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Synthesis of 4,6-dideoxy-3-fluoro-2-keto-β-D-glucopyranosyl analogues of 5-fluorouracil, N^6-benzoyl adenine, uracil, thymine, N^4-benzoyl cytosine and evaluation of their antitumor activities

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ABSTRACT

The synthesis of the unsaturated 4,6-dideoxy-3-fluoro-2-keto-β-D-glucopyranosyl nucleosides of 5-fluorouracil (**6a**), N^6-benzoyl adenine (**6b**), uracil (**6c**), thymine (**6d**) and N^4-benzoyl cytosine (**6e**), is described. Monoiodination of compounds **1a,b**, followed by acetylation, catalytic hydrogenation and finally regioselective 2′-*O*-deacylation afforded the partially acetylated dideoxynucleoside analogues of 5-fluorouracil (**5a**) and N^6-benzoyl adenine (**5b**), respectively. Direct oxidation of the free hydroxyl group at the 2′-position of **5a,b**, with simultaneous elimination reaction of the β-acetoxyl group, afforded the desired unsaturated 4,6-dideoxy-3-fluoro-2-keto-β-D-glucopyranosyl derivatives **6a,b**. Compounds **1c–e** were used as starting materials for the synthesis of the dideoxy unsaturated carbonyl nucleosides of uracil (**6c**), thymine (**6d**) and N^4-benzoyl cytosine (**6e**). Similarly a protection-selective deprotection sequence followed by oxidation of the free hydroxyl group at the 2′-position of the dideoxy benzoylated analogues **9c–e** with simultaneous elimination reaction of the β-benzoyl group, gave the desired nucleosides **6c–e**. None of the compounds was inhibitory to a broad spectrum of DNA and RNA viruses at subtoxic concentrations. The 5-fluorouracil derivative **6a** was more cytostatic (50% inhibitory concentration ranging between 0.2 and 12 μM) than the other compounds.

1. Introduction

Nucleoside analogues have emerged as important therapeutic agents for the development of antiviral and antitumor drugs [1–4]. Antiviral nucleoside analogues inhibit replication of the viral genome, whereas anticancer nucleoside analogues inhibit cellular DNA replication and repair [5]. Attachment of fluorine atoms on the sugar and heterocyclic moieties of such analogues is known to be of advantage both for an improved activity, higher bioavailability as well as for causing a retarded catabolism of several drugs [6–9]. Therefore, specific fluorination at the 2′- and/or 3′-position of the sugar moiety of the nucleosides has been studied in the pursuit of safe, effective and chemically stable antiviral agents [9–15].

The last decades, nucleosides containing pyranosyl rings instead of furanosyl ones have been evaluated for their potential antiviral [16–22], antioxidant [23] and antibiotic [24] properties and as building blocks in nucleic acid synthesis [25,26]. The ketohexose unsaturated nucleosides are a series of sugar modified pyranonucleosides, which not only were found to have significant *in vitro* and *in vivo* inhibitory activity against various types of cancer cells [27,28] but they also appeared to be highly reactive sulfhydryl blocking agents [29]. In this regard, we have been actively engaged in the study of various unsaturated ketopyranonucleosides [22,30–34] and have developed stereoselective synthetic methods for these biologically important targets in medicinal chemistry.

In the course of our studies, we have prepared the unsaturated 3′-fluoro-4′- and 2′-ketonucleosides [30,31], which proved to be efficient as tumor cell growth inhibitors and had a promising potential in combating rotaviral infections. Our recent research also disclosed that the 6′-protected analogues were endowed with higher cytotoxic and antiviral potency, indicating that the presence of a primary hydroxyl group might not be critical for biological activity. This observation encouraged us to develop the synthesis of the dideoxy unsaturated 3′-fluoro-4′-ketopyranonucleoside analogues bearing a methyl group in the 5′-position of the sugar moiety [32]. As expected, these 6′-OH-lacking analogues have emerged as more effective inhibitory agents against a variety of tumor cell lines.

With the above applications in mind and in order to elucidate the structural requirements for biological activity of unsaturated ketonucleosides, we decided to design and synthesize the dideoxy

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unsaturated 3′-fluoro-2′-ketopyranonucleosides of 5-fluorouracil, N^6-benzoyl adenine, uracil, thymine, and N^4-benzoyl cytosine respectively, bearing a methyl group in the 5′-position of the sugar moiety. Chemical synthesis and biological activity of these compounds are presented herein.

2. Experimental

2.1. General methods

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash column chromatography was performed using silica gel (240–400 mesh, Merck). UV–Vis spectra were recorded on a PG T70 UV–VIS spectrometer and mass spectra were obtained with a Micromass Platform LC (ESI-MS). Optical rotations were measured using Autopol I polarimeter. ^{1}H, ^{19}F and ^{13}C NMR spectra were obtained at room temperature with a Bruker 400 spectrometer at 400, 376 and 100 MHz, respectively using $CDCl_3$ and methanol-d_4 (CD_3OD) with internal tetramethylsilane (TMS) for ^{1}H and ^{13}C and internal trifluorotoluene (TFT) for ^{19}F.

The chemical shifts are expressed in parts per million (δ) and following abbreviations were used: s = singlet; br s = broad singlet; d = doublet; dd = doublet doublet; dtr = doublet triplet; m = multiplet. All reactions sensitive to oxygen or moisture were carried out under nitrogen atmosphere with dry solvents. Dimethyl sulfoxide was stored over 3 Å molecular sieves. Pyridine was stored over pellets of potassium hydroxide. Tetrahydrofuran was freshly distilled under nitrogen from sodium/benzophenone before use.

2.2. Synthesis of 1-(4,6-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)5-fluorouracil (**6a**)

2.2.1. 1-(3-Deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)5-fluorouracil (**2a**)

A mixture of 1-(3-deoxy-3-fluoro-β-D-glucopyranosyl)5-fluorouracil (**1a**) [32] (2.5 g, 8.5 mmol), imidazole (1.16 g, 17.0 mmol), triphenylphosphine (3.34 g, 12.75 mmol) and iodine (3.24 g, 12.75 mmol) in tetrahydrofuran (83.2 mL) was stirred under reflux at a bath temperature of 80 °C for 1 h. The reaction mixture was then cooled to room temperature and concentrated under vacuum. Purification by flash chromatography (hexane/AcOEt, 2:8), gave **2a** (2.34 g, 68%, R_f = 0.36 in hexane/AcOEt, 2:8) as a syrup. $[\alpha]_D^{22} - 2.0$ (*c* 0.50, MeOH); λ_{max} 262 nm (ε 10801); ^{1}H NMR (CD_3OD): δ 7.89 (d, 1H, $J_{6,F5}$ = 6.3 Hz, H-6), 5.64 (d, 1H, $J_{1',2'}$ = 9.6 Hz, H-1′), 4.42 (dtr, 1H, $J_{F,3'}$ = 52.5 Hz, $J_{2',3'}$ = 8.7 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 3.87 (m, 1H, H-2′), 3.65–3.41 (m, 3H, H-4′ and H-6a′,6b′), 3.23 (m, 1H, H-5′); Anal. Calcd for $C_{10}H_{11}F_2IN_2O_5$: C, 29.72; H, 2.74; N, 6.93. Found: C, 29.84; H, 2.63; N, 7.15. ESI-MS (*m/z*): 405.10 ($M+H^+$).

