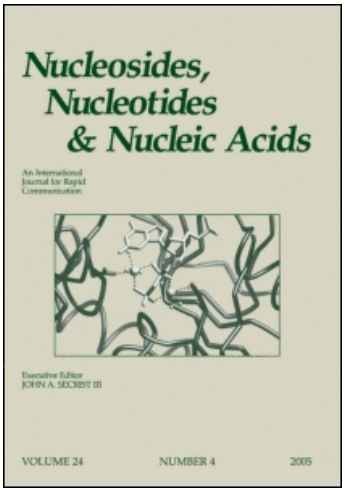


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The 5'-Hydrogen Phosphonate Analog as a Potential Prodrug of 3'-Azido-2',3'-dideoxythymidine (AZT)

Jila H. Boal[ab]; Radhakrishnan P. Iyer[ac]; William Egan[a]
[a] OB/GYN Research Division, University of Rochester Medical School, Rochester, New York [b] Science Park Research Division, The University of Texas, M. D. Anderson Cancer Center, Smithville, Texas [c] Biophysics Laboratory, Center for Biologics Evaluation and Research, Bethesda, Maryland, U.S.A.

To cite this Article Boal, Jila H. , Iyer, Radhakrishnan P. and Egan, William(1993) 'The 5'-Hydrogen Phosphonate Analog as a Potential Prodrug of 3'-Azido-2',3'-dideoxythymidine (AZT)', Nucleosides, Nucleotides and Nucleic Acids, 12: 10, 1075 — 1084
To link to this Article: DOI: 10.1080/07328319308016205
URL: http://dx.doi.org/10.1080/07328319308016205

NUCLEOSIDES & NUCLEOTIDES, 12(10), 1075-1084 (1993)

THE 5'-HYDROGEN PHOSPHONATE ANALOG AS A POTENTIAL PRODRUG OF 3'-AZIDO-2',3'-DIDEOXYTHYMIDINE (AZT)*

Jila H. Boal,[a] Radhakrishnan P. Iyer,[b] and William Egan
Biophysics Laboratory, Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, Maryland 20892, U.S.A.

Abstract. A novel synthesis of AZT-5'-H-phosphonate **2**, involving decarboxylation of the phosphonoformate intermediate **7**, is reported herein. Studies of the metabolism of **2** in U937 cells indicate that **2** is readily converted to AZT-5'-monophosphate.

Nucleoside analogs that are selective inhibitors of reverse transcriptase (RT) of human immunodeficiency virus (HIV-1) have attracted much attention as anti-HIV agents. Amongst the most potent of these is 3'-azido-2',3'-dideoxy thymidine (**1**, AZT), a 2',3'-dideoxynucleoside analog of thymidine.[1] It is now well recognized that both the *in vitro* and *in vivo* RT inhibitory activity and anti-HIV activity of these nucleoside analogs is essentially contingent upon their intracellular conversion to the corresponding 5'-triphosphates.[2-4] Specifically, the conversion of AZT to AZT-triphosphates (AZT-5'-TP) is brought about by sequential catalysis involving cellular kinases. Thus, only in its triphosphate form can AZT compete with deoxythymidine-5'-triphosphate (dT-5'-TP) for the binding site of RT, thereby causing inhibition of DNA synthesis mediated by RT. This imposes limitations on the efficacy of AZT and other analogs. Thus eg., AZT is ineffective in blocking viral proliferation in human peripheral blood T cells and human monocyte-derived macrophages which contain low levels of intracellular thymidine kinases.[5] In

---

**This research was supported in part by the Office of the Director, NIH, with funds earmarked for AIDS -targeted Antiviral Research. The authors thank the referees of this paper for their comments. RPI dedicates this paper to Professor Alex Nickon, Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland on the occasion of his 66th birthday.*

[a]Current address: OB/GYN Research Division, University of Rochester Medical School, Box 668, Rochester, New York 14642.

