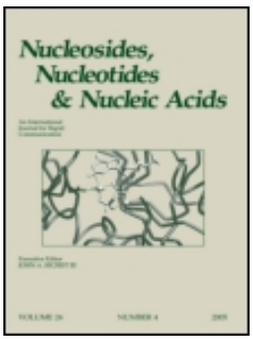


Nucleosides, Nucleotides and Nucleic Acids

ISSN: 1525-7770 (Print) 1532-2335 (Online) Journal homepage: http://www.tandfonline.com/loi/lncn20

UNSATURATED FLUOROKETONUCLEOSIDES AS ANTICANCER AGENTS: THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-FLUORO-1-(3,4-DI-DEOXY-3-FLUORO-6-O-TRITYL-β-D-GLYCERO-HEX-3-ENO-PYRANOS-2-ULOSYL) URACIL

M. J. Egron , B. I. Dorange , K. Antonakis , J. Herscovici & A. P. Ollapally

To cite this article: M. J. Egron , B. I. Dorange , K. Antonakis , J. Herscovici & A. P. Ollapally (2002) UNSATURATED FLUOROKETONUCLEOSIDES AS ANTICANCER AGENTS: THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-FLUORO-1-(3,4-DI-DEOXY-3-FLUORO-6-O-TRITYL-β-D-GLYCERO-HEX-3-ENO-PYRANOS-2-ULOSYL) URACIL, Nucleosides, Nucleotides and Nucleic Acids, 21:4-5, 327-334, DOI: 10.1081/NCN-120006828

To link to this article: http://dx.doi.org/10.1081/NCN-120006828

Published online: 31 Aug 2006.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 21(4&5), 327–334 (2002)

UNSATURATED FLUOROKETONUCLEOSIDES AS ANTICANCER AGENTS: THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-FLUORO-1-(3,4-DI-DEOXY-3-FLUORO-6-O-TRITYL-β-D-*GLYCERO*-HEX-3-ENO-PYRANOS-2-ULOSYL) URACIL

M. J. Egron,[1] B. I. Dorange,[2] K. Antonakis,[1] J. Herscovici,[1] and A. P. Ollapally[2]

[1]Ecole Nationale Supžrieure de Chimie, CNRS-UMR 7001;
11, Rue P.et M. Curie-75005 Paris, France
[2]Department of Chemistry, Florida A&M University,
Tallahassee, FL 32307, USA

ABSTRACT

Direct oxidation of 5-fluoro-1- (4-*O*-acetyl-3-deoxy-3-fluoro-6-*O*-trityl-β-D-glucopyranosyl) uracil **9** led to the title compound **10** after a β-elimination reaction. The formation of the hydrate of ketone **10** due to the highly electronegative fluorine atom in the α position to the carbonyl group, prompted us to carry out a comparative study of different methods of oxidation and to define the best strategy for the synthesis of such molecules. Results of in vitro and in vivo biological evaluations are reported.

