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Short communication

Unsaturated dideoxy fluoro-ketopyranosyl nucleosides as new cytostatic agents: A convenient synthesis of 2,6-dideoxy-3-fluoro-4-keto-β-D-glucopyranosyl analogues of uracil, 5-fluorouracil, thymine, N^4-benzoyl cytosine and N^6-benzoyl adenine

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ABSTRACT

The β-protected nucleosides of uracil (**2a**), 5-fluorouracil (**2b**), thymine (**2c**), N^4-benzoyl cytosine (**2d**) and N^6-benzoyl adenine (**2e**) were synthesized by condensation of the peracetylated 3-deoxy-3-fluoro-D-glucopyranose (**1**) with the corresponding silylated bases. The nucleosides were deacetylated and several subsequent protection and deprotection steps afforded the partially acetylated analogues **6a–e**. Selective iodination followed by hydrogenation gave the acetylated dideoxy analogues of uracil (**8a**), 5-fluorouracil (**8b**), thymine (**8c**), N^4-benzoyl cytosine (**8d**) and N^6-benzoyl adenine (**8e**), respectively. Finally, direct oxidation of the free hydroxyl group at the 4′-position of **8a–e**, and simultaneous elimination reaction of the β-acetoxyl group, afforded the desired unsaturated 2,6-dideoxy-3-fluoro-4-keto-β-D-glucopyranosyl derivatives **9a–e**. The new analogues were evaluated for antiviral and cytostatic activity. Compounds **9a–e** were not active against a broad panel of DNA and RNA viruses at subtoxic concentrations. However, they were markedly cytostatic against a variety of tumor cell lines. The compounds should be regarded as potential new lead compounds to be further investigated for anticancer therapy.

1. Introduction

Nucleoside analogues constitute an important class of antimetabolites used in the treatment of hematological malignancies and in solid tumors [1,2]. These therapeutic compounds are frequently used to inhibit DNA biosynthesis, a process that is essential for cell growth and viral replication [3,4]. Substitution of hydrogen atoms or hydroxyl groups for fluorine has been extensively employed in the design of such analogues, as this can dramatically affect the electronic structure of a nucleoside without significantly altering its size and shape [5]. Thus, specific fluorination at the 2′- and/or 3′-position of the sugar moiety of the nucleoside analogues has been studied in the pursuit of safe, effective and chemically stable antiviral agents [6–17].

Lately, nucleosides containing pyranosyl rings instead of furanosyl ones have been evaluated for their potential antiviral [18–21], antioxidant [22] and antibiotic [23] properties and as building blocks in nucleic acid synthesis [24,25]. Among them, the unsaturated ketonucleosides are a series of cytostatic drugs, which were found to be highly cytotoxic *in vitro* [26–28] and to exert powerful inhibitory action against L1210 leukemia *in vivo* [29,30]. These agents are known to inhibit DNA, RNA, and protein synthesis [31] and to interact with sulfhydryl groups of cellular proteins and enzymes [32]. Moreover, the absence of a genotoxic effect [33] makes some of these compounds particularly interesting and indicates that they act by a mechanism that is probably different from that associated with alkylating or intercalating antitumor drugs.

Based on the above observations, we have previously reported the synthesis and biological evaluation of a series of unsaturated fluoro ketopyranonucleoside analogues, which proved to be efficient as tumor cell growth inhibitors and showed to have a promising potential in combating rotaviral infections. Our studies indicated, *inter alia*, that the biological activity appeared to be independent of the presence of a primary hydroxyl group since the 6′-protected analogues exhibited the most promising cytotoxic and antiviral properties [34–36].

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In view of these facts, it was envisaged that a new class of unsaturated 3′-fluoro-4′-ketopyranonucleoside analogues of uracil, 5-fluorouracil, thymine, N^4-benzoyl cytosine and N^6-benzoyl adenine, respectively, bearing a methyl group in the 5′-position of the sugar moiety, could be of significant biological interest. In the present study we attempt to describe the synthesis and biological importance of these novel molecules.

2. Results and discussion

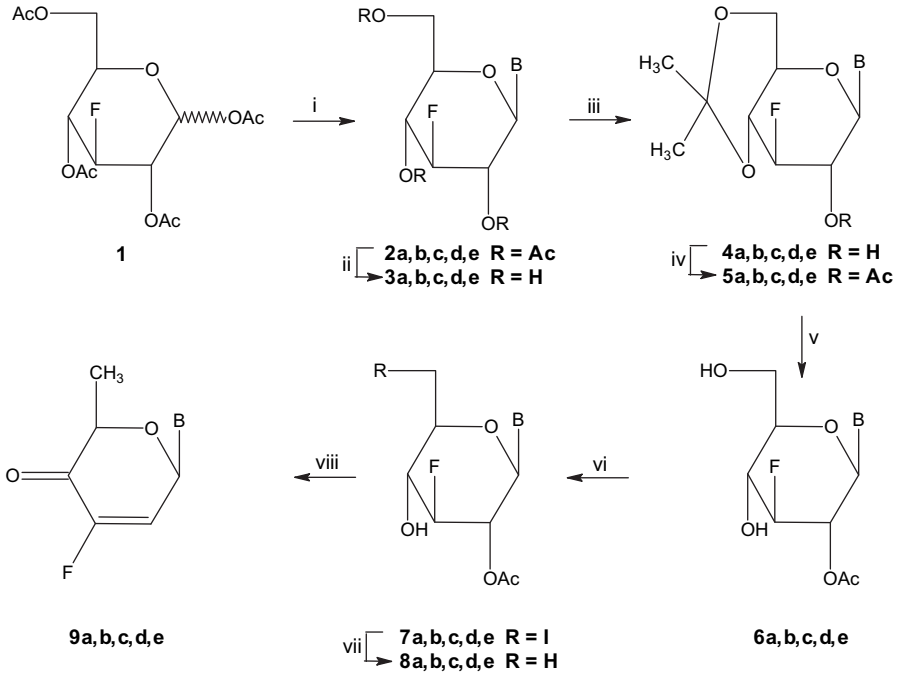
2.1. Chemistry

Our synthetic route to the target compounds **9a–e** is shown in Scheme 1. The protected 1-(2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl) nucleosides of uracil (**2a**), 5-fluorouracil (**2b**) [37], thymine (**2c**), N^4-benzoyl cytosine (**2d**) [34] and 9-(2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl) nucleoside of N^6-benzoyl adenine (**2e**) [35], were obtained upon condensation of 1,2,4,6-tetra-*O*-acetyl-3-deoxy-3-fluoro-glucopyranose (**1**) [38,39] with silylated uracil, 5-fluorouracil, thymine, N^4-benzoyl cytosine and N^6-benzoyl adenine, respectively, in the presence of trimethylsilyl trifluoromethane-sulfonate or tin chloride, in refluxing acetonitrile. The β configuration of these nucleosides was clearly established by their ^{1}H NMR spectra. Removal of all *O*-acetyl protecting groups of the aforementioned compounds gave **3a–e**, in excellent yields [34,35,40]. Specific acetalation of the unprotected analogues using 2,2-dimethoxypropane in *N*,*N*-dimethylformamide (DMF) led to the 4′,6′-*O*-isopropylidene derivatives **4a–e**, respectively. Acetylation of the free hydroxyl group and finally deisopropylidenation, afforded the partially acetylated analogues **6a–e** [34,35]. Replacement of the primary hydroxyl group of compounds **6a–e** by an iodine atom using triphenylphosphine together with iodine and imidazole [41–44], led to the desired iodo derivatives **7a–e** which were reduced to form the deoxy nucleosides **8a–e** by hydrogen in the presence of palladium-on-carbon. In the final step, oxidation of the fluoro acetylated dideoxy precursors **8a–e** performed by the acetic anhydride/dimethyl sulfoxide system [45] afforded, after a β-elimination reaction, the desired unsaturated 2,6-dideoxy-3-fluoro-β-D-*glycero*-hex-2-enopyranosyl-4-ulose derivatives of uracil (**9a**), 5-fluorouracil (**9b**), thymine (**9c**), N^4-benzoyl cytosine (**9d**) and N^6-benzoyl adenine (**9e**), respectively. It should therefore be mentioned that all attempts to remove the benzoyl group of the target nucleoside analogues **9d** and **9e** were unsuccessful and only degradation products were obtained [46–49].

