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Fluoro-ketopyranosyl nucleosides: Synthesis and biological evaluation of 3-fluoro-2-keto-β-D-glucopyranosyl derivatives of N^4-benzoyl cytosine

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Abstract—1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose on mild oxidation, reduction, fluorination, and deisopropylidenation followed by acetylation gave peracetylated 3-deoxy-3-fluoro-D-glucopyranose. This was coupled with silylated N^4-benzoyl cytosine. The nucleoside was deacetylated and after several subsequent protection and deprotection steps afforded the desired 3-fluoro-2-keto-β-D-glucopyranosyl derivatives. These novel synthesized compounds were evaluated for antiviral and cytotoxic activities against rotavirus, vesicular stomatitis virus, and the human colon adenocarcinoma cell line Caco-2, and have a promising potential in combating the rotaviral infections and in the treatment of colon cancer. As compared to AZT, a nucleoside analogue of reverse transcriptase inhibitor, the novel synthesized 1-(3,4-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^4-benzoyl cytosine showed to be more effective at lower concentrations in inhibition of rotavirus infection as well as in the same range of antitumor activity.
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1. Introduction

Nucleoside analogues display a wide range of biological activities as antitumor, antiviral, and chemotherapeutic agents.[1–4] A number of fluorine-substituted nucleoside analogues have demonstrated their potent antiviral activity as well as favorable chemical and pharmacological properties by virtue of the small size and highly electronegative nature of the fluorine atom, which is also capable of participating in hydrogen bonding.[5–7] It appears that the high strength of the C–F bond may hinder metabolism pathways and may increase the effective lifetime of the active molecule. Furthermore, introduction of a fluorine atom in the sugar moiety raises the lipophilicity and makes the penetration of the drug through the cell membrane easier.[8–11]

In recent years a number of five-membered cytosine nucleoside analogues, such as 1-(2-deoxy-2-methylene-β-D-*erythro*-pentofuranosyl) cytosine (DMDC),[12] 2′-deoxy-2′,2′-difluorocytidine (gemcitabine),[13] and 1-(2-*C*-cyano-2-deoxy-β-D-*arabino*-pentofuranosyl) cytosine (CNDAC),[14] have been developed as potent antitumor agents, which are effective not only on leukemias and lymphomas, but also on a wide variety of solid tumors in vitro as well as in vivo. Moreover, cytosine unsaturated nucleoside analogues, such as D-2′,3′-didehydro-2′,3′-dideoxy-5-fluorocytidine (D-d4FC)[15] and its L-enantiomer (L-Fd4C),[16] have been identified as antiHIV agents. Structure–activity relationships of L-3′-fluoro-2′,3′-unsaturated nucleosides showed potent activity in the cytosine and 5-fluorocytosine derivatives (EC_{50} = 0.089 and 0.018 μM, respectively).[17]

The last decades, nucleosides with a six-membered carbohydrate moiety have been evaluated for their potential antiviral[18–21] and antibiotic[22] properties and as building blocks in nucleic acid synthesis.[23,24] Among them, the unsaturated ketonucleosides are well-established for their antineoplastic activity and immunosuppressive

Keywords: Unsaturated fluoro ketonucleosides; β-elimination reaction; Gem-diol; Antiviral; Antitumor activity.
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effects.[25–27] It appeared that these nucleosides not only exhibit growth inhibitory activity against a variety of tumor cells[28,29] in vitro and L1210 leukemia[30,31] in vivo, but they also may constitute important synthetic intermediates in the nucleoside field owing to their chemical reactivity in various media.[32,33] Furthermore, fluorine introduction in 3′-position of an unsaturated 4′-ketonucleoside leads to an important difference in the toxicity toward neoplastic and normal cells.[34]

The above observations and the continuous demand for new antiviral and anticancer agents prompted us to design and synthesize a new class of unsaturated fluoro-ketonucleosides, that of N^4-benzoyl cytosine. This conjugation reported for the first time appeared to us as a challenge and the first biological results confirm our hypothesis.

2. Results and discussion

2.1. Synthesis

Commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**) was readily transformed in five steps into the corresponding 3-deoxy-3-fluoro-1,2,4,6-tetra-*O*-acetyl-glucopyranose (**2**)[35,36] (Scheme 1). Condensation of **2** with silylated N^4-benzoyl cytosine in presence of trimethylsilyl trifluomethane-sulfonate yielded the desired β-protected nucleoside **3**. Selective deprotection of **3** using sodium hydroxide (NaOH)/ethanol (EtOH)/pyridine[37] gave benzoylated derivative **4** in excellent yield. However, when compound **3** was treated by methanolic ammonia,[38] or potassium carbonate (K_2CO_3)/methanol (MeOH),[39] or guanidine,[40] the fully unprotected nucleoside was obtained. Treatment of **4** with 2,2-dimethoxypropane in dry *N*,*N*-dimethylformamide (DMF) gave the 4′,6′-isopropylidene derivative **5** as a bright yellow oil.

