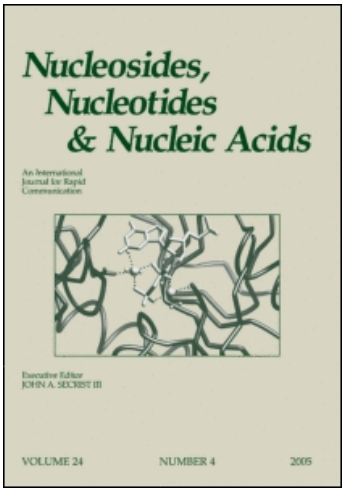


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# 5'-Hydrogenphosphonates and 5'-Methylphosphonates of Sugar Modified Pyrimidine Nucleosides as Potential Anti-HIV-1 Agents.[1]

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NUCLEOSIDES & NUCLEOTIDES, 11(2-4), 177-196 (1992)

# 5'-HYDROGENPHOSPHONATES AND 5'-METHYLPHOSPHONATES OF SUGAR MODIFIED PYRIMIDINE NUCLEOSIDES AS POTENTIAL ANTI-HIV-1 AGENTS.[1]

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**Abstract.** A number of nucleoside 5'-hydrogenphosphonates and nucleoside 5'-methylphosphonates were prepared, to study their ability to inhibit replication of HIV-1. Two compounds, the 5'-hydrogenphosphonate of 3'-azido-3'-deoxythymidine (AZT-HP, **IVc**) and of 3'-deoxy-3'-fluorothymidine (FLT-HP, **IVa**), exhibit potent anti-HIV-1 activity with selectivity indices similar to or better than those of their parent nucleosides.

## Introduction.

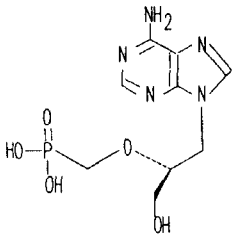
The only clinical agent approved in the United States for the treatment of AIDS is 3'-azido-3'-deoxy-thymidine (AZT).[2,3] Several 2',3'-dideoxy-[4,5] and 2',3'-didehydro-2',3'-dideoxy[6,7] as well as some other types of nucleosides[8-11] are also reported to be active against human immunodeficiency virus (HIV), the pathogen responsible for causing AIDS. The molecular mechanism(s) of action of these nucleosides includes conversion into their corresponding 5'-monophosphates by the action of cellular nucleoside kinase(s), followed by stepwise phosphorylation catalyzed by cellular nucleotide kinases to the corresponding 5'-triphosphates. These inhibit proviral DNA synthesis,[3,12,13] catalyzed by HIV reverse transcriptase (RT), by incorporation to the 3' end of the growing DNA chain.[3,7]

This paper is dedicated to the memory of Professor Tohru Ueda.

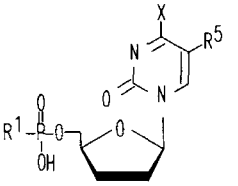
Many nucleosides are poor substrates for deoxynucleoside kinase(s), due to rather restricted structural requirements of the enzyme(s). Nucleosides that are very poor substrates for cellular kinases exhibit little or no activity against HIV infected cells.[12,13] However, nucleoside 5'-triphosphates in general, which are usually formed readily from their corresponding 5'-monophosphates in the cell, inhibit purified RT in cell free systems.[14,15] For example, 2',3'-dideoxycytidine (ddC) is phosphorylated by deoxycytidine kinase[16,17] to its 5'-monophosphate (ddCMP) which is further converted into the triphosphate (ddCTP) and inhibits RT.[3,18] In this case the rate determining step is apparently the ddCMP formation.[19] On the other hand, AZT[3] and FLT[20] are converted by cytosolic thymidine kinase to their 5'-monophosphate (AZT-MP and FLT-MP) which are further converted stepwise into the di- and triphosphate (AZT-DP, -TP, and FLT-DP, -TP, respectively) presumably by the host cell thymidylate kinase. AZT-TP competes about 100-fold better for the HIV RT than for the cellular DNA polymerase $\alpha$.[3] The intracellular levels of AZT-MP and FLT-MP are high compared with the levels of their corresponding nucleoside-DP and -TP's.[3,20] AZT-MP inhibits thymidylate kinase reducing the intracellular level of dTTP needed for DNA synthesis.[3] More recently, AZT was reported to be reduced to 3'-amino-3'-deoxythymidine[21] which is highly toxic to bone marrow cells.[22]

The catabolic pattern of nucleoside phosphonates may be different from that of the parent nucleosides. Also, nucleoside-5'-O-hydrogenphosphonates (H-phosphonates), unlike strongly acidic nucleoside 5'-phosphates (which cannot enter the cell[23]), may penetrate the cell membrane due to their weakly acidic nature, and may be converted into the triphosphate analogues, pyrophosphorylhydrogenphosphonates, which then inhibit viral DNA synthesis catalyzed by the RT, or oxidized to their corresponding phosphates and then further converted into the corresponding triphosphates in the cell. However, unlike AZT-MP, these phosphonates may not inhibit the dTTP formation until they are oxidized. Therefore, the toxicity of the anti-HIV nucleosides may be reduced by formation of their corresponding 5'-phosphonates.

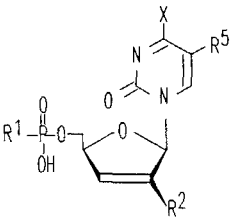
Recently, Holý et al. reported[24] that (S)-9-([3-hydroxy-2-phosphonylmethoxy]propyl)adenine [(S)-HPMPA, Figure 1] was active against several DNA and RNA viruses, including HIV, in cell systems. The H-phosphonate of 9-($\beta$-D-arabinofuranosyl)adenine, ara-A-5'-O-[H-phosphonate], was also



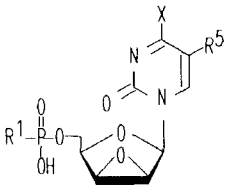
(S)-HPMPA



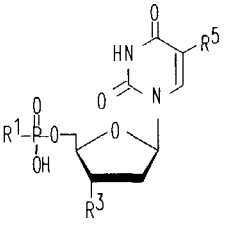
| | X | $R^1$ | $R^5$ |
|---|---|---|---|
| Ia | $NH_2$ | H | H |
| Ib | $NH_2$ | Me | H |
| Ic | OH | H | Me |
| Id | OH | Me | Me |
| Ie | OH | H | H |
| If | OH | Me | H |