2.2.2. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)5-fluorouracil (**3a**)

To a solution of **2a** (2.34 g, 5.78 mmol) in dry pyridine (28.9 mL) was added acetic anhydride (1.36 mL, 14.45 mmol) and the resulted mixture was stirred at room temperature for 2 h. MeOH (0.7 mL) was added to quench the reaction and the mixture was concentrated under high vacuum to remove the solvents. Purification by flash chromatography (hexane/AcOEt, 1:1) afforded **3a** (2.51 g, 89%, R_f = 0.67 in hexane/AcOEt, 3:7) as a yellow oil. $[\alpha]_D^{22} + 2.0$ (*c* 0.26, $CHCl_3$); λ_{max} 266 nm (ε 8727); ^{1}H NMR ($CDCl_3$): δ 8.94 (br s, 1H, NH), 7.46 (d, 1H, $J_{6,F5}$ = 5.6 Hz, H-6), 5.85 (d, 1H, $J_{1',2'}$ = 9.3 Hz, H-1′), 5.25–5.17 (m, 2H, H-2′ and H-4′), 4.77 (dtr, 1H, $J_{F,3'}$ = 51.6 Hz, $J_{2',3'} = J_{3',4'}$ = 9.1 Hz, H-3′), 3.54 (m, 1H, H-5′), 3.40–3.19 (m, 2H, H-6a′,6b′), 2.19 and 2.09 (2s, 6H, 2OAc); Anal. Calcd for $C_{14}H_{15}F_2IN_2O_7$: C, 34.44; H, 3.10; N, 5.74. Found: C, 34.69; H, 2.92; N, 5.85. ESI-MS (*m/z*): 489.19 ($M+H^+$).

2.2.3. 1-(2,4-Di-O-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)5-fluorouracil (**4a**)

After two vacuum/H_2 cycles to remove air from the reaction tube, the stirred mixture of **3a** (2.51 g, 5.14 mmol), 10% Pd/C (0.70 g) and triethylamine (1.42 mL, 10.28 mmol) in AcOEt (157.3 mL) and EtOH (157.3 mL) was hydrogenated at ambient pressure (balloon) and temperature (ca. 20 °C) for 24 h. The reaction mixture was filtered and the filtrate was concentrated in vacuum. The residue was purified by flash chromatography (hexane/AcOEt, 6:4) and compound **4a** (1.21 g, 65%, R_f = 0.31 in hexane/AcOEt, 6:4) was obtained as a white foam. $[\alpha]_D^{22} + 12.0$ (*c* 0.50, $CHCl_3$); λ_{max} 265 nm (ε 9574); ^{1}H NMR ($CDCl_3$): δ 8.76 (br s, 1H, NH), 7.44 (d, 1H, $J_{6,F5}$ = 5.7 Hz, H-6), 5.73 (d, 1H, $J_{1',2'}$ = 9.4 Hz, H-1′), 5.17 (m, 1H, H-2′), 5.04 (m, 1H, H-4′), 4.69 (dtr, 1H, $J_{F,3'}$ = 51.8 Hz, $J_{2',3'} = J_{3',4'}$ = 9.1 Hz, H-3′), 3.71 (m, 1H, H-5′), 2.16 and 2.08 (2s, 6H, 2OAc), 1.28 (d, 3H, $J_{5',6'}$ = 6.1 Hz, H-6′); Anal. Calcd for $C_{14}H_{16}F_2N_2O_7$: C, 46.41; H, 4.45; N, 7.73. Found: C, 46.64; H, 4.34; N, 7.92. ESI-MS (*m/z*): 363.27 ($M+H^+$).

2.2.4. 1-(4-O-Acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)5-fluorouracil (**5a**)

To a solution of **4a** (1.21 g, 3.34 mmol) in pyridine (19 mL) was added hydroxylamine hydrochloride (0.93 g, 13.4 mmol) and anhydrous sodium acetate (1.1 g, 13.4 mmol) with vigorous stirring at 20 °C. After 12 h acetone (0.95 mL) was added and the solvents were removed under high vacuum. Purification by flash chromatography (hexane/AcOEt, 6:4) gave **5a** (0.53 g, 50%, R_f = 0.17 in hexane/AcOEt, 6:4) as a colourless oil. $[\alpha]_D^{22} + 6.0$ (*c* 0.50, $CHCl_3$); λ_{max} 266 nm (ε 5701); ^{1}H NMR ($CDCl_3$): δ 9.85 (br s, 1H, NH), 7.43 (d, 1H, $J_{6,F5}$ = 5.7 Hz, H-6), 5.66 (d, 1H, $J_{1',2'}$ = 9.0 Hz, H-1′), 4.95 (m, 1H, H-4′), 4.63 (dtr, 1H, $J_{F,3'}$ = 52.0 Hz, $J_{2',3'}$ = 8.8 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 3.85 (m, 1H, H-2′), 3.74 (m, 1H, H-5′), 2.15 (s, 3H, OAc), 1.25 (d, 3H, $J_{5',6'}$ = 6.1 Hz, H-6′); Anal. Calcd for $C_{12}H_{14}F_2N_2O_6$: C, 45.01; H, 4.41; N, 8.75. Found: C, 45.19; H, 4.29; N, 8.57. ESI-MS (*m/z*): 321.24 ($M+H^+$).

2.2.5. 1-(4,6-Dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)5-fluorouracil (**6a**)

To a solution of **5a** (0.53 g, 1.67 mmol) in dimethyl sulfoxide (8.18 mL) was added acetic anhydride (4.08 mL, 43.25 mmol). The mixture was heated at 100 °C for 10 min, then cooled to room temperature, diluted with AcOEt and washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2/MeOH, 9.5:0.5) and compound **6a** (0.24 g, 55%, R_f = 0.43 in CH_2Cl_2/MeOH, 9.5:0.5) was obtained as a white powder: mp. 197–199 °C; $[\alpha]_D^{22} - 20.0$ (*c* 0.50, $CHCl_3$); λ_{max} 263 nm (ε 1078); ^{1}H NMR ($CDCl_3$): δ 10.47 (br s, 1H, NH), 7.48 (d, 1H, $J_{6,F5}$ = 5.6 Hz, H-6), 7.16 (d, 1H, $J_{F,1'}$ = 5.5 Hz, H-1′), 6.59 (dd, 1H, $J_{F,4'}$ = 11.3 Hz, $J_{4',5'}$ = 1.3 Hz, H-4′), 4.94 (m, 1H, H-5′), 1.51 (d, 3H, $J_{5',6'}$ = 6.8 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 188.26, 159.32, 157.01, 149.18, 143.91, 125.88, 116.90, 85.37, 69.66, 21.08; ^{19}F NMR: δ −65.0, −65.5. Anal. Calcd for $C_{10}H_8F_2N_2O_4$: C, 46.52; H, 3.12; N, 10.85. Found: C, 46.35; H, 3.40; N, 10.74. ESI-MS (*m/z*): 259.16 ($M+H^+$).

2.3. Synthesis of 9-(4,6-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^6-benzoyl adenine (**6b**)

2.3.1. 9-(3-Deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)-N^6-benzoyl adenine (**2b**)

Adenine derivative **2b** was synthesized from 9-(3-deoxy-3-fluoro-β-D-glucopyranosyl)-N^6-benzoyl adenine (**1b**) [31] by the same

methodology as described for the synthesis of **2a**. Purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) and compound **2b** (2.1 g, 66%, R_f = 0.39 in CH_2Cl_2/MeOH, 9:1) was obtained as a thick syrup. $[\alpha]_D^{22}$ − 4.0 (*c* 0.50, MeOH); λ_{max} 279 nm (ε 6448); ^{1}H NMR (CD_3OD): δ 8.77 and 8.63 (2s, 2H, H-2,8), 8.12–7.58 (m, 5H, Bz), 5.85 (d, 1H, $J_{1',2'}$ = 8.8 Hz, H-1′), 4.64–4.50 (m, 2H, H-2′ and H-3′), 3.77 (m, 1H, H-4′), 3.65–3.45 (m, 3H, H-5′ and H-6a′,6b′); Anal. Calcd for $C_{18}H_{17}FIN_5O_4$: C, 42.12; H, 3.34; N, 13.64. Found: C, 42.30; H, 3.17; N, 13.86. ESI-MS (*m*/*z*): 514.25 (M+H$^+$).