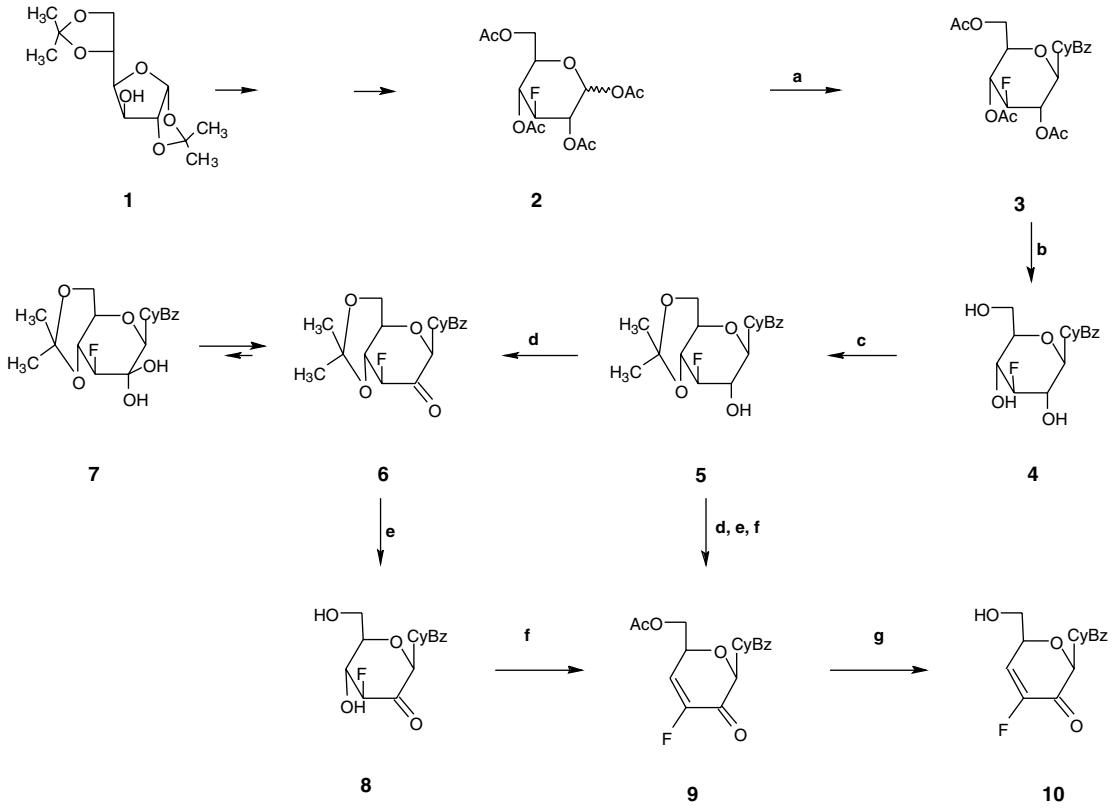
The oxidation of nucleoside **5** was a crucial step because of the instability of the molecule possessing the keto–fluoro system. The presence of an electron-withdrawing fluorine atom α to the carbonyl causes its easy hydration and gem-diol formation.[34] Different oxidation methods have been studied. The use of pyridinium dichromate (PDC)/3E molecular sieves,[41] dimethylsulfoxide (DMSO)/acetic anhydride,[42] and DMSO/oxalyl chloride[43] gave invariably a mixture of the desired 2′-ketonucleoside, 1-(3-deoxy-3-fluoro-4,6-*O*-isopropylidene-β-D-glucopyranosyl-2-ulose)-N^4-benzoyl cytosine (**6**) and its gem-diol **7** in a 7:3 ratio.[44,45] The two derivatives were separated in low yields for characterization of compound **6** by NMR spectroscopy and chemical analysis. Deisopropylidenation of **6** with trifluoroacetic acid afforded the unprotected ketonucleoside **8**, which, upon treatment with acetic anhydride–pyridine gave the unsaturated ketonucleoside **9** after a β-elimination reaction.

However, compound **9** was obtained in a 66% overall yield when **5** was oxidized with PDC–acetic anhydride and the crude reaction mixture was deisopropylidinated without purification and then treated with acetic anhydride–pyridine.[46]

Finally, deacetylation of **9**, which is very unstable under basic conditions, was performed with methanolic hydrogen chloride[47] to afford 1-(3,4-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^4-benzoyl cytosine (**10**).