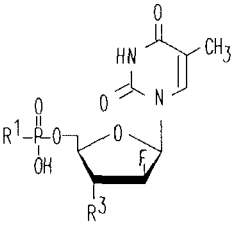
| | X | $R^1$ | $R^2$ | $R^5$ |
|---|---|---|---|---|
| IIa | $NH_2$ | H | H | H |
| IIb | $NH_2$ | Me | H | H |
| IIc | OH | H | H | Me |
| IId | OH | Me | H | Me |
| IIe | OH | H | H | H |
| IIf | OH | Me | H | H |
| IIg | OH | H | F | Me |
| IIh | OH | Me | F | Me |



| | X | $R^1$ | $R^5$ |
|---|---|---|---|
| IIIa | $NH_2$ | H | H |
| IIIb | $NH_2$ | Me | H |
| IIIc | OH | H | F |
| IIId | OH | Me | F |
| IIIe | OH | H | H |



| | $R^1$ | $R^3$ | $R^5$ |
|---|---|---|---|
| IVa | H | F | Me |
| IVb | Me | F | Me |
| IVc | H | $N_3$ | Me |
| IVd | Me | $N_3$ | Me |
| IVe | H | $N_3$ | H |
| IVf | Me | $N_3$ | H |



| | $R^1$ | $R^3$ |
|---|---|---|
| Va | H | H |
| Vb | Me | H |
| Vc | H | $N_3$ |
| Vd | Me | $N_3$ |

*Figure 1*

reported to be active against several DNA viruses in cells.[25] The H-phosphonate, methyl phosphonate, and methylenediphosphonate of AZT were synthesized and found to inhibit replication of HIV in cells.[26,27]

We synthesized H-phosphonates and methylphosphonates of a number of carbohydrate-modified pyrimidine nucleosides, to test their ability to inhibit replication of HIV. These nucleoside phosphonates can be divided into five categories (Figure 1): 2',3'-dideoxynucleosides **I**, 2',3'-didehydro-2',3'-dideoxynucleosides **II**, 2',3'-anhydrolyxofuranosylnucleosides **III**, 2',3'-dideoxy-3'-substituted-nucleosides **IV**, and 2'-β-fluorinated nucleosides **V**.

These nucleoside 5'-phosphonates were prepared by using a modified method of Chen and Benkovic,[28] isolated as their ammonium salts, and used directly in the anti-HIV-1 assay in the H9 cell system. In this way, we were able to prepare a large number of derivatives in a relatively short period of time for initial screening. All the compounds that showed activity were prepared in larger amounts by the modified method of Takaku et al.,[29] and were isolated as their sodium salts. The purity of the sodium salts was from 99.6 to 99.9% by HPLC analyses. These compounds were rescreened in the H9 and MT4 cell systems. $IC_{50}$ and $EC_{50}$ values (the concentrations required to inhibit cell growth by 50% and to inhibit HIV replication by 50%, respectively) were determined on compounds that were repurified by HPLC, and showed that they contain less than 0.01% of the parent nucleosides.

These nucleosides with 10-fold serial dilution were screened preliminally by indirect immunofluorescence assay (IFA) against the HTLV-$III_B$ strain of HIV-I, using H9 cells as the target and $10^3$ tissue culture infectious $doses_{50}$ ($TCID_{50}$) of virus. HIV antigens were detected. The $IC_{50}$ values (for cytotoxicity) of compounds that exhibited activity in the screening were determined. The detailed dose-effect relationships and $EC_{50}$ values (for efficacy) of those compounds were then measured in MT4 cells, using the antigen ELISA kit,[4] and inhibition of the RT release.[5,30]

## Experimental Section

$^1H$ NMR spectra were recorded on a JEOL PFT-100 or JEOL FX90 spectrometer with $Me_4Si$ as the internal standard. TLC was performed on Uniplates (Analtech Co., Newark, DE) and preparative LC on Uniplates 20 x 20 cm, using isopropanol : 25% ammonium hydroxide : water (7:1:2 v/v). Mass

spectral data were recorded by Dr. B. T. Chait of the Mass Spectrometric Biotechnology Resource, Rockefeller University.

**1-(3-Azido-3-deoxy-5-O-[hydrogenphosphonyl]-β-D-erythro-pentofuranosyl)-thymine Ammonium Salt (IVc).** To a solution of 3'-azido-3'-deoxythymidine (53 mg, 0.2 mmol) in pyridine (2 mL) were added 0.6 M solution of phosphorous acid mono-n-butylammonium salt in pyridine (0.5 mL) and N,N'-dicyclohexylcarbodiimide (125 mg, 0.6 mmol). The mixture was stirred for 4 h at room temperature and was then centrifuged for 10 min. The supernatant was removed by decantation, and the solid twice washed by dispersion in $H_2O$ (1 mL each) followed by centrifugation. The combined supernatants were concentrated to dryness in vacuo. The residue was dissolved in a minimal amount of pyridine and applied to a silica gel plate (20 x 20 x 0.15 cm), and the plate was developed in solvent 1 (see Table 1). The UV absorbing band corresponding to the nucleoside-5'-phosphonate was scraped and then extracted with solvent 1 (30 mL). The solvent was removed by evaporation in vacuo. The residue was reevaporated with water (2 mL), and then dried azeotropically by evaporation with EtOH (2 mL x 2) in vacuo to give **IVc** (58 mg, 84% yield) as a colorless foam.

By following the same procedure but using the corresponding nucleosides instead of 3'-azido-3'-deoxythymidine, the following nucleoside-5'-hydrogenphosphonate ammonium salts were prepared:

1-(2,3-Dideoxy-5-O-[hydrogenphosphonyl]-β-D-glycero-pentofuranosyl)-cytosine (**Ia**), -thymine (**Ic**), -uracil (**Ie**); 1-(2,3-dideoxy-2,3-didehydro-5-O-[hydrogenphosphonyl]-β-D-glycero-pentofuranosyl)cytosine (**IIa**), -thymine (**IIc**), -uracil (**IIe**), 1-(2,3-dideoxy-2,3-didehydro-2-fluoro-5-O-[hydrogenphosphonyl]-β-D-glycero-pentofuranosyl)thymine (**IIg**); 1-(2,3-anhydro-5-O-[hydrogenphosphonyl]-β-D-lyxofuranosyl)cytosine (**IIIa**), -5-fluorouracil (**IIIc**), -uracil (**IIIe**), 1-(2,3-dideoxy-3-fluoro-5-O-[hydrogenphosphonyl]-β-D-erythro-pentofuranosyl)thymine(**IVa**),1-(3-azido-2,3-dideoxy-5-O-[hydrogenphosphonyl]-β-D-erythro-pentofuranosyl)thymine (**IVc**), -uracil (**IVe**); 1-(2,3-dideoxy-2-fluoro-5-O-[hydrogenphosphonyl]-β-D-threo-pentofuranosyl)thymine (**Va**), 1-(3-azido-2,3-dideoxy-2-fluoro-5-O-[hydrogenphosphonyl]-β-D-arabinofuranosyl)thymine (**Vc**).

