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Synthesis and biological activity of a fluoroketonucleoside: 7-(3-deoxy-3-fluoro-β-D-*glycero*-hex-2-enopyranosyl-4-ulose)theophylline*

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ABSTRACT

The title compound **5** was synthesized by direct oxidation of the fluoro precursor and not from an epoxyketo intermediate according to the Paulsen procedure, which has been used until now to obtain bromo- and chloro-ketonucleosides. Antineoplastic activity and immunosuppressive effect of **5** were studied on murin splenic lymphocytes (steady state or stimulated by PHA), and on RAJI and DAUDI cells, and compared with the activity of 3-(3-deoxy-β-D-*glycero*-hex-2-enopyranosyl-4-ulose)thymine. IC_{50} values showed that the fluoroderivative **5** has a higher antineoplastic activity and a lower immunosuppressive effect.

INTRODUCTION

In previous papers, the significant *in vitro* and *in vivo* inhibitory activity of unsaturated ketonucleosides against various types of cancer cells has been demonstrated[1–3]. In developing structure–activity relationships, it was found that the introduction of a bromine atom at the double bond of **8** noticeably increased the toxicity but not the specificity towards cancer cells[1–3]. In order to obtain a better target for cancer cells, we have undertaken the synthesis of a molecule containing a fluorine instead of a bromine atom. Indeed, this atom is the most electronegative that can be introduced in an organic compound. Moreover, the introduction of a fluorine atom in a molecule is known to confer to it a low toxicity and to make easier the permeability of drugs through the cell by increasing the lipophilicity; it was also shown to give rise to dramatic changes in biological activity[4].

Until now, the syntheses of unsaturated halogenoketonucleosides were accomplished[5] by oxidation of a hydroxyl group in a 1,2 position to an epoxy bridge, followed by the action of a lithium halide on the ketoepoxy intermediate, according to the procedure of Paulsen *et al.*[6] We describe herein the first synthesis of an unsaturated fluoroketonucleoside by direct oxidation of the parent fluoronucleoside.

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* Dedicated to Professor Serge David on the occasion of his 70th birthday.
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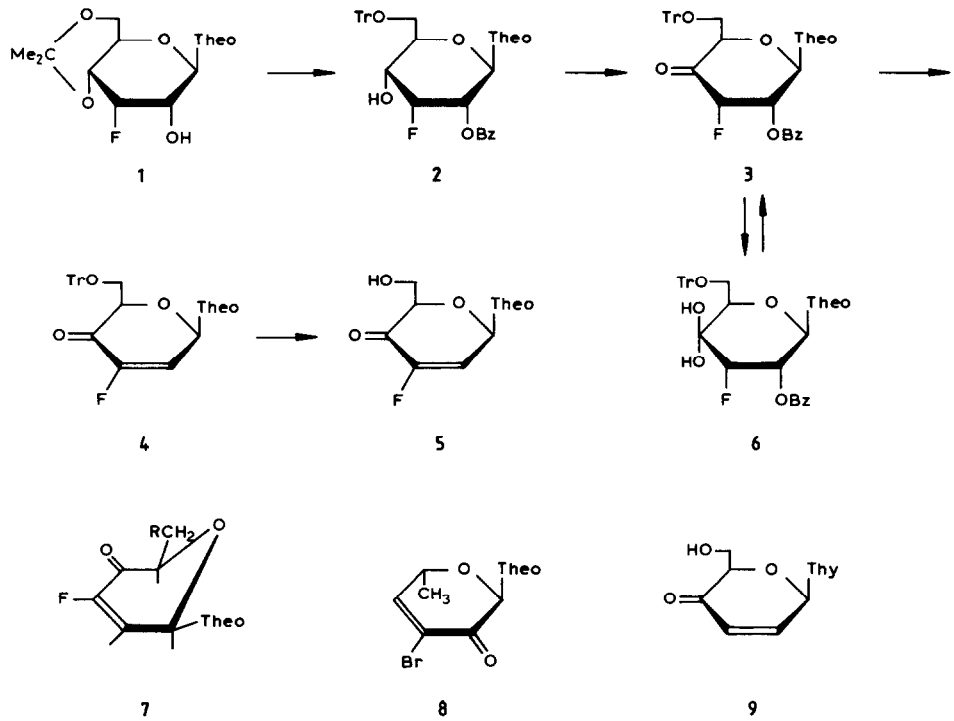
RESULT AND DISCUSSION

