Synthesis of ^{2}H-Labeled Alkoxyethyl Phosphodiester (AZT) Derivatives for Solid-State ^{2}H-NMR Studies

M. Koufaki*, T. Calogeropoulou, T. Mavromoustakos, E. Theodoropoulou

Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., GR-11635 Athens, Greece

Andrew Tsotinis

Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopoli Zografou, GR-15T71 Athens, Greece.

Alexandros Makriyannis*

Departments of Pharmaceutical Sciences, Molecular and Cell Biology and Institute of Materials Science, Storrs, Connecticut 06269, USA

Received February 13 1996

In memory of Professor Nicholas Alexandrou

Two specifically ^{2}H-labeled alkoxyethyl phosphodiester AZT derivatives **9** and **14** were synthesized. Pilot [^{2}H]-solid-state nmr experiments on conjugate **14** demonstrated their usefulness in studying interactions with model or biological membranes.

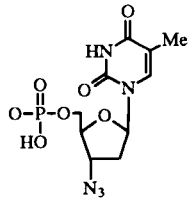
J. Heterocyclic Chem., **33**, 619 (1996).

Introduction.

3'-Azido-3'-deoxythymidine (AZT) is the most extensively used drug today against HIV infections, the etiologic agent of the acquired immunodeficiency syndrome (AIDS) [1,2]. To interact with its target enzyme, the HIV-associated reverse transcriptase, AZT must be phosphorylated intracellularly to its 5'-triphosphate derivative [3,4]. However, the therapeutic potential of AZT is hampered by serious adverse reactions, particularly bone marrow suppression. An attractive approach to overcome the drawbacks of AZT and improve its efficacy is the synthesis of 5'-lipophilic phosphate derivatives of this nucleoside [5-9]. These derivatives are of particular interest since they can penetrate the membranes of virally infected cells and deliver, after hydrolysis, the 5'-mononucleotide, thus, bypassing the first phosphorylation activation step of AZT.

We are especially interested in exploring the stereoelectronic requirements for optimal effectiveness of the lipid component of ether lipid-AZT conjugates and their wider applicability in the antiviral field. We have recently reported [10,11] the synthesis and biological evaluation of new AZT conjugates with alkyl, oxyalkyl and oxyaryl ether phospholipids **1a-m** (Figure 1). Although, these derivatives were found to exhibit significant anti-HIV activity, hydrolysis studies indicated that they do not deliver AZT or AZT-monophosphate intracellularly, but rather act as intact entities with the cellular membrane playing an important role in their mechanism of action.

We have thus initiated studies aiming at understanding the interactions of these conjugates with membranes using deuterium [^{2}H]-solid state nmr, the method of choice for such experiments. For this purpose we have synthesized



1a R = $CH_3(CH_2)_{11}$
1b R = $CH_3(CH_2)_{13}$
1c R = $CH_3(CH_2)_{15}$
1d R = $CH_3(CH_2)_{11}O(CH_2)_2$
1e R = $CH_3(CH_2)_{13}O(CH_2)_2$
1f R = $CH_3(CH_2)_{15}O(CH_2)_2$
1g R = $CH_3(CH_2)_{11}OCH(CH_3)CH_2$ (*R*)
1h R = $CH_3(CH_2)_{11}OCH(CH_3)CH_2$ (*S*)
1i R = $CH_3(CH_2)_{11}OCH_2CH(CH_3)$ (*R*)
1j R = $CH_3(CH_2)_{11}OCH_2CH(CH_3)$ (*S*)
1k R = $PhOCH_2CH_2$
1l R = β-naphthyl-OCH_2CH_2
1m R = α-naphthyl-OCH_2CH_2

