*Carbohydrate Research*, 248 (1993) 143–150
Elsevier Science Publishers B.V., Amsterdam



# Synthesis and antineoplastic properties of 3′-deoxy-3′-fluoroketonucleoside derivatives. Correlations between structure and biological activity

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(Received December 19th, 1992; accepted May 3rd, 1993)

## ABSTRACT

Three fluoroketonucleosides (**6**, **8**, and **11**) have been synthesized by direct oxidation of the fluoro precursors. The presence of the highly electronegative fluorine atom in the $\alpha$ position to the carbonyl group favours hydration leading to the *gem*-diol form so that the $\beta$-elimination process to afford **6** and **8** was made difficult and failed in the case of the difluoro compound **11**. The biological activity of compounds **6**, **8**, and **11** was tested on human peripherical blood lymphocytes stimulated by PHA, and on RAJI and DAUDI cells. The $IC_{50}$ values showed that, surprisingly, the 3′-enopyranosyl-2′-uloses **6** and **8** have much better antineoplastic activities than their 2′-enopyranosyl-4′-ulose analogues **14** and **15** obtained previously. Moreover, compound **11**, which is difluorinated at C-3′ and C-6′ but does not have a C=C–C=O group in its structure, is also very active. These results emphasize the important biological role played by the fluorine atom in this family of compounds and suggest a peculiar mechanism of action which is until now unspecified.

## INTRODUCTION

In recent years, considerable interest in fluoropentonucleosides has been generated in order to find new antitumor, antiviral, or anti-HIV drugs with better selectivity and less immunosuppressive effects compared with known clinically used compounds[1]. The unusual biological activity of certain fluorinated compounds may be due to several causes not yet well understood. Although substitution of a fluorine atom for a hydroxyl group is sterically conservative, the high strength of the C–F bond may hinder metabolism pathways, and thus increases the effective lifetime of the active molecule. In addition, because fluorine is more electronegative, it should be capable of accepting, albeit weakly, a hydrogen bond and such electronegativity may also have important mechanistic consequences when the substitution is adjacent to a reaction center. The introduction of a fluorine atom also raises the lipophilicity of the compound and makes the permeability of the drug through the cell membrane easier.

In our laboratory, various unsaturated ketohexopyranosyl nucleosides have been synthesized in the last few years and their significant in vitro and in vivo inhibitory activity against various types of cancer cells has been clearly demonstrated[2]. In order to lessen their toxicity and to increase their lipophilicity, we undertook to synthesize ketonucleosides containing fluorine.

In a recent paper[3], we reported the first synthesis of an unsaturated fluoroketonucleoside. This 3′-deoxy-3′-fluoro-4′-ketonucleoside (**14**) showed a much better antineoplastic activity and a lower immunosuppressive effect than its analogue **15** towards splenic lymphocytes (steady state or stimulated by PHA) and to RAJI and DAUDI cells. We now describe the synthesis of compounds **6**, **8**, and **11** in order to examine their structure–activity relationship and to compare the activity of 2′- and 4′-keto analogues.

