Aryl Phosphoramidate Derivatives of d4T Have Improved Anti-HIV Efficacy in Tissue Culture and May Act by the Generation of a Novel Intracellular Metabolite

Christopher McGuigan,* Dominique Cahard, Hendrika M. Sheeka,[†] Erik De Clercq,[‡] and Jan Balzarini[‡]

Welsh School of Pharmacy, University of Wales Cardiff, King Edward VII Avenue, Cardiff, CF1 3XF, U.K.

Received August 14, 1995[⊗]

New phosphate derivatives of the anti-HIV nucleoside analogue d4T were prepared as potential membrane-soluble prodrugs of the bioactive free nucleotide. The enhanced antiviral potency and/or reduced cytotoxicity of the derivatives leads to an increase in selectivity relative to the parent nucleoside analogue. Moreover, the derivatives appear to bypass the dependence of the nucleoside on thymidine kinase-mediated activation, retaining full activity in thymidine kinase-deficient cells. This strongly suggests the successful intracellular delivery of free nucleotides by the masked phosphate triester prodrugs. This is further confirmed by studies using radiolabeled compound which clearly demonstrate the generation of d4T mono-, di- and triphosphates from the prodrug, even in thymidine kinase-deficient cells. Moreover, we herein report the generation of a new metabolite, a partially hydrolyzed phosphate diester, alaninyl d4T monophosphate. We suggest that at least part of the antiviral action of the prodrugs derives from the intracellular generation of such novel diesters which may add considerable weight to the suggested further preclinical development of the phosphate prodrugs.

Introduction

Several members of the 2′,3′-dideoxynucleoside (ddN) series are potent inhibitors of human immunodeficiency virus (HIV) in cell culture.[1−5] The 5′-triphosphates of these nucleoside analogues are potent inhibitors of HIV reverse transcriptase.[6−8] As a rule, the activation (phosphorylation) of these nucleosides is in most cases accomplished by cellular nucleoside and nucleotide kinases. Thus, in contrast to other antiviral agents (e.g. acyclovir) where (herpes simplex) virus-specific thymidine kinase mediates the first step of the conversion of the drug to the intracellular active species, the 2′,3′-dideoxynucleoside analogues depend on cellular nucleoside kinases for their phosphorylation. However, in many cases the ddN derivatives have a poor affinity for nucleoside kinases (i.e. 2′,3′-dideoxycytidine for deoxycytidine kinase, 2′,3′-didehydro-2′,3′-dideoxythymidine and 2′,3′-dideoxyuridine for thymidine kinase, 2′,3′-dideoxyadenosine for adenosine kinase and deoxycytidine kinase, and 2′,3′-dideoxyinosine for 5′-nucleotidase).[9−13] Moreover, the dependence on phosphorylation for activation of the particular nucleoside analogue may be a particular problem in cells where the nucleoside kinase activity is known to be low or even lacking (i.e. monocyte/macrophages).[14] Therefore, we have sought to overcome this dependence on nucleoside kinase activation by the development of a suitable nucleotide delivery strategy. The viability of such an approach is entirely based on the ability to suitably modify the phosphate structure of a membrane-soluble masked nucleotide to enable intracellular delivery and release of the free phosphate form. We have previously noted the success of this strategy with (aryloxy)phosphoramidates (**2**) derived from AZT (**1**).[15] These phosphoramidates retained good activity in thymidine kinase-deficient (TK^-) cells, by comparison to thymidine kinase-competent cells, indicating a (partial) independence of thymidine kinase activation. We were particularly keen to apply this technology to the 2′,3′-dideoxy-2′,3′-didehydro analogue of thymidine (d4T) (**3**) for several reasons. This nucleoside analogue has been noted to be a potent inhibitor of HIV, but displays reduced cytotoxicity in certain cells (e.g. bone marrow progenitor cells) compared to AZT.[3,16,17] Furthermore, it is known that the kinetics of the three phosphorylation steps from the nucleoside analogue to the (bioactive) triphosphate differ in the case of d4T, by comparison to AZT and other 3′-modified nucleoside analogues. In particular, the rate-limiting step for AZT appears to be the conversion of mono- to diphosphate, whereas the conversion of nucleoside to monophosphate may well be rate-limiting for d4T.[7] It could follow that the intracellular delivery of preformed d4T monophosphate (d4TMP) may be more useful than the delivery of AZT monophosphate. From a series of blocked phosphate esters of d4T we have noted significant and selective anti-HIV activity for a phosphoramidate derivative So324 which we herein describe. Particularly interesting is the observation that this material may act by an additional mechanism of action.