Table 1 lists the reaction conditions, yields, and chromatographic and UV characteristics of some of these nucleoside-5'-hydrogenphosphonates.

Table 1 Experimental conditions for the synthesis of 5'-H-phosphonates

| Compound | solvent (mL) | time (hrs) | yield (%) | chromatography solvent 1 Rf | chromatography solvent 2 Rf | UV absorption in $H_2O$ (nm) max | min | pH |
|---|---|---|---|---|---|---|---|---|
| Ia | pyridine (2) | 12 | 47 | 0.76 | 0.74 | 261 | 230 | 7.0 |
| Ic | MeCN (2)[*1] | 38 | 61 | 0.70 | 0.66 | 268 | 234 | 7.0 |
| Ie | MeCN (2)[*2] | 8[*3] | 36 | 0.54 | 0.48 | 270 | 247 | 7.0 |
| | | | | | | 277 | 237 | 1.0 |
| IIa | $(MeO)_3PO$ (2) | 4[*3] | 36 | 0.73 | 0.73 | 270 | 247 | 7.0 |
| | | | | | | 277 | 238 | 1.0 |
| IIc | $(MeO)_3PO$ (2) | 8[*3] | 40 | 0.63 | 0.60 | 266 | 234 | 7.0 |
| IIe | MeCN/$(MeO)_3PO$ (1)/(0.5) | 4 | 52 | 0.54 | 0.53 | 261 | 230 | 7.0 |
| IIIa | $(MeO)_3PO$ (1) | 14 | 46 | 0.37 | 0.34 | 270 | 247 | 7.0 |
| | | | | | | 277 | 238 | 1.0 |
| IIIc | $(MeO)_3PO$ (0.5) | 12 | 58 | 0.45 | 0.45 | 268 | 234 | 7.0 |
| IIIe | $(MeO)_3PO$ (0.5) | 12 | 52 | 0.43 | 0.54 | 261 | 230 | 7.0 |
| IVa | pyridine (2) | 6 | 78 | 0.62 | 0.56 | 266 | 234 | 7.0 |
| IVc | pyridine (2) | 12 | 84 | 0.68 | 0.55 | 266 | 234 | 7.0 |
| Va | pyridine (2) | 4 | 84 | 0.88 | 0.80 | 266 | 234 | 7.0 |
| Vc | pyridine (2) | 8 | 76 | 0.80 | 0.80 | 266 | 234 | 7.0 |

Solvent 1: iPrOH : 25% $NH_4OH$ : $H_2O$ (7:1:2 v/v)
Solvent 2: dioxane : iPrOH : 25% $NH_4OH$ : $H_2O$ (6:5:1:4 v/v)

[*1] with 0.4 mL of N-methylimidazole
[*2] with 0.5 mL of $(MeO)_3PO$
[*3] beyond this time, side reactions take place

Table 3 lists the $^1H$ NMR parameters for some of these nucleoside-5'-hydrogenphosphonates.

Table 5 lists the $^{31}P$ NMR data for some representative nucleoside-5'-phosphonates.

Table 6 lists mass spectral data of nucleoside 5'-hydrogenphosphonates.

**1-(2,3-Dideoxy-5-O-[methylphosphonyl]-β-D-glycero-pento-furanosyl)cytosine Ammonium Salt (Ib).** To a solution of 1-(2,3-dideoxy-β-D-glycero-pento-furanosyl)cytosine (43 mg, 0.2 mmol) in $(MeO)_3PO$ (2.0 mL) were added at 0

Table 2. Experimental conditions for the synthesis of 5'-Me-phosphonates

| Compound | $(MeO)_3PO$:MeCN (mL) | (mL) | time (hr) at 0°C | time (hr) at rt | yield (%) | chromatography solvent (1) | chromatography solvent (2) | UV absorption in $H_2O$ (nm) max | min | pH |
|---|---|---|---|---|---|---|---|---|---|---|
| **Ib** | 1.5 | 0 | 18 | 0 | 47 | 0.56 | 0.52 | 270 | 247 | 7.0 |
| | | | | | | | | 277 | 238 | 1.0 |
| **Id** | 2 | 0 | 1 | 4 | 86 | 0.53 | 0.50 | 266 | 234 | 7.0 |
| **If** | 0.5 | 2 | 14 | 4 | 54 | 0.73 | 0.70 | 261 | 232 | 7.0 |
| **IIb** | 2 | 0 | 1 | 4 | 42 | 0.71 | 0.68 | 270 | 247 | 7.0 |
| | | | | | | | | 277 | 238 | 1.0 |
| **IId** | 1.5 | 0 | 1 | 4 | 85 | 0.70 | 0.68 | 266 | 234 | 7.0 |
| **IIf** | 0.5 | 0 | 14 | 0 | 67 | 0.50 | 0.46 | 261 | 232 | 7.0 |
| **IIIb** | 1.5 | 0 | 6 | 12 | 52 | 0.42 | 0.40 | 270 | 247 | 7.0 |
| | | | | | | | | 277 | 238 | 1.0 |
| **IIId** | 0.5 | 0 | 21 | 18 | 46 | 0.87 | 0.80 | 269 | 234 | 7.0 |
| **IVb** | 0.2 | 2 | 14 | 6 | 64 | 0.73 | 0.70 | 261 | 232 | 7.0 |
| **IVd** | 0.3 | 1 | 6 | 2 | 78 | 0.85 | 0.75 | 266 | 234 | 7.0 |
| **Vb** | 0.3 | 1 | 6 | 8 | 57 | 0.65 | 0.54 | 265 | 234 | 7.0 |
| **Vd** | 0.3 | 2 | 14 | 0 | 58 | 0.87 | 0.75 | 265 | 235 | 7.0 |

Solvent 1: iPrOH : 25% $NH_4OH$ : $H_2O$ (7:1:2 v/v)

Solvent 2: dioxane : iPrOH : 25% $NH_4OH$ : $H_2O$ (6:5:1:4 v/v)

°C, successively, dichloromethylphosphoryl oxide (80 mg, 0.6 mmol) and 1,2,4-tetrazole (20 mg). The mixture was stirred at 0 °C for 1 h and then at room temperature for 4 h. The mixture was cooled to 0 °C, and the reaction quenched by addition of $Et_3N$ (0.2 mL) and $H_2O$ (0.2 mL). The mixture was stirred for 2 h at 4 °C and then concentrated in vacuo. Compound **Ib** (29 mg, 42% yield, as a colorless foam) was isolated by preparative TLC on a silica gel plate (solvent 1).