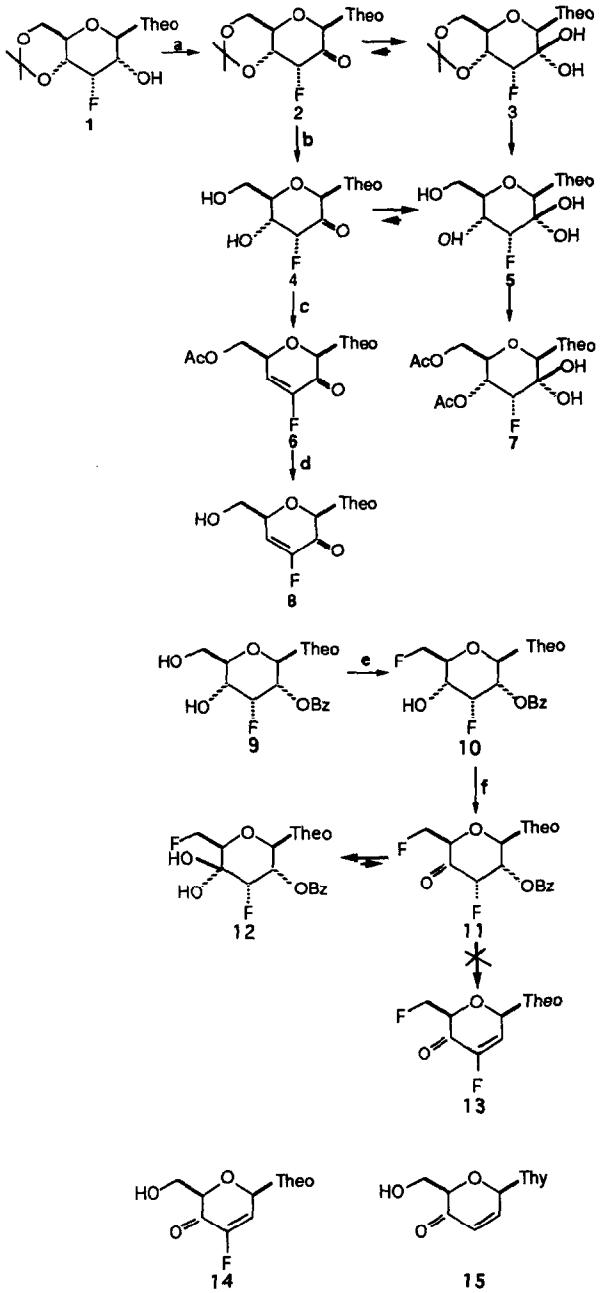
## RESULTS AND DISCUSSION

Compound **1**[4] was oxidized at C-2 with pyridinium chlorochromate (PDC)—molecular sieves[5], giving rise to compound **2**, which was quickly transformed into the hydrate **3** during the purification processes. The presence of an electron-withdrawing fluorine atom $\alpha$ to the carbonyl causes its easy hydration[3]. Fluoropyruvate is known to exist as a *gem*-diol in crystalline form as well as in aqueous solution[6]. Even when kept under dry nitrogen until the next step, it is very difficult to obtain pure **2** and prolonged heating of **3** in vacuo did not result in pure **2**. When a mixture of **2** and **3** was deacetonated and then treated with acetic anhydride—pyridine, the diacetate **7** was mainly obtained with only a small amount of the unsaturated **6**.

In order to obtain better yields of **2**, we oxidized **1** with $Me_2SO$–oxalyl chloride[7] and, after concentration, the mixture was directly deacetonated and then treated with pyridine–acetic anhydride to obtain **4** and **6** in good yield. Deacetylation of **6**, which is very unstable under basic conditions, was performed with methanolic hydrogen chloride to afford **8**[8].

The 3,6-difluoro compound **10** was obtained in 50% yield by reaction of **9**[3] with 4 equivalents of diethylaminosulfur trifluoride (DAST) in dichloromethane. Oxidation of **10** with PDC–molecular sieves or with oxalyl chloride in dichloromethane afforded **11**, which is made so reactive by the presence of the two fluorine atoms that it is already hydrated as the *gem*-diol **12**. So, attempts to obtain the alkene **13** from **11** by different methods failed.

The biological activity of the fluoroketonucleosides **6** and **8** was tested on human peripherical blood lymphocytes stimulated by PHA, and on RAJI and DAUDI cells both derived from Burkitt lymphomas that induce tumors in *nude* mice and express the EBV (Epstein–Barr virus) antigens. The experimental procedure was exactly the same as that described in a previous paper[3]. 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




Scheme 1. (a) PCC, 3A molecular sieves, $CH_2Cl_2$, 20°C, 90 min or oxalyl chloride, $Me_2SO$, $CH_2Cl_2$, −70°C, 30 min, $Et_3N$; (b) Amberlite IR-120 ($H^+$) resin, 60°C, 90 min, Amberlite IR-45 ($HO^-$) resin; (c) pyridine–acetic anhydride, 20°C, 3 h; (d) dry methanolic hydrogen chloride, $CH_2Cl_2$, 20°C, 18 h; (e) DAST, $CH_2Cl_2$, 0°C, 4 h, 20°C, 15 h; (f) PCC, 3A molecular sieves, $CH_2Cl_2$, 20°C, 3 h.