[b]Current address: The University of Texas, M. D. Anderson Cancer Center, Science Park Research Division, P. O. Box 389, Smithville, Texas 78957.

addition, side effects such as bone-marrow toxicity and development of resistance to AZT is presumably due to its conversion or lack thereof to AZT-5'-TP.[6-10] As expected, AZT-5'-TP itself is poorly absorbed when administered externally and is readily dephosphorylated in the extracellular environment. Potentially, these problems can be circumvented using an analog of AZT that could serve as an intracellular slow-release depot to the active AZT-5'-TP. We considered the evaluation of AZT-5'-H-phosphonate **2** as a potential prodrug analog of AZT-5'-TP. While our work was in progress, a report [11] appeared regarding the evaluation of AZT-5'-H-phosphonate as an anti-HIV agent. We report here our results on the synthesis and intracellular metabolic fate of **2**.

EXPERIMENTAL SECTION

Materials and Methods: Analytical grade reagents, solvents and chromatographic media were obtained from commercial suppliers. Toluene, triethylamine, tetrahydrofuran and acetonitrile were distilled from calcium hydride after first refluxing for several hours. Pyridine was distilled from potassium hydroxide pellets following several hours reflux. Anhydrous ether (Mallinckrodt Chemicals) was passed through a column of activated alumina to remove peroxides. Methanol was dried by fractional distillation in the presence of a small amount of sodium metal. Trimethylsilyl chloride was purified by distillation over calcium hydride. Prior to their use in synthesis, the requisite chloroformates were distilled under atmospheric pressure or *in vacuo* as the case warranted.

Melting points (uncorrected) were determined on a Buchi 510 melting point apparatus. Analytical thin-layer chromatography (TLC) was carried out using silica gel 60 F_{254} pre-coated sheets (layer thickness, 0.2 mm). Materials were visualized on TLC plates by either ultraviolet light, iodine vapors, HCl fumes or charring following treatment with sulfuric acid.

Nuclear magnetic resonance (NMR) spectra were obtained on either JEOL GSX-500 or General Electric GN-300 spectrometers. Proton- and Carbon-NMR spectra are reported in parts per million (*ppm*) relative to tetramethylsilane (TMS) for organic solutions and sodium trimethylsilyl-2,2,3,3-d_4 propionate (TSP) for aqueous solutions. ^{31}P-NMR are reported in *ppm* relative to external trimethyl phosphate.

Synthesis of sodium salt of AZT-5'-ethoxycarbonylphosphonate (6):

The pyridinium salt **5** prepared from **3**, as reported previously[12] was rendered anhydrous by repeated evaporation *in vacuo* from pyridine. The salt thus obtained (270 mg, 1.16 mmol) was taken up in anhydrous pyridine (75 mL) and cooled to ambient temperature in a water bath. AZT (309 mg, 1.16 mmol) was then added followed by 1,3-dicyclohexylcarbodimide (DCC) (478 mg, 2.32 mmol) and stirred under anhydrous

conditions at ambient temperature in an atmosphere of argon. The reaction was periodically monitored (TLC, silica gel, $CHCl_3$:MeOH, 75:25). After about 26 hours, the reaction was kept at 5°C for an hour and the precipitated dicyclohexyl urea filtered and washed with pyridine. The combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was taken up in minimum pyridine:water (80:20) and loaded on AG 50-MP1(Cl^- form) column. The column was eluted with water (100 mL), followed by NaCl (0.15 M) The fractions containing the AZT ester **6**, detected by TLC (silica gel, $CHCl_3$:MeOH, 75:25, Rf = 0.2) were combined and evaporated to dryness *in vacuo*. The resulting solid was repeatedly triturated with 98% ethanol (100 mL) filtered to remove NaCl and the ethanolic filtrate concentrated *in vacuo* and dried to give the sodium salt **6** (502 mg, 89% yield) as a white hygroscopic solid.

^{31}P-NMR (D_2O): δ -7.8 *ppm*

^{1}H-NMR (D_2O): δ 1.3 (*td*,3H, $^3J_{H-H}$ =8 *Hz*, $^3J_{H-P}$ = 2.5 *Hz*), 1.9 (*s*, 3H), 2.5 (*m*, 2H), 4.1-4.35 (*m*, 6H), 6.3 (*t*, 1H, J = 7 *Hz*), 7.7 (*s*, 1H) *ppm*.