2.3.2. 9-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)-N^6-benzoyl adenine (**3b**)

Adenine derivative **3b** was synthesized from **2b** by the same methodology as described for the synthesis of **3a**. Purified by flash chromatography (hexane/AcOEt, 2:8) and compound **3b** (2.0 g, 82%, R_f = 0.4 in AcOEt) was obtained as a yellowish syrup. $[\alpha]_D^{22}$ − 2.0 (*c* 0.50, $CHCl_3$); λ_{max} 280 nm (ε 12921); ^{1}H NMR ($CDCl_3$): δ 8.86 and 8.32 (2s, 2H, H-2,8), 8.08–7.55 (m, 5H, Bz), 5.99 (d, 1H, $J_{1',2'}$ = 9.3 Hz, H-1′), 5.71 (m, 1H, H-2′), 5.35 (m, 1H, H-4′), 4.88 (dtr, 1H, $J_{F,3'}$ = 51.6 Hz, $J_{2',3'} = J_{3',4'}$ = 8.9 Hz, H-3′), 3.71 (m, 1H, H-5′), 3.42–3.23 (m, 2H, H-6a′,6b′), 2.23 and 1.87 (2s, 6H, 2OAc); Anal. Calcd for $C_{22}H_{21}FIN_5O_6$: C, 44.24; H, 3.54; N, 11.72. Found: C, 44.09; H, 3.24; N, 11.91. ESI-MS (*m*/*z*): 598.35 (M+H$^+$).

2.3.3. 9-(2,4-Di-O-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-N^6-benzoyl adenine (**4b**)

Adenine derivative **4b** was synthesized from **3b** by the same methodology as described for the synthesis of **4a**. Purified by flash chromatography (hexane/AcOEt, 2:8) and compound **4b** (1.17 g, 74%, R_f = 0.39 in AcOEt) was obtained as a white foam. $[\alpha]_D^{22}$ − 3.0 (*c* 0.50, $CHCl_3$); λ_{max} 280 nm (ε 17712); ^{1}H NMR ($CDCl_3$): δ 8.83 and 8.26 (2s, 2H, H-2,8), 8.05–7.53 (m, 5H, Bz), 5.87 (d, 1H, $J_{1',2'}$ = 9.3 Hz, H-1′), 5.66 (m, 1H, H-2′), 5.19 (m, 1H, H-4′), 4.78 (dtr, 1H, $J_{F,3'}$ = 51.8 Hz, $J_{2',3'} = J_{3',4'}$ = 8.9 Hz, H-3′), 3.83 (m, 1H, H-5′), 2.18 and 1.83 (2s, 6H, 2OAc), 1.31 (d, 3H, $J_{5',6'}$ = 5.5 Hz, H-6′); Anal. Calcd for $C_{22}H_{22}FN_5O_6$: C, 56.05; H, 4.70; N, 14.86. Found: C, 55.83; H, 4.83; N, 14.61. ESI-MS (*m*/*z*): 472.42 (M+H$^+$).

2.3.4. 9-(4-O-Acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-N^6-benzoyl adenine (**5b**)

Adenine derivative **5b** was synthesized from **4b** by the same methodology as described for the synthesis of **5a**. Purified by flash chromatography (AcOEt) and compound **5b** (0.56 g, 53%, R_f = 0.29 in AcOEt) was obtained as an oil. $[\alpha]_D^{22}$ + 16.0 (*c* 0.50, $CHCl_3$); λ_{max} 280 nm (ε 14468); ^{1}H NMR ($CDCl_3$): δ 8.52 and 8.24 (2s, 2H, H-2,8), 7.99–7.50 (m, 5H, Bz), 5.73 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1′), 5.17 (m, 1H, H-4′), 4.80–4.66 (m, 2H, H-2′ and H-3′), 3.79 (m, 1H, H-5′), 2.19 (s, 3H, OAc), 1.28 (d, 3H, $J_{5',6'}$ = 5.4 Hz, H-6′); Anal. Calcd for $C_{20}H_{20}FN_5O_5$: C, 55.94; H, 4.69; N, 16.31. Found: C, 55.67; H, 4.80; N, 16.19. ESI-MS (*m*/*z*): 430.42 (M+H$^+$).

2.3.5. 9-(4,6-Dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^6-benzoyl adenine (**6b**)

Adenine derivative **6b** was synthesized from **5b** by the same methodology as described for the synthesis of **6a**. Purified by flash chromatography (AcOEt) and compound **6b** (0.29 g, 60%, R_f = 0.26 in AcOEt) was obtained as a yellow powder: mp. 211–213 °C; $[\alpha]_D^{22}$ − 12.0 (*c* 0.50, $CHCl_3$); λ_{max} 280 nm (ε 10491); ^{1}H NMR ($CDCl_3$): δ 8.81 and 8.12 (2s, 2H, H-2,8), 8.08–7.55 (m, 5H, Bz), 6.69 (d, 1H, $J_{F,4'}$ = 10.7 Hz, H-4′), 6.53 (s, 1H, H-1′), 5.08 (m, 1H, H-5′), 1.55 (d, 3H, $J_{5',6'}$ = 5.3 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 190.31, 164.28, 156.35, 157.29, 151.60, 150.01, 147.32, 136.11, 133.27, 129.28, 127.69, 124.33, 116.37, 85.91, 67.39, 22.15; ^{19}F NMR: δ −63.2; Anal. Calcd for $C_{18}H_{14}FN_5O_3$: C, 58.85; H, 3.84. N, 19.07. Found: C, 59.07; H, 3.73; N, 19.38. ESI-MS (*m*/*z*): 368.34 (M+H$^+$).

2.4. Synthesis of 1-(4,6-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)uracil (**6c**)

2.4.1. 1-(3-Deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)uracil (**2c**)

Uracil derivative **2c** was synthesized from 1-(3-deoxy-3-fluoro-β-D-glucopyranosyl)uracil (**1c**) [32] by the same methodology as described for the synthesis of **2a**. Purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) and compound **2c** (1.26 g, 60%, R_f = 0.11 in CH_2Cl_2/MeOH, 9:1) was obtained as a yellow foam. $[\alpha]_D^{22}$ + 2.0 (*c* 0.31, MeOH); λ_{max} 257 nm (ε 3220); ^{1}H NMR (CD_3OD): δ 8.33 (br s, 1H, NH), 7.67 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 5.76 (d, 1H, H-5), 5.65 (d, 1H, $J_{1',2'}$ = 9.4 Hz, H-1′), 4.44 (dtr, 1H, $J_{F,3'}$ = 52.4 Hz, $J_{2',3'} = J_{3',4'}$ = 8.7 Hz, H-3′), 3.87 (m, 1H, H-2′), 3.65–3.43 (m, 3H, H-4′ and H-6a′,6b′), 3.22 (m, 1H, H-5′); Anal. Calcd for $C_{10}H_{12}FIN_2O_5$: C, 31.11; H, 3.13; N, 7.26. Found: C, 30.89; H, 3.27; N, 7.07. ESI-MS (*m*/*z*): 387.13 (M+H$^+$).