INTRODUCTION

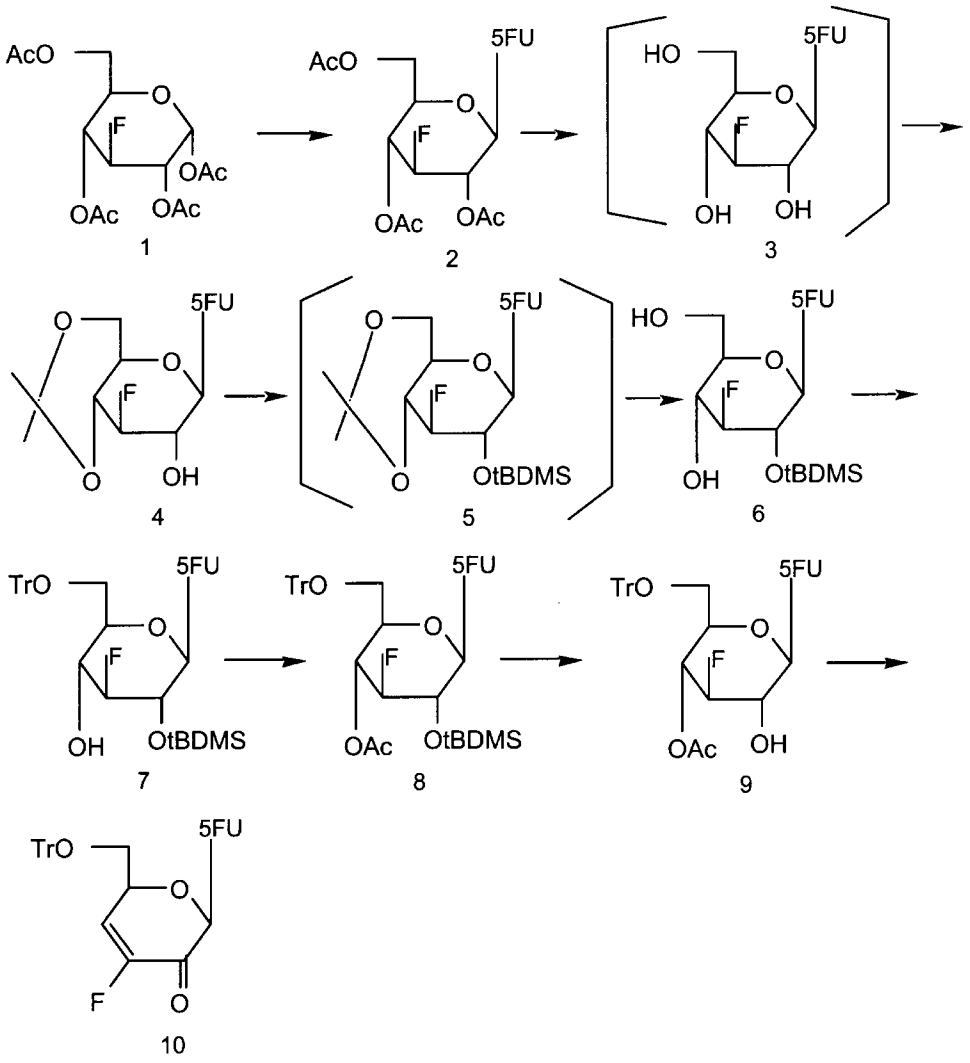
The introduction of a fluorine atom in a molecule is known to confer on it low toxicity and increased permeability through the cell membrane by increasing the lipophilicity. Although substitution of a fluorine atom for a hydroxyl group is sterically conservative, the high strength of the C-F bond

may hinder metabolism pathways and thus increase the effective lifetime of the active molecule.[1–4] The syntheses of several fluoroketonucleosides possessing significant antineoplastic activity and immunosuppressive effect have been reported.[5–7] These studies on various normal and tumor cells showed that the introduction of a fluorine atom in 3′-position of an unsaturated 4′-ketonucleoside leads to an important difference in the toxicity toward neoplastic and normal cells, [6] the cytotoxicity was shown to be significantly higher for the neoplastic than the normal cells. In addition, the comparison of the antineoplastic activity and the immunosuppressive effect of this fluoro-4′-ketonucleoside with an analog devoid of the fluorine atom showed a higher antineoplastic activity and a lower immunosuppressive effect (for steady state cells) for the fluorocompounds.[5–6] To further confirm this observation with other analogs, the synthesis and the in vitro antitumor activity of fluoro analogs of unsaturated 2′-ketonucleosides has recently been reported.[7]