The isopropylidene group of 7-(3-deoxy-3-fluoro-4,6-*O*-isopropylidene-β-D-allopyranosyl)theophylline[7] (**1**) was removed, followed by protection of O-2 and O-6, with a benzoyl and a trityl group, respectively, to give **2**. This compound was oxidized at O-4 but, in spite of a good oxidation-yield, the obtention of pure **3** was not possible because of hydration that was due to the presence of an electron-drawing fluorine atom in 1,2-position to the carbonyl group. Purification of the reaction mixture by flash chromatography on silica gel gave mainly the gem-diol **6**. To avoid this hydration, the crude oxidation mixture was directly treated with acetic anhydride in pyridine to afford **4** by a β-elimination mechanism, and detritylation gave **5**.



1 2 3 4 5 6 7 8 9

The presence of CO-4 was ascertained by the disappearance, in the ^{1}H-n.m.r. spectrum, of the signal for H-4 and the deshielding effect of other protons, especially of the allylic proton H-2 in compounds **4** and **5**, as previously observed for other (2-deoxy-β-D-hex-2-enopyranosyl-4-ulose)nucleosides[8]. The allylic coupling constants $^4J_{2,5}$ were determined for **4** as 1.8 and for **5** as 2.0 Hz. According to these results, the half-chair conformation[8] **7** was assigned to compounds **4** and **5**. Few $^3J_{F,H}$ and $^4J_{F,H}$ coupling constants have been observed for compounds where a fluorine atom is attached to a sp^2-hybridized carbon atom[9] and, to our knowledge, never in 1,2 position to a carbonyl group. We found $^3J_{F\text{-}3,2}$ values of 7.4 for **4**, and 7.1 Hz for **5**, and $^4J_{F\text{-}3,1}$ values of 10.4 for **4**,

TABLE I

^{13}C-N.m.r. data for compounds **2** and **4**[a].

Compounds	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	$J_{F-3',C-2'}$	$J_{F-3',C-3'}$	$J_{F-3',C-4'}$
2	80.2	67.2	89.8	70.5	74.8	62.8	18.0	180.5	18.0
4	79.9	120.5	150.5	184.5	78.1	61.3	1	172.2	16.2

[a] For a solution in $CDCl_3$ (internal standard, C_6F_6). *J* values in Hz.

and 10.3 Hz for **5**. Surprisingly, in these cases, the values found for $^3J_{F,H}$ and $^4J_{F,H}$ were large and the $^4J_{F,H}$ value was higher than the $^3J_{F,H}$ value. As previously observed for other unsaturated 4-ketonucleosides[8], the ^{13}C-n.m.r. data (Table I) showed that C-2, C-3, and C-4 were markedly deshielded in **4**, as compared with the parent compound **2**. For **2**, the $^2J_{F,C}$ and $^3J_{F,C}$ values were in accordance with previous results[7,9]. The value of 18.0 Hz for $J_{F-3,2}$ and $J_{F-3,4}$ was in the range previously observed when the oxygen atom linked to the coupled carbon atom is *gauche* to the coupled fluorine atom. For compound **4**, the $^2J_{F-3,3}$ was found to be 172.2 Hz and the $^2J_{F-3,4}$ value 16.2 Hz. Surprisingly, the $^3J_{F-3,4}$ value was smaller than 1 Hz and could not be measured.