2.4.2. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)uracil (**3c**)

Uracil derivative **3c** was synthesized from **2c** by the same methodology as described for the synthesis of **3a**. Purified by flash chromatography (hexane/AcOEt, 4:6) and compound **3c** (1.32 g, 86%, R_f = 0.62 in AcOEt) was obtained as a white solid: mp. 138–140 °C; $[\alpha]_D^{22}$ − 1.0 (*c* 0.16, $CHCl_3$); λ_{max} 256 nm (ε 4882); ^{1}H NMR ($CDCl_3$): δ 8.32 (br s, 1H, NH), 7.34 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.85 (m, 2H, H-5 and H-1′), 5.30–5.14 (m, 2H, H-2′ and H-4′), 4.75 (dtr, 1H, $J_{F,3'}$ = 51.6 Hz, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 9.1 Hz, H-3′), 3.50 (m, 1H, H-5′), 3.40–3.16 (m, 2H, H-6a′,6b′), 2.18 and 2.08 (2s, 6H, 2OAc); Anal. Calcd for $C_{14}H_{16}FIN_2O_7$: C, 35.76; H, 3.43; N, 5.96. Found: C, 36.05; H, 3.18; N, 6.10. ESI-MS (*m*/*z*): 471.18 (M+H$^+$).

2.4.3. 1-(2,4-Di-O-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)uracil (**4c**)

Uracil derivative **4c** was synthesized from **3c** by the same methodology as described for the synthesis of **4a**. Purified by flash chromatography (hexane/AcOEt, 1:1) and compound **4c** (0.67 g, 70%, R_f = 0.22 in hexane/AcOEt, 1:1) was obtained as a white foam. $[\alpha]_D^{22}$ + 4.0 (*c* 0.50, $CHCl_3$); λ_{max} 255 nm (ε 7832); ^{1}H NMR ($CDCl_3$): δ 8.45 (br s, 1H, NH), 7.34 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.82 (d, 1H, H-5), 5.75 (d, 1H, $J_{1',2'}$ = 9.6 Hz, H-1′), 5.22 (m, 1H, H-2′), 5.02 (m, 1H, H-4′), 4.68 (dtr, 1H, $J_{F,3'}$ = 51.8 Hz, $J_{2',3'} = J_{3',4'}$ = 9.1 Hz, H-3′), 3.70 (m, 1H, H-5′), 2.16 and 2.07 (2s, 6H, 2OAc), 1.27 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); Anal. Calcd for $C_{14}H_{17}FN_2O_7$: C, 48.84; H, 4.98; N, 8.14. Found: C, 48.52; H, 5.14; N, 8.45. ESI-MS (*m*/*z*): 345.28 (M+H$^+$).

2.4.4. 1-(2-O-Acetyl-4-O-benzoyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)uracil (**8c**)

To a stirred solution of 1-(2-*O*-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)uracil (**7c**) [32] (1.50 g, 4.96 mmol) in pyridine (24.8 mL) was added benzoyl chloride (BzCl) (2.88 mL, 24.8 mmol). The reaction mixture was stirred at room temperature for 2 h, diluted with AcOEt and washed several times with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (hexane/AcOEt, 1:1) and compound **8c** (1.59 g, 79%, R_f = 0.33 in hexane/AcOEt, 1:1) was obtained as a white foam. $[\alpha]_D^{22}$ − 6.0 (*c* 0.50, $CHCl_3$); λ_{max} 255 nm (ε 19627); ^{1}H NMR ($CDCl_3$): δ 8.13–7.94 (m, 5H, Bz), 7.53 (d, 1H, $J_{5,6}$ = 8.3 Hz, H-6), 5.98 (d, 1H, H-5), 5.82 (d, 1H, $J_{1',2'}$ = 9.3 Hz, H-1′), 5.36–5.26 (m, 2H, H-2′ and H-4′), 4.90 (dtr, 1H, $J_{F,3'}$ = 51.8 Hz, $J_{2',3'} = J_{3',4'}$ = 9.0 Hz, H-3′), 3.88 (m, 1H, H-5′), 2.07 (s, 3H, OAc), 1.37 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); Anal. Calcd for $C_{19}H_{19}FN_2O_7$: C, 56.16; H, 4.71; N, 6.89. Found: C, 56.28; H, 4.59; N, 7.02. ESI-MS (*m*/*z*): 407.35 (M+H$^+$).

*2.4.5. 1-(4-O-Benzoyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)uracil (**9c**)*

Compound **8c** (1.59 g, 3.92 mmol) was dissolved in EtOH-pyridine (78.4 + 35.5 mL), 2 M NaOH (2.75 mL) was added and the mixture stirred at room temperature for 30 min. Amberlite IR-120 (H^+) was added to neutralize the base. The suspension was filtered, the resin was washed with EtOH and pyridine (30 + 30 mL) and the filtrate was evaporated. The solid residue was purified by flash chromatography (hexane/AcOEt, 3:7) and compound **9c** (0.93 g, 65%, R_f = 0.34 in hexane/AcOEt, 3:7) was obtained as a yellowish foam. $[\alpha]_D^{22} + 4.0$ (*c* 0.50, $CHCl_3$); λ_{max} 261 nm (ε 3936); ^{1}H NMR ($CDCl_3$): δ 8.07–7.63 (m, 5H, Bz), 7.37 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.78 (m, 2H, $J_{1',2'}$ = 9.7 Hz, H-5 and H-1′), 5.22 (m, 1H, H-4′), 4.81 (dtr, 1H, $J_{F,3'}$ = 52.1 Hz, $J_{2',3'} = J_{3',4'}$ = 9.0 Hz, H-3′), 4.01–3.85 (m, 2H, H-2′ and H-5′), 1.29 (d, 3H, $J_{5',6'}$ = 6.1 Hz, H-6′); Anal. Calcd for $C_{17}H_{17}FN_2O_6$: C, 56.04; H, 4.70; N, 7.69. Found: C, 56.15; H, 4.55; N, 7.88. ESI-MS (*m*/*z*): 365.34 ($M+H^+$).

*2.4.6. 1-(4,6-Dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)uracil (**6c**)*

Uracil derivative **6c** was synthesized from **9c** by the same methodology as described for the synthesis of **6a**. Purified by flash chromatography (hexane/AcOEt, 3:7) and compound **6c** (0.37 g, 60%, R_f = 0.35 in CH_2Cl_2/MeOH, 9.5:0.5) was obtained as a white powder: mp. 174–176 °C; $[\alpha]_D^{22} - 6.0$ (*c* 0.50, $CHCl_3$); λ_{max} 260 nm (ε 1224); ^{1}H NMR ($CDCl_3$): δ 8.31 (br s, 1H, NH), 7.82 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 7.07 (d, 1H, $J_{F,1'}$ = 8.1 Hz, H-1′), 6.58 (dd, 1H, $J_{F,4'}$ = 11.4 Hz, $J_{4',5'}$ = 1.4 Hz, H-4′), 5.79 (d, 1H, H-5), 4.94 (m, 1H, H-5′), 1.51 (d, 3H, $J_{5',6'}$ = 6.7 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 187.05, 164.31, 157.26, 148.37, 140.02, 117.35, 103.09, 86.28, 68.71, 20.19; ^{19}F NMR: δ −64.3; Anal. Calcd for $C_{10}H_9FN_2O_4$: C, 50.01; H, 3.78; N, 11.66. Found: C, 50.12; H, 3.57; N, 11.85. ESI-MS (*m*/*z*): 241.17 ($M+H^+$).

