_6) δ 46.8 (q, C-3′, $^2J_{C,F} = 25.9$), 64.8 (C-5′), 73.2 (C-2′), 76.5 (C-4′), 91.0 (C-1′), 120.0 (C-5), 126.4 (q, CF_3, $^1J_{C,F} = 278.3$), 129.6–134.4 (C-Arom), 140.9 (C-8), 149.8 (C-4), 153.6 (C-2), 157.0 (C-6), 166.2 (CO); ^{19}F NMR (DMSO-d_6) δ −61.2 (d, CF_3, $J_{F,H} = 9.7$) ; *m*/*z* (FAB > 0) 424 $(M+H)^+$, 136 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; *m*/*z* (FAB < 0) 422 $(M-H)^-$, 134 $(B)^-$, 121 $(C_6H_5CO_2)^-$. Anal. calcd for $C_{18}H_{16}F_3N_5O_4$: C, 51.07; H, 3.81; N, 16.54; F, 13.46. Found: C, 51.20; H, 3.71; N, 16.28; F, 13.30.

9-(5-*O*-Benzoyl-2,3-dideoxy-3-*C*-trifluoromethyl-β-D-*erythro*-pentofuranosyl)adenine (5). To a stirred solution of **4** (0.450 g, 1.06 mmol) in dry acetonitrile (15 mL) were added successively DMAP (1.16 g, 9.57 mmol) and phenoxy(thiocarbonyl) chloride (0.30 mL, 2.12 mmol). After 14 h, the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and the organic layer was washed with hydrochloric acid 0.2 N (3 × 50 mL), dried over sodium sulfate, and evaporated to dryness. The resulting crude material was co-evaporated with dry toluene, then dissolved in the same solvent (21 mL) and α,α′-azoisobutyronitrile (0.052 g, 0.31 mmol) and tris(trimethylsilyl)silane (0.4 mL, 1.27 mmol) were added. The resultant solution was heated under reflux for 4 h. After cooling to room temperature, the solvent was removed under reduced pressure. Chromatography of the residue on a silica gel column using as eluent a stepwise gradient of methanol (0–5%) in dichloromethane afforded the title compound **5** as a white foam (0.3 g, 70%): $[\alpha]_D^{20} = -31$ (*c* 0.95 in DMSO); UV λ_{max} (EtOH)/nm 260 nm (ε 15,100), 232 nm (ε 16,000), λ_{min} (EtOH)/nm 245 (ε 11,400); ^{1}H NMR (DMSO-d_6) δ 2.75 (1H, ddd, H-2′α, $J_{2',3'} = 7.3$, $J_{2',1'} = 7.4$, $J_{2'\alpha,2'\beta} = 14.0$), 3.13 (ddd, H-2′β, $J_{2',1'} = 4.8$, $J_{2',3'} = 9.2$), 3.99 (1H, m, H-3′), 4.40 (1H, m, H-5′), 4.54 (2H, m, H-4′ and H-5″), 6.36 (1H, dd, H-1′), 7.30 (2H, br s, NH_2), 7.46–7.88 (5H, m, C_6H_5CO), 8.11 (1H, s, H-2), 8.32 (1H, s, H-8); ^{13}C NMR (DMSO-d_6) δ 31.4 (C-2′), 43.9 (q, C-3′, $^2J_{C,F} = 27.6$), 65.4 (C-5′), 77.5 (C-4′), 84.6 (C-1′), 120.1 (C-5), 127.7 (q, CF_3, $^1J_{C,F} = 277.4$), 129.0–134.3 (C-Arom), 140.7 (C-8), 149.7 (C-4), 153.5 (C-2), 157.0 (C-6), 166.2 (CO); ^{19}F NMR (DMSO-d_6) δ −67.9 (d, CF_3, $J_{F,H} = 9.4$); *m*/*z* (FAB > 0) 408 $(M+H)^+$, 136 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; *m*/*z* (FAB < 0) 406 $(M-H)^-$, 134 $(B)^-$. Anal. calcd for $C_{18}H_{16}F_3N_5O_3 \cdot 0.9\ CH_3OH$: C, 52.04; H, 4.53; N, 16.06, F, 13.07. Found: C, 52.21; H, 4.17; N, 16.39; F, 12.71.