Figure 1

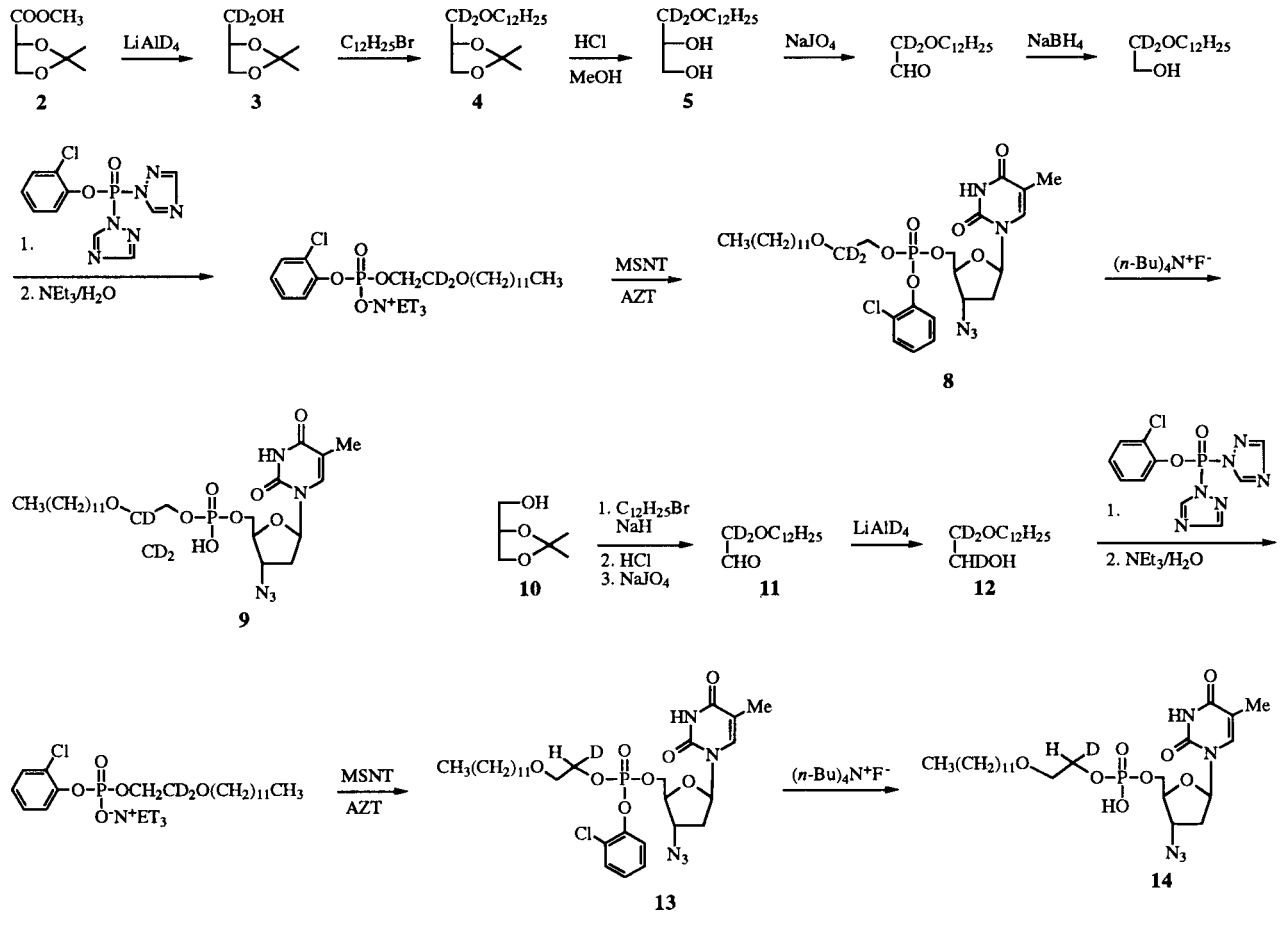
two specifically ^{2}H-labeled analogs **9** and **14** which could then be introduced into model or biological membranes.

In the present paper we outline the synthesis of analogs **9, 14** and describe the pilot [^{2}H]-solid state nmr experiments employing **14**, in order to demonstrate the usefulness of these ^{2}H-labeled AZT-conjugates for our studies on their interactions with membranes.

Results and Discussion.

The synthetic strategy followed for the preparation of **9** and **14** is delineated in Scheme 1. Thus, commercially available methyl α,β-isopropylidene-*L*-glycerate (**2**) was reduced with lithium aluminum deuteride to the alcohol **3**. The latter was alkylated with bromododecane to ether **4** which was then converted to diol **5** upon treatment with hydrochloric acid in methanol. Oxidation of **5** with sodium periodate resulted in the formation of aldehyde **6** which was reduced to the primary alcohol **7**. The synthesis of the desired phosphodiester **9** was effected by the method we have previously published for the preparation of **1d** [10].