The synthesized compounds were well characterized by NMR, UV and IR spectroscopies, mass spectrometry and elemental analysis. ^{1}H NMR data obtained for the starting nucleosides **2a–e** ($J_{1',2'}$, $J_{2',3'}$, $J_{3',4'} \geq 9.0$ Hz) revealed that, as expected, these compounds had the β configuration and that it existed in the $^4C_1$ conformation. Finally, in compounds **9a–e**, the presence of the C(O)CF=CH-system was ascertained by the disappearance of the signal for H-4′ in the ^{1}H NMR spectra and the deshielding of other protons, especially the olefinic proton H-2′ as previously observed for other (2-deoxy-β-D-hex-2-enopyranosyl-4-ulose) nucleosides [50–52].

2.2. Antiviral, cytostatic and cytotoxic activity

The compounds **9a–e** were evaluated against a broad variety of DNA and RNA viruses but found to be inactive at subtoxic concentrations. The cytostatic/cytotoxic activity measurements were based on (i) the inhibition of tumor cell proliferation of murine leukemia L1210, murine mammary carcinoma FM3A, human lymphocyte Molt4/C8 and CEM cells, or (ii) microscopically detectable alteration of human embryonic lung (HEL), monkey kidney (Vero), and human cervix carcinoma (HeLa) cell morphology or (iii) viability staining of Madin–Darby canine kidney cells by the colorimetric formasan-based



Scheme 1. Reagents and conditions: i) silylated base, CH_3CN, trimethylsilyl trifluomethane-sulfonate or tin chloride; ii) methanolic ammonia or ethanol, pyridine, NaOH, 0 °C, 30 min, Amberlite IR-120 (H^+) resin; iii) 2,2-dimethoxypropane, *p*-toluenesulfonic acid, DMF; iv) pyridine, acetic anhydride; v) 90% trifluoroacetic acid in methanol, 20 °C, 10 min; vi) iodine, triphenylphosphine, imidazole, tetrahydrofuran, 80 °C, 1 h; vii) H_2, 10% Pd/C, triethylamine, ethyl acetate, ethanol, 20 °C, 24 h; viii) acetic anhydride/dimethyl sulfoxide, 100 °C, 10 min.

MTS assay. Compounds **9a–e** showed a similar cytotoxic activity against several monolayer cell types (MIC: ~20 μM). However, the compounds markedly inhibited cell proliferation of suspension tumor cells at IC_{50} values that ranged between 0.49 and 16 μM (Table 1). There was no significant difference between the compounds in terms of potency of inhibition, except for **9a–c** that were more cytostatic for MDCK cells when evaluated with the MTS dye staining method. Also, when compounds such as **9d** and **9e** were evaluated on their inhibitory potential against macromolecule (DNA, RNA, protein) synthesis in L1210 and CEM cells, they remarkably inhibited radiolabeled thymidine (DNA synthesis), uridine (RNA synthesis) and leucine (protein synthesis) incorporation into methanol-insoluble cell material to a similar extent (EC_{50}: 7.9–14 μM) (Table 2) upon incubation of the cell suspensions with the radiolabeled precursors and the test compounds for 20 h.

3. Conclusion

In conclusion, the synthesis of the unsaturated dideoxy fluoro-ketopyranosyl nucleoside analogues bearing uracil, 5-fluorouracil, thymine, N^4-benzoyl cytosine and N^6-benzoyl adenine, respectively was undertaken, and the target nucleosides were evaluated for their antiviral and cytostatic/cytotoxic activity. Compounds **9a–e** were not active against a broad panel of DNA and RNA viruses at subtoxic concentrations. However, they were markedly inhibitory against the proliferation of a variety of tumor cell lines. The mechanism of antiproliferative activity is thus far unclear. It would therefore be interesting to further explore the structure–activity relationship by modifying the base part as well as replacing the fluorine or methyl group by different chemical entities. We believe that unsaturated dideoxy fluoro-ketopyranosyl nucleoside analogues should be considered as novel lead compounds that have to be further explored for antiproliferative (i.e. antitumor) activity.

4. Experimental part

4.1. Chemistry

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). ^1H, ^{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 dimethyl sulfoxide-d_6 (DMSO-d_6) with internal tetramethylsilane (TMS) for ^1H and ^{13}C and internal trifluorotoluene (TFT) for ^{19}F.

Table 1
Cytostatic and cytotoxic activity of **9a–e** against a panel of tumor cell lines.

Compound	IC_{50}[a] (μM)				CC_{50}[b] (μM)	MIC[c] (μM)		
	L1210	FM3A	Molt4/C8	CEM	MDCK	HEL	Vero	HeLa
9a	2.9 ± 1.1	16 ± 2	2.5 ± 1.2	2.2 ± 0.9	0.6	20	20	20
9b	0.82 ± 0.47	0.49 ± 0.45	3.3 ± 0.2	3.7 ± 0.6	0.6	20	20	20
9c	2.8 ± 1.0	10 ± 8	2.5 ± 1.3	2.6 ± 1.1	0.7	20	20	20
9d	1.4 ± 0.0	11.9 ± 5.2	1.6 ± 0.2	1.7 ± 0.5	8.2	20	20	20
9e	2.4 ± 0.7	6 ± 1	5.0 ± 3.9	4.3 ± 3.4	12	20	20	100

[a] 50% inhibitory concentration, or compound concentration required to inhibit cell proliferation by 50%.
[b] 50% cytotoxic concentration, or compound concentration required to reduce cell viability by 50% as measured by MTS dye staining.
[c] Minimal inhibitory concentration, or compound concentration required to induce a microscopically visible alteration of cell morphology.

Table 2
Inhibitory activity of **9d** and **9e** against the incorporation of [^{3}H]dThd, [^{3}H]Urd and [^{3}H]Leu into cellular macromolecules.

Compound	IC_{50}[a] (μM) for incorporation into cellular macromolecules					
	[^{3}H]dThd		[^{3}H]Urd		[^{3}H]Leu	
	L1210	CEM	L1210	CEM	L1210	CEM
9d	12	11	10	12	12	7.9
9e	12	13	9.8	13	13	14

[a] 50% inhibitory concentration required to inhibit incorporation of radiolabeled precursor in methanol-insoluble material.