Results and Discussion

Chemistry. D4T (**3**) was prepared from thymidine essentially by the method of Horwitz et al.,[18] noting the later comments of Mansuri et al.[19] Then, phenyl methoxyalaninyl phosphorochloridate[20] was allowed to react with d4T using THF/*N*-methylimidazole to give compound **4a** in good yield (88). As anticipated, this material displayed two closely spaced signals in the ^{31}P NMR (δ_P ca. 3.3 ppm),[21] corresponding to the presence of diastereoisomers, resulting from mixed stereochemistry at the phosphate center. Similar diastereomeric splitting, and phosphorus coupling where appropriate,

[†] Department of Chemistry, University of Southampton, Southampton, SO9 5NH, U.K.
[‡] Rega Institute for Medical Research, Katholieke Universiteit of Leuven, B-3000, Leuven, Belgium.
* Author for correspondence. Tel. (+44) 1222 874537, FAX (+44) 1222 874180.
[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1996.

0022-2623/96/1839-1748$12.00/0 © 1996 American Chemical Society

Table Aand Anti-HIV-2 Activity and Cytotoxicity of Test Compounds **1**, **3**, **4a**−**f**, **5a**−**e**, and **6a,b** *in vitro*[a]and Anti-HIV-2 Activity and Cytotoxicity of Test Compounds **1**, **3**, **4a**−**f**, **5a**−**e**, and **6a,b** *in vitro*[a]

	EC_{50} (μM)					CC50 (μM)	
compd	MT-4 HIV-1	MT-4 HIV-2	CEM/0 HIV-1	CEM/0 HIV-2	CEM/TK$^-$ HIV-1	MT4	CEM/0
1	0.002	0.003	0.003	0.004	>100	6	>500
3	0.651	0.770	0.800	0.775	33.3	19	174
4a	0.066	0.067	0.182	0.200	0.075	>100	100
4b	1.34	6.71	6	6	7	≥250	≥250
4c	3.58	11.2	12.5	12.5	4	>250	>250
4d	0.2	0.47	1.1	2.23	0.4	61.5	≥250
4e	0.19	0.38	0.8	1.35	0.33	34.1	216
4f	0.23	0.38	0.6	0.8	0.34	22	≥250
5a	ND	ND	0.04	0.055	0.025	ND	16
5b	0.046	0.11	0.15	0.15	0.11	9.75	29.8
5c	1.72	4.07	2.5	3.7	8.5	82.2	115
5d	0.037	0.12	0.11	0.15	0.11	10.5	26.9
5e	0.057	0.063	0.07	0.16	0.06	>100	60
6a	25.4	50.9	20	50	>250	>250	>250
6b	28.5	62.5	48	240	48	≥250	≥250

[a] EC_{50} is the 50% effective concentration or compound concentration required to protect MT-4 or CEM cells against the cytopathogenicity of HIV by 50%. Data are the mean of two to four independent experiments. CC_{50} is the 50% cytotoxic concentration or compound concentration required to reduce MT-4 or CEM cell viability by 50%.

were also noted in the H-decoupled ^{13}C spectrum. The presence of diastereoisomers was also apparent from ^1H NMR spectroscopy, and analytical HPLC studies on **4a**.

Similarly prepared were the analogues of **4a** with other amino acids: glycine **4b**, valine **4c**, leucine **4d**, phenylalanine **4e**, and methionine **4f**. Each of the analogues was isolated as a mixture of [phosphate] diastereoisomers, as evidenced from spectroscopic and chromatographic data.