By following the same procedure but using the corresponding nucleosides instead of 1-(2,3-dideoxy-β-D-glycero-pentofuranosyl)cytosine, the following nucleoside-5'-methylphosphonate ammonium salts were prepared:

Table 3. $^1$H NMR parameters for 5'-hydrogenphosphonates in $D_2O$.*[1]

| Compound | H1' | H2' | H3' | H4' | H5',5" | H-5 | H-6 | 5Me | H-P |
|---|---|---|---|---|---|---|---|---|---|
| **Va** | 6.11dt (6.1, 0.4) | 5.6-5.0m | | 4.50m | 4.05m | | 7.68s | 1.89d (0.4) | 6.79d (637.7) |
| **Vc** | 6.18t (6.2) | 5.34dt (5.6, 5.1) | | 4.58m | 4.14m | | 7.62s | 1.87d (0.7) | 6.29d (639.4) |
| **IVa** | 6.21t (4.7) | 4.56m | 5.63t (8.2) | 5.06t (8.2) | 4.15t (4.9) | | 7.62d (1.1) | 1.88d (0.8) | 6.80d (639.7) |
| **IVc** | 6.21t (6.6) | 2.46t (6.2) | 4.63t (5.45) | 3.88s | 4.20m | | 7.65d (0.5) | 1.87d (0.8) | 7.11d (637.5) |
| **Ia** | 6.4m | 2.15m | 3.52dd (10.7,10.7) | 4.20m | 3.99m | | 8.04d (7.7) | | 6.71d (629.8) |
| **Ic** | 6.40t (6.6) | 2.3-2.1m | | 4.42m | 4.06m | | 7.78d (1.1) | 1.74d (1.1) | 6.80d (636.3) |
| **Ie** | 6.08dd (6.8,6.0) | 2.17m | 3.36m | 4.60m | 4.02m | 5.86d (6.6) | 7.91d (6.6) | | 6.73d (637.5) |
| **IIa** | 6.95t (5.0) | 4.98m | 6-4.5m | 4.1-4m | 3.7-3.6m | 6.45d (7.3) | 7.72d (7.3) | | 6.48d (638.3) |
| **IIc** | 6.1-6.0m | 4.5-4.3m | 5.03t (10.7) | 4.4-4.0m | 3.62q (6.55) | | 7.98s | | 6.74d (637.7) |
| **IIIa** | 6.20s | 4.2---------- | | | -2.9m | 6.08d (7.7) | 7.85d (7.7) | | 7.14d (641.1) |
| **IIIc** | 6.20s | 5.9---------- | | -4.0m | 4.3-4.2m | | 7.86d (8.2) | | 6.75d (645.3) |
| **IIIe** | 6.25m | 4.5 ---------- | | | -4.8m | | 8.03d (6.6) | | 6.78d (641.1) |

*[1] Chemical shifts in ppm ($\delta$). Signal description by apparent shape (e.g., t or q). Coupling constants in Hz in parentheses right below chemical shifts first order. For $HP(O)(OH)_2$, $\delta$ 6.88d (672.0 Hz).

1-(2,3-Dideoxy-5-O-[methylphosphonyl]-β-D-glycero-pentofuranosyl)-thymine (**Id**), -uracil (**If**); 1-(2,3-dideoxy-2,3-didehydro-5-O-[methylphosphonyl]-β-D-glycero-pentofuranosyl)cytosine (**IIb**), -thymine (**IId**), -uracil (**IIf**); 1-(2,3-dideoxy-2,3-didehydro-3-fluoro-5-O-[methylphosphonyl]-β-D-glycero-pentofuranosyl)thymine (**IIh**); 1-(2,3-anhydro-5-O-[methylphosphonyl]-β-D-lyxofuranosyl)cytosine (**IIIb**), -5-fluorouracil (**IIId**); 1-(2,3-dideoxy-3-fluoro-5-O-[methylphosphonyl]-β-D-erythro-

Table 4. $^1$H NMR parameters for 5'-methylphosphonates in $D_2O$[*1]

| Compound | H1' | H2' | H3' | H4' | H5',5" | H5 | H-6 | 5Me | Me-P |
|---|---|---|---|---|---|---|---|---|---|
| **Ib** | 6.05m | 1.78m | 3.58d (10.7) | 4.12m | 4.02m | | 8.58d (7.7) | | 1.31d (16.5) |
| **Id** | 6.25m | 2.03m | 3.12m | 5.02m | 4.0-3.8m | | 8.13s | 1.41d (1.1) | 1.29d (16.5) |
| **If** | 6.11t (11.2) | 2.13m | 3.12m | 5.02m | 4.1-3.5m | 5.86d (8.2) | 7.91d (8.2) | | 1.27d (14.5) |
| **IVb** | 6.40t (8.8) | 4.53m | 5.72d (4.1) | 5.16m | 3.7-2.6m | | 7.75s | 1.93s | 1.33d (16.2) |
| **IVd** | 6.53t (6.6) | 2.80t (6.3) | 4.48m | 3.88d (5.4) | 4.4-4.3m | | 7.99d (1.1) | 2.21d (0.5) | 1.22d (16.1) |
| **Vb** | 6.12t (6.1) | | | | | | 7.68d (1.2) | 1.89d (1.2) | 1.36d (13.2) |
| **Vd** | 6.52t (4.6) | 5.72t (8.4) | 4.78m | 3.54t (7.4) | 4.5-4.4m | | 7.97d (1.2) | 2.23d (1.2) | 1.69d (16.4) |
| **IIb** | 6.93m | 5.05d (5.0) | 5.98m | 3.96m | 3.88 (10.4) | 6.43d (8.5) | 7.79d (8.5) | | 1.16d (16.5) |
| **IId** | 6.94m | 4.53m | 4.94t (10.4) | 3.98m | 3.61m | | 6.59d (1.0) | 1.87d (1.0) | 1.16d (16.4) |
| **IIf** | 6.81m | 5.83m | 6.50m | 4.78m | 4.01t (5.5) | 6.53d (8.0) | 7.30d (8.0) | | 1.22d (16.5) |
| **IIIb** | 6.19s | 4.6---------- | | | ----------3.5m | 6.15d (8.5) | 8.75d (8.5) | | 1.29d (16.5) |
| **IIId** | 6.25t | 4.3---------- | | | ----------3.2m | | 8.09d (6.6) | | 1.36d (16.5) |

[*1] Chemical shifts in ppm ($\delta$). Signal description by apparent shape (e.g., t or q). Coupling constants in Hz in parentheses right below chemical shifts first order.

pentofuranosyl)thymine (**IVb**), 1-(3-azido-2,3-dideoxy-5-O-[methylphosphonyl]-$\beta$-D-erythro-pentofuranosyl)thymine (**IVd**); -uracil (**IVf**); 1-(2,3-di-deoxy-2-fluoro-5-O-[methylphosphonyl]-$\beta$-D-threo-pentofuranosyl)thymine (**Vb**); 1-(3-azido-2,3-dideoxy-2-fluoro-5-O-[methylphosphonyl]-$\beta$-D-arabinofuranosyl)thymine (**Vd**).