Scheme 1



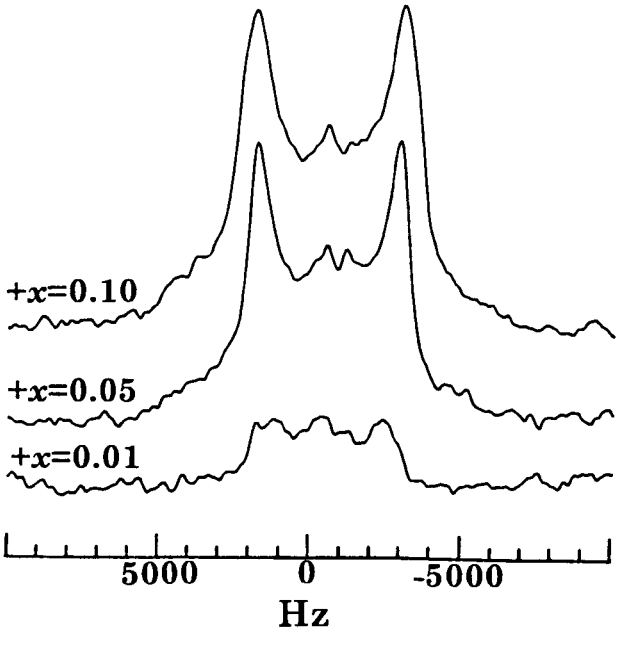


Figure 2. Solid state ^{2}H-nmr spectra from hydrated dimyrostoylphosphatidylcholine bilayer preparations containing derivative **1** (x = 0.01, 0.05, 0.10).

The monodeuterated alcohol **12** was prepared by reducing aldehyde **11** which was obtained from the commercially available isopropylidene glycerol (**10**) according to the method of Baumann *et al.* [12]. The desired monodeuterated phosphodiester **14** was prepared as its counterpart **9**.

In order to obtain direct information on the amount of drug molecule present in the bilayer we performed solid state ^{2}H-nmr experiments. The experiments were conducted on a stationary sample using the deuterated analog **14** and dimyrostoylphosphatidylcholine. The spectrum due to the ^{2}H-labeled compound **14** is expected to appear either as a relatively narrow singlet if the molecule present in solution is undergoing isotropic motions or as a characteristic Pake pattern [13,14], if it is embedded in the anisotropic environment of the bilayer. Figure 2 depicts the solid state ^{2}H-nmr spectra of dimyrostoylphosphatidylcholine bilayer preparations containing **14** at 27°, in three different concentrations (x = 0.01, 0.05 and 0.10 molar ratios). Since the same amount of dimyrostoylphosphatidylcholine was used in all the samples and the spectra were obtained using identical experimental parameters, we are able to correlate the spectral intensities with the

amount of drug incorporated in the bilayer. In all of the spectra the observed doublet is due to the -CHD group located in the α-position next to the phosphate group. The spectra show that **14** is fully incorporated at a concentration of x = 0.05 molar ratio. At x = 0.10, the intensity of the doublet remains essentially unchanged from that of x = 0.05 indicating that no additional AZT-conjugate is incorporated into the bilayer. The excess drug thus appears to aggregate outside the bilayer as is undetected under the experimental conditions utilized. The method thus allows us to detect the degree of incorporation of the lipid conjugate into the membrane. Experimental details and additional biophysical experiments with analogs **9** and **14** will be described elsewhere.

EXPERIMENTAL

All reactions were carried out under scrupulously dry conditions. The ^1H nmr spectra were recorded on a Bruker AC 300 spectrometer operating at 300 MHz and are reported in units of relative δ to internal chloroform at 7.24 ppm. ^{13}C nmr spectra were recorded on a Bruker AC 300 spectrometer operating at 75.43 MHz. Shifts are expressed in units of δ relative to deuteriochloroform at 77.00 ppm. ^{31}P nmr spectra were recorded on a Bruker AC 300 (121.44 MHz) instrument and are reported in units of δ relative to 85% phosphoric acid as external standard; positive shifts are downfield. Both phosphorus-31 and carbon-13 nmr spectra were proton noise decoupled. [^2H]-Solid state nmr experiments were recorded on a Bruker MSL-400 spectrometer. Silica gel plates (Merck F254) were used for thin layer chromatography. Chromatographic purification was performed using silica gel (200-400 mesh). Molecular weights were obtained by CI mass spectrometry on a Finnigan MAT TSQ 7000 instrument. Elemental analyses were carried out by the microanalytical section of the Institute of Organic and Pharmaceutical Chemistry of the National Hellenic Research Foundation.