We were also interested in studying the structure−activity relationships operating in the aryl region of the phosphoramidates. In particular, we had previously noted that, for diaryl phosphates of AZT, the introduction of electron-withdrawing groups within the aryl moieties leads to a substantial increase in antiviral activity.[22] This was taken as corresponding to the increased ability of such systems to undergo [intracellular] liberation of the aryl moiety and release of the phosphate diester *en route* to the free nucleotides. Thus, we prepared the 2,4-dibromo- (**5a**), 3-trifluoromethyl (**5b**), pentafluoro (**5c**), and 3,5-dichloro (**5d**) analogues, each with some degree of electron withdrawal. For purposes of comparison, we also prepared the 4-ethyl compound **5e** which lacks any electron-withdrawing nature.

Finally, we prepared the analogues lacking the aryl moiety entirely, and instead carrying a methyl group (**6a**) or an ethyl group (**6b**). These were designed to simply probe the necessity for antiviral action of an aryloxy group.

Antiviral Activity. The parent nucleoside d4T (**3**) and phosphoramidates (**4a**−**f, 5a**−**e,** and **6a,b**) were tested for their ability to inhibit the replication of HIV, as previously described,[23] and the results obtained using HIV-1- or HIV-2-infected CEM and MT4 cells are displayed in Table 1. The clinically used nucleoside analogue AZT (**1**) was included as reference material, and tests were also conducted in thymidine kinase-deficient CEM cells.

It is notable that the phosphoramidate derivative **4a** is approximately 4−10-fold more potent than d4T itself against HIV-1 and HIV-2 in MT-4 and CEM cells. Moreover, the >5-fold reduced cytotoxicity of **4a** by comparison to d4T **3** in MT-4 cells leads to a 50-fold improved selectivity index for the phosphoramidate. Furthermore, it is particularly striking that, whereas d4T is virtually inactive in thymidine kinase-deficient CEM cells, the phosphoramidate **4a** retains *full* activity, being ca. 400-fold more potent than d4T in these cells. Similarly, while AZT is inherently more potent than either d4T or the d4T phosphate in thymidine kinase-competent CEM cells, the phosphoramidate derivative **4a** is >1300 times more active than AZT in the kinase-deficient CEM cell line.

In terms of structure-activity relationships in operation, it is apparent that relatively small changes in the amino acid region lead to significant changes in activity. Thus, of the series **4a**−**f**, the alanine compound **4a** is the most potent, with leucine **4d**, phenylalanine **4e**, and methionine **4f** analogues being approximately 10-fold less active, depending on the virus and cell line in question. The glycine compound **4b** is less active still, and the valine analogue **4c** is the least active of the series, being approximately 100-fold less potent than the lead alanine compound **4a**. However, throughout this series, it is notable that full activity is always retained in TK$^-$ cells by comparison to the activity shown in TK$^+$ cells.

Similarly, there is some variation in activity with varying substitution in the aryloxy moiety, studying the series **4a**, and **5a**−**e**. The dibromophenyl analogue **5a** is perhaps slightly more active than the parent phenyl compound **4a**, although this is at the cost of some cytotoxicity in CEM cells. The (trifluoromethyl)phenyl (**5b**), dichlorophenyl (**5d**), and the ethylphenyl (**5e**) compounds are approximately equipotent with the parent phenyl system, while the pentafluorophenyl analogue **5c** is approximately 50-fold less potent. Again, unlike the parent phenyl compound, some of the substituted aryl analogues appear to exhibit significant cytotoxicity. As a result, the highest selectivity index is displayed by the parent phenyl compound **4a**.

Finally, the importance of an aryl moiety for antiviral activity is most evident noting the data for the methyl (**6a**) and ethyl (**6b**) phosphates. These show very little antiviral activity, being approximately 1000-fold less potent than the lead compound **4a**.