The antineoplastic activity and immunosuppressive effect of compound **5** were studied on steady-state (LY) or stimulated (PHA-LY) murin splenic lymphocytes, and on RAJI and DAUDI cells; they were compared with the activity of compound **9** the sugar residue of which differs from that of **5** by the presence of a fluorine atom; previous results[1-3] have shown that the role of the heterocyclic base with regard to the biological activity was not predominant. The technique used was based on the inhibition of tritiated thymidine incorporation by dividing cells, which allows a good estimation of DNA synthesis. An inhibition of thymidine incorporation reflects, therefore, the anti-proliferative properties of the tested drug. The results are given in Tables II and III, and in Figs. 1 and 2. They show that **9** is more cytotoxic for PHA-stimulated lymphocytes than for steady state lymphocytes, and RAJI and DAUDI cells. Fluoroketonucleoside **5** showed a high toxicity for DAUDI and RAJI cells, but the most significative feature was the important difference in the toxicity of this compound toward neoplastic and normal cells. It showed a higher antineoplastic activity than the analog **9** and, for an equivalent antineoplastic activity, a lower immunosuppressive effect and the most lower toxicity for steady state cells was observed.

TABLE II

IC_{50} (μM) values for compounds **9** and **5** for murin splenic lymphocytes stimulated by PHA (PHA-LY) or not (LY), and for RAJI and DAUDI cells.

Cpd.	*LY*	*PHA-LY*	*RAJI*	*DAUDI*
9	210	27	220	290
5	115	23	45	65

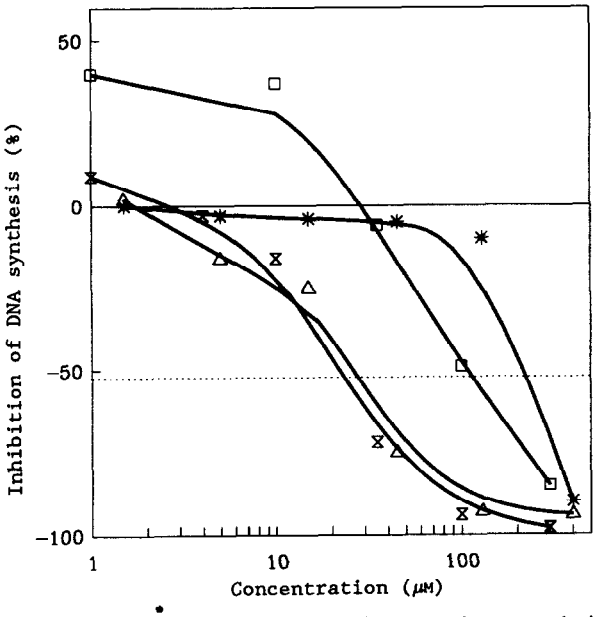


Fig. 1. Effects of ketonucleosides **9** and **5** on steady-state and stimulated lymphocytes. Compounds **9**, (*) LY and (△) PHA-LY. Compound **5**, (□) LY and (X) PHA-LY.

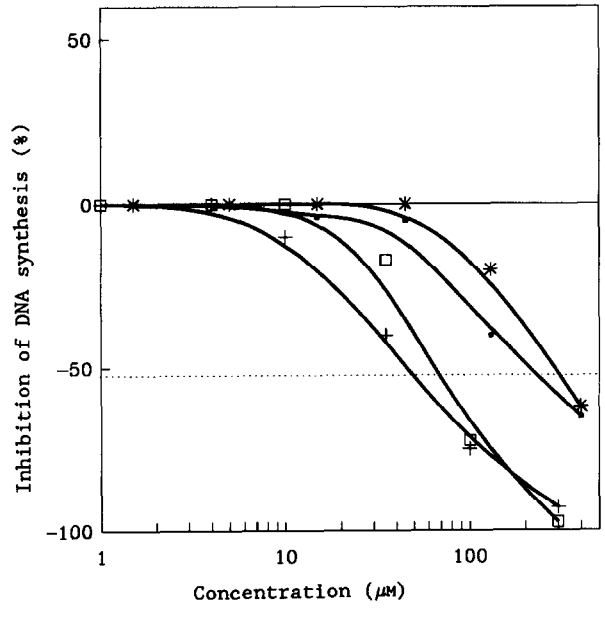


Fig. 2. Effects of ketonucleosides **9** and **5** on RAJI and DAUDI cells. Compound **9**, (□) RAJI and (*) DAUDI. Compound **5**, (+) RAJI and (□) DAUDI.