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, ddd = doublet doublet doublet, dtr = doublet triplet and m = multiplet. Mass spectra were obtained with a Micromass Platform LC (ESI-MS). Infrared spectra were obtained with a Nicolet 6700 FT-IR spectrometer. Optical rotations were measured using Autopol I polarimeter. All reactions sensitive to oxygen or moisture were carried out under nitrogen atmosphere with dry solvents. Acetonitrile was distilled from calcium hydride and stored over 3 Å molecular sieves. DMF and dimethyl sulfoxide were also 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.

4.2. Synthesis of 1-(2,6-dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)uracil (**9a**)

4.2.1. 1-(2,4,6-Tri-O-acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)uracil (**2a**)

A mixture of uracil (1.79 g, 15.99 mmol), hexamethyldisilazane (HMDS) (4.18 mL, 19.83 mmol) and saccharine (0.14 g, 0.74 mmol) in anhydrous CH_3CN (56 mL) was refluxed for 30 min under nitrogen. Tetraacetylated 3-deoxy-3-fluoro-D-glucose (**1**) [38,39] (4.00 g, 11.42 mmol) and trimethylsilyl trifluoromethane-sulfonate (2.89 mL, 15.99 mmol) were then added and the reaction mixture was refluxed for 3 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH_2Cl_2 (1000 mL). The organic layer was washed with water (3 × 20 mL) and dried over anhydrous sodium sulfate, evaporated to dryness, finally purified by flash column chromatography using ethyl acetate–*n*-hexane (7:3) as eluant to give compound **2a** as a solid. Yield: 3.40 g (74%); R_f = 0.3 in ethyl acetate–*n*-hexane (7:3); m.p. 115–117 °C; $[\alpha]_D^{22}$ −5.3 (c 0.10, $CHCl_3$); UV ($CHCl_3$): λ_{max} 260 nm (ε 7068); ^1H NMR ($CDCl_3$): δ 8.50 (br s, NH), 7.35 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.84 (d, 1H, H-5), 5.79 (d, 1H, $J_{1',2'}$ = 9.6 Hz, H-1′), 5.30–5.22 (m, 2H, H-2′ and H-4′), 4.74 (dtr, 1H, $J_{F,3'}$ = 51.7 Hz, $J_{2',3'}$ = 9.1 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 4.30–4.11 (m, 2H, H-6a′,6b′), 3.83 (m, 1H, H-5′), 2.14 and 2.10 and 2.08 (3s, 9H, 3OAc); ^{19}F NMR: δ −65.0; Anal. Calcd for $C_{16}H_{19}FN_2O_9$: C, 47.76; H, 4.76; N, 6.96. Found: C, 47.65; H, 4.87; N, 6.84; ESI-MS (*m*/*z*): 403.34 ($M + H^+$).

4.2.2. 1-(3,4-Dideoxy-3-fluoro-β-D-glucopyranosyl)uracil (**3a**)

A mixture of methanolic ammonia and compound **2a** (3.40 g, 8.45 mmol) stirred for 4 h at room temperature. The reaction mixture was concentrated and crude **3a** was obtained as a colorless oil and it was used without further purification. Yield: 2.05 g (88%); $[\alpha]_D^{22}$ + 5.3 (c 0.10, MeOH); UV (MeOH): λ_{max} 260 nm (ε 6235); Anal. Calcd for $C_{10}H_{13}FN_2O_6$: C, 43.48; H, 4.74; N, 10.14. Found: C, 43.39; H, 4.86; N, 9.97; ESI-MS (*m*/*z*): 277.23 ($M + H^+$).

4.2.3. 1-(3-Deoxy-3-fluoro-4,6-O-isopropylidene-β-D-glucopyranosyl)uracil (**4a**)

Compound **3a** (2.05 g, 7.44 mmol) was dissolved in a mixture of 29.8 mL of 2,2-dimethoxypropane and 94.1 mL of dry DMF. To this

was added *p*-toluenesulfonic acid (1.70 g, 8.93 mmol) and the mixture stirred at room temperature for 2 h. The reaction mixture was neutralized with triethylamine so that pH did not exceed 7. The mixture was concentrated under high vacuum to eliminate the DMF. Purification by flash column chromatography using ethyl acetate–*n*-hexane (8:2) as eluant gave **4a** as a yellowish oil. Yield: 1.53 g (65%); $R_f = 0.25$ in ethyl acetate–*n*-hexane (8:2); $[\alpha]_D^{22}$ −7.0 (c 0.33, $CHCl_3$); UV ($CHCl_3$): λ_{max} 257 nm (ε 5704); ^{1}H NMR ($CDCl_3$): δ 9.71 (br s, NH), 7.31 (d, 1H, $J_{5,6} = 8.1$ Hz, H-6), 5.82 (d, 1H, H-5), 5.71 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1′), 4.66 (dtr, 1H, $J_{F,3'} = 53.2$ Hz, $J_{2',3'} = 8.5$ Hz, $J_{3',4'} = 8.8$ Hz, H-3′), 4.02–3.88 (m, 2H, H-2′ and H-4′), 3.86–3.73 (m, 2H, H-6a′,6b′), 3.53 (m, 1H, H-5′), 1.55 and 1.48 (2s, 6H, 2 × CH_3); Anal. Calcd for $C_{13}H_{17}FN_2O_6$: C, 49.37; H, 5.42; N, 8.86. Found: C, 49.15; H, 5.55; N, 9.03; ESI-MS (*m*/*z*): 317.27 ($M + H^+$).

4.2.4. 1-(2-O-Acetyl-3-deoxy-3-fluoro-4,6-O-isopropylidene-β-D-glucopyranosyl)uracil (**5a**)

To a solution of **4a** (1.53 g, 4.84 mmol) in dry pyridine (24.2 mL) was added acetic anhydride (0.91 mL, 9.68 mmol) and the resultant mixture was stirred at room temperature for 1 h. Methanol (0.45 mL) was added to quench the reaction and the mixture was concentrated under high vacuum to remove the solvents. Purification by flash column chromatography using ethyl acetate–*n*-hexane (1:1) as eluant gave **5a** as a white powder. Yield: 1.54 g (89%); $R_f = 0.25$ in ethyl acetate–*n*-hexane (1:1); $[\alpha]_D^{22}$ −2.0 (c 0.39, $CHCl_3$); UV ($CHCl_3$): λ_{max} 255 nm (ε 8165); ^{1}H NMR ($CDCl_3$): δ 8.50 (br s, NH), 7.32 (d, 1H, $J_{5,6} = 8.2$ Hz, H-6), 5.82-5.77 (m, 2H, $J_{1',2'} = 9.2$ Hz, H-5 and H-1′), 5.79 (d, 1H, $J_{1',2'} = 9.0$ Hz, H-1′), 5.23 (m, 1H, H-2′), 4.70 (dtr, 1H, $J_{F,3'} = 53.0$ Hz, $J_{2',3'} = 8.8$ Hz, $J_{3',4'} = 9.0$ Hz, H-3′), 4.03–3.94 (m, 1H, H-4′), 3.91–3.75 (m, 2H, H-6a′,6b′), 3.50 (m, 1H, H-5′), 2.09 (s, 3H, OAc), 1.55 and 1.48 (2s, 6H, 2 × CH_3); Anal. Calcd for $C_{15}H_{19}FN_2O_7$: C, 50.28; H, 5.34; N, 7.82. Found: C, 50.12; H, 5.56; N, 7.50; ESI-MS (*m*/*z*): 359.34 ($M + H^+$).