Table 2 lists the reaction conditions, yields, and chromatographic and UV characteristics of some of these nucleoside-5'-methylphosphonates that are synthesized by the above procedure.

Table 3 lists the $^1$H NMR parameters of these nucleoside-5'-hydrogenphosphonates.

Table 5. $^{31}P$ NMR parameters for 5'-phosphonates in $D_2O$.

| Compounds | Chemical shift ppm ($\delta$) | Coupling constants (Hz) |
|---|---|---|
| **Ia** | 5.65m | 929.9 |
| **Ib** | 19.18m | 541.4, |
| **Ic** | 6.16d | 637.0 |
| **If** | 24.46m | 551.7 |
| **IIIa** | 6.16m | 634.3 |
| **IVb** | 26.72m | 636.6 |
| **IVd** | 6.29d | 637.2 |

Table 4 lists the $^1H$ NMR parameters for these nucleoside-5'-methylphosphonates.

Table 5 lists the $^{31}P$ NMR data for some representative nucleoside-5'-phosphonates.

Table 6 lists mass spectral data of nucleoside 5'-hydrogenphosphonates.

**1-(3-Deoxy-3-fluoro-5-O-[hydrogenphosphonyl]-β-D-erythro-pentofuranosyl)-thymine Sodium Salt (IVa).** To a solution of 3'-deoxy-3'-fluorothymidine (122 mg, 0.50 mmol) in pyridine (5 mL) was added $Et_3N$ (7 μL), and the mixture was cooled in an ice-bath. Tris-(1,1,1,3,3,3-hexafluoro-2-propyl)-phosphite (0.40 g, 0.75 mmol) was added, and the mixture was stirred at room temperature overnight under $N_2$. The reaction was quenched by addition of 2 M $Et_3NH_2CO_3$ (pH 7.5, 2 mL). After stirring for 30 min, the mixture was concentrated in vacuo (bath temperature 40 °C). The residue, after several coevaporations with MeOH, was dissolved in $H_2O$ (10 mL), and the solution was placed on top of a column of DEAE-Sephadex A25 ($HCO_3^-$ form) (16 x 250 mm). The column was washed with $H_2O$, and then the product was eluted using a linear gradient formed from 1.0 L each of $H_2O$ and 0.30 M $Et_3NH_2CO_3$. The UV absorbing fractions were combined, concentrated in vacuo, and the excess buffer was removed by codistillation with MeOH. The residual syrup was dissolved in $H_2O$, and the solution passed through a column of AG 50W x 8 ($Na^+$) to yield, after evaporation, the Na salt of **IVa** (148 mg, 90%).

By following the same procedure but using the corresponding nucleosides instead of 3'-deoxy-3'-fluoro-thymidine, the following nucleoside-5'-hydrogenphosphonate sodium salts were prepared:

**Table 6.** Mass spectral data of 5'-hydrogenphosphonates and 5'-methylphosphonates of pyrimidine nucleosides.

| Compound[a] | | Formula[b] (mol. wt.) | m/z[c] (+)FAB | m/z[c] (-)FAB |
|---|---|---|---|---|
| ddC-HP | **Ia** | $C_9H_{14}N_3O_5P$ (275.20) | 276 | 274 |
| ddT-HP | **Ic** | $C_{10}H_{15}N_2O_6P$ (290.22) | 291 | 289 |
| ddU-HP | **Ie** | $C_9H_{13}N_2O_6P$ (276.19) | 277 | 275 |
| d4C-HP | **IIa** | $C_9H_{12}N_3O_5P$ (273.19) | 274 | 272 |
| d4T-HP | **IIc** | $C_{10}H_{13}N_2O_6P$ (288.20) | 289 | 287 |
| F-d4T-HP | **IIg** | $C_{10}H_{12}FN_2O_6P$(306.19) | 307 | 305 |
| laC-HP | **IIIa** | $C_9H_{12}N_3O_6P$ (289.19) | 290 | 288 |
| laFU-HP | **IIIc** | $C_9H_{10}FN_2O_7P$ (308.16) | 309 | 307 |
| laU-HP | **IIIe** | $C_9H_{11}N_2O_7P$ (290.17) | 291 | 289 |
| AZT-HP | **IVc** | $C_{10}H_{14}N_5O_6P$ (331.23) | 332 | 330 |
| FLT-HP | **IVa** | $C_{10}H_{14}FN_2O_6P$(308.21) | 309 | 307 |
| AZU-HP | **IVe** | $C_9H_{12}N_5O_6P$ (317.20) | 318 | 316 |
| ddC-MeP | **Ib** | $C_{10}H_{16}N_3O_5P$ (289.23) | 290 | 288 |
| ddT-MeP | **Id** | $C_{11}H_{17}N_2O_6P$ (304.24) | 305 | 303 |
| ddU-MeP | **If** | $C_{10}H_{15}N_2O_6P$ (290.22) | 291 | 289 |
| d4C-MeP | **IIb** | $C_{10}H_{14}N_3O_5P$ (287.21) | 288 | 286 |
| F-d4T-MeP | **IIh** | $C_{11}H_{14}FN_2O_6P$(320.22) | 321 | 319 |
| laFU-MeP | **IIId** | $C_{10}H_{12}FN_2O_7P$(322.19) | 323 | 321 |
| laC-MeP | **IIIb** | $C_{10}H_{14}N_3O_6P$ (303.21) | 304 | 302 |
| FLT-MeP | **IVb** | $C_{11}H_{16}FN_2O_6P$(322.23) | 323 | 321 |
| AZT-MeP | **IVd** | $C_{11}H_{16}N_5O_6P$ (345.25) | 346 | 344 |
| AZU-MeP | **IVf** | $C_{10}H_{14}N_5O_6P$ (331.23) | 332 | 330 |
| F-AZT-MeP | **Vd** | $C_{11}H_{15}FN_5O_6P$(363.24) | 364 | 362 |

[a] Phosphonates that showed activity in preliminary screening.
[b] As free acids.
[c] Ammonium salts. Major peak: Xe(+)FAB ($M - NH_3 + H^+$), Xe(-)FAB ($M - NH_3 - H^+$).



1-(2,3-Dideoxy-5-O-hydrogenphosphonyl-β-D-glycero-pentofuranosyl)-cytosine (**Ia**), -thymine (**Ic**), -uracil (**Ie**); 1-(2,3-dideoxy-2,3-didehydro-5-O-hydrogenphosphonyl-β-D-glycero-pento-furanosyl)cytosine (**IIa**), -thymine (**IIb**), -uracil (**IIe**), 1-(2,3-anhydro-5-O-hydrogenphosphonyl-β-D-lyxofuranosyl)-cytosine (**IIIa**), -5-fluorouracil (**IIIc**), -uracil (**IIIe**), 1-(3-azido-2,3-dideoxy-5-O-[hydrogenphosphonyl]-β-D-erythro-pentofuranosyl)thymine (**IVc**).