L-2,3-*O*-Isopropylidene-(1, 1-d_2)-*sn*-glycerol (**3**).

To a solution of **2** (3.2 g, 2.9 ml, 20 mmoles) in dry diethyl ether (360 ml) lithium aluminum deuteride (1.05 g, 25 mmoles) was added at 0°. The resulting mixture was stirred at 0° for 30 minutes and at room temperature for 2 hours. The reaction was subsequently cooled to 0° and quenched by sequentially adding 1 ml of tetrahydrofuran:water (80:20, v/v), 8 ml of tetrahydrofuran:water (50:50, v/v), and 2 ml of water. Sodium sulfate was then added and the salts were filtered. The filtrate was evaporated to dryness to give 2.41 g (90%) of the deuterated alcohol **3** as a viscous oil; ^1H nmr (deuteriochloroform): δ 1.21 (s, 3H), 1.27 (s, 3H), 3.12 (br s, 1H), 3.60 (dd, J = 6.5, 8.2 Hz, 1H), 3.87 (dd, J = 6.5, 8.2 Hz, 1H), 4.05 (t, J = 6.5 Hz, 1H); ^{13}C nmr (deuteriochloroform): δ 25.0, 26.4, 62.0 (q, J_{CD} = 22.0 Hz), 65.6, 75.9, 109.0; ms: m/z 135 $(M+1)^+$.

1-*O*-Dodecyl-(1,1-d_2)-*sn*-glycerol (**5**).

A solution of **3** (2.01 g, 15 mmoles) in dry toluene (40 ml) was slowly added to a suspension of sodium hydride (1.08 g, 45 mmoles) in toluene (20 ml) at 0° for 30 minutes. The mixture was then warmed to room temperature and bromododecane (5.6 g, 22.5 mmoles) and potassium iodide (0.25 g, 1.5 mmoles) were added and the mixture was stirred at 110° overnight. The reaction mixture was quenched with methanol at 0° and diluted with ethyl acetate. The organic layer was washed with water, brine, dried (sodium sulfate) and evaporated to dryness. The crude deuterated ether **4** was used without any further purification for the following step.

To a solution of crude ether **4** in methanol (50 ml) concentrated hydrochloric acid (4.1 ml) was added and the resulting mixture was refluxed for 45 minutes. The solvent was then evaporated and the residue was diluted with diethyl ether. The organic layer was washed with saturated aqueous sodium bicarbonate solution, brine, dried (sodium sulfate) and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography eluting with diethyl ether to give 3.41 g (83%) of **5** as a solid, mp 51-52°; ^1H nmr (deuteriochloroform): δ 0.85 (t, J = 6.5 Hz, 3H), 1.24 (s, 18H), 1.52-1.57 (m, 2H), 2.97 (s, 2H), 3.43 (t, J = 6.6 Hz, 2H), 3.56-3.70 (m, 2H), 3.81-3.84 (m, 1H); ms: m/z 263 $(M+1)^+$.

1-*O*-Dodecyl-(2,2-d_2)-acetaldehyde (**6**).

To a solution of **5** (2.7 g, 9.82 mmoles) in methanol (100 ml) sodium periodate (3 g, 14 mmoles) was added at 0°. The mixture was then allowed to reach room temperature and left stirring for 3 hours. The suspension was then filtered, the filtrate concentrated *in vacuo* and the resulting residue was diluted with methylene chloride. The organic layer was washed with water and brine, dried (sodium sulfate) and evaporated to dryness to give 2.19 g (92%) of **6** as a liquid; ^1H nmr (deuteriochloroform): δ 0.82 (t, J = 6.1 Hz, 3H), 1.19 (s, 18H), 1.51-1.59 (m, 2H), 3.44 (t, J = 6.7 Hz, 2H), 9.66 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 13.9, 22.5, 25.9, 29.2, 29.5, 31.8, 71.9, 75.4 (q, J_{CD} = 22.0 Hz), 201.2; ms: m/z 231 $(M+1)^+$.

2-*O*-Dodecyloxy-(2,2-d_2)-ethane-1-ol (**7**).