4.2.5. 1-(2-O-Acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)uracil (**6a**)

Product **5a** (1.54 g, 4.31 mmol), obtained from the previous procedure, was dissolved in 21.6 mL of 90% trifluoroacetic acid in methanol. The solution was stirred for 10 min at room temperature and then concentrated at 40 °C under high vacuum in order to remove traces of trifluoroacetic acid. Purification by flash column chromatography using ethyl acetate as eluant gave **6a** as a foam. Yield: 1.23 g (90%); $R_f = 0.23$ in ethyl acetate; $[\alpha]_D^{22}$ + 7.0 (c 0.50, MeOH); UV (MeOH): λ_{max} 257 nm (ε 3530); Anal. Calcd for $C_{12}H_{15}FN_2O_7$: C, 45.29; H, 4.75; N, 8.80. Found: C, 45.64; H, 4.57; N, 9.19; ESI-MS (*m*/*z*): 319.27 ($M + H^+$).

4.2.6. 1-(2-O-Acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)uracil (**7a**)

A mixture of **6a** (1.23 g, 3.88 mmol), imidazole (0.53 g, 7.76 mmol), triphenylphosphine (1.53 g, 5.82 mmol) and iodine (1.48 g, 5.82 mmol) in tetrahydrofuran (38 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 in vacuum. Purification by flash column chromatography using ethyl acetate–*n*-hexane (6:4) as eluant gave **7a** as a syrup. Yield: 1.13 g (68%); $R_f = 0.24$ in ethyl acetate–*n*-hexane (6:4); $[\alpha]_D^{22}$ −3.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 256 nm (ε 5621); ^{1}H NMR ($CDCl_3$): δ 8.03 (br s, NH), 7.36 (d, 1H, $J_{5,6} = 8.4$ Hz, H-6), 5.87–5.81 (m, 2H, $J_{1',2'} = 9.2$ Hz, H-5 and H-1′), 5.18 (m, 1H, H-2′), 4.70 (dtr, 1H, $J_{F,3'} = 52.3$ Hz, $J_{2',3'} = 8.5$ Hz, $J_{3',4'} = 8.9$ Hz, H-3′), 3.89 (m, 1H, H-4′), 3.56 (m, 2H, H-6a′,6b′), 3.17 (m, 1H, H-5′), 2.09 (s, 3H, OAc); Anal. Calcd for $C_{12}H_{14}FIN_2O_6$: C, 33.66; H, 3.30; N, 6.54. Found: C, 33.93; H, 3.52; N, 6.30; ESI-MS (*m*/*z*): 429.17 ($M + H^+$).

4.2.7. 1-(2-O-Acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)uracil (**8a**)

After two vacuum/H_2 cycles to remove air from the reaction tube, the stirred mixture of **7a** (1.13 g, 2.64 mmol), 10% Pd/C (0.36 g) and triethylamine (0.73 mL, 5.28 mmol) in ethyl acetate (80.8 mL) and ethanol (80.8 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 column chromatography using ethyl acetate–*n*-hexane (7:3) as eluant to give **8a** as a white foam. Yield: 0.60 g (75%); $R_f = 0.38$ in ethyl acetate–*n*-hexane (7:3); $[\alpha]_D^{22}$ +7.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 256 nm (ε 6812); ^{1}H NMR ($CDCl_3$): δ 8.34 (br s, NH), 7.33 (d, 1H, $J_{5,6} = 8.2$ Hz, H-6), 5.80 (d, 1H, H-5), 5.73 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1′), 5.16 (m, 1H, H-2′), 4.59 (dtr, 1H, $J_{F,3'} = 52.4$ Hz, $J_{2',3'} = 8.6$ Hz, $J_{3',4'} = 8.9$ Hz, H-3′), 3.68–3.50 (m, 2H, H-4′ and H-5′), 2.08 (s, 3H, OAc), 1.39 (d, 3H, $J_{5',6'} = 5.7$ Hz, H-6′); ^{19}F NMR: δ −65.5; Anal. Calcd for $C_{12}H_{15}FN_2O_6$: C, 47.68; H, 5.00; N, 9.27. Found: C, 47.97; H, 5.18; N, 8.91; ESI-MS (*m*/*z*): 303.24 ($M + H^+$).

4.2.8. 1-(2,6-Dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)uracil (**9a**)

To a solution of **8a** (0.60 g, 1.98 mmol) in dimethyl sulfoxide (9.7 mL) was added acetic anhydride (4.84 mL, 51.28 mmol). The mixture was heated at 100 °C for 10 min, then cooled to room temperature, diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash column chromatography using ethyl acetate–*n*-hexane (6:4) as eluant to afford pure **9a** as a white solid. Yield: 0.31 g (65%); $R_f = 0.4$ in ethyl acetate–*n*-hexane (6:4); m.p. 247–250 °C; $[\alpha]_D^{22}$ −2.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 255 nm (ε 10814); ^{1}H NMR (DMSO-d_6): δ 7.69 (d, 1H, $J_{5,6} = 8.0$ Hz, H-6), 7.01 (dd, 1H, $J_{F,1'} = 12.5$ Hz, $J_{1',2'} = 1.3$ Hz, H-1′), 6.81 (dd, 1H, $J_{F,2'} = 6.4$ Hz, H-2′), 5.67 (d, 1H, H-5), 4.69 (m, 1H, H-5′), 1.30 (d, 3H, $J_{5',6'} = 6.5$ Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 189.05 (C-4′); 163.00 (C-4); 153.54 (C-3′); 150.06 (C-2); 141.71 (C-6); 123.36 (C-2′); 102.79 (C-5); 77.32 (C-1′); 75.85 (C-5′); 14.81 (C-6′); ^{19}F NMR: δ −64.3; IR (Neat, cm^{-1}): 1711.10 (keto group); Anal. Calcd for $C_{10}H_9FN_2O_4$: C, 50.01; H, 3.78; N, 11.66. Found: C, 50.19; H, 3.56; N, 11.39; ESI-MS (*m*/*z*): 241.17 ($M + H^+$).

4.3. Synthesis of 1-(2,6-dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)5-fluorouracil (**9b**)

The isopropylidene 5-fluorouracil derivative **4b** was synthesized by condensation of peracetylated 3-deoxy-3-fluoro-D-glucopyranose (**1**) [38,39] with silylated 5-fluorouracil, followed by deprotection of the β-nucleoside formed **2b** and finally by specific acetalation of the fully unprotected analogue **3b**, as previously described [37].