TABLE III

Comparative ratio of drug cytotoxicity for compounds **9** and **5**.

Cpd.	*Normal cells (Ly)*	*PHA-LY*	*Neoplastic cells*
9	1	10	1
5	2	12	5

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured for solutions in methanol (*c* 0.1). ^{1}H-, ^{13}C- and ^{19}F-N.m.r. spectra were recorded with a Brucker MSL 300 spectrometer using tetramethylsilane and hexafluorobenzene, respectively, as internal reference. Flash-column chromatography was performed on Silica gel 60 (230–400 mesh, S.D.S.).

7-(2-O-Benzoyl-3-deoxy-3-fluoro-6-O-trityl-β-D-allopyranosyl)theophylline (**2**). — To a solution of **1**[7] (1 mmol, 384 mg) in pyridine (5 mL) was added benzoyl chloride (1.2 mmol, 139.2 μL) at room temperature. After 10 min, the mixture was concentrated and worked-up in the usual manner. The syrupy residue crystallized from methanol to give *7-(2-O-benzoyl-3-deoxy-3-fluoro-4,6-O-isopropylidene-β-D-allopyranosyl)theophylline* (366 mg, 75%), m.p. 210°, $[\alpha]_D^{20}$ −75°; ^{1}H-n.m.r. ($CDCl_3$): δ 7.94–7.38 (m, 5 H, C_6H_5) and 5.24 (d, 1 H, $J_{F,3'}$ 54.7 Hz, H-3'); ^{19}F-n.m.r. ($CDCl_3$): δ −40.8.

Anal. Calc. for $C_{23}H_{25}FN_4O_7$: C, 56.55; H, 5.12; N, 11.47; F, 3.89. Found: C, 56.04; H, 5.10; N, 11.22; F, 3.11.

Heating at reflux of a methanolic solution of the aforementioned compound with Amberlite IR-120 (H^+) cation-exchange resin for 0.5 h and neutralization with Amberlite IR-45 (OH^-) anion-exchange resin gave, after filtration and concentration, *7-(2-O-benzoyl-3-deoxy-3-fluoro-β-D-allopyranosyl)theophylline* (75%) in a semi-crystalline form from ethyl ether, m.p. 120–130°, $[\alpha]_D^{20}$ −25°; ^{1}H-n.m.r. ($CDCl_3$): δ 7.90–6.75 (m, 5 H, C_6H_5), 6.19 (d, 1 H, $J_{1',2'}$ 9.15, H-1'), 5.78 (dd, 1 H, $J_{1',2'}$ 9.23, $J_{F,2'}$ 27.23 Hz, H-2'), 5.35 (d, 1 H, $J_{F,3'}$ 53.9 Hz, H-3'), 4.35 (dd, 1 H, $J_{3',4'}$ 9.85, $J_{F,4'}$ 29.15 Hz, H-4'), and 4.13–3.66 (m, 3 H, H-5',6a',6b'); ^{19}F-n.m.r. ($CDCl_3$): δ −42.9.

Anal. Calc. for $C_{20}H_{21}FN_4O_7 \cdot 0.5H_2O$: C, 52.51; H, 4.81; F, 4.15; N, 12.25. Found: C, 52.48; H, 4.98; F, 3.59; N, 11.77.