Synthesis of 3'-azido-2',3'-dideoxy thymidine-5'-H-phosphonate (2):

The ester **6** (298 mg, 0.62 mmol) was taken up in distilled water (12 mL) and aqueous NaOH solution (1M, 1 mL) was then added and the contents stirred. The progress of the hydrolysis was monitored by ^{31}P-NMR and TLC. After 15 minutes of reaction, pH of the solution was adjusted to 7 using aqueous NH_4Cl (1N). The solution was concentrated *in vacuo* to give the residue A. At this stage, a portion of the residue was used for isolating the acid **7** as below:

The residue was dissolved in minimum water and loaded on C-18 SEP-PAK cartridges (Millipore). Elution was done with distilled water and the UV-absorbing fractions (λ_{254}) were combined and lyophilized to yield the white crystalline ammonium salt of **7**. The corresponding sodium salt was obtained by ion-exchange chromatography on AG 50-WX8 (Na^+ form) and characterized as below:

^{31}P-NMR (D_2O): δ -2 (*t*, J = 5 *Hz*) *ppm*.

^{1}H-NMR (D_2O): δ 1.9 (*s*, 3H), 2.5 (*m*, 2H), 4.1-4.2(*m*, 3H), 4.5-4.7 (*m*, 1H), 6.3 (*t*, 1H, J = 7 *Hz*), 7.8 (*s*, 1H) *ppm*.

^{13}C-NMR (D_2O): δ 8.8, 33.5, 58.0, 61.6(*d*, J = 5 *Hz*), 80.4 (*d*, J = 7 Hz), 82.1, 108.9, 134.6, 148.8, 163.7, 174.1 (*d*, J = 230 *Hz*) *ppm*.

To obtain the H-phosphonate **2**, the residue **A**, obtained as above, was dissolved in water (10 mL) and HCl (2 mL, 1% *v/v*) and stirred at ambient temperature. The conversion of the acid **7** to the H-phosphonate **2** was best monitored by ^{31}P-NMR (**7** at -2

ppm and **2** at + 3.3 *ppm*). After about 2 hours, the solution was carefully adjusted to pH 7, using dilute NaOH solution (0.5% *w/v*), concentrated *in vacuo* and loaded on C-18 SEP-PAK cartridges. The UV absorbing fractions (λ_{254}) were combined and concentrated *in vacuo* and passed through an ion-exchange column AG 50W-X8 (Na^+ form). Concentration of the eluent *in vacuo* and drying of the residue gave the sodium salt of the H-phosphonate **2** in overall yields of about 70% from **6**.

^{31}P-NMR (D_2O): δ +3.3 (*dt*, $^1J_{P-H}$ = 629 *Hz*, $^3J_{P-H}$ = 6 *Hz*) *ppm*

^{1}H-NMR (D_2O): δ 1.95 (*s*, 3H), 2.5 (*m*, 2H), 4.1-4.3 (*m*, 3H), 4.45-4.6 (*m*, 1H), 6.25 (*t*, 1H, J = 7 *Hz*), 6.8 (*d*, 1H, J = 629 *Hz*), 7.7 (*s*, 1H) *ppm*.

^{13}C-NMR (D_2O): δ 8.6, 33.2, 57.4, 59.9 (*d*, J = 3 *Hz*), 79.8 (*d*, J = 8 *Hz*), 81.8, 108.5, 134.1, 148.5, 163.3 *ppm*.

The intracellular conversion of AZT-5'-H-phosphonate (2) to AZT-5'-MP:

The origin and growth characteristics of human U937 cell line is well described and documented elsewhere.[13] Cells were cultured in RPMI-1640 (BIOFLUIDS) supplemented with penicillin and streptomycin (1%, BIOFLUIDS) and 10% fetal calf serum (HYCLONE). Cell cultures were split one day before drug treatment. The U937 cells (plated at 3 X 10^5 cells/mL) were incubated with AZT (200 μM) and AZT-5'-H-phosphonate (200 μM) for 8 and 16 hours. AZT metabolism by these cells was included as a control in our assay and also for comparing levels of AZT-5'-MP resulting from metabolism of AZT and AZT-5'-H-phosphonate. At the end of the incubation period, the cell viability was checked by trypan blue exclusion and the cell count determined. Cells were harvested by centrifugation, washed twice with Hank's balanced salt solution (without Ca^{++} and Mg^{++} and phenol red) (BIOFLUIDS) and the metabolites extracted with aqueous methanol (5 mL, 60%) at -20°C, over a period of 10 hours. The extract was subjected to centrifugation to remove cell debris and the supernatant lyophilized. The resulting dried extract was reconstituted in 500 μL deionized water. Samples were stored frozen at -80°C before use.