4.3.1. 1-(2-O-Acetyl-3-deoxy-3-fluoro-4,6-O-isopropylidene-β-D-glucopyranosyl)5-fluorouracil (**5b**)

5-Fluorouracil derivative **5b** was synthesized from 1-(3-deoxy-3-fluoro-4,6-*O*-isopropylidene-β-D-glucopyranosyl)5-fluorouracil (**4b**) [37] by the same methodology as described for the synthesis of **5a**. Compound **5b** was obtained as a white powder following purification by flash column chromatography using ethyl acetate–*n*-hexane (1:1) as eluant. Yield: 1.80 g (80%); $R_f = 0.29$ in ethyl acetate–*n*-hexane (1:1); $[\alpha]_D^{22}$ + 2.0 (c 0.27, $CHCl_3$); UV ($CHCl_3$): λ_{max} 261 nm (ε 2556); ^{1}H NMR ($CDCl_3$): δ 8.30 (br s, NH), 7.38 (d, 1H, $J_{6,F5} = 5.6$ Hz, H-6), 5.75 (dd, 1H, $J_{1',F5} = 1.3$ Hz, $J_{1',2'} = 9.3$ Hz, H-1′), 5.16 (m, 1H, H-2′), 4.70 (dtr, 1H, $J_{F,3'} = 52.9$ Hz, $J_{2',3'} = J_{3',4'} = 9.0$ Hz, H-3′), 3.98 (m, 1H, H-4′), 3.93–3.78 (m, 2H, H-6a′,6b′), 3.50 (m, 1H,

H-5′), 2.10 (s, 3H, OAc), 1.56 and 1.48 (2s, 6H, 2 × CH_3); Anal. Calcd for $C_{15}H_{18}F_2N_2O_7$: C, 47.88; H, 4.82; N, 7.44. Found: C, 47.75; H, 5.15; N, 7.67; ESI-MS (*m*/*z*): 377.32 ($M + H^+$).

4.3.2. 1-(2-O-Acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)5-fluorouracil (**6b**)

5-Fluorouracil derivative **6b** was synthesized from **5b** by the same methodology as described for the synthesis of **6a**. Compound **6b** was obtained as a foam following purification by flash column chromatography using ethyl acetate–*n*-hexane (8:2) as eluant. Yield: 1.39 g (87%); $R_f = 0.26$ in ethyl acetate–*n*-hexane (8:2); $[\alpha]_D^{22}$ +14.0 (c 0.50, MeOH); UV (MeOH): λ_{max} 262 nm (ε 6394); Anal. Calcd for $C_{12}H_{14}F_2N_2O_7$: C, 42.86; H, 4.20; N, 8.33. Found: C, 43.04; H, 4.09; N, 8.10; ESI-MS (*m*/*z*): 337.23 ($M + H^+$).

4.3.3. 1-(2-O-Acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)5-fluorouracil (**7b**)

5-Fluorouracil derivative **7b** was synthesized from **6b** by the same methodology as described for the synthesis of **7a**. Compound **7b** was obtained as an oil following purification by flash column chromatography using dichloromethane–methanol (9.5:0.5) as eluant. Yield: 1.04 g (56%); $R_f = 0.20$ in dichloromethane–methanol (9.5:0.5); $[\alpha]_D^{22}$ −2.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 263 nm (ε 6140); ^{1}H NMR ($CDCl_3$): δ 8.35 (br s, NH), 7.43 (d, 1H, $J_{6,F5} = 5.6$ Hz, H-6), 5.82 (dd, 1H, $J_{1',F5} = 1.0$ Hz, $J_{1',2'} = 9.7$ Hz, H-1′), 5.11 (m, 1H, H-2′), 4.70 (dtr, 1H, $J_{F,3'} = 52.0$ Hz, $J_{2',3'} = 8.9$ Hz, $J_{3',4'} = 9.1$ Hz, H-3′), 3.89 (m, 1H, H-4′), 3.56 (m, 2H, H-6a′,6b′), 3.19 (m, 1H, H-5′), 2.10 (s, 3H, OAc); Anal. Calcd for $C_{12}H_{13}F_2IN_2O_6$: C, 32.31; H, 2.94; N, 6.28. Found: C, 32.13; H, 3.25; N, 6.40; ESI-MS (*m*/*z*): 447.13 ($M + H^+$).

4.3.4. 1-(2-O-Acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)5-fluorouracil (**8b**)

5-Fluorouracil derivative **8b** was synthesized from **7b** by the same methodology as described for the synthesis of **8a**. Compound **8b** was obtained as a foam following purification by flash column chromatography using ethyl acetate–*n*-hexane (2:8) as eluant. Yield: 0.51 g (68%); $R_f = 0.10$ in ethyl acetate–*n*-hexane (2:8); $[\alpha]_D^{22}$ +12.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 263 nm (ε 7708); ^{1}H NMR ($CDCl_3$): δ 8.55 (br s, NH), 7.40 (d, 1H, $J_{6,F5} = 5.7$ Hz, H-6), 5.70 (d, 1H, $J_{1',2'} = 9.6$ Hz, H-1′), 5.10 (m, 1H, H-2′), 4.60 (dtr, 1H, $J_{F,3'} = 52.0$ Hz, $J_{2',3'} = 8.5$ Hz, $J_{3',4'} = 8.9$ Hz, H-3′), 3.69–3.51 (m, 2H, H-4′ and H-5′), 2.09 (s, 3H, OAc), 1.41 (d, 3H, $J_{5',6'} = 5.6$ Hz, H-6′); ^{19}F NMR: δ −64.3, −63.2; Anal. Calcd for $C_{12}H_{14}F_2N_2O_6$: C, 45.01; H, 4.41; N, 8.75. Found: C, 44.90; H, 4.65; N, 8.89; ESI-MS (*m*/*z*): 321.26 ($M + H^+$).

4.3.5. 1-(2,6-Dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)5-fluorouracil (**9b**)

5-Fluorouracil derivative **9b** was synthesized from **8b** by the same methodology as described for the synthesis of **9a**. Compound **9b** was obtained as a white powder following purification by flash column chromatography using ethyl acetate–*n*-hexane (2:8) as eluant. Yield: 0.22 g (55%); $R_f = 0.16$ in ethyl acetate–*n*-hexane (2:8); $[\alpha]_D^{22}$ +6.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 259 nm (ε 9433); ^{1}H NMR (DMSO-d_6): δ 8.15 (d, 1H, $J_{6,F5} = 6.7$ Hz, H-6), 6.93 (d, 1H, $J_{F,1'} = 12.4$ Hz, H-1′), 6.81 (d, 1H, $J_{F,2'} = 5.2$ Hz, H-2′), 4.69 (m, 1H, H-5′), 1.31 (d, 3H, $J_{5',6'} = 6.5$ Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 189.03 (C-4′); 153.66 (C-4); 151.41 (C-3′); 148.79 (C-2); 139.41 (C-5); 125.96; (C-6); 122.69 (C-2′); 77.52 (C-1′); 75.79 (C-5′); 14.82 (C-6′); ^{19}F NMR: δ −65.0, −65.5; IR (Neat, cm^{-1}): 1714.45 (keto group); Anal. Calcd for $C_{10}H_8F_2N_2O_4$: C, 46.52; H, 3.12; N, 10.85. Found: C, 46.38; H, 3.34; N, 10.74; ESI-MS (*m*/*z*): 259.16 ($M + H^+$).