9-(2,3-Dideoxy-3-*C*-trifluoromethyl-β-D-*erythro*-pentofuranosyl)adenine (6). A solution of **5** (0.250 g, 0.61 mmol) in methanolic ammonia (previously saturated at −10 °C and tightly stoppered) (15 mL) was stirred for 14 h at room temperature, then evaporated to dryness. The residue was subjected to silica gel column chromatography, with a stepwise gradient of methanol (0–10%) in dichloromethane to afford the title compound **6** (0.155 g, 83%) which was crystallized from methanol/water: mp 218 °C; $[\alpha]_D^{20} = -29$ (*c* 1.04 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 15,400) ; ^{1}H NMR (DMSO-d_6) δ 2.66 (1H, m, H-2′), 2.95 (1H, m, H-2″), 3.45–3.74 (3H, m, H-3′, H-5′ and H-5″), 4.22 (1H, m, H-4′), 5.35 (1H, t, OH-5′, $J = 5.3$), 6.30 (1H, t, H-1′, $J = 6.5$), 7.35 (1H, br s, NH_2), 8.14 (1H, s, H-2), 8.36 (1H, s, H-8) ; ^{13}C NMR (DMSO-d_6) δ 31.9 (C-2′), 43.6 (q, C-3′, $^2J_{C,F} = 27.3$), 63.1 (C-5′), 80.9 (C-4′), 84.7 (C-1′), 120.1 (C-5), 128.1 (q, CF_3, $^1J_{C,F} = 277.8$), 140.4 (C-8), 149.7 (C-4), 153.4 (C-2), 157.0 (C-6); ^{19}F NMR (DMSO-d_6) δ −68.3 (d, CF_3, $J_{F,H} = 9.9$); *m*/*z* (FAB > 0) 607 $(2M+H)^+$, 304 $(M+H)^+$, 136 $(BH_2)^+$; *m*/*z* (FAB < 0) 605 $(2M-H)^-$, 302 $(M-H)^-$, 134 $(B)^-$; HPLC t_R 21.9 min. Anal. calcd for $C_{11}H_{12}F_3N_5O_2 \cdot 0.4H_2O$: C, 42.56; H, 4.16; N, 22.56; F, 18.36. Found: C, 42.76; H, 4.09; N, 22.69; F, 18.36.

9-(5-*O*-Benzoyl-2,3-dideoxy-3-*C*-trifluoromethyl-β-D-*glycero*-pent-2-eno-furanosyl)adenine (7). Methanesulfonyl chloride (0.173 mL, 2.23 mmol) was added to a solution of nucleoside **4** (0.315 g, 0.75 mmol) in dry pyridine (7.5 mL). The resultant solution was stirred at room temperature for 7 h, and a solution of saturated sodium hydrogen carbonate (15 mL) was then added. The mixture was extracted with dichloromethane (2 × 15 mL). The organic phase was dried over sodium sulfate, evaporated under reduced pressure and co-evaporated with toluene. The crude material was then dissolved in anhydrous THF (15 mL), and a solution of TBAF in THF (1 M) (1.5 mL, 1.5 mmol) was added. The mixture was stirred for 21 h at 50 °C, then evaporated to dryness. Chloroform (30 mL) and water (30 mL) were added. The organic phase was separated, dried and evaporated to dryness under reduced pressure. The residue was subjected to silica gel column chromatography, with a stepwise gradient of methanol (0–3%) in dichloromethane to afford the title compound **7** (0.242 g, 80%) as a white foam: $[\alpha]_D^{20} = -45$ (*c* 1.00 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 15,000), 231 (ε 16,100), λ_{min} (EtOH)/nm 245 (ε 11,400) ; ^{1}H NMR (DMSO-d_6) δ 4.48 (1H, dd, H-5′, $J_{5',4'} = 4.2$, $J_{5',5''} = 12.4$), 4.63 (1H, dd, H-5″, $J_{5'',4'} = 2.8$), 5.49 (1H, m, H-4′), 7.12 (1H, br s,