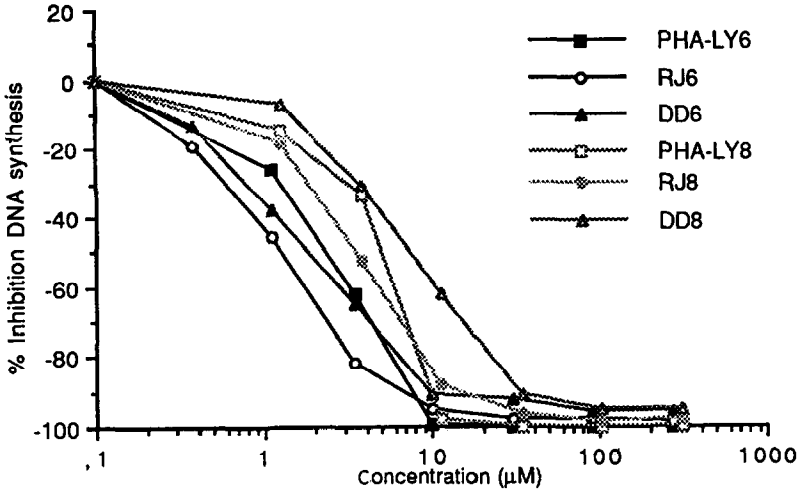



Fig. 1. Effects of ketonucleosides **6** and **8** on human peripherical blood lymphocytes stimulated by PHA (PHA-LY), and RAJI (RJ) and DAUDI (DD) cells.

thymidine incorporation reflects, therefore, the antiproliferative properties of the test drug.

The results are given in Figs. 1 and 2, and in Table I. Fig. 1 shows that compound **6**, possessing an *O*-acetyl group in the 6′ position, is between 2 and 3 times more active than **8** whose 6′-hydroxyl is unblocked. We have noticed before[2]

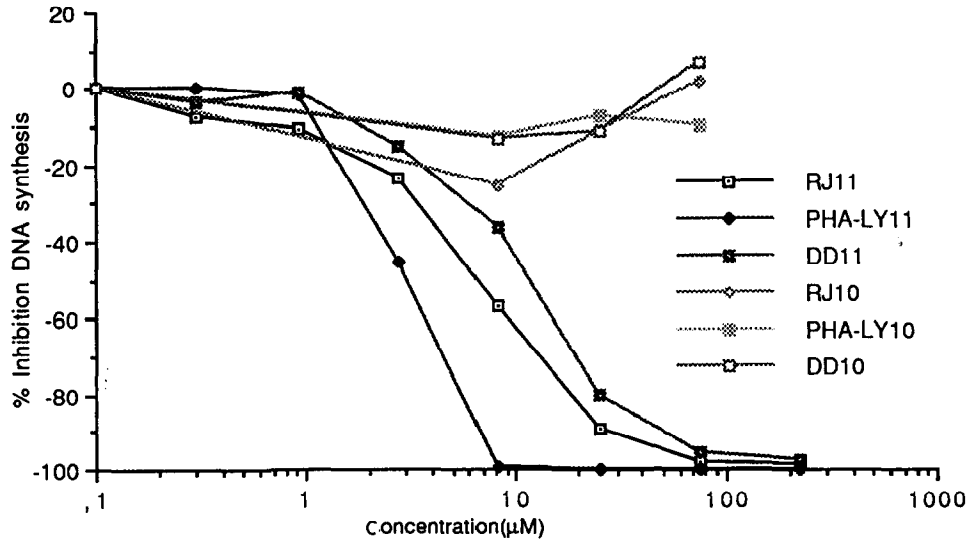



Fig. 2. Effects of ketonucleosides **10** and **11** on human peripherical blood lymphocytes stimulated by PHA (PHA-LY), and on RA(RJ) and DAUDI(DD) cells.

TABLE I

$IC_{50}$ ($\mu$M) values for compounds **6**, **8**, **14**, **15**, **11**, and **10** for lymphocytes stimulated by PHA (PHA-LY), and for RAJI and DAUDI cells

| Compound | PHA-LY | RAJI | DAUDI |
|---|---|---|---|
| **6** [a] | 2.2 | 1.2 | 1.8 |
| **8** [a] | 4.5 | 3.5 | 7.4 |
| **14** [b] | 23 | 45 | 65 |
| **15** [b] | 27 | 220 | 290 |
| **11** [a] | 2.7 | 6.5 | 11.2 |
| **10** [a] | > 100 | > 100 | > 100 |

[a] Tested on human peripherical blood lymphocytes. [b] Tested on murine splenic lymphocytes: see ref 3. Differences of activity of the compounds towards murine and human lymphocytes have been tested (unpublished results) and never exceeded 20%.

that compounds with 6′-$CH_3$ or 6′-$CH_2OR$ were more active than those with free $CH_2OH$. On the other hand, we were very surprised to notice that the 2′-ketofluoro nucleoside **8** was much more active (between 1 and 30 times) than its 4′-keto analogue **14** (Table I), whereas the contrary is usually observed for non-fluorinated ketohexonucleosides[2].