4.4. Synthesis of 1-(2,6-dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)thymine (**9c**)

4.4.1. 1-(2,4,6-Tri-O-acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)thymine (**2c**)

A mixture of thymine (2.02 g, 15.99 mmol), HMDS (4.18 mL, 19.83 mmol) and saccharine (0.13 g, 0.74 mmol) in anhydrous CH_3CN (56 mL) was refluxed for 30 min, under nitrogen. Tetraacetylated 3-deoxy-3-fluoro-D-glucose (**1**) [38,39] (4.00 g, 11.42 mmol) and tin chloride (1.87 mL, 15.99 mmol) were then added and the reaction mixture was refluxed for 2 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH_2Cl_2 (1000 mL). The organic layer was washed with water (3 × 20 mL) and dried over anhydrous sodium sulfate, evaporated to dryness, finally purified by flash column chromatography using ethyl acetate–*n*-hexane (8:2) as eluant to give compound **2c** as a solid. Yield: 3.42 g (72%); $R_f = 0.35$ in ethyl acetate–*n*-hexane (8:2); m.p. 180–182 °C; $[\alpha]_D^{22}$ −10.5 (c 0.10, $CHCl_3$); UV ($CHCl_3$): λ_{max} 260 nm (ε 7894); ^{1}H NMR ($CDCl_3$): δ 8.20 (br s, NH), 7.16 (s, 1H, H-6), 5.78 (d, 1H, $J_{1',2'} = 9.6$ Hz, H-1′), 5.33–5.22 (m, 2H, H-2′ and H-4′), 4.73 (dtr, 1H, $J_{F,3'} = 51.8$ Hz, $J_{2',3'} = 9.1$ Hz, $J_{3',4'} = 9.0$ Hz, H-3′), 4.30–4.12 (m, 2H, H-6a′,6b′), 3.85–3.80 (m, 1H, H-5′), 2.15 and 2.10 and 2.08 (3s, 9H, 3OAc), 1.97 (s, 3H, 5-CH_3); ^{19}F NMR: δ −65.0; Anal. Calcd for $C_{17}H_{21}FN_2O_9$: C, 49.04; H, 5.08; N, 6.73. Found: C, 49.17; H, 4.92; N, 6.58; ESI-MS (*m*/*z*): 417.35 ($M + H^+$).

4.4.2. 1-(3,4-Dideoxy-3-fluoro-β-D-glucopyranosyl)thymine (**3c**)

Thymine derivative **3c** was synthesized from **2c** by the same methodology as described for the synthesis of **3a**. Compound **3c** was obtained as a colorless oil and it was used without further purification. Yield: 2.14 g (90%); $[\alpha]_D^{22}$ +5.3 (c 0.10, MeOH); UV (MeOH): λ_{max} 260 nm (ε 6794); Anal. Calcd for $C_{11}H_{15}FN_2O_6$: C, 45.52; H, 5.21; N, 9.65. Found: C, 45.35; H, 5.14; N, 9.53; ESI-MS (*m*/*z*): 291.26 ($M + H^+$).

4.4.3. 1-(3-Deoxy-3-fluoro-4,6-O-isopropylidene-β-D-glucopyranosyl)thymine (**4c**)

Thymine derivative **4c** was synthesized from **3c** by the same methodology as described for the synthesis of **4a**. Compound **4c** was obtained as a yellowish oil following purification by flash column chromatography using ethyl acetate–*n*-hexane (7:3) as eluant. Yield: 1.76 g (72%); $R_f = 0.25$ in ethyl acetate–*n*-hexane (7:3); $[\alpha]_D^{22}$ −6.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 263 nm (ε 5156); ^{1}H NMR ($CDCl_3$): δ 8.00 (br s, NH), 7.11 (s, 1H, H-6), 5.76 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 4.71 (dtr, 1H, $J_{F,3'} = 53.3$ Hz, $J_{2',3'} = 8.6$ Hz, $J_{3',4'} = 8.7$ Hz, H-3′), 4.00–3.87 (m, 2H, H-2′ and H-4′), 3.84–3.73 (m, 2H, H-6a′,6b′), 3.56 (m, 1H, H-5′), 2.96 and 2.88 (2s, 6H, 2 × CH_3), 1.88 (s, 3H, 5-CH_3); Anal. Calcd for $C_{14}H_{19}FN_2O_6$: C, 50.91; H, 5.80; N, 8.48. Found: C, 50.72; H, 6.08; N, 8.80; ESI-MS (*m*/*z*): 331.32 ($M + H^+$).

4.4.4. 1-(2-O-Acetyl-3-deoxy-3-fluoro-4,6-O-isopropylidene-β-D-glucopyranosyl)thymine (**5c**)

Thymine derivative **5c** was synthesized from **4c** by the same methodology as described for the synthesis of **5a**. Compound **5c** was obtained as a white foam following purification by flash column chromatography using ethyl acetate–*n*-hexane (7:3) as eluant. Yield: 1.63 g (82%); $R_f = 0.37$ in ethyl acetate–*n*-hexane (7:3); $[\alpha]_D^{22}$ −2.0 (c 0.28, $CHCl_3$); UV ($CHCl_3$): λ_{max} 261 nm (ε 3756); ^{1}H NMR ($CDCl_3$): δ 8.38 (br s, NH), 7.12 (s, 1H, H-6), 5.78 (d, 1H, $J_{1',2'} = 9.5$ Hz, H-1′), 5.25 (m, 1H, H-2′), 4.69 (dtr, 1H, $J_{F,3'} = 53.0$ Hz, $J_{2',3'} = 8.8$ Hz, $J_{3',4'} = 8.9$ Hz, H-3′), 4.00–3.77 (m, 3H, H-4′ and H-6a′,6b′), 3.50 (m, 1H, H-5′), 2.08 (s, 3H, OAc), 1.95 (s, 3H, 5-CH_3), 1.56 and 1.48 (2s, 6H, 2 × CH_3); Anal. Calcd for $C_{16}H_{21}FN_2O_7$: C, 51.61; H, 5.68; N, 7.52. Found: C, 51.75; H, 5.46; N, 7.63; ESI-MS (*m*/*z*): 373.33 ($M + H^+$).

4.4.5. 1-(2-O-Acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)thymine (**6c**)

Thymine derivative **6c** was synthesized from **5c** by the same methodology as described for the synthesis of **6a**. Compound **6c** was obtained as a powder following purification by flash column chromatography using ethyl acetate as eluant. Yield: 1.31 g (90%); $R_f = 0.19$ in ethyl acetate; $[\alpha]_D^{22} + 12.0$ (c 0.50, MeOH); UV (MeOH): λ_{max} 262 nm (ε 7427); Anal. Calcd for $C_{13}H_{17}FN_2O_7$: C, 46.99; H, 5.16; N, 8.43. Found: C, 47.23; H, 4.98; N, 8.54; ESI-MS (*m*/*z*): 333.27 ($M + H^+$).

4.4.6. 1-(2-O-Acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)thymine (**7c**)

Thymine derivative **7c** was synthesized from **6c** by the same methodology as described for the synthesis of **7a**. Compound **7c** was obtained as a syrup following purification by flash column chromatography using ethyl acetate–*n*-hexane (4:6) as eluant. Yield: 1.13 g (65%); $R_f = 0.16$ in ethyl acetate–*n*-hexane (4:6); $[\alpha]_D^{22} + 3.0$ (c 0.28, $CHCl_3$); UV ($CHCl_3$): λ_{max} 261 nm (ε 5102); ^{1}H NMR ($CDCl_3$): δ 8.53 (br s, NH), 7.19 (s, 1H, H-6), 5.78 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 5.53 (m, 1H, H-2′), 4.98 (m, 1H, H-4′), 4.27 (ddd, 1H, $J_{F,3'} = 48.9$ Hz, $J_{2',3'} = 8.8$ Hz, $J_{3',4'} = 8.9$ Hz, H-3′), 3.43-3.23 (m, 2H, H-6a′,6b′), 3.14 (m, 1H, H-5′), 2.07 (s, 3H, OAc), 1.99 (s, 3H, 5-CH_3); Anal. Calcd for $C_{13}H_{16}FIN_2O_6$: C, 35.31; H, 3.65; N, 6.34. Found: C, 34.99; H, 3.77; N, 6.48; ESI-MS (*m*/*z*): 443.19 ($M + H^+$).