These observations prompted us to synthesize a keto fluoronucleoside possessing a fluoro group in the 3′-position and the keto group in the 2′-position. Syntheses of unsaturated 4′-ketonucleosides containing fluoro groups in the 3′-position possessing either a purine or a pyrimidine base have been reported.[5, 6] It appears that the crucial problem of these syntheses is the oxidation of OH groups in the sugar moiety[8] and the nature of the base. Although the synthesis of the fluoro 4′-ketonucleosides containing purine as well as pyrimidine bases could be carried out without many difficulties, that of fluoro-2′-ketonucleosides of pyrimidines has proved to be delicate, giving unforeseen results. In a recent note,[7] we reported briefly the synthesis of an unsaturated 3′-fluoro-2′-ketonucleoside of 5-fluorouracil. We now report a complete comparative study of the different routes to this biologically important molecule.

RESULTS AND DISCUSSION

The free 3′-fluoro derivative of fluorouracil **3** (see the Scheme) has been prepared from 1,2,4,6-tetra-*O*-acetyl-3-deoxy-3-fluoroglucose **1** by a recently described method.[7] The molecule was protected in 4′,6′-position with a isopropylidene group, using 2,2-dimethoxypropane in dry DMF. This partially protected compound **4** was obtained pure as a crystalline material but the yields were relatively low. The isopropylidene derivative 4 should be isolated from the organic layer by extraction. However, this method gave better results than that involving methoxyethylidene as the blocking group. The protection of the 2′-hydroxyl was performed using t-butylchlorodimethylsilane with imidazole to give the 2′-*O*-t-butyldimethylsilyl derivative **5** in more than 90% yield, which could be deacetalated directly with IR 120 resin in methanol to give **6** in excellent yield. Some other methods of acid hydrolysis of acetals have been examined. In particular the



Scheme.

method using catalytic amounts of p-toluenesulfonic acid led to a mixture containing the starting material. The specific protection of 6′-hydroxyl group was accomplished with triphenylmethyl chloride in the presence of dimethylaminopyridine (DMAP) in pyridine. The 6′-trityl derivative **7** was easily isolated pure in 60% yield. The following step consisted of the acetylation of 4′-hydroxyl with acetic anhydride in pyridine in the presence of DMAP. This easily gave the desired **8** in 95% yield. The conditions for the specific removal of the 2′-t-butyldimethylsilyl group were established after a careful study of the action of tetrabutylammonium fluoride trihydrate (TBAF) on **8** in THF. After 30 min in dry conditions and under nitrogen, the mixture was extracted

with CH_2Cl_2 to give pure **9** in excellent yields. The oxidation of nucleoside structures such as **9** is a crucial problem depending on many factors difficult to predict.[8–11] Different oxidation methods have been studied. The use of PDC/3 Å molecular sieves[11] gave pure ketonucleoside **10** in poor yields (10–12%). After a chromatographic study, we were able to find the best conditions and improved the yields to only up to 20% by stopping the reaction after 2 h. The low yields are probably due to the long reaction time and possibly the formation of small quantities of *gem*-diol. The chromatographic study showed that the ketonucleoside appeared after one hour of reaction and no degradation of the compound appeared before 2 h. After this time a little degradation appeared but also the filtration of the mixture on silicagel might have contributed to a further degradation.

An alternative approach consisted of the use of the DMSO/acetic anhydride at room temperature for 48 h. This method led to an approximately 1:1 mixture of the desired **10** and its *gem*-diol. The same method at 40°C for 6 h gave the ketonucleoside and its gem-diol in a 7:3 ratio, but difficulties in the separation of the derivatives led finally to low yields of pure **10** (15%) characterized by NMR and physical data.

Another method based on the use of oxalyl chloride, appeared to be promising since we obtained pure **10** in about 20% yields, without resorting to tedious purifications. We believe that improvements in the technical conditions of this approach, such as temperature and time of reaction, may significantly improve the yields.

The in vitro biological activity of fluoroketonucleoside **10** was tested on HT29 human colon carcinoma cells and KB human epidermoid carcinoma cells in comparison with **9** both possessing a 6′-*O*-trityl group. This study clearly confirmed previous results, that the introduction of an enone system in a nucleosidic structure, increases the anti-tumor properties of the molecule.