2.2. Biological activity

To investigate the biological activity of ketonucleosides, we used in vitro tests mimicking the intestinal tract. To examine the potential antiviral properties of the ketonucleosides, two cell models were used. First one, a small intestinal pig IPEC-J2 cell line infected with VSV and second one, embryonic monkey kidney MA 104 cell line and gastrointestinal rotavirus were used as model virus. Three different approaches were followed to test the possible inhibitory activity on VSV and rotavirus infectivity as described in experimental part. Experiments demonstrated that all compounds may have the antiviral activity, but the effect is virus selective. While no significant protection was observed in the case of VSV for compounds **8** and **9** when compounds were added before, during or after infection of IPEC-J2 cells, compound **10** was able partially to inhibit virus infectivity following virus attachment at the concentration of 7 μg/mL (Fig. 1). It could well be that more concentrated compounds would elicit the inhibitory activity but the concentrations over the 0.02 mg/mL caused cytotoxicity of the IPEC-J2 cell line. Those findings are in accordance with previously published results showing that nitro-derivatives but not amino-derivatives of ketonucleosides inhibit replication of murine leukemia virus but at cytotoxic doses.[48,49] In spite of the fact that higher concentrations of all tested compounds were toxic to the MA 104 cells, the concentrations below 0.02 mg/mL inhibited the rotavirus infectivity (Table 1 and Fig. 2). Although the compound **8** was less toxic to the MA 104 cells even at higher concentrations (Table 1 and Fig. 2), the IC_{50} value was 0.07 mg/mL. This value was similar to the one of AZT, which inhibited rotavirus infection of the cells at the concentration of 0.06 mg/mL (Table 1). This was significantly higher than for the compounds **9** and **10** against rotavirus infection either in inhibiting infectivity following virus attachment or neutralizing the virus in solution before its attachment to the cell surface (Table 1 and Fig. 2). The best IC_{50} value was obtained for the compounds **9** and **10** (0.02 mg/mL) in inhibiting infectivity following virus attachment by 60% and 75%, respectively (Fig. 2). On the contrary, only the compound **9** did not elicit inhibitory activity in neutralization of the virus in solution before the rotavirus attachment (Table 1). None of the tested compounds was able to trigger the antiviral state in the IPEC-J2 and MA 104 cells significantly, whereas AZT showed positive results at the concentrations as low as 0.004 mg/mL (Fig. 3 and Table 1). Interestingly, all three compounds analyzed, at the concentration of 30 ng/mL, stimulated the proliferation of MA 104 cells increasing the cell number for 40% (compound **8**), 55% (compound **9**), and 70% (compound **10**) as compared to the control, non-treated cells (Fig. 4), respectively.



Scheme 1. Reagents and conditions: (a) N^4-benzoyl cytosine, trimethylsilyl trifluoromethane-sulfonate, HMDS, saccharine, CH_3CN, 120 °C, 5 h, reflux; (b) EtOH, pyridine, 0 °C, 30 min, Amberlite IR-120 (H^+) resin; (c) 2,2-dimethoxypropane, *p*-toluenesulfonic acid, DMF, 60 °C, 1 h; (d) PDC, acetic anhydride, CH_2Cl_2, 20 °C, 3 h; (e) 90% trifluoroacetic acid in MeOH, 20 °C, 10 min; (f) pyridine–acetic anhydride, 0 °C, 1 h; (g) dry methanolic hydrogen chloride, CH_2Cl_2, 20 °C, 24 h.

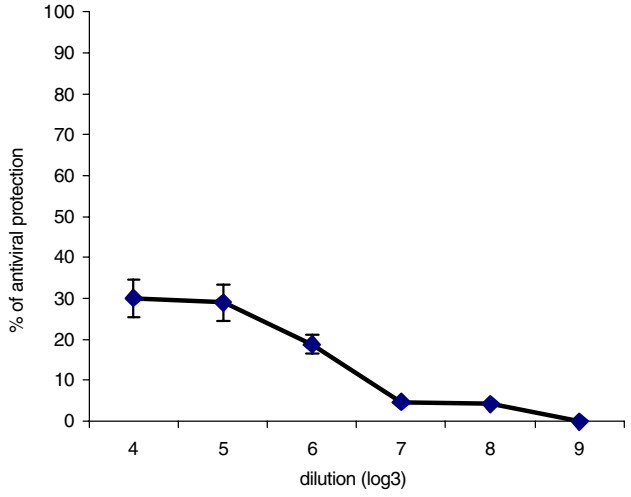


Figure 1. Compound **10** inhibition of the VSV infectivity following virus attachment to IPEC-J2 cells. Cell monolayer was first co-incubated with VSV for 1 h, after the remaining virus was washed off the monolayers, threefold serial dilutions of the compound **10** were performed in culture medium without serum. Following 24 h incubation at 37 °C in a humidified atmosphere of 5% CO_2, the plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by mean ratios (%, ±SD) of absorbances in treated wells ($n = 6$) to those in control wells ($n = 6$).