4.4.7. 1-(2-O-Acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)thymine (**8c**)

Thymine derivative **8c** was synthesized from **7c** by the same methodology as described for the synthesis of **8a**. Compound **8c** was obtained as a colorless oil following purification by flash column chromatography using ethyl acetate–*n*-hexane (4:6) as eluant. Yield: 0.58 g (72%); $R_f = 0.12$ in ethyl acetate–*n*-hexane (4:6); $[\alpha]_D^{22} + 10.0$ (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 262 nm (ε 5106); ^{1}H NMR ($CDCl_3$): δ 8.03 (br s, NH), 7.18 (s, 1H, H-6), 5.64 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 5.05 (m, 1H, H-2′), 4.77 (m, 1H, H-3′), 3.80 (m, 1H, H-4′), 2.33 (m, 1H, H-5′), 2.06 (s, 3H, OAc), 1.96 (s, 3H, 5-CH_3), 1.34 (d, 3H, $J_{5',6'} = 6.2$ Hz, H-6′); ^{19}F NMR: –65.5; Anal. Calcd for $C_{13}H_{17}FN_2O_6$: C, 49.37; H, 5.42; N, 8.86. Found: C, 49.65; H, 5.29; N, 8.75; ESI-MS (*m*/*z*): 317.26 ($M + H^+$).

4.4.8. 1-(2,6-Dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)thymine (**9c**)

Thymine derivative **9c** was synthesized from **8c** by the same methodology as described for the synthesis of **9a**. Compound **9c** was obtained as a white solid following purification by flash column chromatography using ethyl acetate–*n*-hexane (4:6) as eluant. Yield: 0.27 g (58%); $R_f = 0.11$ in ethyl acetate–*n*-hexane (4:6); m.p. 112-115 °C; $[\alpha]_D^{22} + 13.0$ (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 259 nm (ε 11,335); ^{1}H NMR (DMSO-d_6): δ 7.56 (s, 1H, H-6), 6.98 (d, 1H, $J_{F,1'} = 12.5$ Hz, H-1′), 6.80 (dd, 1H, $J_{F,2'} = 6.5$ Hz, $J_{1',2'} = 1.4$ Hz, H-2′), 4.67 (m, 1H, H-5′), 1.77 (s, 3H, 5-CH_3), 1.29 (d, 3H, $J_{5',6'} = 6.5$ Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 198.07 (C-4′); 163.83 (C-4); 151.42 (C-3′); 150.14 (C-2); 136.98 (C-6); 123.60 (C-5); 110.60 (C-2′); 77.14 (C-1′); 75.83 (C-5′); 14.81 (C-6′); 11.84 (CH_3); ^{19}F NMR: δ –63.2; IR (Neat, cm^{-1}): 1651.83 (keto group); Anal. Calcd for $C_{11}H_{11}FN_2O_4$: C, 51.97; H, 4.36; N, 11.02. Found: C, 51.79; H, 4.25; N, 11.21; ESI-MS (*m*/*z*): 255.23 ($M + H^+$).

4.5. Synthesis of 1-(2,6-dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)-N^4-benzoyl cytosine (**9d**)

The partially protected cytosine derivative **6d** was prepared by the coupling reaction of tetraacetylated 3-deoxy-3-fluoro-D-glucopyranose (**1**) [38,39] with silylated N^4-benzoyl cytosine followed by selective deprotection of the β-nucleoside formed **2d**, specific acetalation of the base protected analogue **3d**, conventional acetylation of the partially protected derivative **4d** and finally by deisopropylidenation of the acetylated analogue **5d**, as previously described [34,35].

4.5.1. 1-(2-O-Acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**7d**)

Cytosine derivative **7d** was synthesized from 1-(2-*O*-acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**6d**) [34,35] by the same methodology as described for the synthesis of **7a**. Compound **7d** was obtained as a yellow syrup following purification by flash column chromatography using dichloromethane–methanol (9.5:0.5) as eluant. Yield: 1.56 g (62%); $R_f = 0.35$ in dichloromethane–methanol (9.5:0.5); $[\alpha]_D^{22}$ +6.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 260 nm (ε 18038); ^{1}H NMR ($CDCl_3$) δ 8.77 (br s, 1H, NH), 7.90–7.50 (m, 7H, Bz, H-5 and H-6), 6.14 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1′), 5.18 (m, 1H, H-2′), 4.80 (dtr, 1H, $J_{F,3'} = 52.2$ Hz, $J_{2',3'} = J_{3',4'} = 8.9$ Hz, H-3′), 3.91 (m, 1H, H-4′) 3.62-3.56 (m, 2H, H-6a′,6b′), 3.26 (m, 1H, H-5′), 2.06 (s, 3H, OAc); ^{19}F NMR: δ –65.0. Anal. Calcd for $C_{19}H_{19}FIN_3O_6$: C, 42.95; H, 3.60; N, 7.91. Found: C, 43.15; H, 3.47; N, 8.23; ESI-MS (*m*/*z*): 532.29 ($M + H^+$).

4.5.2. 1-(2-O-Acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-N^4-benzoyl cytosine (**8d**)

Cytosine derivative **8d** was synthesized from **7d** by the same methodology as described for the synthesis of **8a**. Compound **8d** was obtained as a yellowish foam following purification by flash column chromatography using ethyl acetate as eluant. Yield: 0.66 g (55%); $R_f = 0.24$ in ethyl acetate; $[\alpha]_D^{22} + 26.0$ (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 260 nm (ε 18145); ^{1}H NMR ($CDCl_3$) δ 7.91–7.45 (m, 7H, Bz, H-5 and H-6), 6.00 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 5.18 (m, 1H, H-2′), 4.71 (dtr, 1H, $J_{F,3'} = 52.2$ Hz, $J_{2',3'} = J_{3',4'} = 8.7$ Hz, H-3′), 3.70–3.57 (m, 2H, H-4′ and H-5′), 2.04 (s, 3H, OAc), 1.41 (d, 3H, $J_{5',6'} = 5.8$ Hz, H-6′); ^{19}F NMR: δ –65.5. Anal. Calcd for $C_{19}H_{20}FN_3O_6$: C, 56.29; H, 4.97; N, 10.37. Found: C, 56.61; H, 4.85; N, 10.63; ESI-MS (*m*/*z*): 406.36 ($M + H^+$).