To determine if enzymatic conversion of AZT-H-phosphonate to AZT occurs, **2** (0.7 μmol) was dissolved in 20 μl sterile water, and 20 μl of 5'-nucleotidase (225 units/mL) (from *Crotalus adamanteus* venom, SIGMA), in Tris-HCl buffer (0.1 M, 0.01 mM $MgCl_2$, pH 9.0) was added and the reaction mixture was incubated at 37°C for 3, 8 and 24 hours. Aliquots were stored frozen at -70°C until ready for HPLC analysis (*vide infra*). Adenosine-5'-monophosphate (SIGMA) was used as the positive control.

HPLC analysis was done on a Hewlett-Packard 1050 with an HPLC integrator, UV detector set at 254 *nm*. The extracts were loaded on a SAX column (Whatman Partsil 4.6 X 250 mm, 10 μ) and eluted with a linear gradient of ammonium phosphate (10 mM, pH

3.5) in 7% ethanol to 700 mM of the same buffer over 60 min. AZT-5'-MP was synthesized according to published procedure[14] and had a retention time of 10.7 minutes under the above conditions.

RESULTS

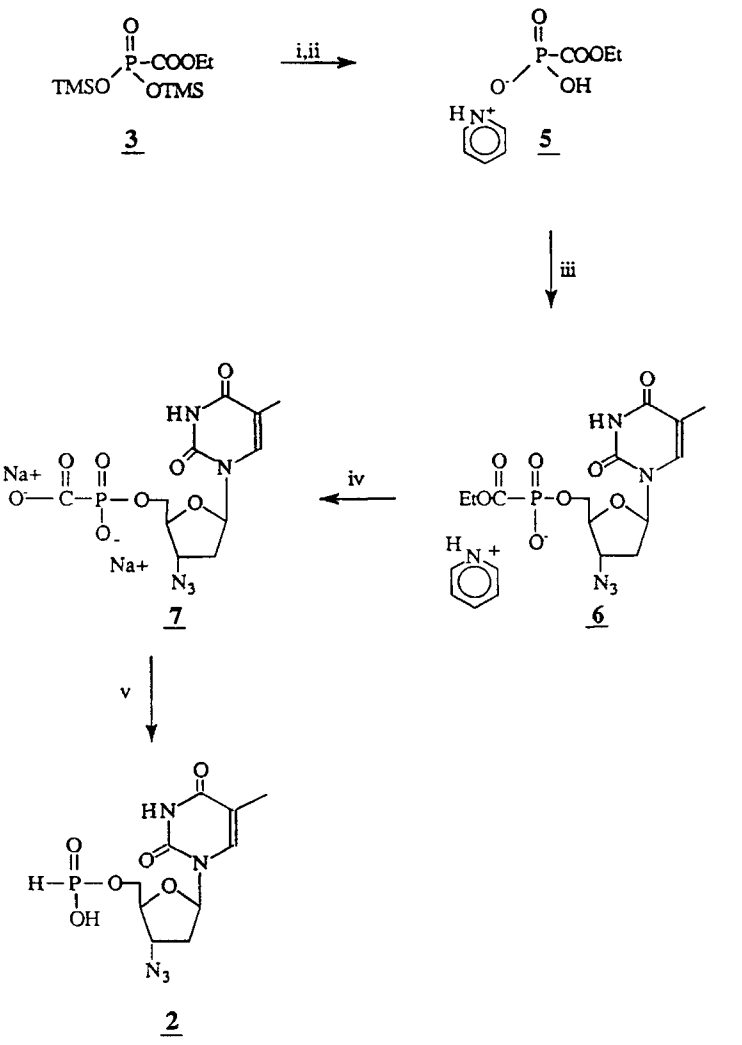
Synthesis of the H-phosphonate 2:

The apparent simplicity of the target phosphonate **2** prompted us to undertake a rather direct synthetic route to it. However attempted coupling of AZT with phosphorous acid in pyridine in presence of excess 1,3-dicyclohexylcarbodimide (DCC) gave a complex mixture from which isolation and purification of the desired **2** proved quite tedious. Direct phosphitylation of the 5'-OH group of AZT, using β-cyanoethyl N,N-diisopropylchlorophosphoramidite, followed by its conversion to H-phosphonate (tetrazole/H_2O) also gave disappointing results. Quite clearly, the presence of the 3'-azido group in AZT contributed to a variety of side reactions and therefore direct and easy access to H-phosphonate was not feasible at least in our hands.

Our synthetic strategy (Scheme I) for **2** takes advantage of the known propensity of ethoxycarbonyl phosphonates to undergo decarbethoxylation when subjected to sequential base and acid hydrolytic conditions.[15,16] We had ready access to bis(trimethylsilyl)ethoxy carbonyl phosphonate **3** as the starting material.[12] The silyl phosphonate **3** was converted to the dianilinium salt **4** and subsequently to the monopyridinium salt **5** by ion-exchange chromatography over AG-50 W-X8 (pyridinium form). The reaction of **5** with AZT (pyridine-DCC) gave the ester salt **6** in isolated yields greater than 90%.

The conversion of **6** to the H-phosphonate **2** was done by *brief* exposure to 1N NaOH followed by reaction with 2% HCl. The reaction was carefully monitored by ^{31}P-NMR, and was found to proceed by initial conversion to the carboxylic acid **7**. The sodium salt of **7** was isolated and characterized. The carboxylic acid salt **7**, upon treatment with dil. HCl, gave the H-phosphonate **2** in overall yields of 70% from **6**.

While our work was in progress, a report appeared where a direct synthesis of the H-phosponate **2** was claimed using AZT and PCl_3 in presence of imidazole.[17] It is pertinent to mention here that our synthetic methodology can be applied for the preparation of 5'-H-phosphonates of various 2'-deoxy nucleosides, eg. 5'-fluoro-deoxyuridine, without protection of the free 3'-OH group.[18] Additionally, our approach also provides synthetic access to the phosphonic carboxylic acids, such as **7**, which represent novel structures incorporating both a nucleosidic type and a non-nucleosidic type RT inhibitor. Although not evaluated in the present study, **7** could have a potential synergistic anti-HIV effect of AZT and foscarnet. Recently it has been reported that AZT H-phosphonate **2**, though less active as an anti-HIV agent as compared to AZT, was nevertheless much less toxic than AZT itself.[11]



i)MeOH, aniline ii) AG50 X8 (pyridinium) iii) AZT, pyridine, DCC, 26h

iv) 1N NaOH, 15 min v)1% HCl, 2h.

Scheme I

Metabolic fate of the H-phosphonate 2:

In order to check whether the H-phosphonate **2** can undergo intracellular conversion to the corresponding monophosphate, we incubated human U937 cell lines in presence of **2**. At the end of the incubation period (8 and 16 hours), the resulting metabolites were extracted and analyzed by HPLC (FIG.1). As is evident, **2** undergoes facile conversion to the corresponding monophosphate. The presence of AZT-5'-MP in