Furthermore, we have examined the antiproliferative and cytotoxic potency of ketonucleosides, using human colon adenocarcinoma cell line Caco-2. All compounds analyzed, diminished the growth of Caco-2 cells as determined by colony numbers after 10 days of incubation (Table 2) in a concentration-dependent manner with the IC_{50} value being 2.5 mg/mL for the compound **8**, 1.0 mg/mL for the compound **9**, and 0.2 mg/mL for the compound **10**, respectively. As compared to AZT, compounds **9** and **10** showed similar antiproliferative activity to AZT, while compound **8** was active only at significantly higher concentrations (2.5 mg/mL) (Table 2). Moreover, the compounds **9** and **10** showed the highest cytotoxic potency with the IC_{50} value being 0.2 mg/mL equally, whereas significantly higher concentration (1.5 mg/mL) was needed for the compound **8** to elicit the cytotoxic effect on Caco-2 cells after 72 h of co-incubation (Table 2). Our results confirmed previously published data where it was reported that keto-*C*-glycosides and methylene nucleoside analogues inhibit the growth of some tumor cell lines.[49,25]

3. Conclusion

In conclusion, fluoro-ketopyranosyl nucleosides, in particular compound **10**, are potential antiadenocarcinoma agents and moreover, compound **10** is a promising can-

Table 1. IC_{50} (mg/mL) of compounds **8**, **9**, **10**, and **AZT** for the antiviral activity against rotavirus RF strain

	IC_{50} (mg/mL)			
Compound	**8**	**9**	**10**	**AZT**
Antiviral state	0	0	0	0.004
Neutralization of the virus in the solution before its attachment	0.07	0.02	0.02	0.06
Inhibition of infectivity following virus attachment	0.07	0	0.0008	0.015

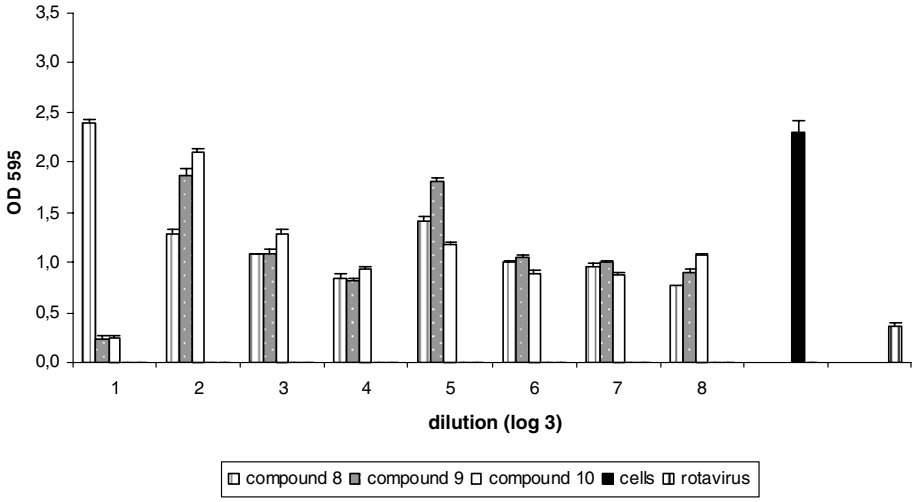


Figure 2. Inhibition of the rotavirus infectivity following virus attachment to MA 104 cells. Cell monolayer was first co-incubated with rotavirus RF strain for 1 h, after, the remaining virus was washed off the monolayers, threefold serial dilutions of each compound were performed in culture medium without serum. Following further 72 h incubation at 37 °C in a humidified atmosphere of 5% CO_2, the plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by mean ratios (%, ±SD) of absorbances in treated wells ($n = 6$) to those in control wells ($n = 6$).