H-1′), 7.23 (1H, br s, H-2′), 7.31 (2H, NH_2), 7.48–7.86 (5H, m, C_6H_5CO), 8.09 (1H, s, H-8), 8.14 (1H, s, H-2); ^{13}C NMR (DMSO-d_6) δ 64.9 (C-5′), 82.4 (C-4′), 87.9 (C-1′), 119.5 (C-5), 121.9 (q, CF_3, $^1J_{C,F}=270.0$), 129.7–129.9 (C-Arom), 133.7 (q, C-3′, $^2J_{C,F}=34.6$), 134.2 (q, C-2′, $^3J_{C,F}=4.8$), 134.5 (C-Arom), 139.6 (C-8), 150.0 (C-4), 153.9 (C-2), 156.9 (C-6), 166.2 (CO) ; ^{19}F NMR (DMSO-d_6) δ−61.4 (s, CF_3); *m/z* (FAB > 0) 406 $(M+H)^+$, 136 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; *m/z* (FAB < 0) 404 $(M-H)^-$, 134 $(B)^-$, 121 $(C_6H_5CO_2)^-$. Anal. calcd for $C_{18}H_{14}F_3N_5O_3$•0.7 CH_3OH: C, 52.51; H, 3.96; N, 16.37; F, 13.32. Found: C, 52.25, H, 3.64; N, 16.33; F, 13.23.

9-(2,3-Dideoxy-3-*C*-trifluoromethyl-β-D-*glycero*-pent-2-enofuranosyl)adenine (8). A solution of **7** (0.210 g, 0.51 mmol) in methanolic ammonia (previously saturated at −10 °C and tightly stoppered) (13 mL) was stirred for 14 h at room temperature, then evaporated to dryness. The residue was subjected to silica gel column chromatography, with a stepwise gradient of methanol (0–10%) in dichloromethane to afford the title compound **8** (0.110 g, 70%) which was crystallized from methanol/water: mp 229 °C; $[\alpha]_D^{20}=+63.3$ (*c* 1.01 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 14,200); ^1H NMR (DMSO-d_6) δ 3.61–3.69 (2H, m, H-5′ and H-5″), 5.12 (1H, m, H-4′), 5.36 (1H, t, OH-5′, *J* = 5.0), 6.99 (1H, d, H-2′, $J_{2',1'}=1.7$), 7.06 (1H, br s, H-1′), 7.31 (1H, br s, NH_2), 8.14 (1H, s, H-2), 8.20 (1H, s, H-8); ^{13}C NMR (DMSO-d_6) δ 62.1 (C-5′), 86.1 (C-4′), 87.5 (C-1′), 117.9 (C-5), 121.9 (q, CF_3, $^1J_{C,F}=277.0$), 133.0 (q, C-2′, $^3J_{C,F}=4.7$), 135.1 (q, C-3′, $^2J_{C,F}=34.6$), 140.2 (C-8), 149.8 (C-4), 153.6 (C-2), 156.8 (C-6); ^{19}F NMR (DMSO-d_6) δ−61.4 (s, CF_3); *m/z* (FAB > 0) 603 $(2M+H)^+$, 302 $(M+H)^+$, 136 $(BH_2)^+$; *m/z* (FAB < 0) 601 $(2M-H)^-$, 300 $(M-H)^-$, 134 $(B)^-$; HPLC t_R 21.2 min. Anal. calcd for $C_{11}H_{10}F_3N_5O_2$•0.8H_2O: C, 41.86; H, 3.70; N, 22.19; F, 18.06. Found: C, 42.25; H, 3.44; N, 21.73; F, 17.95.