IC50 (μM) Values for Compounds **9** and **10** for KB and HT29 Cells

	KB	HT29
9	10	15
10	0.7	5

The in-vivo tests performed on mice bearing RXF 393 renal tumor, showed moderate activity for high tolerate doses (7.50 mg/kg/dose), the compound **10** was well tolerated and no loss of body weight was observed in comparison to the control mice. Further investigations with various tumors will be undertaken.

EXPERIMENTAL

General methods: Melting points (uncorrected) were recorded using a Mel-Temp apparatus. TLC was performed on Silica Gel 60 (240–400 mesh, Merck). 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 over CaH_2 under reduced pressure and stored over 3Å molecular sieves. Oxalyl chloride was freshly distilled under N_2 and kept in a sealed bottle.

5-Fluoro-1-(3-deoxy-3-fluoro-2, 4, 6-tri-*O*-acetyl-β-D-glucopyranosyl)uracil (2). In a flask, 3.55 g (27.3 mmol) of 5-fluorouracil and a small quantity of saccharin were dried by dissolving in benzene and concentrating, then 53 mL of dried acetonitrile and 19.1 mL (2 equiv) of hexamethyldisilazane were added. The solution was heated at 80°C for 30 min under reflux, then concentrated. 7.85 g (22.4 mmol) of the tetraacetylated fluoro-D-glucose **1** in anhydrous acetonitrile (2 mL) was added to the previous flask containing silylated 5-fluorouracil. The mixture was kept at 0°C and then was added a solution of 1.44 mL (1.2 equiv) of trimethylsilyl trifluoromethanesulfonate in 2 mL anhydrous acetonitrile. It was heated at 85°C for 3 h, then cooled to ambient temperature and stirred overnight. After evaporation of the solvent, cold water (0°C) was added and the mixture was neutralized with aq. $NaHCO_3$ (10%), at 0°C and then extracted with CH_2Cl_2 (3×50 mL). This product was purified by flash chromatography, eluting with ethyl acetate/heptane, (6:4 v/v).

Crystallization from ethanol, gave **2** in 92% yield: mp and elemental analysis agreed with the data previously reported.[7]

5-Fluoro-1-(3-deoxy-3-fluoro-β-D-glucopyranosyl)uracil (3). To a solution of 8.5 g (20.23 mmol) of **2** in 80 mL of anhydrous methanol was added 8 mL of 2 N MeONa. After 1.5 h, the solution was neutralized with IR 120 resin and concentrated to give 5.64 g of **3** as an oil (yield 95%), which was used directly in the next step.

5-Fluoro-1-(3-deoxy-3-fluoro-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-uracil (4). To 7.69 g (21 mmol) of **3** dissolved in a mixture of 19 mL of dry 2, 2-dimethoxypropane and 50 mL of DMF were added 20 drops of conc. H_2SO_4. This mixture was stirred for 5 h and then neutralized with $NaHCO_3$. The mixture was filtered and the liquid phase was concentrated under high vacuum to remove the DMF. Pure crystalline **4** was obtained from a mixture of heptane and ethanol, in 60% yield: mp 152–154°C. Anal. Calcd for $C_{13}H_{16}O_8N_2F_2$ $(1/2C_7H_{16})$: C, 47.59; H, 5.77; N, 6.73; Found: C, 47.92; H, 5.61; N, 7.16.

5-Fluoro-1-(2-*O*-t-butyldimethylsilyl-3-deoxy-3-fluoro-4, 6-*O*-isopropylidene-β-D-glucopyranosyl)uracil (5). A mixture of 5.16 g (14.1 mmol) of **4**, 4.13 g. (27.53 mmol, 1.2 equiv) of t-butylchlorodimethylsilane and 2.39 g (2.5 equiv) of imidazole, were dried with benzene. DMF (9.6 mL) were then added and the solution was stirred for 14 h at 35°C. The mixture was concentrated under high vacuum and the residue extracted with CH_2Cl_2. Flash chromatography (heptane/ethyl acetate 6:4 v/v), gave 6.09 g of **5** in 90% yield as an oil which was used directly in the next step.