To a solution of **6** (1.01 g, 4.40 mmoles) in ether (80 ml) lithium aluminum hydride (0.235 g, 6.20 mmoles) was added at 0°. The suspension was stirred at 0° for 30 minutes and at room temperature for 2 hours. The resulting mixture was worked up as described for **3**. The crude product was purified by flash column chromatography eluting with petroleum ether:diethyl ether (75:25, v/v) to give 0.9 g (90%) of **7** as a viscous liquid; ^1H nmr (deuteriochloroform): δ 0.85 (t, J = 6.5 Hz, 3H), 1.23 (s, 18H), 1.51-1.57 (m, 2H), 2.43 (br s, 1H), 3.43 (t, J = 6.7 Hz, 2H), 3.67 (s, 2H); ^{13}C nmr (deuteriochloroform): δ 14.0, 22.6, 26.0, 29.3, 29.4, 29.6, 31.8, 61.6, 71.3, 71.3 (q, J_{CD} = 22.0 Hz); ms: m/z 233 $(M+1)^+$.

Anal. Calcd. for $C_{14}H_{32}O_2$: C, 72.35; H, 13.88. Found: C, 72.94; H, 13.30.

3'-Azido-3'-deoxythymidine-5'-[2-dodecyloxy-(2,2-d_2)-ethyl] Phosphate (**9**).

This compound was prepared from **7** (0.232 g, 1 mmole) *via* triester **8** according to the procedure previously described for **1d** [11], yield 0.140 g (30%), viscous oil; ^1H nmr (deuteriochloroform): δ 0.85 (t, J = 6.7 Hz, 3H), 1.23 (s, 18H), 1.51-1.56 (m, 2H), 1.88 (s, 3H), 2.36-2.41 (m, 2H), 3.44 (t, J = 6.8 Hz, 2H), 3.98-4.00 (m, 1H), 4.14 (d, J_{HP} = 7.6 Hz, 2H), 4.34-4.38 (m, 3H), 6.15 (t, J = 6.4 Hz, 1H), 7.41 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 12.2, 14.1, 22.6, 26.0, 29.3 29.4, 29.6, 31.9, 37.5, 60.2, 66.0, 66.7, 67.5 (q, J_{CD} = 22.0 Hz), 71.6, 82.5 (d, J_{CP} =7.4 Hz), 85.3, 111.3, 135.9, 150.2, 164.1; ^{31}p nmr (deuteriochloroform): δ -1.1; ms: m/z 562 $(M+1)^+$.

Anal. Calcd. for $C_{24}H_{44}N_5O_8P$: C, 51.33; H, 7.90. Found: C, 51.59; H, 7.88.

2-Dodecyloxy-(1-d_2)-ethane-1-ol (**12**).

To a solution of aldehyde **11** (lit 12) (1.01 g, 4.43 mmoles) in dry ether (80 ml), lithium aluminum deuteride (0.235 g, 5.54 mmoles) was added. The preparation followed the procedure described under the synthesis of **3**. The crude product was purified by flash column chromatography eluting with petroleum ether:diethyl ether (75:25, v/v) to give 0.94 g (92%) of **12** as a viscous oil; ^{1}H nmr (deuteriochloroform): δ 0.82 (t, J = 6.4 Hz, 3H), 1.20 (s, 18H), 1.50-1.55 (m, 2H), 2.74 (s, 1H), 3.40 (t, J = 6.7 Hz, 2H), 3.45 (d, J = 4.6 Hz, 2H), 3.51-3.63 (br s, 1H); ^{13}C nmr (deuteriochloroform): δ 14.0, 22.6, 26.0, 29.3, 29.4, 29.5, 31.8, 61.3 (d, J_{CD} = 21.7 Hz), 71.4, 71.8; ms: m/z 232 $(M+1)^+$.

Anal. Calcd. for $C_{14}H_{31}O_2$: C, 72.67; H, 13.50. Found: C, 73.02; H, 13.41.

3'-Azido-3'-deoxythymidine-5'-[2-dodecyloxy-(1-d_2)-ethyl] Phosphate (**14**).