*2.5. Synthesis of 1-(4,6-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)thymine (**6d**)*

*2.5.1. 1-(3-Deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)thymine (**2d**)*

Thymine derivative **2d** was synthesized from 1-(3-deoxy-3-fluoro-β-D-glucopyranosyl)thymine (**1d**) [32] by the same methodology as described for the synthesis of **2a**. Purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) and compound **2d** (1.43 g, 69%, R_f = 0.4 in CH_2Cl_2/MeOH, 9:1) was obtained as a white foam. $[\alpha]_D^{22} + 2.0$ (*c* 0.50, MeOH); λ_{max} 262 nm (ε 5394); ^{1}H NMR (CD_3OD): δ 8.06 (br s, 1H, NH), 7.50 (s, 1H, H-6), 5.63 (d, 1H, $J_{1',2'}$ = 9.4 Hz, H-1′), 4.43 (dtr, 1H, $J_{F,3'}$ = 52.1 Hz, $J_{2',3'}$ = 8.8 Hz, $J_{3',4'}$ = 9.1 Hz, H-3′), 3.91 (m, 1H, H-2′), 3.65–3.41 (m, 3H, H-4′ and H-6a′,6b′), 3.22 (m, 1H, H-5′), 1.93 (s, 3H, 5-CH_3); Anal. Calcd for $C_{11}H_{14}FIN_2O_5$: C, 33.02; H, 3.53; N, 7.00. Found: C, 33.26; H, 3.36; N, 7.18. ESI-MS (*m*/*z*): 401.15 ($M+H^+$).

*2.5.2. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)thymine (**3d**)*

Thymine derivative **3d** was synthesized from **2d** by the same methodology as described for the synthesis of **3a**. Purified by flash chromatography (hexane/AcOEt, 6:4) and compound **3d** (1.43 g, 83%, R_f = 0.5 in hexane/AcOEt, 4:6) was obtained as a white powder: mp. 160–162 °C; $[\alpha]_D^{22} + 2.0$ (*c* 0.44, $CHCl_3$); λ_{max} 261 nm (ε 7617); ^{1}H NMR ($CDCl_3$): δ 8.37 (br s, 1H, NH), 7.17 (s, 1H, H-6), 5.85 (d, 1H, $J_{1',2'}$ = 9.5 Hz, H-1′), 5.32–5.14 (m, 2H, H-2′ and H-4′), 4.74 (dtr, 1H, $J_{F,3'}$ = 51.7 Hz, $J_{2',3'} = J_{3',4'}$ = 9.1 Hz, H-3′), 3.51 (m, 1H, H-5′), 3.39–3.16 (m, 2H, H-6a′,6b′), 2.18 and 2.07 (2s, 6H, 2OAc), 1.97 (s, 3H, 5-CH_3); Anal. Calcd for $C_{15}H_{18}FIN_2O_7$: C, 37.21; H, 3.75; N, 5.79. Found: C, 37.51; H, 3.63; N, 5.98. ESI-MS (*m*/*z*): 485.20 ($M+H^+$).

*2.5.3. 1-(2,4-Di-O-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)thymine (**4d**)*

Thymine derivative **4d** was synthesized from **3d** by the same methodology as described for the synthesis of **4a**. Purified by flash chromatography (hexane/AcOEt, 6:4) and compound **4d** (0.71 g, 67%, R_f = 0.5 in hexane/AcOEt, 4:6) was obtained as a white foam. $[\alpha]_D^{22} + 2.0$ (*c* 0.50, $CHCl_3$); λ_{max} 261 nm (ε 10114); ^{1}H NMR ($CDCl_3$): δ 8.43 (br s, 1H, NH), 7.16 (s, 1H, H-6), 5.75 (d, 1H, $J_{1',2'}$ = 9.5 Hz, H-1′), 5.25 (m, 1H, H-2′), 5.04 (m, 1H, H-4′), 4.68 (dtr, 1H, $J_{F,3'}$ = 51.9 Hz, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 9.1 Hz, H-3′), 3.70 (m, 1H, H-5′), 2.16 and 2.06 (2s, 6H, 2OAc), 1.96 (s, 3H, 5-CH_3), 1.28 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); Anal. Calcd for $C_{15}H_{19}FN_2O_7$: C, 50.28; H, 5.34; N, 7.82. Found: C, 50.47; H, 5.12; N, 7.98. ESI-MS (*m*/*z*): 359.31 ($M+H^+$).

*2.5.4. 1-(2-O-Acetyl-4-O-benzoyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)thymine (**8d**)*

Thymine derivative **8d** was synthesized from 1-(2-*O*-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)thymine (**7d**) [32] by the same methodology as described for the synthesis of **8c**. Purified by flash chromatography (hexane/AcOEt, 6:4) and compound **8d** (1.45 g, 73%, R_f = 0.65 in hexane/AcOEt, 4:6) was obtained as a white solid: mp. 288–290 °C; $[\alpha]_D^{22} - 14.0$ (*c* 0.50, $CHCl_3$); λ_{max} 260 nm (ε 18654); ^{1}H NMR ($CDCl_3$): δ 8.25 (br s, 1H, NH), 8.09–7.49 (m, 5H, Bz), 7.22 (s, 1H, H-6), 5.84 (d, 1H, $J_{1',2'}$ = 9.5 Hz, H-1′), 5.38–5.29 (m, 2H, H-2′ and H-4′), 4.88 (dtr, 1H, $J_{F,3'}$ = 52.0 Hz, $J_{2',3'} = J_{3',4'}$ = 9.0 Hz, H-3′), 3.87 (m, 1H, H-5′), 2.09 (s, 3H, OAc), 2.00 (s, 3H, 5-CH_3), 1.35 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); Anal. Calcd for $C_{20}H_{21}FN_2O_7$: C, 57.14; H, 5.04; N, 6.66. Found: C, 56.89; H, 4.93; N, 6.88. ESI-MS (*m*/*z*): 421.37 ($M+H^+$).

*2.5.5. 1-(4-O-Benzoyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)thymine (**9d**)*

Thymine derivative **9d** was synthesized from **8d** by the same methodology as described for the synthesis of **9c**. Purified by flash chromatography (hexane/AcOEt, 4:6) and compound **9d** (0.89 g, 68%, R_f = 0.23 in hexane/AcOEt, 4:6) was obtained as a clear viscous oil. $[\alpha]_D^{22} + 3.0$ (*c* 0.50, $CHCl_3$); λ_{max} 263 nm (ε 7658); ^{1}H NMR ($CDCl_3$): δ 9.95 (br s, 1H, NH), 8.07–7.41 (m, 5H, Bz), 7.22 (s, 1H, H-6), 5.81 (d, 1H, $J_{1',2'}$ = 9.2 Hz, H-1′), 5.26 (m, 1H, H-4′), 4.88 (dtr, 1H, $J_{F,3'}$ = 52.0 Hz, $J_{2',3'}$ = 8.7 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 4.06–3.89 (m, 2H, H-2′ and H-5′), 1.93 (s, 3H, 5-CH_3), 1.31 (d, 3H, $J_{5',6'}$ = 6.0 Hz, H-6′); Anal. Calcd for $C_{18}H_{19}FN_2O_6$: C, 57.14; H, 5.06; N, 7.40. Found: C, 57.44; H, 4.93; N, 7.58. ESI-MS (*m*/*z*): 379.36 ($M+H^+$).

*2.5.6. 1-(4,6-Dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)thymine (**6d**)*

Thymine derivative **6d** was synthesized from **9d** by the same methodology as described for the synthesis of **6a**. Purified by flash chromatography (CH_2Cl_2/MeOH, 9.5:0.5) and compound **6d** (0.35 g, 58%, R_f = 0.36 in CH_2Cl_2/MeOH, 9.5:0.5) was obtained as a white foam. $[\alpha]_D^{22} - 40.0$ (*c* 0.50, $CHCl_3$); λ_{max} 260 nm (ε 2542); ^{1}H NMR ($CDCl_3$): δ 8.84 (s, 1H, H-6), 6.88 (s, 1H, H-1′), 6.58 (d, 1H, $J_{F,4'}$ = 11.1 Hz, H-4′), 4.94 (m, 1H, H-5′), 1.93 (s, 3H, 5-CH_3), 1.51 (d, 3H, $J_{5',6'}$ = 6.8 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 189.36, 162.98, 156.01, 149.00, 136.12, 116.31, 111.90, 84.92, 69.26, 20.21, 11.98; ^{19}F NMR: δ −65.5; Anal. Calcd for $C_{11}H_{11}FN_2O_4$: C, 51.97; H, 4.36; N, 11.02. Found: C, 51.83; H, 4.58; N, 10.77. ESI-MS (*m*/*z*): 255.22 ($M+H^+$).