**1-(2,3-Didehydro-2,3-dideoxy-2-fluoro-5-O-[methylphosphonyl]-β-D-glycero-pentofuranosyl)thymine Sodium Salt (IIg).** To an ice-cold solution of

methylphosphonyl dichloride (359 mg, 2.7 mmol) in anhydrous pyridine (2.7 mL) was added dropwise a 1 M solution of $H_2O$ in pyridine (2.7 mL). The suspension was stirred at room temperature for 15 min and then centrifuged. The clear supernatant (2.7 mL, 3 equiv) was added to predried 2'-deoxy-2',3'-didehydro-2'-fluorothymidinene (108 mg, 0.45 mmol), and the homogeneous solution was kept at room temperature for 20 h. The reaction was quenched by addition of 2M $Et_3NH_2CO_3$ (4 mL), and the mixture was concentrated to dryness in vacuo. After several coevaporations with MeOH, the residue was dissolved in $H_2O$ (10 mL) and chromatographed on a DEAE-Sephadex A-25 column (16 x 250 mm) using, at first, $H_2O$ as the eluent and then a linear gradient from $H_2O$ and 0.3M $Et_3NH_2CO_3$ (1 L each). Fractions containing the desired product were combined, and concentrated in vacuo. The residue was dissolved in $H_2O$, and the solution passed through a column of AG 50W x 8 ($Na^+$ form). The UV absorbing fractions were collected, concentrated, and the residue triturated with EtOH to obtain crystalline **IIg**. An additional amount of **IIg** was obtained from the mother liquor by concentration, reprecipitation with MeOH-$Et_2O$, and centrifugation. The combined solid product was dried in vacuo over $P_2O_5$ to afford **IIg** (104 mg, 73 %).

**Purification of Nucleoside 5'-phosphonates by HPLC.** Analytical and semipreparative HPLC were performed on an HPLC system (Rainin) in a gradient system on Dynamax-300A 5 μm reverse phase column (10 x 250 mm) or Dynamax Macro HPLC C18 column (16 x 300 mm) (both from Rainin), using the 0.1M TEAB-$H_2O$-MeCN system as the mobile phase (flow rate 2 mL/min). Retention time for a phosphonate is always shorter than for the parent nucleoside (e.g., FLT-HP 5.8 min, FLT 7.9 min). Fractions containing the desired product were collected, concentrated in vacuo, and the residue, after several co-evaporations with MeOH, was dried in vacuo over $P_2O_5$. The solid residue was dissolved in $H_2O$, the solution was filtered through a Millipore filter (0.2 μm), and the concentration of the compound was determined spectrophotometrically. This solution was directly used for anti-HIV-1 assay. The nucleoside phosphonates were stable in solution at room temperature for at least 3 weeks as measured by HPLC.

**Anti-HIV-I Assay. Preliminary Screening using H9 Cells.** Experiments were performed in 24-well tissue culture plates, using 5 x $10^5$ cells in a total

volume of 1 mL. The cells were pre-incubated with 10 serial dilutions of nucleoside for 60 min before incubation with $10^3$ $TCID_{50}$ of virus. The cultures were refed every 3 to 4 days with fresh medium containing the appropriate concentrations of each drug. On day 8, cells were harvested and HIV antigens were detected by immunofluorescence assay (IFA),[30] using human serum containing high titers of anti-HIV antibodies. Cells were assessed for drug toxicity by trypan blue dye exclusion. Results are expressed as percent inhibition of infection in cultures containing experimental drugs, compared with control (untreated) cultures. Zidovudine was routinely used as a positive control for inhibition.

**Anti-HIV-I Assay using MT4 Cells.** MT4 cells were infected with HIV-1 at 200 $TCID_{50}$ viruses per $10^6$ cells. After an adsorption period of 1 h at 37 °C, 5% $CO_2$, unabsorbed virus was removed by washing once with fresh medium. The cells were then suspended in fresh medium to make a cell suspension of $10^6$ cells/3mL. The cell suspension was distributed into 12-well plates (3 mL/well), and various concentrations of the test compounds were added immediately. After 5 days of incubation at 37 °C, 5% $CO_2$, HIV-1 P24 core antigens in the supernatants of the cell cultures were determined. Untreated and uninfected cell control, and untreated but infected cell control were included in each experiment. Cell-free supernatant fluids of the cell cultures were assayed by HIV-1 P24 core antigen ELISA[30] (Du Pont-NEN Research Products, Boston, MA), directly employing the procedure described in the ELISA kit. AZT was routinely used as a positive control for inhibition.

**Reverse Transcriptase (RT) Assay.** The presence of HIV-1 RT in the supernatants of infected, drug-treated and infected, and non drug-treated MT4 cell cultures was assayed by a modification of the method of Spira et al.[31] as described by Hartshorn et al.[30] Cell culture fluids were clarified at 1000 rpm for 10 min to remove cellular debris. The supernatant samples (20 μL) were transferred in duplicate to 12 x 75 mm plastic Falcon tubes (Becton Dickinson & Co., NJ). Ten μL of virus solubilization buffer [(0.5% Triton x-100 in 0.8M NaCl, 0.5 mM phenylmethylsulfonylfluoride, 20% (v/v) glycerol, and 50 mL Tris-HCl, pH 7.8)] was then added to each tube, to solubilize the viral particles in the supernatant samples and to release HIV-1 RT located in the viral core. These sample tubes were kept

in an ice bath for 15 min. To these tubes was added a mixture (170 μL, cocktail I) [52 mM Tris (pH 7.8), 10 mM $MgCl_2$, 2 mM dithiothreitol, 5 μg/mL poly(rA)-oligo(dT), 83 μg/mL dATP, and 3 μCi/mL [$^3$H]dTTP], and the mixture was incubated at 37 °C for 2 h. Ten μL of yeast tRNA (2.5 mg/mL in 10 mM Tris - 0.1 M NaCl -1 mM EDTA) was added to each tube to cause the strands of DNA to clump together. Three mL of cold 10% TCA was added to the mixture to precipitate DNA, which was then collected by filtration (27 mm glass fiber filter in a Millipore sampling manifold). The filter was washed (5% TCA followed by 70% EtOH). Radioactivity of the DNA on the filter was determined by a scintillation counter (Tri-Carb 1900 CA liquid scintillation analyzer, Packard Instrument).