Data in TK$^-$ versus TK$^+$ cells clearly demonstrate that the antiviral activity of the phosphoramidates **4a**−**f** and **5a**−**e** is independent of thymidine kinase-mediated phosphorylation. The most obvious mechanism of action consistent with this observation is of intracellular delivery of the free nucleotide d4TMP from the phosphoramidates, and further phosphorylation to generate the active metabolite d4T triphosphate (d4TTP). In order to test this hypothesis, we incubated CEM cells with ^3H-labeled compound **4a** and subsequently studied the formation of radiolabeled intracellular metabolites by HPLC.[24] Markers of authentic d4T mono-, di-, and triphosphate were included, along with d4T (**3**) and blocked phosphoramidate **4a**. The study was compared with labeled d4T (**3**), and both experiments were also performed using thymidine kinase-deficient CEM cells. Measurable levels of d4T mono-, di-, and triphosphate were formed from either d4T incubation or incubation with **4a** using kinase-competent cells. However, with kinase-deficient cells there was no detectable phospho-

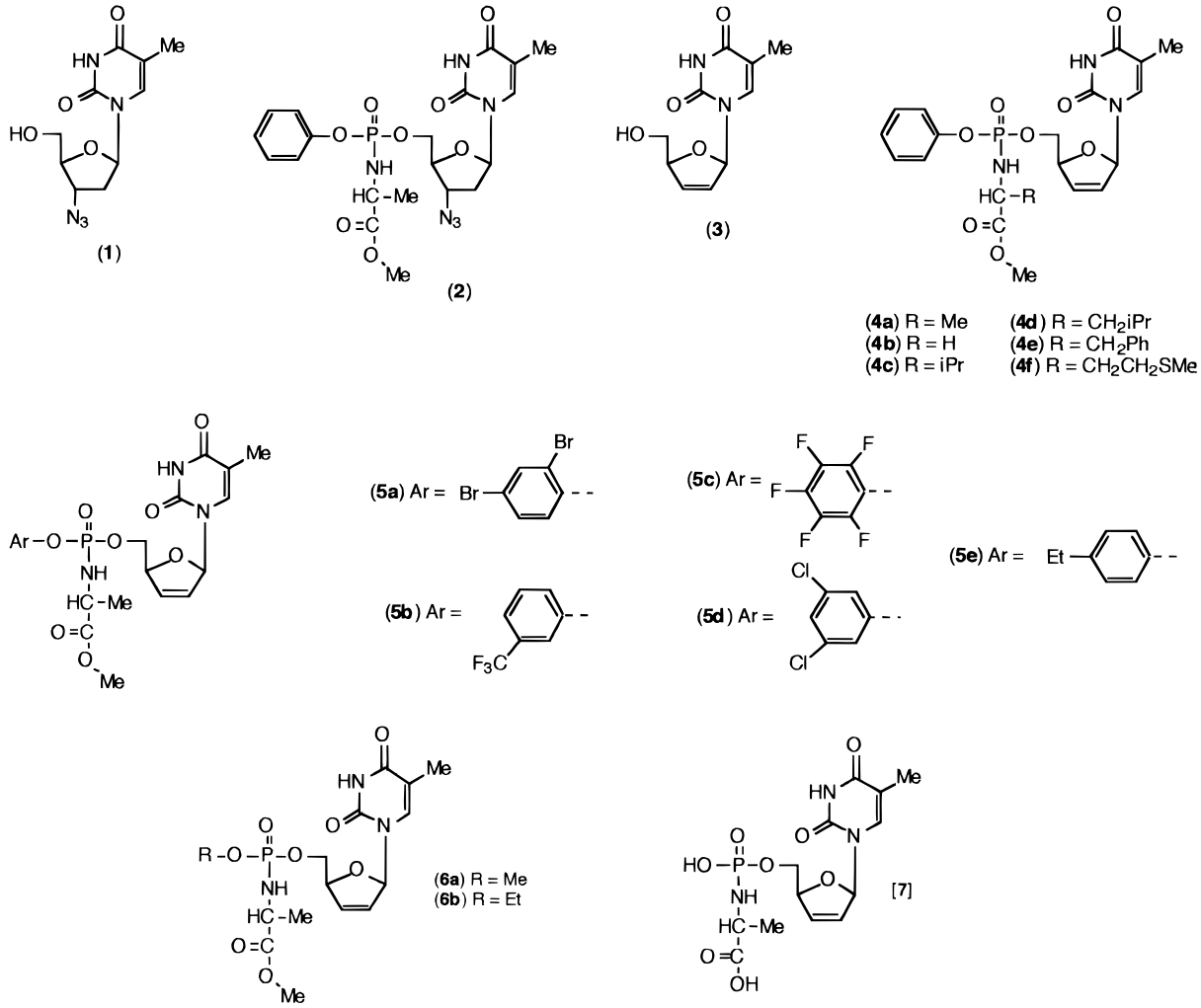

Figure 1.

rylation of d4T. On the other hand, levels of d4T triphosphate generated by **4a** incubation were entirely maintained in the thymidine kinase-deficient cells. Indeed, CEM (wild type) and CEM-TK$^-$ cells generate 0.25 and 0.48 nmol/10^9 cells of d4TTP respectively after 20 h of incubation with 0.2 μM **4a**. There was thus firm evidence that **4a** could give rise to significant levels of the bioactive metabolite d4T triphosphate, by a mechanism which was entirely independent of thymidine kinase, unlike d4T which could give similar levels of the triphosphate, but by an entirely thymidine kinase-dependent process. This is consistent with the suggested intracellular hydrolysis of phosphoramidates such as **4a** to give d4T monophosphate and the thymidylate kinase-mediated phosphorylation of this material to the higher phosphates. The alternative hydrolysis of **4a** to give d4T could not play a major role in the activation pathway, otherwise the generation of d4T triphosphate (and the antiviral activity) would have been significantly reduced in thymidine kinase-deficient cells.[24]