5-Fluoro-1-(2-*O*-t-butyldimethylsilyl-3-deoxy-3-fluoro-β-D-glucopyranosyl)-uracil (6). Compound **5** was deacetalated by heating at reflux for 1.5 h in methanol containing IR 120 resin. The mixture was then neutralized with IR 45 resin. Pure **6** was isolated as a solid with CH_2Cl_2 in 90% yield: mp 128–132°C.

Anal. Calcd for $C_{16}H_{26}O_6SiN_2F_2$: C, 47.05; H, 6.37; N, 6.94: Found: C, 46.88; H, 6.54; N, 6.86.

5-Fluoro-1-(2-*O*-t-butyldimethylsilyl-3-deoxy-3-fluoro-6-*O*-trityl-β-D-gluco-pyranosyl)uracil (7). To 5 g (11.11 mmol) of **6** was dissolved in 55 mL of anhydrous pyridine was added, 4.046 g of triphenylmethylchloride and a catalytic amount of DMAP. The solution was stirred for 24 h at room temperature and then extracted with CH_2Cl_2 and concentrated. The tritylated product was purified (60% yield) by flash chromatography (ethyl acetate/heptane 6:4 v/v) and crystallized from ethanol: mp 224–226°C. Anal. Calcd for $C_{35}H_{40}O_6SiN_2F_2$: C, 64.61; H, 6.15; N, 4.30. Found: C, 64.49; H, 6.18; N, 4.24. ^{1}HNMR ($CDCl_3$): δ 7.49–7.20 (m, 15 H, $3C_6H_5$), 5.65 (d, 1H, $J_{1',2'} = 8.81$ Hz, H1′), 4.45 (dt, 1H, $J_{F,3'} = 51.88$ Hz, $J_{F,4'} = 16.89$ Hz, $J_{3',4'} = 9.31$ Hz, H3′), 3.90 (m, 1H $J_{4',5'} = 3.94$ Hz, H4′), 3.63 (m, 2H, $J_{5',6'b} = 3.73$ Hz, H2′, H5′), 3.46 (2H, $J_{6'a,6'b}$, H6′).

5-Fluoro-1-(4-*O*-acetyl-2-*O*-t-butyldimethylsilyl-3-deoxy-3-fluoro-6-*O*-tritryl-β-D-glucopyranosyl)uracil (8). To 7.97 g (12.25 mmol) of **7** in 100 mL of CH_2Cl_2 were added 2 mL (1.2 equiv) of pyridine, 2.3 mL (1.2 equiv) of acetic anhydride and a small quantity of DMAP. After 30 min the solvents were evaporated in vacuo. Purification by flash chromatography (heptane/ethyl acetate 6:4 v/v) and crystallization from isopropylalcohol gave pure **8** in 95% yield: mp 212–214°C. Anal Calcd for **8** $C_{37}H_{42}O_7N_2F_2H_2O$: C, 62.41; H, 5.03; N, 4.69; Found: C, 62.07; H, 5.35; N, 4.45. ^{1}HNMR ($CDCl_3$): δ 7.49–7.20 (m, 15H, 3 C_6H_5), 5.66 (d, 1H, $J_{1',2'} = 8.6$ Hz, H1′), 5.28 (m, 1H, $J_{4',5'} = 10.5$ Hz, $J_{F,4'} = 22.04$ Hz, $J_{3',4'} = 10$ Hz, H4′), 4.48 (t, 1H, $J_{F,3',2'} = 51.48$ Hz, $J_{3',2'} = 8.78$ Hz, H3′), 3.77 (m 2H, $J_{5',6'b} = 4.64$ Hz, H2′, H5′), 3.16 (dm, 2H, $J_{6a',6'b} = 10.86$ Hz).