**Cytotoxicity Assay.** The cytotoxicity in terms of cell growth inhibition of the agents was determined in duplicate in 96-well microplates, by XTT-microculture tetrazolium assay.[32] 2',3'-Bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) was prepared at 1 mg/mL in prewarmed (37 °C) medium without serum. Phenazine methosulfate (PMS) was prepared at 5 mM (1.53 mg/mL) in PBS. Fresh XTT and PMS were mixed together to form an 0.075 mM PMS-XTT solution (25 μL of the stock PMS was added per 5 mL of 1 mg/mL XTT). Fifty μL of this mixture was added to each well of the cell culture after 4-day exposure to the agents. After incubation at 37 °C for 6 h, the 96-well plates were mixed, and absorbance at 450 nm and 630 nm was measured with a microplate reader (EL340, Bio-TEK Instruments, Winooski, VT).

**Experimental Data Analysis.** The dose-effect relationships of at least five different concentrations of each compound (plus drugless control) were analyzed by the median-effect plot[33,34] using computer software[35] for automated analysis. The analysis provided anti-HIV-1 $EC_{50}$s (median-effect concentrations), cytotoxicity $IC_{50}$ (median-inhibitory concentrations), and other dose-effect related parameters.

## Results and Discussion

All the phosphonates initially showing activity are derivatives of active nucleosides. Due to their nature as the salts of weak acids and weak base, they did not give consistent elemental analytical results.

Table 7. Anti-HIV-1 effect and cytotoxicity of 5'-H- and 5'-Me-phosphonates of pyrimidine nucleosides in MT4 cells.

| Compound[a] | | Anti-HIV[b] activity $EC_{50}$ ($\mu$M) | Anti-cell growth[c] activity $IC_{50}$ ($\mu$M) | Selectivity index SI $IC_{50}/EC_{50}$ |
|---|---|---|---|---|
| ddC-HP | **Ia** | 3.91 | 2,320 | 590 |
| ddT-HP | **Ic** | >100 | >5,000 | ---- |
| ddU-HP | **Ie** | 5.34 | >5,000 | >920 |
| d4C-HP | **IIa** | 24.2 | >5,000 | >210 |
| d4T-HP | **IIc** | 11.98 | >5,000 | >410 |
| F-d4T-HP | **IIg** | >50 | >5,000 | ---- |
| laC-HP | **IIIa** | 38.1 | 1,280 | 34 |
| laFU-HP | **IIIc** | >100 | >5,000 | ---- |
| laU-HP | **IIIe** | >100 | >5,000 | ---- |
| AZT-HP | **IVc** | **0.072** | **2,500** | **34,700** |
| FLT-HP | **IVa** | **0.088** | **>4,170** | **>56,810** |
| AZU-HP | **IVe** | 10.65 | >5,000 | >470 |
| ddC-MeP | **Ib** | 10.03 | >5,000 | >499 |
| ddT-MeP | **Id** | >100 | >5,000 | ---- |
| ddU-MeP | **If** | 2.7 | 3,580 | 1,326 |
| d4C-MeP | **IIb** | 16.1 | >5,000 | >310 |
| F-d4T-MeP | **IIh** | >50 | >5,000 | ---- |
| laFU-MeP | **IIId** | >100 | >5,000 | ---- |
| laC-MeP | **IIIb** | >100 | 3,250 | <33 |
| FLT-MeP | **IVb** | 2.62 | 4,600 | 1,756 |
| AZT-MeP | **IVd** | 133 | >5,000 | >37 |
| AZU-MeP | **IVf** | 35 | >5,000 | >143 |
| F-AZT-MeP | **Vd** | 2.67 | >5,000 | >1,873 |
| AZT | | 0.005 | 154 | 30,800 |
| FLT | | 0.069 | 190 | 27,530 |
| ddC | | 0.29 | 2,280 | 7,860 |
| ddT | | 1.88 | >5,000 | >2,660 |
| ddA | | 5.04 | 1,493 | 300 |

[a] Phosphonates that showed activity in preliminary screening.
HP = 5'-(H-phosphonate), MeP = 5'-(Methylphosphonate)
ddC = 2',3'-dideoxycytidine;
ddT = 3'-deoxythymidine;
ddU = 2',3'-dideoxyuridine;
d4C = 2',3'-dihehydro-2',3'- dideoxycytidine (cytidinene);
d4T = 2',3'-dideoxy-2',3'-didehydrothymidine;
F-d4T = 2'-fluoro-d4T;
laC = 1-(2,3-anhydro-ß-D-lyxofuranosyl)cytosine;
laFU = 1-(2,3-anhydro-ß-D-lyxofuranosyl)-5-fluorouracil;
laU = 1-(2,3-anhydro-ß-D-lyxofuranosyl)uracil;
AZT = 3'-azido-3'-deoxythymidine; F-AZT = 2'-$\beta$-fluoro-AZT;
FLT = 3'-fluorothymidine; AZU = 3'-azido-dideoxyuridine;

[b] 50% effective concentration of inhibiting HIV-1 replication, based on P24-ELISA.[4,30] [c] 50% inhibitory concentration of MT4 cell growth, based on XTT-microculture tetrazolium assay.[32]

Table 8. Anti-HIV-1 activity of AZT-HP and FLT-HP based on RT assay on day-4 in MT4 cells.

| Compound | Anti-HIV $EC_{50}$ ($\mu M$)[a] | Anti-cell growth $IC_{50}$ ($\mu M$)[b] | $IC_{50}/EC_{50}$ |
|---|---|---|---|
| AZT-HP | 0.276 | 2,500 | 9,058 |
| FLT-HP | 0.178 | >5,000 | >28,080 |
| AZT | 0.014 | 154 | 11,000 |
| FLT | 0.008 | 190 | 23,750 |
| ddT | 5.52 | >5,000 | >906 |

[a] 50% effective concentration of inhibiting HIV based on RT assay.[30,31]
[b] 50% inhibitory concentration of MT4 cell growth based on XTT-microculture tetrazolium assay.[32]

Table 9. Dose-effect relationships of inhibiting HIV-1 replication in MT4 cells.