*2.6. Synthesis of 1-(4,6-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^4-benzoyl cytosine (**6e**)*

*2.6.1. 1-(3-Deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**2e**)*

Cytosine derivative **2e** was synthesized from 1-(3-deoxy-3-fluoro-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**1e**) [30] by the

same methodology as described for the synthesis of **2a**. Purified by flash chromatography (CH_2Cl_2/MeOH, 9:1) and compound **2e** (1.14 g, 59%, R_f = 0.27 in CH_2Cl_2/MeOH, 9:1) was obtained as a yellowish foam. $[\alpha]_D^{22}$ + 1.0 (*c* 0.50, MeOH); λ_{max} 261 nm (ε 6517); ^{1}H NMR (CD_3OD): δ 8.68 (br s, 1H, NH), 7.79–7.38 (m, 7H, Bz, H-5 and H-6), 5.89 (d, 1H, $J_{1',2'}$ = 9.2 Hz, H-1′), 4.67–4.43 (m, 2H, H-2′ and H-3′), 3.67 (m, 1H, H-4′), 3.59–3.37 (m, 2H, H-6a′,6b′), 3.22 (m, 1H, H-5′); Anal. Calcd for $C_{17}H_{17}FIN_3O_5$: C, 41.73; H, 3.50; N, 8.59. Found: C, 42.09; H, 3.64; N, 8.42. ESI-MS (*m*/*z*): 490.22 ($M+H^+$).

2.6.2. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**3e**)

Cytosine derivative **3e** was synthesized from **2e** by the same methodology as described for the synthesis of **3a**. Purified by flash chromatography (hexane/AcOEt, 4:6) and compound **3e** (1.1 g, 82%, R_f = 0.62 in AcOEt) was obtained as a yellow oil. $[\alpha]_D^{22}$ + 16.0 (*c* 0.50, $CHCl_3$); λ_{max} 262 nm (ε 28431); ^{1}H NMR ($CDCl_3$): δ 8.76 (br s, 1H, NH), 7.91–7.43 (m, 7H, Bz, H-5 and H-6), 6.14 (d, 1H, $J_{1',2'}$ = 9.4 Hz, H-1′), 5.31–5.19 (m, 2H, H-2′ and H-4′), 4.80 (dtr, 1H, $J_{F,3'}$ = 51.6 Hz, $J_{2',3'}$ = $J_{3',4'}$ = 9.1 Hz, H-3′), 3.53 (m, 1H, H-5′), 3.42–3.19 (m, 2H, H-6a′,6b′), 2.19 and 2.06 (2s, 6H, 2OAc); Anal. Calcd for $C_{21}H_{21}FIN_3O_7$: C, 43.99; H, 3.69; N, 7.33. Found: C, 44.24; H, 3.55; N, 7.21. ESI-MS (*m*/*z*): 574.32 ($M+H^+$).

2.6.3. 1-(2,4-Di-O-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**4e**)

Cytosine derivative **4e** was synthesized from **3e** by the same methodology as described for the synthesis of **4a**. Purified by flash chromatography (hexane/AcOEt, 2:8) and compound **4e** (0.59 g, 69%, R_f = 0.39 in AcOEt) was obtained as a white foam. $[\alpha]_D^{22}$ + 22.0 (*c* 0.50, $CHCl_3$); λ_{max} 262 nm (ε 20073); ^{1}H NMR ($CDCl_3$): δ 8.65 (br s, 1H, NH), 7.90–7.50 (m, 7H, Bz, H-5 and H-6), 6.04 (d, 1H, $J_{1',2'}$ = 9.4 Hz, H-1′), 5.24 (m, 1H, H-2′), 5.07 (m, 1H, H-4′), 4.73 (dtr, 1H, $J_{F,3'}$ = 51.8 Hz, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 9.1 Hz, H-3′), 3.74 (m, 1H, H-5′), 2.17 and 2.05 (2s, 6H, 2OAc), 1.29 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); Anal. Calcd for $C_{21}H_{22}FN_3O_7$: C, 56.37; H, 4.96; N, 9.39. Found: C, 56.65; H, 4.85; N, 9.26. ESI-MS (*m*/*z*): 448.43 ($M+H^+$).

2.6.4. 1-(2-O-Acetyl-4-O-benzoyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**8e**)

Cytosine derivative **8e** was synthesized from 1-(2-*O*-acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-*N*^{4}-benzoyl cytosine (**7e**) [32] by the same methodology as described for the synthesis of **8c**. Purified by flash chromatography (hexane/AcOEt, 3:7) and compound **8e** (1.51 g, 80%, R_f = 0.26 in hexane/AcOEt, 3:7) was obtained as a white solid: mp. 308–310 °C; $[\alpha]_D^{22}$ + 7.0 (*c* 0.50, $CHCl_3$); λ_{max} 262 nm (ε 18578); ^{1}H NMR ($CDCl_3$): δ 8.65 (br s, 1H, NH), 8.09–7.47 (m, 12H, 2Bz, H-5 and H-6), 6.11 (d, 1H, $J_{1',2'}$ = 9.5 Hz, H-1′), 5.40–5.27 (m, 2H, H-2′ and H-4′), 4.91 (dtr, 1H, $J_{F,3'}$ = 52.0 Hz, $J_{2',3'}$ = 9.3 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 3.90 (m, 1H, H-5′), 2.07 (s, 3H, OAc), 1.35 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); Anal. Calcd for $C_{26}H_{24}FN_3O_7$: C, 61.29; H, 4.75; N, 8.25. Found: C, 61.47; H, 4.64; N, 8.12. ESI-MS (*m*/*z*): 510.49 ($M+H^+$).

2.6.5. 1-(4-O-Benzoyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**9e**)

Cytosine derivative **9e** was synthesized from **8e** by the same methodology as described for the synthesis of **9c**. Purified by flash chromatography (hexane/AcOEt, 2:8) and compound **9e** (0.95 g, 69%, R_f = 0.32 in hexane/AcOEt, 2:8) was obtained as a yellowish foam. $[\alpha]_D^{22}$ + 2.0 (*c* 0.48, $CHCl_3$); λ_{max} 260 nm (ε 11403); ^{1}H NMR ($CDCl_3$): δ 8.70 (br s, 1H, NH), 8.08–7.40 (m, 12H, 2Bz, H-5 and H-6), 5.98 (d, 1H, $J_{1',2'}$ = 9.2 Hz, H-1′), 5.28 (m, 1H, H-4′), 4.83 (dtr, 1H, $J_{F,3'}$ = 51.9 Hz, $J_{2',3'}$ = 8.6 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 4.03–3.87 (m, 2H, H-2′ and H-5′), 1.33 (d, 3H, $J_{5',6'}$ = 6.2 Hz, H-6′); Anal. Calcd for $C_{24}H_{22}FN_3O_6$: C, 61.67; H, 4.74; N, 8.99. Found: C, 61.95; H, 4.63; N, 9.17. ESI-MS (*m*/*z*): 468.46 ($M+H^+$).