This compound was prepared from **12** (0.231, 1 mmole) *via* triester **13** according to the procedure previously described for **1d**, yield 0.093 g (20%), viscous oil; ^{1}H nmr (deuteriochloroform): δ 0.85 (t, J = 6.6 Hz, 3H), 1.23 (s, 18H), 1.51-1.55 (m, 2H), 1.88 (s, 3H), 2.35-2.41 (m, 2H), 3.43 (t, J = 6.7 Hz, 2H), 3.60 (d, J = 4.5 Hz, 2H), 3.99-4.00 (m, 1H), 4.124.37 (m, 4H), 6.16 (t, J = 6.3 Hz, 1H), 7.43 (s, 1H); ^{13}C nmr (deuteriochloroform): δ 12.3, 14.1, 22.7, 26.0, 29.3. 29.5, 29.61, 31.9, 37.6, 60.1, 66.1, 66.7 (t, J_{CD} = 22.0 Hz), 69.3, 71.7, 82.5 (d, J_{CP} = 7.4 Hz), 85.2, 111.3, 135.8, 150.1, 164.0; ^{31}P nmr (deuteriochloroform): δ -1.016; ms: m/z 561 $(M+1)^+$.

Anal. Calcd. for $C_{24}H_{43}N_5O_8P$: C, 51.42; H, 7.73. Found: C, 51.42; H, 7.71.

Acknowledgment.

This work was supported in part by KESY (Central Health Council of Greece) and by the National Drug Organization of Greece. It was also supported by NHRF and grants NIDA DA 3801, DA 7215 and DA 9158. We would like to express our gratitude to Dr. E. Kalatzis for performing the analytical work.

REFERENCES AND NOTES

[1] H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. Nusinoff-Lehrman, R. C. Gallo, D. P. Bolognesi, D. W. Barry, and S. Broder, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 7096 (1985).

[2] M. Baba, R. Pauwels, P. Herdewijn, E. De Clercq, J. Desmyter and M. Vandeputte, *Biochem. Biophys. Res. Commun.*, **142**, 128 (1987).

[3] P. A. Furman, J. A. Fyfe, M. H. St. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. Nusinoff-Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya and D. W. Barry, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8333 (1986).

[4] J. Balzarini, P. Herdewijn and E. De Clercq, *J. Biol. Chem.*, **264**, 6127 (1989).

[5] G. M. T. van Wijk, K. Y. Hostetler, E. Kroneman, D. D. Richman, C. N. Sridhar, R. Kumar and H. van der Bosch, *Chem. Phys. Lipids*, **70**, 213 (1994).

[6] Y. Henin, C. Gouyette, O. Schwartz, J.-C. Debouzy, J.-M. Neumann and T. Huynh-Dinh, *J. Med. Chem.*, **34**, 1830 (1991).

[7] C. Piantadosi, C. J. Marasco Jr., S. L. Morris-Natschke, K. L. Meyer, F. Gumus, J. R. Surles, K. Ishaq, L. S. Kucera, N. Iyer, C. A. Wallen, S. Piantadosi and E. Modest, *J. Med. Chem.*, **34**, 1408 (1991).

[8] C. McGuigan, R. N. Pathirana, M. P. H. Davies, J. Balzarini and E. De Clercq, *Biorg. Med. Chem. Letters*, **4**, 427 (1994).

[9] W. Thomson, D. Nicholls, W. J. Irwin, J. Al-Mushadani, S. Freeman, A. Karpas, J. Petrik, N. Mahmood and A. J. Hay, *J. Chem. Soc., Perkin Trans. 1*, 1239 (1993).

[10] T. Calogeropoulou, M. Koufaki, A. Tsotinis, J. Balzarini, E. De Clercq and A. Makriyannis, *Antiv. Chem. Chemother.*, **6**, 43, (1995).

[11] A. Tsotinis, T. Calogeropoulou, M. Koufaki, Ch. Souli, J. Balzarini, E. De Clercq and A. Makriyannis, *J. Med. Chem.*, in press (1996).

[12] W. J. Baumann, H. O. Schmid, H. W. Ulshöfer and H. K. Mangold, *Biochim. Biophys. Acta*, **144**, 355 (1967).

[13] J. H. Davis, K. R. Jeffrey, M. Bloom, M. J. Valic and T. P. Higgs, *Chem. Phys. Letters*, **42**, 390 (1976).

[14] T. Mavromoustakos, D. P. Yang and A. Makriyannis, *Biochim. Biophys. Acta*, **227**, 1231 (1995).