5-Fluoro-1-(4-*O*-acetyl-3-deoxy-3-fluoro-6-*O*-trityl-β-D-glucopyranosyl)-uracil (9). Tetrabutylammonium fluoride trihydrate was used to cleave the 2′-tert-butyldimethylsilyl group. This was done using 692 mg (1.00 mmol) of **8** and 1.039 g (3.3 mmol) of dry tetrabutylammonium fluoride trihydrate (TBAF) in anhydrous THF under nitrogen. After 30 min, the mixture was concentrated in vacuo and extracted with CH_2Cl_2. The compound **9** was obtained in 92% yield as a powder from diisopropyl ether: mp 133–136°C. Anal. Calcd for $CH_{31}H_{28}C_7N_2F_2H_2O$: C, 62.41; H, 5.03; N, 4.69; Found: C, 62.07; H, 5.35; N, 4.45. ^{1}HNMR ($CDCl_3$): δ 7.49–7.20 (m, 15H, $3C_6H_5$), 5.76 (d, 1H, $J_{1',2'} = 8.6$ Hz, H1′), 5.38 (m, 1H, $J_{F,4'} = 21.4$ Hz, $J_{4',5'} = 10.45$ Hz, $J_{3',4'} = 9.67$ Hz, H4′), 4.65 (dt, 1H, $J_{F,4'} = 21.45$ Hz, $J_{F,3'} = 52.46$ Hz, $J_{3',2'} =$ 8.9 Hz, H3′), 3.86 (m, 2H, $J_{5',6'b} = 3.97$ Hz, H2′, H5′), 3.25 (m, 2H, $J_{6'a,6'b} =$ 10.77 Hz, H6′), 1.87(1s, 3H, $COCH_3$)

5-Fluoro-1-(3,4-dideoxy-3-fluoro-6-*O*-trityl-β-D-glycero-hex-3-enopyranos-2-ulosyl)uracil (10). Method 1 (with PDC/molecular sieves): 3 g (5.26 mmol) of **9** and 3.9 g of PDC were dried with dry benzene. Then 100 mL of dry CH_2Cl_2, freshly activated 3 Å molecular sieves and 5 drops of acetic acid were added and the mixture was stirred during 5 h at room temperature. The mixture was then filtered through silica gel G, washed twice with CH_2Cl_2. The combined filtrate and washings were concentrated in vacuo. Then it was purified by Flash chromatography: The yields were low (10–12%). A chromatographic study showed that after 2 h the keto compound was formed in about 60% yield with 35% of the starting material remaining. Finally stopping the reaction after 2 h we could isolate the **10** in 20% yield: Anal. Calcd for $C_{29}H_{22}O_5N_2F_2$: C, 67.31; H, 4.45; N, 5.41; Found: C, 67.24; H, 5.01; N, 4.70. ^{1}HNMR ($CDCl_3$): δ 7.50–7.25 (m, 15H, 3 C_6H_5), 6.71 (m, 1H, $J_{F,4'} = 11.4$ Hz, $J_{4',5'} = 1.73$ Hz, H4′), 6.34 (s, 1H, H-1′), 4.88 (dd, 1H, $J_{5',6'a} = 4.89$ Hz, $J_{5',6'b} = 5.25$ Hz, H5′), 3.43 (dq, 2H, $J'_{6'a,6'b} = 9.43$ Hz, H6′).