1-(2-*O*-Acetyl-5-*O*-benzoyl-3-deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)uracil (9). A mixture of uracil (1.14 g, 10.2 mmol), hexamethyldisilazane (51 mL) and a catalytic amount of ammonium sulfate was refluxed for 14 h. The resultant clear solution was concentrated to dryness under reduced pressure. TMSOTf (1.98 mL, 10.2 mmol) was added to a solution of sugar **1** (1 g, 2.56 mmol) and silylated base in dry 1,2-dichloroethane (25 mL). The reaction mixture was stirred for 8 h at room temperature, poured into a solution of saturated sodium hydrogen carbonate and extracted with chloroform (3 × 30 mL). The organic phase was dried over sodium sulfate and evaporated to dryness. Chromatography of the residue on a silica gel column using as eluent a stepwise gradient of ethyl acetate (0–60%) in petroleum ether afforded the title compound **9** as a white foam (0.710 g, 63%) which was crystallized from ethanol: mp 126–127 °C (lit.[5] 116–16.9 °C); $[\alpha]_D^{20}=-8.7$ (*c* 1.15 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 10,800), 231 (ε 16600), λ_{min} (EtOH)/nm 247 (ε 10,000) ; *m/z* (FAB > 0) 885 $(2M+H)^+$, 443 $(M+H)^+$, 331 $(S)^+$, 113 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; *m/z* (FAB < 0) 883 $(2M-H)^-$, 441 $(M-H)^-$, 121 $(C_6H_5CO_2)^-$, 111 $(B)^-$. ^1H, ^{13}C and ^{19}F NMR spectra (DMSO-d_6) were similar to those previously reported. Anal. calcd for $C_{19}H_{17}F_3N_2O_7$: C, 51.59; H, 3.87; N, 6.33; F, 12.88. Found: C, 51.67; H, 3.94; N, 6.39; F, 12.92.

1-(3-Deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)cytosine (10). A solution of compound **9** (0.5 g, 1.13 mmol) and 1-methylpyrrolidine (1.12 mL, 10.85 mmol) in anhydrous acetonitrile (5.6 mL) was cooled down to 0 °C. Trifluoroacetic anhydride (0.4 mL, 2.82 mmol) was then added. After 45 min at 0 °C, 4-nitrophenol (0.47 g, 3.39 mmol) was added to the solution. After stirring for 12 h at 0 °C, the solution was then poured into a solution of saturated sodium hydrogen carbonate (30 mL), and the resultant mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure. The residue was dissolved in dioxane (5.6 mL) and concentrated aqueous ammonia (*d* 0.89, 1.13 mL) was added. The reaction mixture was stirred at 55 °C for 3 h. The resulting yellow solution was concentrated under reduced pressure and directly treated with methanolic ammonia (previously saturated at −10 °C and tightly stoppered) (40 mL) for 14 h at room temperature. After evaporation to dryness under reduced pressure, the residue was subjected to silica gel column chromatography, with a stepwise gradient of methanol (2–10%) in dichloromethane to afford the title compound **10** (0.240 g, 72%) which was crystallized from acetonitrile: mp 177 °C ; $[\alpha]_D^{20}=+8.6$ (*c* 1.05 in DMSO); UV λ_{max} (EtOH)/nm 272 (ε 10,100) ; ^1H NMR (DMSO-d_6) δ 3.07 (1H, m, H-3′), 3.50 (1H, m, H-5′), 3.78 (1H, m, H-5″), 4.33 (1H, m, H-4′), 4.41 (1H, m, H-2′), 5.28 (1H, t, OH-5′, *J* = 5.0), 5.68 (2H, m, H-1′ and H-5), 6.14 (1H, d, OH-2′, *J* = 5.6), 7.15 (2H, br d, NH_2), 7.88 (1H, d, H-6, $J_{6,5}=7.4$); ^{13}C NMR (DMSO-d_6) δ 43.2 (C-3′, q, $^2J_{C,F}=25.5$), 59.5 (C-5′), 72.4 (C-2′), 77.6 (C-4′), 90.2 (C-1′), 92.6 (C-5), 123.2 (q, CF_3, $^1J_{C,F}=277.9$), 140.1 (C-6), 154.1 (C-2), 164.6 (C-4); ^{19}F NMR (DMSO-d_6) δ−61.1 (d, CF_3, $J_{F,H}=9.7$) ; *m/z* (FAB > 0) 296 $(M+H)^+$, 112 $(BH_2)^+$; *m/z* (FAB < 0) 294 $(M-H)^-$. Anal. calcd for $C_{10}H_{12}F_3N_3O_4$•0.3 CH_3CN: C, 41.16; H, 4.27; N, 14.94. Found: C, 40.90; H, 4.37; N, 15.10.