4.5.3. 1-(2,6-Dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)-N^4-benzoyl cytosine (**9d**)

Cytosine derivative **9d** was synthesized from **8d** by the same methodology as described for the synthesis of **9a**. Compound **9d** was obtained as a white foam following purification by flash column chromatography using ethyl acetate–*n*-hexane (9:1) as eluant. Yield: 0.28 g (50%); $R_f = 0.30$ in ethyl acetate–*n*-hexane (9:1); $[\alpha]_D^{22} + 61.0$ (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 260 nm (ε 16459); ^{1}H NMR (DMSO-d_6): δ 8.19 (br s, 1H, NH), 8.00–7.39 (m, 7H, Bz, H-5 and H-6), 7.07 (d, 1H, $J_{F,1'} = 12.3$ Hz, H-1′), 7.00 (d, 1H, $J_{F,2'} = 6.0$ Hz, H-2′), 4.75 (m, 1H, H-5′), 1.33 (d, 3H, $J_{5',6'} = 6.5$ Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 189.06 (C-4′); 167.52 (C=O); 163.99 (C-4); 153.48 (C-3′); 151.32 (C-2); 146.87 (C-6); 135.60 (C_{arom}); 135.05 (CH_{arom}); 132.95 ($2CH_{arom}$); 128.53 ($2CH_{arom}$); 123.52 (C-2′); 97.45 (C-5); 78.76 (C-1′); 76.18 (C-5′); 14.86 (C-6′); ^{19}F NMR: δ –64.3; IR (Neat, cm^{-1}): 1719.29 (keto group); Anal. Calcd for $C_{17}H_{14}FN_3O_4$: C, 59.47; H, 4.11; N, 12.24. Found: C, 59.63; H, 3.94; N, 12.56; ESI-MS (*m*/*z*): 344.32 ($M + H^+$).

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

The partially protected adenine derivative **6e** was prepared by the coupling reaction of peracetylated 3-deoxy-3-fluoro-D-glucopyranose (**1**) [38,39] with silylated N^6-benzoyl adenine followed by selective deprotection of the β-nucleoside formed **2e**, specific acetalation of the base protected analogue **3e**, conventional acetylation of the partially protected derivative **4e** and finally by

deisopropylidenation of the acetylated analogue **5e**, as previously described [35].

4.6.1. 9-(2-O-Acetyl-3-deoxy-3-fluoro-6-iodo-β-D-glucopyranosyl)-N^6-benzoyl adenine (**7e**)

Adenine derivative **7e** was synthesized from 9-(2-*O*-acetyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)-N^6-benzoyl adenine (**6e**) [35] by the same methodology as described for the synthesis of **7a**. Compound **7e** was obtained as a white solid following purification by flash column chromatography using dichloromethane–methanol (9:1) as eluant. Yield: 1.62 g (65%); $R_f = 0.48$ in dichloromethane–methanol (9:1), m.p. 95–98 °C; $[\alpha]_D^{22}$ −6.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 279 nm (ε 7288); ^{1}H NMR ($CDCl_3$) δ 9.25 (br s, 1H, NH), 8.84 and 8.26 (2s, 2H, H-2,8), 8.05-7.45 (m, 5H, Bz), 5.98 (d, 1H, $J_{1',2'} = 9.3$ Hz, H-1′), 5.58 (m, 1H, H-2′), 4.79 (dtr, 1H, $J_{F,3'} = 52.1$ Hz, $J_{2',3'} = 8.4$ Hz, $J_{3',4'} = 8.9$ Hz, H-3′), 4.02 (m, 1H, H-4′), 3.57 (m, 2H, H-6a′,6b′), 3.37 (m, 1H, H-5′), 1.84 (s, 3H, OAc); ^{19}F NMR: δ −65.5. Anal. Calcd for $C_{20}H_{19}FIN_5O_5$: C, 43.26; H, 3.45; N, 12.61. Found: C, 43.38; H, 3.67; N, 12.42; ESI-MS (*m*/*z*): 556.28 ($M + H^+$).

4.6.2. 9-(2-O-Acetyl-3,6-dideoxy-3-fluoro-β-D-glucopyranosyl)-N^6-benzoyl adenine (**8e**)

Adenine derivative **8e** was synthesized from **7e** by the same methodology as described for the synthesis of **8a**. Compound **8e** was obtained as a clear viscous oil following purification by flash column chromatography using ethyl acetate as eluant. Yield: 0.88 g (70%); $R_f = 0.19$ in ethyl acetate; $[\alpha]_D^{22}$ +4.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 274 nm (ε 6699); ^{1}H NMR ($CDCl_3$) δ 9.08 (br s, 1H, NH), 8.84 and 8.22 (2s, 2H, H-2,8), 8.03–7.45 (m, 5H, Bz), 5.85 (d, 1H, $J_{1',2'} = 9.5$ Hz, H-1′), 5.59 (m, 1H, H-2′), 4.68 (dtr, 1H, $J_{F,3'} = 52.1$ Hz, $J_{2',3'} = J_{3',4'} = 8.4$ Hz, H-3′), 3.78–3.69 (m, 2H, H-4′ and H-5′), 1.83 (s, 3H, OAc), 1.43 (d, 3H, $J_{5',6'} = 5.0$ Hz, H-6′); ^{19}F NMR: δ −65.0. Anal. Calcd for $C_{20}H_{20}FN_5O_5$: C, 55.94; H, 4.69; N, 16.31. Found: C, 56.19; H, 4.81; N, 16.16; ESI-MS (*m*/*z*): 430.42 ($M + H^+$).

4.6.3. 9-(2,6-Dideoxy-3-fluoro-β-D-glycero-hex-2-enopyranosyl-4-ulose)-N^6-benzoyl adenine (**9e**)

Adenine derivative **9e** was synthesized from **8e** by the same methodology as described for the synthesis of **9a**. Compound **9e** was obtained as a white solid following purification by flash column chromatography using ethyl acetate–*n*-hexane (9:1) as eluant. Yield: 0.41 g (55%); $R_f = 0.20$ in ethyl acetate–*n*-hexane (9:1), m.p. 205-207 °C; $[\alpha]_D^{22}$ + 9.0 (c 0.50, $CHCl_3$); UV ($CHCl_3$): λ_{max} 279 nm (ε 9038); ^{1}H NMR (DMSO-d_6): δ 8.80 and 8.71 (2s, 2H, H-2,8), 8.05-7.54 (m, 5H, Bz), 7.34 (d, 1H, $J_{F,1'} = 12.3$ Hz, H-1′), 7.15 (d, 1H, $J_{F,2'} = 6.2$ Hz, H-2′), 4.85 (m, 1H, H-5′), 1.32 (d, 3H, $J_{5',6'} = 6.5$ Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 189.32 (C-4′); 165.75 (C=O); 153.21 (C-3′); 152.19 (C-2); 151.05 (C-6); 150.60 (C-4); 143.38 (C-8); 133.26 (C_{arom}); 132.77 (CH_{arom}); 132.58 ($2CH_{arom}$); 128.54 ($2CH_{arom}$); 125.12 (C-5); 123.10 (C-2′); 76.45 (C-1′); 76.19 (C-5′); 14.85 (C-6′); ^{19}F NMR: δ −63.2; IR (Neat, cm^{-1}): 1714.45 (keto group); Anal. Calcd for $C_{18}H_{14}FN_5O_3$: C, 58.85; H, 3.84; N, 19.07. Found: C, 58.98; H, 3.62; N, 19.18; ESI-MS (*m*/*z*): 368.35 ($M + H^+$).

4.7. 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 and vesicular stomatitis virus], 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 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.

4.8. Cytostatic/toxic activity assays

Murine leukemia L1210, murine mammary carcinoma FM3A, and human lymphocyte Molt4/C8 and CEM cells were seeded in 96-well microtiter plates at 50,000 (L1210, FM3A) or 75,000 (Molt, CEM) cells per 200-μL well in the presence of different concentrations of the test compounds. After 2 (L1210, FM3A) or 3 (Molt, CEM) 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%.

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