Method 2 (with DMSO/Acetic anhydride): The reaction of 0.11 mmol of nucleoside **9** in DMSO (4 mL) and acetic anhydride (0.25 mL), after two days at room temperature, gave a 1:1 mixture of the ketonucleoside and the corresponding *gem*-diol detected by chromatography. This is due to the formation of hydrogen bonding favored by the method. *Improvement*: 0.11 mmol of **9**, 0.4 mL of DMSO and 0.3 mL of acetic anhydride stirred for 6 h at 40°C, led to a 7:3 mixture of the keto nucleoside **10** and its *gem*-diol. The reaction was followed by NMR analysis. The *gem*-diol was obtained in semi-crystalline form. Anal. Calcd for $C_{29}H_{24}O_6N_2F_2$: C, 65.06; H, 4.49; N, 5.24; Found: C, 64.56; H, 5.01; N, 4.57.

Method 3 (with oxalyl chloride): This method was carried out with 578 mg (1 mmole) of nucleoside **9**.

In first flask, 3.6 mL of dried CH_2Cl_2, and 91.68 mL (1.05 mmol) of freshly distilled oxalyl chloride were added and kept at −70°C. In a second flask: 1 mL of dried CH_2Cl_2, and 156 mL (2.2 mmol) of dried DMSO were

injected. The solution was kept at 40°C for 5 min then the contents of the first flask, kept at −70°C, was injected very slowly into the second flask. In a third flask, 1 mmol of **9** was dissolved in 1 mL of CH_2Cl_2 and kept at −40°C for 15 min then the contents of this flask were injected into the second flask. After 30 min the mixture was neutralized with 557 mL (3.7 equiv) of triethylamine and removing the DMSO and the remaining triethylamine yielded **10** (characterized by NMR) in 15–20% yield. This method was selected for the oxidation step by virtue of the ease with which the final fluoroenone compound could be isolated.

ACKNOWLEDGMENT

This work was supported by the National Institutes of Health through the MBRS program GM08111 and by the 3M Foundation, St Paul, Minnesota for basic research. We thank also the French Association pour la Recherche sur le Cancer (ARC) for financial support. The authors appreciate the excellent secretarial assistance rendered by Mrs. Glory Brown of the College of Pharmacy and Pharmaceutical Sciences.

REFERENCES

1. Van Aerschot, A.; Herdewijn, P.; Balzarini, J.; De Clercq, E. J. Med. Chem. **1989**, *32*, 1743–1749.
2. Blandin, M.; Son, T.-D.; Catlin, J.; Guschlbauer, J. Biochim. Biophys. Acta **1974**, *361*, 249–253.
3. Morton, G.; Lancaster, J.; Van Lear, G.; Fulmor, W.; Meyer, W. J. Amer. Chem. Soc. **1969**, *91*, 1535–1537.
4. Lipnick, R.; Fissekis, J. Biochm. Biophys Acta **1980**, *608*, 96–102.
5. Egron, M.J.; Leclercq, F.; Antonakis, K.; Bennani-Baiti, I.; Frayssinet, C. Carbohydr. Res. **1993**, *248*, 143–150.
6. Leclercq, F.; Egron, M.J.; Antonakis, K.; Bennani-Baiti, I.; Frayssinet, C. Carbohydr. Res. **1992**, *228*, 95–102.
7. Ollapally, A.P.; Dorange, B.I.; Whitaker, Jr., T.L. Nucleosides and Nucleotides **1999**, *18* (4&5), 703–705.
8. Crich, D.; Neelamkavil, S. J. Am. Chem. Soc. **2001**, *123*, 7449–7450.
9. Alaoui, M.; Egron, M.J.; Bessodes, M.; Antonakis, K. and Chouroulinkov, I. Eur. J. Med. Chem. **1987**, *22*, 305–310.
10. Komiotis, D.; Delatre, S.; Holt, S.; Ollapally, A.P.; Balzarini, J.; De Clercq, E.; Masuaki, I. Nucleosides and Nucleotides **1991**, *110*, 431–455.
11. Herscovici, J.; Antonakis, K. J. Chem. Soc. Chem. Commun. **1979**, *12*, 561–563.

Received November 1, 2001
Accepted April 30, 2002