2.6.6. 1-(4,6-Dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^4-benzoyl cytosine (**6e**)

Cytosine derivative **6e** was synthesized from **9e** by the same methodology as described for the synthesis of **6a**. Purified by flash chromatography (hexane/AcOEt, 2:8) and compound **6e** (0.40 g, 58%, R_f = 0.62 in CH_2Cl_2/MeOH, 9.5:0.5) was obtained as a white powder: mp. 226–228 °C; $[\alpha]_D^{22}$ − 16.0 (*c* 0.50, $CHCl_3$); λ_{max} 261 nm (ε 18216); ^{1}H NMR ($CDCl_3$): δ 8.66 (br s, 1H, NH), 8.12–7.44 (m, 7H, Bz, H-5 and H-6), 6.58 (dd, 2H, $J_{F,4'}$ = 11.4 Hz, $J_{4',5'}$ = 1.7 Hz, H-4′ and H-1′), 4.98 (m, 1H, H-5′), 1.53 (d, 3H, $J_{5',6'}$ = 6.8 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 191.36, 166.25, 165.22, 157.01, 155.19, 142.93, 134.13, 131.87, 129.43, 127.89, 117.07, 101.32, 83.96, 66.04, 22.66; ^{19}F NMR: δ −65.0; Anal. Calcd for $C_{17}H_{14}FN_3O_4$: C, 59.47; H, 4.11; N, 12.24. Found: C, 59.65; H, 3.99; N, 11.96. ESI-MS (*m*/*z*): 344.30 ($M+H^+$).

2.7. Antiviral activity assays

The antiviral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV) and varicella-zoster virus (VZV)], Vero (parainfluenza-3, reovirus-1, sindbis virus and coxsackie B4), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) or MDCK [influenza A (H1N1; H3N2) and influenza B] cell cultures. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures). After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, ..., μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/cm^3) cells were infected with 100 $CCID_{50}$ of HIV(III_B) or HIV-2(ROD)/mL and seeded in 200-μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

2.8. Cytostatic/toxic activity assays

Murine leukemia L1210, murine mammary carcinoma FM3A, and human lymphocyte CEM and human cervix carcinoma HeLa cells were seeded in 96-well microtiter plates at 50,000 (L1210, FM3A), 75,000 (CEM) or 20,000 (HeLa) cells per 200 μL-well in the presence of different concentrations of the test compounds. After 2 (L1210, FM3A), 3 (CEM) or 4 (HeLa) days, the viable cell number was counted using a Coulter counter apparatus. The 50% cytostatic concentration (CC_{50}) was defined as the compound concentration required to inhibit tumor cell proliferation by 50%.

3. Results and discussion

3.1. Synthesis

Retrosynthetic analysis of the target unsaturated dideoxy fluoroketopyranosyl nucleosides **6a–e** suggested that the β-nucleosides

1a–e [30–32] were the most promising starting materials (Fig. 1). Therefore, our synthetic route was based on the assumption that the target molecules **6a–e** could be reached from the corresponding 6-bromo-4-benzoates **II** by hydrogenation and subsequent oxidation (Fig. 1). The latter analogues could be obtained from 4,6-*O*-benzylidene nucleosides **I**, since the benzylidene acetals could undergo smooth ring opening by the action of *N*-bromosuccinimide [35–38]. Although, direct benzylidenation of nucleosides **1a–e** [30–32] with α,α-dimethoxytoluene, under Evans [39] conditions afforded the desired analogues **I**, the Hanessian–Hullar reaction on 4,6-*O*-benzylidene acetals failed and only decomposition products were obtained. It seems that the presence of the electron-withdrawing fluorine atom α to the benzylidene acetal ring causes destabilization of the benzylidene radical formed and therefore the ring opening cannot take place.

Since the *N*-bromosuccinimide-mediated fragmentation of benzylidene derivatives **I** failed, an alternative and more versatile plan was then decided to acquire the target unsaturated carbonyl nucleosides **6a–e** (Fig. 2). Thus, monoiodination of the primary hydroxyl group of fluoro nucleosides **1a–e** [30–32] using triphenylphosphine, iodine and imidazole [40–43], led to the desired iodo derivatives **2a–e**. Acetylation of the free hydroxyl groups in the 2′- and 4′-position of the sugar moiety of the aforementioned nucleosides with acetic anhydride/pyridine afforded the desired acetylated derivatives **3a–e** in very good yields (**3a**, 89%, **3b**, 82%, **3c**, 86%, **3d**, 83% and **3e**, 82%). Catalytic hydrogenation of **3a–e** in the presence of palladium-on-carbon furnished the deoxy nucleosides **4a–e**. However, when regioselectively 2′-*O*-deacylation of the fully protected deoxy nucleosides **4a–e** with hydroxylamine hydrochloride and sodium acetate [44] was employed, only the partially acetylated analogues of 5-fluorouracil (**5a**) and N^6-benzoyl adenine (**5b**) were formed. It is noteworthy that treatment of the deoxy nucleosides of uracil (**5c**), thymine (**5d**) and N^4-benzoyl cytosine (**5e**) either with hydroxylamine hydrochloride [44] or hydrazine hydrate [45], resulted in intractable products. In the final step, oxidation of the fluoro acetylated dideoxy precursors **5a,b** performed by the acetic anhydride/dimethyl sulfoxide system [46] gave, after a β-elimination reaction, the desired unsaturated 4,6-dideoxy-3-fluoro-β-D-*glycero*-hex-3-

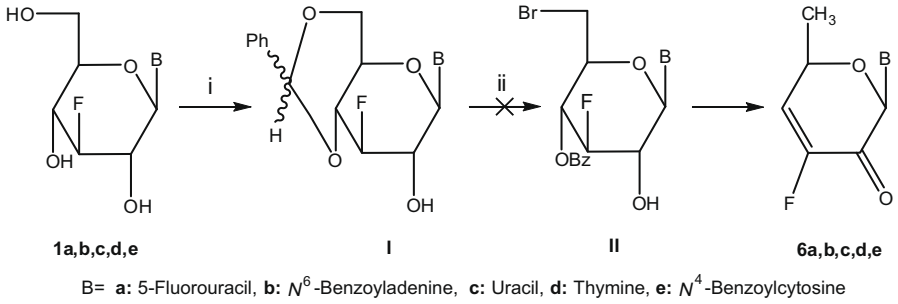

Fig. 1. Rationale for the design of the target molecules **6a**–**e**. Reagents and conditions: (i) *N*,*N*-dimethylformamide, α,α-dimethoxytoluene, 68 °C, 1 h. (ii) $BaCO_3$, *N*-bromosuccinimide, CCl_4, reflux, 3 h.