1-(5-*O*-Benzoyl-3-deoxy-3-*C*-trifluoromethyl-β-D-ribofuranosyl)uracil (11). Hydrazine hydrate (0.16 mL, 3.29 mmol) was added to a stirred solution of **9** (0.48 g, 1.09 mmol) in a acetic acid–pyridine (v/v 1:4, 10.4 mL). After 3 days, acetone (5 mL) was added and the solution was stirred at room temperature for 30 min. A solution of saturated sodium hydrogen carbonate (30 mL) was then added, and the aqueous phase was extracted with dichloromethane (2 × 30 mL). The organic phase was washed with water (2 × 30 mL), dried over sodium sulfate and evaporated to dryness. Column chromatography of the residue on silica gel using a stepwise gradient of methanol (0–2%) in dichloromethane afforded the title compound **11** as a white foam (0.42 g, 96%) which was crystallized from diethyl ether: mp 182 °C (lit.[5] 177–178.5 °C); $[\alpha]_D^{20}=-17.9$ (*c* 1.17 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 10,700), 231 (ε 15,900), λ_{min} (EtOH)/nm 247 (ε 8700) ; *m/z* (FAB > 0) 401 $(M+H)^+$, 289 $(S)^+$, 105 $(C_6H_5CO)^+$; *m/z* (FAB < 0) 399 $(M-H)^-$,

121 $(C_6H_5CO_2)^-$, 111 $(B)^-$. ^1H, ^{13}C and ^{19}F NMR spectra (DMSO-d_6) were similar to those previously reported. Anal. calcd for $C_{17}H_{15}F_3N_2O_6$: C, 51.01; H, 3.78; N, 7.00; F, 14.24. Found: C, 50.86; H, 3.79, N, 7.12; F, 14.40.

1-(5-*O*-Benzoyl-2,3-dideoxy-3-*C*-trifluoromethyl-β-D-*erythro*-pentofuranosyl)uracil (12). To a stirred solution of **11** (0.5 g, 1.25 mmol) in dry dichloromethane (7.5 mL) were added pyridine (0.38 mL, 4.75 mmol) and phenoxy(thiocarbonyl) chloride (0.19 mL, 1.37 mmol) successively. After 4 h, the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and the organic layer was washed with water (2 × 50 mL), dried over sodium sulfate and evaporated to dryness. The resulting crude material was co-evaporated with dry toluene, then dissolved in the same solvent (25 mL) and α,α′-azoisobutyronitrile (0.102 g, 0.62 mmol) and tris(trimethylsilyl)silane (0.77 mL, 2.5 mmol) were added. The resultant solution was stirred under reflux for 17 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was purified on a silica gel column using methanol/dichloromethane (1:99) as eluent to afford the title compound **12** (0.384 g, 80%) as a white foam which was crystallized from ethanol/water: mp 140 °C; (lit.[5] 80.0–82.0 °C); $[\alpha]_D^{20} = -15.3$ (*c* 0.98 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 10,000), 231 (ε 15,000), λ_{min} (EtOH)/nm 247 (ε 8400); *m*/*z* (FAB > 0) 385 $(M+H)^+$, 273 $(S)^+$, 113 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; *m*/*z* (FAB < 0) 383 $(M-H)^-$, 121 $(C_6H_5CO_2)^-$, 111 $(B)^-$. ^1H, ^{13}C and ^{19}F NMR spectra (DMSO-d_6) were similar to those previously reported. Anal. calcd for $C_{17}H_{15}F_3N_2O_5 \cdot 0.9H_2O$: C, 50.98; H, 4.23; N, 6.99. Found: C, 51.08; H, 3.82; N, 7.23.