To a solution of the aforementioned compound (1 mmol, 448 mg) in pyridine (5 mL) was added chlorotriphenylmethane (1.2 mmol, 333 mg) and a catalytic amount of 4-dimethylaminopyridine. The mixture was stirred at room temperature for 24 h and then concentrated. After extraction with dichloromethane and concentration, a flash chromatography of the residue (1:1 ethyl acetate–hexane) gave pure **2** (415 mg, 60%), m.p. 135° (from ethanol), $[\alpha]_D^{20}$ −25°; ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 7.85–7.24 (m, 15 H, 3 C_6H_5), 5.65 (d, 1 H, $J_{1',2'}$ 5.6 Hz, H-1'), and 5.27 (d, 1 H, $J_{F,3'}$ 54.06 Hz, H-3'); ^{19}F-n.m.r. ($CDCl_3$): δ −42.9.

Anal. Calc. for $C_{39}H_{35}FN_4O_7 \cdot H_2O$: C, 66.10; H, 5.22; F, 2.68; N, 7.90. Found: C, 66.17; H, 5.13; F, 2.79; N, 7.82.

*7-(2-O-Benzoyl-3-deoxy-3-fluoro-6-O-trityl-β-*D*-ribo-hexopyranosyl-4-ulose) theophylline (***3***) and gem-diol* **6**. — A mixture of **2** (1 mmol, 690 mg; dried by coevaporation with toluene), pyridinium dichromate (2 mmol, 752 mg), 3A molecular sieves (1 g), and acetic acid (5 drops) were stirred in dry dichloromethane (10 mL) for 1 h. The mixture was then filtered on Silica gel G (Merck), and the silica gel was washed with dichloromethane (125 mL), followed by ethyl acetate (125 mL). The washings were concentrated *in vacuo* and the resulting syrup was purified by flash chromatography (1:1 ethyl acetate–hexane). T.l.c. plates, developed with the same mixture, showed the formation of two products, the keto compound **3** having R_F 0.33 and the hydrate **6** having R_F 0.5. The equilibrium between both compounds could be displaced toward **3** by heating the mixture while **6** could be obtained in pure form from the methanolic solution.

Compound **3**. Oil, $[\alpha]_D^{20}$ −7.5°.

Compound **6**. M.p. 88–95° (from methanol), $[\alpha]_D^{20}$ 0°.

Anal. Calc. for $C_{39}H_{35}FN_4O_8$: C, 66.28; H, 4.95; F, 2.69; N, 7.93. Found: C, 66.00; H, 5.41; F, 2.41; N, 7.45.

*7-(3-Deoxy-3-fluoro-6-*O*-trityl-β-*D*-*glycero*-hex-2-enopyranosyl-4-ulose) theophylline* (**4**). — Compound **2** (1 mmol, 690 mg) was oxidized as described for **3**. After filtration on Silica gel G and concentration, the syrup was dissolved without further purification in 2:1 pyridine–acetic anhydride (3 mL) and stirred for 75 min. Concentration and purification by flash chromatography (1:1 ethyl acetate–hexane) afforded pure **4** which crystallized from ethanol (277 mg, 50%), m.p. 115–118°, $[\alpha]_D^{20}$ −15°; ^{1}H-n.m.r. ($CDCl_3$): δ 7.43–7.18 (m, 16 H, 3 C_6H_5 and H-2'), 6.66 (dd, 1 H, $J_{1',2'}$ 1.7, $J_{F,1'}$ 10.4 Hz, H-1'), and 4.53 (dt, 1 H, $J_{2',5'}$ 1.8, $J_{5',6a'}$ 4.0, $J_{5',6b'}$ 6.0 Hz, H-5'); ^{19}F-n.m.r. ($CDCl_3$): δ −36.7 (dd, $J_{F,1'}$ 10.4, $J_{F,2'}$ 7.4 Hz).

Anal. Calc. for $C_{31}H_{27}FN_4O_5$. C: 67.15; H, 4.87; F, 3.42; N, 10.11. Found: C, 66.92; H, 5.03; F, 3.10; N, 9.59.