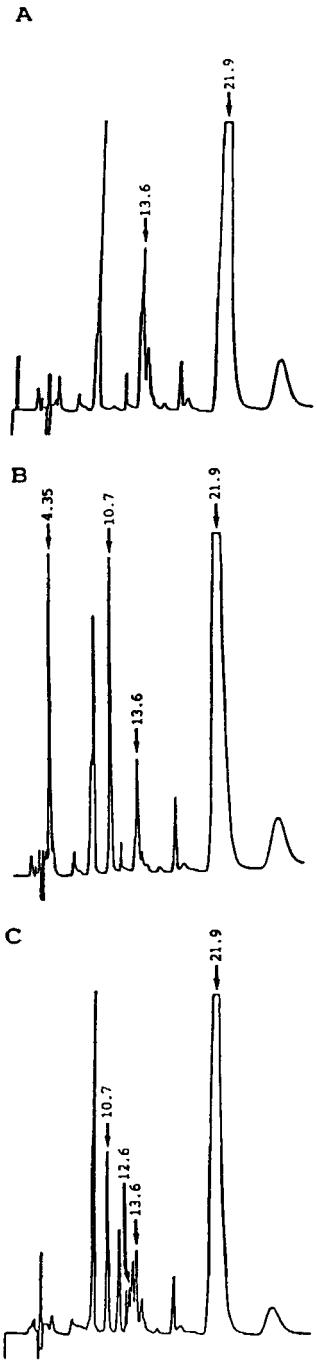


Figure 1: HPLC profile of the metabolism of AZT-5'-H-phosphonate in U937 cells. Methanolic extract of U937 cells (control) (panel A); following incubation with 200 mM AZT for 8 hours at 37°C (panel B); following incubation with 200 mM AZT-5'-H-phosphonate for 8 hours at 37°C (panel C); Retention times (min): AZT (4.35), AZT-5'-monophosphate (10.7) AZT-5'-H-phosphonate (12.6), Adenosine-5'-diphosphate (13.6), Adenosine-5'-triphosphate (21.9).

TABLE 1

Intracellular concentration of AZT-5'-MP, derived from HPLC analysis of cell extracts of U937 cells, after treatment with 200 μM AZT or AZT-5'-H-phosphonate at various incubation times.

Compound	Incubation time (h)	AZT-5'-MP (pmol/10^6 cells)
AZT	16	4900
2	16	3350
AZT	8	5300
2	8	1700

extracts of AZT- and AZT H-phosphonate-treated cells were confirmed by co-chromatographic comparison using authentic AZT-5'-MP in the same eluent buffer.

The presence of AZT could not be detected in the extract of the AZT-5'-H-phosphonate-treated cells. This observation rules out the possiblity of prior conversion of **2** to AZT followed by conversion of the latter to AZT-5'-MP (*vide infra*). TABLE 1 shows the relative concentrations of the intracellular AZT-5'-MP in U937 cells after incubations of 8 and 16 hours. AZT 5'-H-phosphonate metabolism in these cells produced AZT-5'-MP ca. three times less than that from metabolism of AZT during 8 hours of incubation, but after 16 hours, the level of AZT-5'-MP was comparable to that produced by AZT.

The possiblity existed that the observed formation of AZT-5'-MP from metabolism of **2**, was due to initial facile conversion of the H-phosphonate **2** to AZT by cellular 5'-nucleotidases, followed by its phosphorylation by thymidine kinase to AZT-5'-MP. Alternatively, one could also conceive of a possible non-enzymatic conversion of **2** to AZT and subsequent intracellular formation of AZT-5'-MP.[19,20] We present the following preliminary evidence to demonstrate the intracellular formation of AZT-5'-MP by a process involving *oxidation of intact* **2**:

(a) the H-phosphonate **2** was found to be stable and intact upon its incubation in cell culture medium (RPMI-1640) as evidenced by HPLC studies (data not shown). (b) Additionally, no conversion of **2** to AZT was observed by prolonged incubation of **2** with 5'-nucleotidase, a ubiquitous plasma membrane-bound enzyme, involved in the hydrolysis of ribonucleotides/deoxyribonucleotides (data not shown). (c) Finally, under the conditions

of our assay, where the formation of AZT-5'-MP from **2** was observed, the intermediate formation of AZT was not observed (FIG. 1).

DISCUSSION:

In conclusion, our preliminary studies lead us to postulate that (a) the cellular metabolism of AZT-5'-H-phosphonate to AZT-5'-MP does occur in U937 cells and (b) the conversion of AZT-5'-H-phosphonate to AZT by 5'-nucleotidase or by other enzymatic processes does not occur. Additional studies are required to elucidate the metabolic pathway involved in the conversion of AZT-H-phosphonate to AZT-5'-MP.

These results demonstrate the possibility of using the hydrogen-phosphonate as a surrogate phosphate group in the design of new antiviral nucleosides. Moreover, in situations where the virus-infected cells lack the presence of essential enzymes, required for the first phosphorylation of the nucleoside analogs such as DDI, DDC and AZT, one could consider the corresponding 5'-H-phosphonate analogs as potential prodrug derivatives.