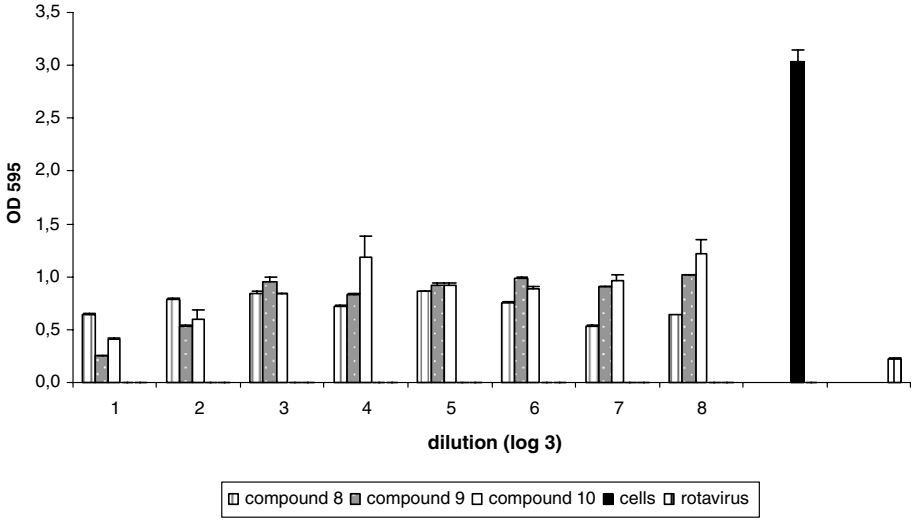


Figure 3. Ability of antiviral state induction in rotavirus-infected MA 104 cells. Washed monolayer of MA104 cells was first incubated with threefold serial dilutions of the compounds for 10 h at 37 °C in the atmosphere of 5% CO_2. After incubation the compounds were washed off with DMEM without supplements and monolayer was challenged immediately with rotavirus (100 μL per well). After 72 h of incubation for rotavirus, the plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) to those in control (only virus-infected) wells ($n = 6$).

didate in development of antirotavirus drugs as (i) inhibitor of infectivity following virus attachment and (ii) neutralizing agent of the virus in solution before attachment to the cell surface. Moreover, as compared to AZT, a nucleoside analogue of reverse transcriptase inhibitor, 1-(3,4-dideoxy-3-fluoro-β-D-glycero-hex-3-

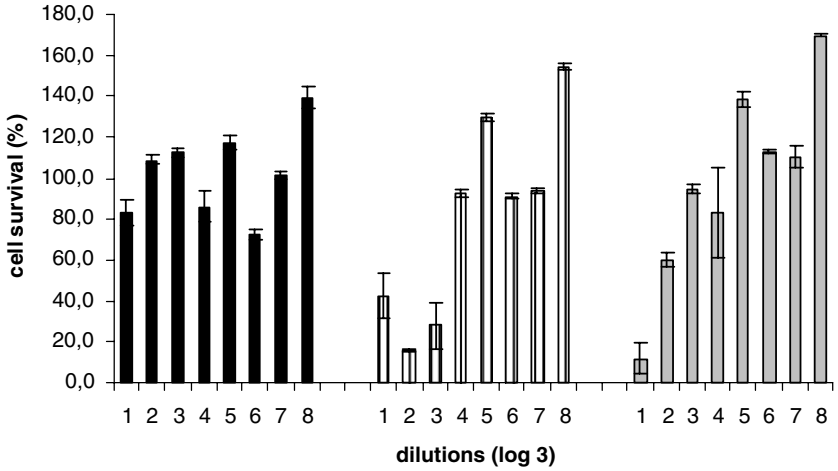


Figure 4. Cytotoxicity of compound **8** (black box), compound **9** (dashed box), and compound **10** (gray box) to MA 104 cells. Each of the compounds at the initial concentration of 0.2 mg/mL was serially diluted in cell culture medium and left on MA104 cell monolayer seeded in 96-well plates. Following 72 h incubation at 37 °C in a humidified atmosphere of 5% CO_2, the plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by mean ratios (%, ±SD) of absorbances in treated wells ($n = 4$) to those in control wells ($n = 8$).

Table 2. IC_{50} (mg/mL) of compounds **8**, **9**, **10**, and **AZT** for growth inhibition and cytotoxic activity on Caco-2 cells

	IC_{50} (mg/mL)			
Compound	**8**	**9**	**10**	**AZT**
Growth inhibition	2.5	1.0	0.2	0.015
Cytotoxicity	1.5	0.2	0.2	0.015

enopyranosyl-2-ulose)-N^4-benzoyl cytosine (**10**), showed to be more effective at lower concentrations in inhibition of rotavirus infection as well as in the same range of antitumor activity.

4. Experimental

4.1. General procedure

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Silica gel (240–400 mesh, Merck). NMR spectra were recorded at room temperature with a Brucker 250 MHz spectrometer in chloroform-d ($CDCl_3$) with internal tetramethylsilane (TMS). All reactions were carried out in dry solvents. Dichloromethane (CH_2Cl_2) was distilled from phosphorus pentoxide (P_2O_5) and stored over 4E molecular sieves. Acetonitrile (CH_3CN) was distilled from calcium hydride (CaH_2) and stored over 3E molecular sieves. DMF was also stored over 3E molecular sieves. Pyridine was stored over pellets of potassium hydroxide (KOH).