*7-(3-Deoxy-3-fluoro-β-*D*-glycero-hex-2-enopyranosyl-4-ulose) theophylline* (**5**). — Compound **4** (1 mmol, 554 mg) was dissolved in 70% acetic acid (11.5 mL) and heated at 60° for 3 h. After cooling to room temperature, triphenylmethanol was filtered off and washed with 70% acetic acid. The filtrate was concentrated under vacuum to afford **5** as a white crystalline powder which was insoluble in most organic solvents and was purified by decantation from a mixture of methanol and dichloromethane (227 mg, 70%), m.p. 215–219° (dec.), $[\alpha]_D^{20}$ −7.5°; ^{1}H-n.m.r. ($CDCl_3$): δ 6.97 (dt, $J_{1',2'}$ 1.8, 2.0, and 7.1 Hz, H-2'), 6.65 (dd, $J_{1',2'}$ 1.8, $J_{F,1'}$ 10.3 Hz, H-1'), and 4.53 (dt, $J_{2',5'}$ 2.0, $J_{5',6a'}$ 4.0, $J_{2',6b'}$ 6.0 Hz, H-5'); ^{19}F-n.m.r.-n.m.r. ($CDCl_3$): δ -45.0.

Anal. Calc. for $C_{13}H_{13}FN_4O_5$: C, 48.14; H, 4.01; F, 5.86; N, 17.28. Found: C, 48.23; H, 3.90; F, 6.16; N, 16.91.

Cells. — RAJI and DAUDI cells were derived from Burkitt lymphomas. These two human cell lines express the EBV (Epstein–Barr virus) antigens and induce tumors

in *nude* mice. The cells were grown in RPMI 1640 medium, supplemented with 2mM glutamine, 10% of decomplemented calf serum, and antibiotics (100 IU of penicillin/mL and 100 mg of streptomycin/mL). They were usually seeded at 150 000 cells/mL of medium in culture flasks, incubated at 37° in a humidified incubator with 5% CO_2 and subcultured three times a week. For the test, the cell suspension was dispensed in wells (200 μL each) of microtest plates.

Splenic lymphocytes were prepared from spleen of *Balb/c* mice as described earlier[10]. Spleens were aseptically removed, minced with scissors, and filtered through a 100-mesh stainless steel sieve. The cell suspension was collected and centrifuged for 6 min at 1500 r.p.m. The pellet was resuspended in a known volume of RPMI 1640, and the cells were counted on a 50-μL aliquot (diluted 1:10 with Trypan Blue) with a hemocytometer.

Cells (500 000) were seeded in microplate wells in RPMI (150 μL) supplemented with 2 mM glutamine, 10% fetal calf serum (inactivated by heating 30 min at 56°), antibiotics (100 IU of penicillin/mL and 100 μg of streptomycin/mL), and PHA (50 μL; phytohemagglutinin from *Phasoleus vulgaris*, HA 16, Wellcome, 1 μL/mL of cell culture).

To test the cytotoxic activities for stimulated lymphocytes, the drugs were added together with PHA when the cells were seeded. To test the cytotoxicity for steady-state lymphocytes, the cells were first incubated for 2, 4, or 24 h with the drugs, then they were washed three times, centrifuged off, and resuspended in RPMI 1640 medium (150 μL) and PHA (50 μL). In both cases, the cells were incubated for 72 h at 37° in an atmosphere of 19:1 air–CO_2. The ketonucleosides to be tested were diluted with 50% ethanol, and 1 μL of each dilution was added to each well of the microplates. Each experiment was conducted in triplicate and the data presented are the mean of at least three independent experiments. Twelve hours before the cells were harvested, [^{3}H] thymidine (37 kBq) was added to each well. The cultures were harvested and washed with an automated sample harvester (Skatron) on glass-fiber filters. The filters were dried and the radioactivity was counted in Omnifluor (1 mL) in a liquid-scintillation spectrometer (Kontron). A curve was plotted from the results obtained, and the ketonucleoside concentration that inhibits cell growth by half (IC_{50}), as compared with control cells, could be estimated.

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