REFERENCES:

1. Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair M. H.; Lehrman, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. (USA)*, **1985**, *82*, 7096-7100.

2. Furman, P. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Lehrman, S. N.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. *Proc. Natl. Acad. Sci. (USA)*, **1986**, *83*, 8333-8337.

3. St. Clair, M. H.; Richards, C. A.; Spector, T.; Weinhold, K. J.; Miller, W. H.; Langlois, A. J.; Furman, P. A. *Antimicro. Agents Chemoth.*, **1987**, *31*, 1972-1977.

4. Kedar, P. S.; Abbotts, J.; Kovacs, T.; Lesiak, K.; Torrence, P.; Wilson, S. H. *Biochemistry*, **1990**, *29*, 3603-3611.

5. Richman, D. D.; Kornbluth, R. S.; Carson, D. A. *J. Exp. Med.*, **1987**, *166*, 1144-1149.

6. Yarchoan, R.; Pluda, J. M.; Perno, C-F.; Mitsuya, H.; Broder, S. *Blood*, **1991**, *78*, 859-884.

7. Yarchoan, R.; Klecker, R.W.; Weinhold, K. J.; Markham, P. D.; Lyerly, H. K.; Durack, D. T.; Gelmann, E.; Lehrman, S. N.; Blum, R. M.; Barry, D. W.; Shearer, G. M.; Fischl, M. A.; Mitsuya, H.; Gallo, R. C.; Collins, J. M.; Bolognesi, D. P.; Myers, C. E.; Broder, S. *Lancet*, **1986**, *1*, 575-80.

8. Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. M.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; Lehrman, S. N. *New Engl. J. Med.*, **1987**, *317*, 192-97.

9. Dournon, E.; Matheron, S.; Rozenbaum, W.; Gharakhanian, S.; Michon, C.; Girard, P. M.; Perronne, C.; Salmon, D.; De Truchis, P.; LePort, C.; Bouvet, E.; Dazza, M. C.; Levacher, M.; Regnier, B. *Lancet*, **1988**, *2*, 1297-1302.

10. Larder, B. A.; Darby, G.; Richman, D. D. *Science*, **1989**, *243*, 1731-34.

11. Federov, I. I.; Krayevsky, A. A.; Balzarini, J.; DeClercq, E. *Mol. Biol.*, **1992**, *26*, 749-52.

12. Iyer, R. P.; Phillips, L. R.; Biddle, J. A.; Thakker, D. R.; Egan, W.; Aoki, S.; Mitsuya, H. *Tet. Lett.*, **1989**, *30*, 7141-7144.

13. Sundstrom, C.; Nisson, K. Int. J. Cancer, **1976**, *17*, 565-577.

14. Zaitseva, V. E.; Dyatkina, N. B.; Kraevski, A. A.; Skaptsova, N. V.; Turina, O. V.; Gnuchev, N. V.; Gottikh, B. P.; Azhaev, A. V. *Biorg. Chem.*, **1984**, *10*, 369-378.

15. Warren, S.; Willimas, M. R. *J. Chem. Soc., (B)* **1971**, 618-620.

16. Warren, S.; Williams, M. R. *J. Chem. Soc. Chem. Commun.*, **1969**, 180.

17. Tarusova, N. B.; Khorlin, A. A.; Krayevsky, A. A.; Korneeva, M. N.; Nosik, D. N.; Kruglov, N. CV.; Galegov, G. A.; Bibilashvili, R. Sh. **Mol. Biol., 1989,** 23, 1716-1723.

18. Unpublished results.

19. Tarusova, N. B.; Khorlin, A. A.; Kraeskii, A. A.; Korneeva, M. N.; Nosik, D. N.; Kruglov, N. V.; Galegov, G. A.; Bibilashvili, R. Sh. *Mol. Biol.*, **1989**, *23*, 1361-68.

20. Karamov, E. V.; Lukashov, V. V.; Tarusova, N. B.; Kornilaeva, G. V.; Rodina, M. A.; Kukhanova, M. K.; Kraevskii, A. A. *Mol. Biol.*, **1990**, *24*, 1695-1701.

Received 7/20/93
Accepted 9/21/93