1-(2,3-Dideoxy-3-*C*-trifluoromethyl-β-D-*erythro*-pentofuranosyl)cytosine (13). A solution of compound **12** (0.300 g, 0.781 mmol) and 1-methylpyrrolidine (0.780 mL, 7.5 mmol) in anhydrous acetonitrile (3.9 mL) was cooled to 0 °C. Trifluoroacetic anhydride (0.275 mL, 1.95 mmol) was then added. After 45 min at 0 °C, 4-nitrophenol (0.326 g, 2.34 mmol) was added. The solution was stirred for 18 h at 0 °C, then poured into a solution of saturated sodium hydrogen carbonate (30 mL). The resultant mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure. The residue was dissolved in dioxane (3.9 mL) and concentrated aqueous ammonia (*d* 0.89, 0.781 mL) was added. The reaction mixture was stirred at 55 °C for 12 h. The resulting yellow solution was concentrated under reduced pressure and directly treated with methanolic ammonia (previously saturated at −10 °C and tightly stoppered) (40 mL) for 12 h at room temperature. After evaporation to dryness under reduced pressure, the residue was subjected to silica gel column chromatography, with a stepwise gradient of methanol (4–10%) in dichloromethane to afford the title compound **13** (0.170 g, 78%). An analytical sample of **13** was obtained as its chlorohydrate salt: mp 215 °C (lit.[5] 214.8–216 °C); $[\alpha]_D^{20} = +53.3$ (*c* 0.15 DMSO); UV λ_{max} (EtOH)/nm 272 (ε 8500); *m*/*z* (FAB > 0) 280 $(M+H)^+$, 112 $(BH_2)^+$. ^1H, ^{13}C and ^{19}F NMR spectra (DMSO-d_6) were similar to those previously reported. HRMS (FAB > 0, *m*/*z*) 280.0919 (calcd 280.0909).

1-(5-*O*-Benzoyl-2,3-dideoxy-3-*C*-trifluoromethyl-β-D-*glycero*-pent-2-eno-furanosyl)uracil (14). Methanesulfonyl chloride (0.3 mL, 3.75 mmol) was added to a solution of nucleoside **11** (0.5 g, 1.25 mmol) in a mixture of dry pyridine (7.5 mL) and dichloromethane (9.4 mL). The resultant solution was stirred at room temperature for 48 h, and a solution of saturated sodium hydrogen carbonate (15 mL) was then added. The mixture was extracted with dichloromethane (2 × 15 mL). The organic phase was dried over sodium sulfate, evaporated under reduced pressure and co-evaporated with toluene. The crude material was then dissolved in anhydrous THF (25 mL), and a solution of TBAF in THF (1.25 mL, 1.25 mmol) was added. The mixture was stirred for 4 h at 50 °C, then evaporated to dryness. Ethyl acetate (30 mL) and water (30 mL) were added. The organic phase was separated, dried over sodium sulfate and evaporated to dryness under reduced pressure. The residue was subjected to silica gel column chromatography using methanol/dichloromethane (1:99) as eluent to afford the title compound **14** (0.383 g, 80%) as a white foam which was crystallized from diethyl ether: mp 153 °C; $[\alpha]_D^{20} = -100$ (*c* 1.05 in DMSO); UV λ_{max} (EtOH)/nm 260 (ε 10,100), 230 (ε 16,400), λ_{min} (EtOH)/nm 247 (ε 8800); ^1H NMR (DMSO-d_6) δ 4.42 (1H, dd, H-5′, $J_{5',4'} = 3.5$, $J_{5',5''} = 12.8$), 4.70 (1H, dd, H-5″, $J_{5'',4'} = 2.7$), 5.08 (1H, d, H-5, $J_{5,6} = 8.0$), 5.39 (1H, br s, H-4′), 6.92 (1H, d, H-1′, $J_{1',2'} = 1.6$), 6.99 (1H, br s, H-2′), 7.39 (1H, d, H-6), 7.54–7.91 (5H, m, C_6H_5CO), 11.45 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 64.5 (C-5′), 81.8 (C-4′), 89.4 (C-1′), 102.8 (C-5), 121.7 (q, CF_3, $^1J_{C,F} = 270.2$), 129.8–130.0 (C-arom), 133.7 (q, C-3′, $^2J_{C,F} = 34.8$), 134.4 (q, C-2′, $^3J_{C,F} = 4.7$), 134.7 (C-Arom), 141.4 (C-6), 151.1 (C-2), 163.6 (C-4), 166.1 (CO); ^{19}F NMR (DMSO-d_6) δ −61.5 (s, CF_3); *m*/*z* (FAB > 0) 765 $(2M+H)^+$, 475 $(M+G+H)^+$, 383 $(M+H)^+$, 113 $(BH_2)^+$, 105 $(C_6H_5CO)^+$; *m*/*z* (FAB < 0) 473$(M+G-H)^-$, 381 $(M-H)^-$, 121 $(C_6H_5CO_2)^-$, 111 $(B)^-$. Anal. calcd for $C_{17}H_{13}F_3N_2O_5$: C, 53.41; H, 3.43; N, 7.33; F, 14.91. Found: C, 53.58; H, 3.56; N, 7.43; F, 14.47.