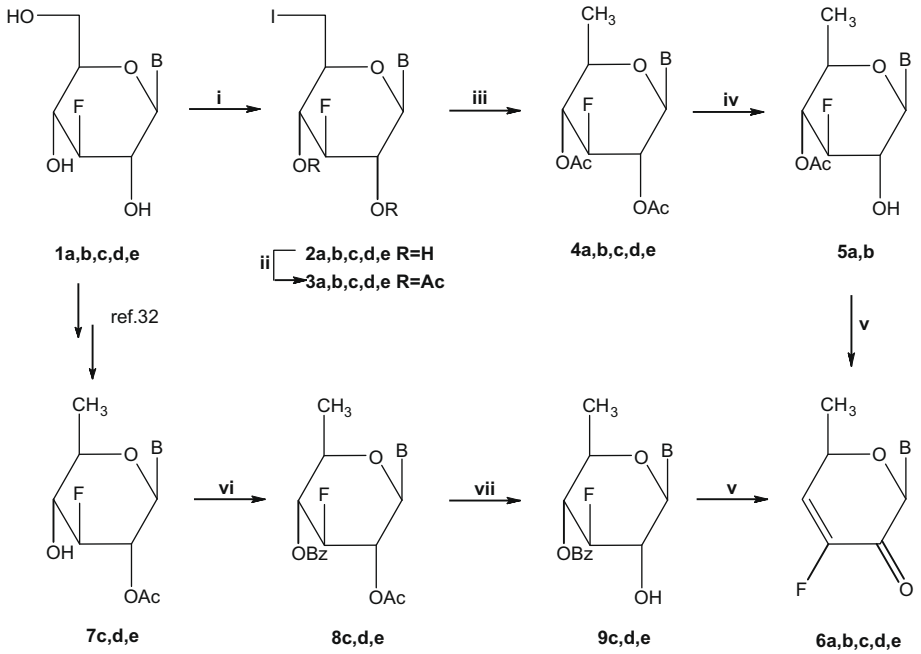

Fig. 2. Reagents and conditions: (i) iodine, triphenylphosphine, imidazole, tetrahydrofuran, 80 °C, 1 h; 68% of **2a**; 66% of **2b**; 60% of **2c**; 69% of **2d**; 59% of **2e**. (ii) Pyridine, acetic anhydride, 2 h; 89% of **3a**; 82% of **3b**; 86% of **3c**; 83% of **3d**; 82% of **3e**. (iii) H_2, 10% Pd/C, triethylamine, AcOEt, EtOH, 20 °C, 24 h; 65% of **4a**; 74% of **4b**; 70% of **4c**; 67% of **4d**; 69% of **4e**. (iv) Hydroxylamine hydrochloride, sodium acetate, pyridine, 12 h; 50% of **5a**; 53% of **5b**. (v) Acetic anhydride/dimethyl sulfoxide, 100 °C, 10 min; 55% of **6a**; 60% of **6b**; 60% of **6c**; 58% of **6d**; 58% of **6e**. (vi) BzCl, pyridine, 2 h; 79% of **8c**; 73% of **8d**; 80% of **8e**. (vii) EtOH, pyridine, NaOH, 30 min, Amberlite IR-120 (H^+) resin; 65% of **9c**; 68% of **9d**; 69% of **9e**.

enopyranosyl-2-ulose derivatives of 5-fluorouracil (**6a**) and N^6-benzoyl adenine (**6b**), in 55% and 60% yield, respectively.

In order to overcome the difficulty encountered in the previous route and to successfully synthesize the desired unsaturated 1-(4,6-dideoxy-3-fluoro-β-D-*glycero*-hex-3-enopyranosyl-2-ulose) nucleosides of uracil (**6c**), thymine (**6d**) and N^4-benzoyl cytosine (**6e**), a different synthetic strategy was then investigated. After several subsequent protection and deprotection steps of compounds **1c–e** [31,32] and finally catalytic hydrogenation, the dideoxy fluoro acetylated nucleosides **7c–e** were formed [32] (Fig. 2). Treatment of the latter analogues with BzCl in pyridine afforded the corresponding 4-benzoates **8c–e**. Deacetylation was performed using NaOH/EtOH/pyridine [47] to give the partially protected derivatives **9c–e**. Finally, oxidation of the fluoro benzoylated dideoxy precursors **9c–e** using the acetic anhydride/dimethyl sulfoxide system [46] afforded, after a β-elimination reaction, the desired unsaturated 4,6-dideoxy-3-fluoro-β-D-*glycero*-hex-3-enopyranosyl-2-ulose derivatives of uracil (**6c**), thymine (**6d**) and N^4-benzoyl cytosine (**6e**) in 60%, 58% and 58% yield, respectively.

All new compounds were well-characterized by NMR and UV spectroscopy, mass spectrometry and elemental analysis. ^{1}H NMR data obtained for the nucleosides **2a–e** ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'} \geqslant 8.7$ Hz), **3a–e** ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'} \geqslant 8.9$ Hz), **4a–e** ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'} \geqslant 8.9$ Hz), **5a,b** ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'} \geqslant 8.1$ Hz), **8c–e** ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'} \geqslant 9.0$ Hz) and **9c–e** ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'} \geqslant 8.6$ Hz) revealed that, as expected, these compounds have the β configuration and that it existed in the $^4C_1$ conformation. The unsaturated dideoxy fluoro ketonucleosides **6a–e** have half-chair conformations [48] and each of the compounds have the bulky substituent at the C-1′ in the equatorial position [49]. Finally, in compounds **6a–e** the presence of the C(O)–CF=CH-system was ascertained by the disappearance of the signal of H-2′ in the ^{1}H NMR spectra and the deshielding of other protons, especially the olefinic proton H-4′.

3.2. Biological activity

Compounds **6a–e** have been evaluated against a broad panel of DNA and RNA viruses in cell culture (mentioned at Experimental part). Unfortunately, none of the compounds inhibited virus infection and replication at subtoxic concentrations. When the compounds **6a–e** were evaluated for their anti-proliferative activity against two murine and two human tumor cell lines, moderate to poor inhibitory activity was noted for **6b–e** (IC_{50}: 7.8 to 219 μM, depending on the nature of the compound and the tumor cell line tested) (Table 1). However, the 5-fluorouracil derivative **6a** showed pronounced cytostatic activity against the murine (L1210, FM3A) and human (HeLa) tumor cell lines (IC_{50}: 0.21–1.2 μM). The compound was moderately inhibitory against CEM cell proliferation (IC_{50}: 12 μM) (Table 1). The new compounds were in general less cytotoxic to confluent adherent tumor cell cultures (Table 2).

It is striking to see that the 2′-keto derivatives presented in this study are markedly less cytostatic against the tumor cell lines than their corresponding 4′-keto derivatives [32] when the uracil, thymine, N^6-benzoyladenine and N^4-benzoylcytosine were present as the heterocyclic base (IC_{50}: 1.4–16 μM for the 4′-keto derivatives [32]). In contrast, the cytostatic activity of the 5-fluorouracil 2′-keto and 4′-keto derivatives were quite similar (IC_{50}: 0.49–3.7 μM for the 4′-keto derivatives [32]). These findings point to an important role of the heterocycle (5-fluorouracil) in the eventual cytostatic activity of this compound.

The explanation for the "detoxifying" role of the 2′-keto, compared to the 4′-keto derivative is currently unclear, but may open interesting perspectives for this type of derivative if antiviral molecules may emerge from this class of compounds. Therefore, novel compounds bearing different heterocyclic base parts should be synthesized in an attempt to reach this goal (antiviral potency combined with poor cytostatic activity).

Table 1
Cytostatic activity of **6a–e** against a panel of tumor cell lines.

Compounds	IC_{50}[a] (μM)			
	L1210	FM3A	HeLa	CEM
6a	1.2 ± 0.1	0.21 ± 0.03	0.84 ± 0.30	12 ± 3
6b	99 ± 63	185 ± 25	71 ± 44	59 ± 21
6c	134 ± 96	219 ± 27	190 ± 6	131 ± 76
6d	101 ± 92	180 ± 89	34 ± 7	35 ± 22
6e	22 ± 21	90 ± 86	12 ± 6	7.8 ± 3.0

[a] 50% Inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%.

Table 2
Cytotoxic activity of **6a–e** against a panel of tumor cell lines.

Compounds	MCC[a]			
	Vero	MDCK	HeLa	HEL
6a	100	≥0.8	100	100
6b	100	20	100	100
6c	>100	100	>100	>100
6d	100	20	100	100
6e	20	4	≥20	20

[a] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

4. Conclusion

In summary, the synthesis of the unsaturated 4,6-dideoxy-3-fluoro-β-D-*glycero*-hex-3-enopyranosyl-2-ulose nucleosides of 5-fluorouracil, N^6-benzoyl adenine, uracil, thymine, and N^4-benzoyl cytosine, respectively was undertaken and the target nucleosides were evaluated for their antiviral and cytostatic/cytotoxic activity. The newly synthesized 2′-ketopyranosyl derivatives were not potent antivirals at subtoxic concentrations, and found to be less inhibitory than the previously synthesized 4′-keto congeners against a panel of tumor cell lines [32]. Therefore, it becomes clear that the transposition of the keto group from C-4′ to C-2′ lowers the cytotoxic activity of this type of molecules.

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