Compound **11**, which contains two fluorine atoms (one of which is adjacent to the 4′-carbonyl group) but is not $\alpha,\beta$-unsaturated, was almost as active as **6** and **8** and much more so than **14** and **15** where the carbonyl group is also in the 4′ position. Compound **10**, which has no carbonyl group, is inactive. Until now, the ketohexonucleosides without unsaturation or an epoxy group $\alpha$ to the carbonyl were little or not active[2]. So, the presence of the two electronegative fluorine atoms confers a peculiar behaviour to this molecule. It can be suggested that, during the metabolisation process, the fluorine atom at C-6′, which is $\beta$ to the carbonyl group, is released leading to an exocyclic unsaturated ketonucleoside.

We are currently carrying out other in vitro experiments with these molecules in order to grasp better the biological features and the antineoplastic potential of this new class of compound.

## EXPERIMENTAL

*General methods.*—TLC was performed on Silica Gel 60 $F_{254}$ (Merck) and flash-column chromatography on Silica Gel 60 (240–400 mesh, Merck). $[\alpha]_D^{20}$ values were determined on solutions in MeOH (*c* 0.1). NMR spectra were recorded at room temperature with a Bruker 300 MSL spectrometer with internal $Me_4Si$ for $^1H$ and internal $C_6F_6$ for $^{19}F$. The positions in carbohydrate moieties are designated by primes. $Me_2SO$ was distilled from $CaH_2$ under reduced pressure and stored over 3A molecular sieves. Oxalyl chloride was freshly distilled under $N_2$ and kept in a sealed bottle.

*7-(3-Deoxy-3-fluoro-4,6-*O*-isopropylidene-β-*D*-ribo-hexopyranosyl-2-ulose)theophylline* (**2**) *and* gem*-diol* **3**.—*Procedure A.* A mixture of **1**[4] (1 mmol, 385 mg; dried

by co-evaporation with toluene), pyridinium chlorochromate (2 mmol, 800 mg), and 3A molecular sieves (1 g) was stirred in dry $CH_2Cl_2$ (15 mL) for 90 min. The mixture was then filtered through silica gel (Merck) which was washed with 200 mL of $CH_2Cl_2$. The $CH_2Cl_2$ was evaporated in vacuo, the resulting syrup was purified by flash chromatography (3:1 EtOAc–hexane), and a mixture (193 mg, 50%) of **2** and **3** was obtained in a semi-crystalline form from EtOAc.

*Procedure B.* All reactions were carried out under scrupulously dry conditions and under an $N_2$ atmosphere. To a stirred solution of 1.05 mmol of oxalyl chloride in $CH_2Cl_2$ (3.6 mL) at −70°C was slowly added a solution of $Me_2SO$ (2.2 mmol) in $CH_2Cl_2$ (1 mL). The mixture was kept at −40°C for 3 min, then cooled to −70°C. A solution of **1** (1 mmol, 385 mg) in $CH_2Cl_2$ (1 mL) was then added dropwise. Stirring at −70°C was continued for 30 min before slow addition of ca. 3.7 equiv of $Et_3N$ so that the pH did not exceed 7. The mixture was then allowed to warm to room temperature and evaporation to dryness was effected under high vacuum. The residue (stored under $N_2$) was used for the next step without further purification (crude yield: 70%); $[\alpha]_D^{20}$ +40°; $\lambda_{max}$ 275 nm ($\epsilon$ 7330); $^1$H NMR ($CDCl_3$): $\delta$ 6.07 (d, 1 H, $J_{1',5'}$ 2.17 Hz, H-1'), 4.73 (d, 1H, $J_{F,3'}$ 51.9 Hz, H-3'), 4.17 (dd, 1H, $J_{F,4'}$ 28.8, $J_{4',5'}$ 9.77 Hz, H-4'), 4.05–3.89 (m, 3 H, H-5',6'a,6'b); $^{19}$F NMR: $\delta$ −36.5. Anal. Calcd for $C_{16}H_{19}FN_4O_6 \cdot H_2O$: C, 48.00; H, 5.25; F, 4.75; N, 14.00. Found: C, 47.47; H, 5.26; F, 4.63; N, 13.64.