4.1.1. 1-(3-Deoxy-3-fluoro-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-N^4-benzoyl cytosine (3). A mixture of N^4-benzoyl cytosine (3.99 g, 18.55 mmol), hexamethyldisilazane (HMDS) (4.8 mL, 23 mmol), and saccharine (0.16 g, 0.85 mmol) in anhydrous CH_3CN (69 mL) was refluxed for 30 min at 120 °C. To this were added tetraacetylated 3-deoxy-3-fluoro-D-glucose[35,36] (**2**) (5.00 g, 14.27 mmol) and trimethylsilyl trifluomethane-sulfonate (3.6 mL, 19.98 mmol). The reaction mixture was refluxed at 120 °C for 5 h, then cooled, neutralized with aqueous sodium bicarbonate ($NaHCO_3$), and extracted with CH_2Cl_2 (1000 mL). The organic layer was washed with water (1× 20 mL) and dried over anhydrous sodium sulfate, evaporated to dryness, and finally purified by flash chromatography (hexane/ethyl acetate (EtOAc), 2:8) to give compound **3** (4.90 g, 68%, $R_f = 0.35$) as a solid. Mp 277 °C.

^{1}HNMR ($CDCl_3$): δ 7.88 (d, 1H, $J_{6,5} = 7.2$ Hz, H-6), 7.84–7.48 (m, 6H, Bz and H-5), 6.10 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1′), 5.39–5.22 (m, 2H, $J_{2',1'} = 9.4$ Hz, $J_{4',3'} = 9.0$ Hz, H-2′ and H-4′), 4.8 (dtr, 1H, $J_{F,3'} = 51.7$ Hz, $J_{3',2'} = 9.1$ Hz, $J_{3',4'} = 9.0$ Hz, H-3′), 4.34–4.11 (m, 2H, H-6a′,6b′), 3.86 (m, 1H, H-5′), 2.16–2.11–2.07 (3s, 9H, 3OAc).

Found: C, 54.70; H, 4.80; F, 3.75; N, 8.42. Calcd for $C_{23}H_{24}FN_3O_9$: C, 54.65; H, 4.79; F, 3.76; N, 8.31.

4.1.2. 1-(3,4-Dideoxy-3-fluoro-β-D-glucopyranosyl)-N^4-benzoyl cytosine (4). Compound **3** (4.90 g, 9.7 mmol) was dissolved in EtOH–pyridine (97 + 29.1 mL), 2 M NaOH (19.4 mL) was added, and the mixture stirred for 30 min at 0 °C. Amberlite IR-120 (H^+) was added to neutralize the base. The suspension was filtered, the resin was washed with EtOH and pyridine (100 + 100 mL), and the filtrate was evaporated. The solid residue was triturated with diethyl ether (2× 30 mL) and

CH_2Cl_2 (2× 30 mL), and filtered. Product **4** (3.31 g, 90%) was used without further purification.

4.1.3. 1-(3-Deoxy-3-fluoro-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-N^4-benzoyl cytosine (5). Compound **4** (3.31 g, 8.73 mmol) was dissolved in a mixture of 34.92 mL of 2,2-dimethoxypropane and 110.4 mL of dry DMF. To this was added (2.65 g, 13.97 mmol) of *p*-toluenesulfonic acid and the mixture stirred at 60 °C for 1 h. The reaction mixture was neutralized with triethylamine so pH did not exceed 7. After that, the mixture was concentrated under high-vacuum pump to eliminate the DMF. Purification with flash chromatography (hexane/EtOAc, 1:9), gave **5** (2.93 g, 80%, $R_f = 0.3$) as a bright yellow oil.

^{1}HNMR ($CDCl_3$): δ 7.88 (d, 1H, $J_{6,5} = 7.9$ Hz, H-6), 7.84–7.43 (m, 6H, Bz and H-5), 5.86 (d, 1H, $J_{1',2'} = 9.2$ Hz, H-1′), 4.68 (dtr, 1H, $J_{F,3'} = 53.2$ Hz, $J_{3',2'} = 8.7$ Hz, $J_{3',4'} = 8.7$ Hz, H-3′), 4.05–3.87 (m, 2H, $J_{2',1'} = 9.2$ Hz, $J_{4',3'} = 8.7$ Hz, H-2′ and H-4′), 3.86–3.73 (m, 2H, H-6a′,6b′), 3.51 (m, 1H, H-5′), 1.53–1.45 (2s, 6H, 2×CH_3).

Found: C, 57.16; H, 5.31; F, 4.24; N, 10.03. Calcd for $C_{20}H_{22}FN_3O_6$: C, 57.28; H, 5.29; F, 4.53; N, 10.02.

4.1.4. 1-(3-Deoxy-3-fluoro-4,6-*O*-isopropylidene-β-D-glucopyranosyl-2-ulose)-N^4-benzoyl cytosine (6). A mixture of **5** (2.93 g, 6.98 mmol; dried by co-evaporation with toluene), PDC (3.15 g, 8.38 mmol), and acetic anhydride (1.98 mL, 20.94 mmol) was stirred in dry CH_2Cl_2 (58.1 mL) for 3 h. After cooling, EtOAc (10 mL) was added and the resulting slurry was transferred on the top of a silica-gel column packed in EtOAc. The solution was filtered through the column and washed with EtOAc (92 mL) until the product was eluted completely. The solvent was evaporated and the residue was rendered free of acetic anhydride and pyridine by co-evaporation with dry toluene (3×) to afford 2.45 g of crude **6**. Purification by flash chromatography (hexane/EtOAc, 2:8) gave pure **6** in 22% yield.