1-(2,3-Dideoxy-3-*C*-trifluoromethyl-β-D-*glycero*-pent-2-enofuranosyl)cytosine (15). A solution of compound **14** (0.333 g, 0.871 mmol) and 1-methylpyrrolidine (0.870 mL, 8.36 mmol) in anhydrous acetonitrile (4.4 mL) was cooled down to 0 °C. Trifluoroacetic anhydride (0.307 mL, 2.17 mmol) was then added. After 45 min at 0 °C, 4-nitrophenol (0.363 g, 2.61 mmol) was added. The solution was stirred for 5 h at 0 °C, then poured into a solution of saturated sodium hydrogen carbonate (30 mL), and the resultant mixture was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure. The residue was dissolved in dioxane (4.4 mL) and concentrated aqueous ammonia (*d* 0.89, 0.871 mL) was added. The reaction mixture was stirred at 55 °C for 12 h. The resulting yellow solution

was concentrated under reduced pressure and directly treated with methanolic ammonia (previously saturated at −10 °C and tightly stoppered) (40 mL) for 12 h at room temperature. After evaporation to dryness under reduced pressure, the residue was subjected to silica gel column chromatography, with a stepwise gradient of methanol (4–10%) in dichloromethane to afford the title compound **13** (0.200 g, 82%) which was crystallized from methanol/water: mp 221 °C (lit.[5] 221.3–221.7 °C); $[\alpha]_D^{20} = +$ 23.4 (*c* 1.07 in DMSO); UV λ_{max} (EtOH)/nm 272 (ε 9000) ; *m/z* (FAB > 0) 370 $(M+G+H)^+$, 278 $(M+H)^+$; *m/z* (FAB) 276 $(M-H)^-$. ^1H, ^{13}C and ^{19}F NMR spectra (DMSO-d_6) were similar to those previously reported. HRMS (FAB > 0, *m/z*) 278.0750 (calcd 278.0753).

Stability studies

For determination of the chemical stability of nucleosides, pH 7.2 phosphate buffer (0.02 M) and pH 2 Glycine/HCl buffer were used. For each compound, a stock solution (1×10^{-3} M in Milli-Q water) was prepared. The study at pH 7.2 buffer was performed in the following manner: 0.1 mL of stock solution was added to a vial and diluted with pH 7.2 buffer to give a final concentration of 5×10^{-5} M; a 0.1 mL aliquot was immediately removed for analysis and the remainder was incubated at 37 °C. Subsequent 0.1 mL samples were taken at predetermined times, then frozen in liquid nitrogen. The concentration of adenine and remaining nucleoside in each sample was determined by HPLC analysis of an 80-μL aliquot. The study at pH 2 buffer was accomplished in the following way: 0.25 mL of stock solution was added to a vial, diluted with pH 2 buffer to give a solution with a concentration of 2.5×10^{-4} M and incubated at 37 °C. Subsequent 20 μL samples were taken at different times, diluted with 80 μL of TEAC to give a final concentration of 5×10^{-5} M, then frozen in liquid nitrogen. Determination of the concentration of base and remaining nucleoside in each sample was determined by HPLC analysis of a 80 μL aliquot.

Antiviral assays

The anti-HIV and anti-HBV assays in cell cultures were performed following previously established procedures.[17,18] Briefly, in MT-4 cells, the determination of the antiviral activity of compounds was based on a reduction of HIV-1_{IIIB}-induced cytopathogenicity, the metabolic activity of the cells being measured by the property of mitochondrial dehydrogenase to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) into formazan. In parallel experiments, cytotoxicity of the test compounds was measured after incubation of uninfected cells for 4 days in their presence using the colorimetric MTT test. 2.2.15 Cells (HBV DNA-transfected human hepatoblastoma-derived Hep-G2 cells) were used for anti-HBV assays. Briefly, cells were cultured and inhibition of HBV extracellular DNA (HBV virion) or HBV intracellular DNA (HBV replicative intermediate, HBV RI) was determined. Cytotoxicity assays were conducted in Hep-G2 cells. Each compound was tested in four concentration in triplicate cultures and the median inhibitory concentration (IC_{50}) was determined.

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