*7-(3-Deoxy-3-fluoro-β-*D*-*ribo*-hexopyranosyl-2-ulose)theophylline* (**4**) *and* gem*-diol* **5**.—Refluxing of a dry methanolic solution of **2** (380 mg, 1 mmol) with Amberlite IR-120 ($H^+$) cation-exchange resin at 60°C for 90 min, neutralization with Amberlite IR-45 ($HO^-$) anion-exchange resin, then filtration, and concentration gave **4** in a semi-crystalline form (240 mg, yield 70%) from ethyl ether; the product must also be stored under dry $N_2$ to avoid an equilibrium with the corresponding *gem*-diol **5**; $[\alpha]_D^{20}$ +45°; $\lambda_{max}$ 275 nm ($\epsilon$ 9900); $^1$H NMR ($CDCl_3$): $\delta$ 6.55 (s, 1 H, H-1'), 4.72 (dd, 1 H, $J_{F,3'}$ 49.75, $J_{3',4'}$ 1.91 Hz, H-3'), 4.28 (ddd, 1 H, $J_{F,4'}$ 19.4, $J_{4',5'}$ 3.11 Hz, H-4'), 4.0–3.55 (m, 3 H, H-5',6'a,6'b). Anal. Calcd for $C_{13}H_{15}FN_4O_6 \cdot 0.5\ H_2O$: C, 44.44; H, 4.56; F, 5.41; N, 15.95. Found: C, 44.84; H, 4.98; F, 5.25; N, 15.11.

*7-(6-*O*-Acetyl-3,4-dideoxy-3-fluoro-β-*D*-*glycero*-hex-3-enopyranosyl-2-ulose)theophylline* (**6**).—Crude **4** (340 mg, 1 mmol), obtained from procedure *B*, was dissolved in a mixture of pyridine (2 mL) and $Ac_2O$ (1 mL) and stirred at room temperature for 3 h. After being diluted with $CH_2Cl_2$, the resulting solution was washed twice with water, dried, and concentrated. Purification by flash chromatography (3:1 EtOAc–hexane) gave **6** in a semi-crystalline form (yield 50%); $^1$H NMR ($CDCl_3$): $\delta$ 6.67 (dd, 1 H, $J_{F,4'}$ 11.2, $J_{4',5'}$ 1.72 Hz, H-4'), 6.57 (s, 1 H, H-1'), 5.10 (m, 1 H, H-5'), 4.37 (m, 2 H, H-6'a,6'b), 2.14 (s, 3 H, OAc); $^{19}$F NMR: −39.5 (q, $J_{F,4'}$ 11.2, $J_{F,5'}$ 7.5 Hz). Anal. Calcd for $C_{15}H_{13}FN_4O_6$: C, 49.18; H, 4.10; F, 5.19; N, 15.30. Found: C, 49.21; H, 4.46; F, 4.36; N, 14.49.

When **4** was obtained from procedure *A*, TLC showed the presence of two compounds which were separated by flash chromatography (3:1 EtOAc–hexane): the faster was **6** (10%) and the slower was identified as **7** (50%) by $^1$H NMR

($CDCl_3$): δ 6.29 (d, 1 H, $J_{1',5'}$ 2.58 Hz, H-1'), 5.18 (dd, 1 H, $J_{3',4'}$ 2.10, $J_{3',F}$ 50.9 Hz, H-3'), 4.10 (ddd, 1 H, $J_{4',5'}$ 7.15, $J_{4',F}$ 14.6 Hz, H-4'), 3.72–3.58 (m, 3 H, H-5',6'a,6'b), 2.16 and 2.07 (2 s, 6 H, 2 $CH_3CO$).