^{1}HNMR ($CDCl_3$): δ 8.05 (d, 1H, $J_{6,5} = 7.6$ Hz, H-6), 8.0–7.44 (m, 6H, Bz and H-5), 5.92 (1s, 1H, H-1′), 4.65 (dd, 1H, $J_{3',4'} = 9.2$ Hz, $J_{F,3'} = 51.4$ Hz, H-3′), 4.13–4.03 (m, 1H, $J_{4',3'} = 9.2$ Hz, H-4′), 3.93–3.77 (m, 2H, H-6a′,6b′), 3.53 (m, 1H, H-5′), 1.54–1.44 (2s, 6H, 2×CH_3).

Found: C, 57.26; H, 4.33; F, 4.92; N, 10.35. Calcd for $C_{20}H_{20}FN_3O_6$: C, 57.55; H, 4.83; F, 4.55; N, 10.07.

4.1.5. 1-(6-*O*-Acetyl-3,4-dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^4-benzoyl cytosine (9). Crude **6** (2.0 g, 4.79 mmol), obtained from the previous procedure, was dissolved in 23.95 mL of 90% trifluoroacetic acid in MeOH. The solution was stirred for 10 min at room temperature and then concentrated at 40 °C under high-vacuum pump to remove traces of trifluoroacetic acid. Crude **8** (1.79 g, 99%) was dissolved in dry pyridine (9.5 mL), acetic anhydride (1.79 mL) was added, and the mixture stirred at 0 °C for 1 h under strictly dry conditions. MeOH (2.0 mL) was added to quench the reaction and the mixture concentrated under high-vacuum pump to remove the solvents. Purification by flash chromatography (hexane/EtOAc, 3:7) gave the title compound **9** (1.85 g, 66% from **5**, $R_f = 0.27$).

^{1}HNMR ($CDCl_3$): δ 7.89 (d, 1H, $J_{6,5} = 7.5$ Hz, H-6) 7.58–7.43 (m, 6H, Bz and H-5), 6.56 (dd, 1H, $J_{F,4'} = 11.0$ Hz, $J_{4',5'} = 1.5$ Hz, H-4′), 5.88 (s, 1H, H-1′), 5.03 (m, 1H, H-5′), 4.35–4.2 (m, 2H, H-6a′,6b′), 2.05 (s, 3H, OAc).

Found: C, 57.03; H, 4.00; F, 4.75; N, 10.45. Calcd for $C_{19}H_{16}FN_3O_6$: C, 56.86; H, 4.02; F, 4.73; N, 10.47.

4.1.6. 1-(3,4-Dideoxy-3-fluoro-β-D-glycero-hex-3-enopyranosyl-2-ulose)-N^4-benzoyl cytosine (10). To a solution of **9** (1.85 g, 4.61 mmol) in dry CH_2Cl_2 (9.2 mL) was added, at room temperature, 9.2 mL of methanolic hydrogen chloride prepared by addition at 0 °C, of acetyl chloride (0.4 mL) to dry MeOH (10 mL). After 24 h, reaction was complete, and the mixture was diluted with CH_2Cl_2 and treated with an excess of $NaHCO_3$. After washing twice with water, drying over anhydrous magnesium sulfate ($MgSO_4$) and concentration, the crude mixture was purified by flash chromatography (EtOAc) to give **10** (0.83 g, 50%, $R_f = 0.29$).

^{1}HNMR ($CDCl_3$): δ 7.89 (d, 1H, $J_{6,5} = 7.5$ Hz, H-6), 7.59–7.43 (m, 6H, Bz and H-5), 6.61 (dd, 1H, $J_{F,4'} = 11.4$ Hz, $J_{4',5'} = 1.1$ Hz, H-4′), 5.22 (s, 1H, H-1′), 4.89 (m, 1H, H-5′), 3.88–3.77 (m, 2H, H-6a′,6b′).

Found: C, 56.72; H, 3.91; F, 5.26; N, 11.73. Calcd for $C_{17}H_{14}FN_3O_5$: C, 56.83; H, 3.93; F, 5.29; N, 11.69.

4.2. Methods for measurement of biological activity

4.2.1. Cells and culture conditions. The IPEC-J2 cell line (Intestinal pig epithelial-cell jejenum) was a generous gift of Prof. Anthony Blikslager (North Carolina State University, USA). Human colon adenocarcinoma Caco-2 and embryonic rhesus monkey kidney MA104 cells were a generous gift of dr. Rene L'Harridon, INRA, VIM, Jouy-en-Josas, France. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma–Aldrich, Grand Island, USA), supplemented with 5% fetal calf serum (Cambrex, Verviers, Belgium), L-glutamine (2 mmol/L, Sigma, St. Louis, USA) penicillin (100 U/mL, Sigma, St. Louis, USA) and streptomycin (1 mg/mL, Fluka, Buchs, Switzerland) at 37 °C in 5% carbon dioxide (CO_2) atmosphere in tissue culture flasks until confluent. Cell culture medium was regularly changed.