| Compounds | ($\mu M$) | % Inhibition P24 ELISA | % Inhibition RT assay | Median-effect plot parameters[a] P24 ELISA | Median-effect plot parameters[a] RT assay |
|---|---|---|---|---|---|
| AZT | 0.1 | 99.20 | 97.89 | Dm: 0.0087 | ; 0.014 |
| | 0.05 | 97.39 | 92.14 | m: 2.08 | ; 1.98 |
| | 0.025 | 91.71 | 72.21 | r: 0.994 | ; 0.999 |
| | 0.0125 | 74.86 | 50.50 | | |
| | 0.00625 | 37.76 | 15.02 | | |
| | 0.00312 | 7.34 | 4.64 | | |
| FLT | 0.08 | 99.63 | 99.58 | Dm: 0.0074 | ; 0.0082 |
| | 0.04 | 98.17 | 97.29 | m: 2.33 | ; 2.22 |
| | 0.02 | 91.13 | 77.76 | r: 0.997 | ; 0.975 |
| | 0.01 | 56.82 | 51.20 | | |
| | 0.005 | 35.24 | 38.51 | | |
| AZT-HP | 1.25 | 99.42 | 98.37 | Dm: 0.072 | ; 0.276 |
| | 0.625 | 97.90 | 94.18 | m: 1.80 | ; 3.17 |
| | 0.312 | 94.58 | 81.28 | r: 0.994 | ; 0.980 |
| | 0.156 | 73.99 | 13.96 | | |
| | 0.078 | 57.13 | 1.0 | | |
| FLT-HP | 1.25 | 99.17 | 98.13 | Dm: 0.135 | ; 0.177 |
| | 0.625 | 98.06 | 93.59 | m: 2.36 | ; 2.05 |
| | 0.312 | 88.38 | 72.94 | r: 0.969 | ; 0.999 |
| | 0.156 | 57.67 | 42.79 | | |
| | 0.078 | 11.35 | 16.54 | | |

[a] Dm (obtained from X-intercept) signifies the potency (the median-effect dose, i.e., $EC_{50}$); m (slope) signifies the shape of the dose-effect curve (m = 1, >1, and <1, indicate hyperbolic, sigmoidal, and negatively sigmoidal, respectively); r (linear correlation coefficient) determines the conformity of the dose-effect data to the median-effect principle of the mass-action law. All parameters are calculated by using a computer software for IBM-PC.[35]

Table 10. Dose-effect relationships of inhibiting HIV-1 replication in H9 cells.

| Compounds | μM | Fractional Inhibition | | Median-effect plot parameters | | |
|---|---|---|---|---|---|---|
| | | P24 ELISA | RT assay | P24 ELISA | | RT assay |
| AZT | 0.312 | 0.9282 | 0.9818 | Dm: | 0.036 μM ; | 0.041 μM |
| | 0.156 | 0.9116 | 0.8814 | m: | 1.51 ; | 1.96 |
| | 0.078 | 0.8805 | 0.8404 | r: | 0.903 ; | 0.957 |
| | 0.039 | 0.6776 | 0.6773 | | | |
| | 0.0195 | 0.1345 | 0.1032 | | | |
| AZT-HP | 1.25 | 0.8927 | 0.8706 | Dm: | 0.144 μM ; | 0.113 μM |
| | 0.625 | 0.8782 | 0.8164 | m: | 1.22 ; | 0.90 |
| | 0.156 | 0.660 | 0.6904 | r: | 0.935 ; | 0.937 |
| | 0.078 | 0.4691 | 0.5245 | | | |
| | 0.039 | 0.0782 | 0.1688 | | | |
| FLT | 0.40 | 0.9167 | 0.8062 | Dm: | 0.005 μM ; | 0.010 μM |
| | 0.20 | 0.7587 | 0.6665 | m: | 1.15 ; | 1.10 |
| | 0.10 | 0.7345 | 0.5639 | r: | 0.973 ; | 0.980 |
| | 0.05 | 0.5473 | 0.2737 | | | |
| | 0.025 | 0.2509 | ND | | | |
| FLT-HP | 0.312 | 0.9120 | 0.8922 | Dm: | 0.016 μM ; | 0.049 μM |
| | 0.156 | 0.8562 | 0.8358 | m: | 0.79 ; | 1.31 |
| | 0.078 | 0.7653 | 0.7417 | r: | 0.977 ; | 0.989 |
| | 0.0195 | 0.6382 | 0.2252 | | | |
| | 0.0098 | 0.340 | 0.0941 | | | |



Their mass spectral data, however, are consonant with the phosphonate structures (Table 6). All the phosphonates showing activity in the initial screening were found to contain from small to significant amounts of their parent nucleosides, as evidenced by HPLC. After rigorous purification (each sample contained less than 0.01% of the parent nucleoside), all compounds except the 5'-H-phosphonates of AZT and FLT showed diminished activity.

We also studied the stability of the 5'-H-phosphonate of FLT (FLT-HP) at 37 °C in $H_2O$, in culture broth containing preheated calf serum but without MT4 cells, and in culture medium with the cells. It was found that both FLT-HP and FLT-MP were both stable in $H_2O$ and in culture broth without the cells for 19 hours. In the culture medium with the cells,

approximately 69% of FLT-HP (retention time 6.8 min for FLT-HP, 13.4 min for FLT: on RP18 ODMP reverse phase column with 20mM $KH_2PO_4$ - MeCN as the mobile phase) was detected in the supernatant as measured by HPLC whereas negligible amount of FLT-MP (retention time 3.2 min) was present in the supernatant. In the cell pack, FLT-HP was found following homogenization and centrif-ugation. These experiments clearly showed that FLT-HP is significantly stable metabolically, and it also penetrates cell membrane of MT4.

FLT-HP gave anti-HIV-1 50% inhibition concentration ($EC_{50}$) of 0.088 $\mu$M, based on p24 core antigen production assay (ELISA), and cytotoxicity 50% inhibition concentration ($IC_{50}$) of >5,000 $\mu$M, based on XTT-microculture tetrazolium assay. The selectivity index (SI) or the $IC_{50}/EC_{50}$ ratio was >56,810. The same assays for the 5'-H-phosphonate of AZT (AZT-HP) showed $EC_{50}$ of 0.072 $\mu$M and $IC_{50}$ of 2500 $\mu$M, with an SI of 34,700. The parent nucleosides, FLT and AZT, gave SI values of 27,530 and 30,800, respectively (Table **7**). When inhibition of RT is used as an indication of suppressing HIV-1 replication, the $EC_{50}$ and $IC_{50}$ of FLT-HP were 0.178 $\mu$M and >5,000 $\mu$M, respectively, which yield the SI value of >28,080, whereas the $EC_{50}$ and $IC_{50}$ of AZT-HP are 0.276 $\mu$M and 2,500 $\mu$M which yield the SI of 9,058 (Table **8**). In contrast, the SI values for RT assays are 23,750 for FLT and 11,000 for AZT (Table **8**). In addition to these data, nucleoside phosphonates are stable in solution at room temperature for at least for 3 weeks. Therefore, the activity of FLT-HP and FLT-HP are apparently not from the parent nucleosides produced by hydrolysis. However, the possibilitis of different metabolism or prodrug mechanism for these nucleoside phosphonates remain to be proven. Typical dose-effect relationships for AZT, AZT-HP, FLT and FLT-HP with p24-ELISA and RT assays in MT4 cells and H9 cells are given in Tables **9** and **10**, respectively.

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**References and Footnotes**

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