*7-(3,4-Dideoxy-3-fluoro-β-*D*-glycero-hex-3-enopyranosyl-2-ulose)theophylline* (**8**). —To a solution of **6** (1 mmol, 366 mg) in dry $CH_2Cl_2$ (2 mL) was added, at room temperature, 2 mL of methanolic hydrogen chloride prepared by addition at 0°C under Ar of acetyl chloride (0.2 mL) to dry MeOH (5 mL). After 18 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 ($MgSO_4$), and concentration, the crude mixture was purified by flash-column chromatography (3:2 EtOAc–hexane) to give **8**; $[\alpha]_D^{20}$ −12.5°; $\lambda_{max}$ 275 nm (ε 7790); $^1$H NMR ($CDCl_3$): δ 6.70 (dd, 1 H, $J_{1',4'}$ 1.60, $J_{F,4'}$ 11.7 Hz, H-4'), 6.36 (d, 1 H, $J_{1',4'}$ 1.60 Hz, H-1'), 5.04 (m, 1 H, H-5'), 3.95–3.82 (m, 2 H, H-6'a,6'b); $^{19}$F NMR: δ −39.2.

*7-(2-O-Benzoyl-3,6-dideoxy-3,6-difluoro-β-*D*-allo-hexopyranosyl)theophylline* (**10**). —To a stirred solution of **9**[3] (1 mmol, 448 mg) in dry $CH_2Cl_2$ (5 mL) were slowly added at −20°C 4 equiv (0.39 mL) of diethylaminosulfur trifluoride (DAST). The temperature was raised slowly to 0°C and maintained thereat for 4 h, and then raised to room temperature for another 15 h. The mixture was then quenched by MeOH (2 mL) and poured into satd aq $NaHCO_3$. Extraction into $CH_2Cl_2$ followed by drying and concentration afforded a colorless syrup. Flash-column chromatography (3:7 hexane–EtOAc) afforded **10** as a white powder from diethyl ether (yield 50%); $[\alpha]_D^{20}$ −35°, $\lambda_{max}$ 275 nm (ε 7400); $^1$H NMR ($CDCl_3$): δ 5.38 (d, 1 H, $J_{3',F}$ 53.6 Hz, H-3'), 4.8 (dd, 2 H, $J_{6',F}$ 47.18, $J_{5',6'a,6'b}$ 1.9 Hz, H-6'a,6'b), 4.17 (m, 1 H, $J_{4',5'}$ 2.73, $J_{5',F}$ 18.2 Hz, H-5'); $^{19}$F NMR: δ −46.5. Anal. Calcd for $C_{20}H_{20}F_2N_4O_6 \cdot 0.5\ H_2O$: C, 52.28; H, 4.57; F, 8.27; N, 12.20. Found: C, 52.41; H, 4.76; F, 7.68; N, 11.73.

*7-(2-O-Benzoyl-3,6-dideoxy-3,6-difluoro-β-*D*-ribo-hexopyranosyl-4-ulose)theophylline* (**11**) *and* gem-*diol* **12**.—Compound **10** (1 mmol, 450 mg) was treated with pyridinium chlorochromate (4 mmol, 860 mg) and 3A molecular sieves (1 g) in dry $CH_2Cl_2$ according to procedure *A* for 3 h. After filtration, washings, and concentration, the resulting syrup was purified on a column of Silica Gel 60 eluted with diethyl ether. The *gem*-diol **12** (225 mg, 50%) crystallized from $CH_2Cl_2$; $[\alpha]_D^{20}$ +5°; mp 126–128°C; $\lambda_{max}$ 275 nm (ε 6950); $^1$H NMR ($CDCl_3$): δ 4.94 (ddd, 2 H, $J_{F\text{-}6,6'}$ 46.3, $J_{5',6'}$ 2.6, $J_{6'a,6'b}$ 10.5 Hz, H-6'a,6'b), 4.91 (d, 1 H, $J_{F\text{-}3',3'}$ 53.3 Hz, H-3'), 4.5 (m, 1 H, $J_{F\text{-}6',5'}$ 20.9 Hz, H-5'). Anal. Calcd for $C_{20}H_{18}F_2N_4O_6 \cdot H_2O$: C, 50.52; H, 4.42; F, 8.00; N, 11.78. Found: C, 51.07; H, 4.72; F, 7.17; N, 12.23.

ACKNOWLEDGMENTS

We thank the Association pour la Recherche sur le Cancer (ARC) for financial support, and the Ligue Nationale de lutte contre le Cancer for a grant to M.I. Bennani-Baiti.

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