4.2.2. Fluoro-ketopyranosyl nucleosides. Compounds **8**–**10** were freshly prepared in sterile DMSO at the concentration of 5 mg/mL. The final concentration of DMSO was below 0.1% of cell culture medium. All solutions were protected against light.

AZT (Retrovir®) GlaxoSmithKline, USA, a drug used for antiretroviral therapy (ART) was used as a standard compound in biological experiments, prepared in the same way as fluoro-ketopyranosyl nucleosides.

4.2.3. Virus propagation. *Vesicular stomatitis virus (VSV)*—Indiana strain was propagated in IPEC-J2 monolayers. Supernatant containing the virus was collected from the flasks when cytopathic effect (CPE) was observed (24–48 h at 37 °C) by microscopy and clarified by centrifugation. Virus was stored at −70 °C until used. For the antiviral assay, virus with 1.436 tissue culture infective dose 50% units per mL ($TCID_{50}$/mL) was used (100 μL per well). *The bovine RF strain* was propagated in MA104 cells in the presence of trypsin as described previously.[50] Virus titers present in the clarified lysed-cell supernatants were determined as above.

4.2.4. Antiviral assay. The potential antiviral activity of the novel synthesized compounds was tested against rotavirus or VSV by investigating:

(a) The ability of antiviral state induction in the infected cells: washed monolayer of MA104 or IPEC-J2 cells was first incubated with threefold serial dilutions of the compounds or AZT for 10 h at 37 °C in the atmosphere of 5% CO_2. After incubation the compounds were washed off with DMEM without supplements and monolayer was challenged immediately with rotavirus or VSV virus (100 μL per well). After 24 h post-infection for VSV and 72 h post-infection for rotavirus, the plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) to those in control (only virus infected) wells ($n = 6$).

(b) The inhibition of infectivity following virus attachment: washed monolayer of MA104 or IPEC-J2 cells was first incubated with rotavirus or VSV for 1 h at 37 °C in the atmosphere of 5% CO_2 (time for virus to attach to cell membrane receptors). After incubation the remaining virus was washed off with DMEM without supplements and monolayer was treated immediately with the F-ketonucleosides or AZT, added in threefold serial dilutions. After 24 h post-infection for VSV and 72 h post-infection for rotavirus, the plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) to those in control (only virus infected) wells ($n = 6$). The minimal inhibitory concentration (IC_{50}) of the tested compounds was obtained from the concentration–effect curve.

(c) The neutralization of the virus in solution before attachment: threefold dilutions of each of the tested compounds or AZT were first co-incubated with rotavirus or VSV in DMEM for 12 h prior to the infection of cell monolayer at 37 °C and 5% CO_2. Residual viral infectivity was measured after 24 h post-infection for VSV and 72 h post-infection for rotavirus. Rotavirus or VSV alone was treated in the same way as the control. After 72 h of incubation, the plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in virus-infected wells ($n = 6$) to those in control (only virus infected) wells ($n = 6$). The minimal inhibitory concentration (IC_{50}) of the tested compounds was obtained from the concentration–effect curve.

4.2.5. Growth inhibition assay. It was performed on Caco-2 cell line by modified method described previously.[51] Briefly, in 96-well plates, six wells of threefold dilutions of each compound or AZT (initial concentration 5 mg/mL) were applied to monolayers of 10 cells/well in Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum. Incubation was performed at 37 °C in the humidified incubator for 10 days. The colonies were counted in each well and the results were expressed, for each dilution, by mean ratios (%, ±SD) of colony number in treated wells ($n = 2$) to those in control wells ($n = 24$). The minimal inhibitory concentration (IC_{50}) of the tested compounds was obtained from the concentration–effect curve.

4.2.6. Cytotoxicity assay. Caco-2 cells (6×10^6 cells per plate) were seeded in P 96 plate and treated with the compounds at threefold serial dilutions of each compound or AZT (initial concentration 5 mg/mL). Then, the cells were incubated at 37 °C in the humidified incubator for 72 h. The plates were stained with Crystal violet in EtOH, rinsed with water, and destained with 10% (v/v) acetic acid. The A_{590} was measured, and the results were expressed, for each dilution, by the mean ratios (%, ±SD) of absorbances in treated wells ($n = 2$) to those in control wells ($n = 24$). The minimal inhibitory concentration (IC_{50}) of the tested compounds was obtained from the concentration–effect curve.

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