However, we noted in the HPLC chromatogram arising from incubation with radiolabeled **4a**, using either kinase-competent or kinase-deficient cells, the formation of high levels of a new metabolite, of apparent polarity intermediary between d4T mono- and diphosphate. The concentration of this material, as estimated from the radiolabel, was approximately 10−200 times the levels of d4T triphosphate generated, depending on the initial concentration of the phosphoramidate **4a**. At its EC_{50} (ca. 0.2 μM) **4a** generates levels of the new metabolite 10−15-fold higher than the levels of d4T triphosphate (d4TTP) generated from d4T administration to the cell cultures over a comparable incubation time (24 h). At longer incubation times (72 h) the new metabolite was still present at ca. 10-fold higher levels than those of d4TTP. Eventually we were able to prepare a synthetic material identical to this metabolite, on the basis of HPLC retention time in two different systems, by the regioselective base-catalyzed hydrolysis of compound **4a**. This product was then identified as the novel phosphate diester **7** arising from cleavage of both the phenyl and methyl ester groups. We noted that this material could also be generated from **4a** using hog liver esterase, incubated for 24 h at 37 °C. Further evidence for the structure of the metabolite, identified as **7**, came from the observation that compounds **4c, 4e,** and **4f** gave products with hog liver esterase with similar, but nonidentical HPLC retention times. This is entirely consistent with the suggestion that the amino acid moiety is retained in the metabolites. Furthermore, the metabolite and the synthetic material **7** were both stable to alkaline phosphatase, as anticipated for the proposed structure. We wondered whether the metabolite **7** could exert antiviral action via release of d4T (at least in TK$^+$ cells) or d4T monophosphate, and subsequent phosphorylation to the triphosphate act in an entirely novel way. Indeed, we suggest that the much greater concentrations of **7** generated, by comparison to the intracellular levels of d4T triphosphate,

indicates that **7** could contribute to the antiviral efficacy of phosphoramidates such as **4a** via intracellular release of d4TMP. However, it is likely that the contribution of each mechanism to the overall antiviral effect will vary with the exact structure of the phosphoramidate, the initial drug concentration, and the cell type studied. It is quite feasible that some of the structure−activity relationships we noted above, particularly involving variations in the aryl moiety, may relate to the efficiency of the intracellular generation of d4T monophosphate and/or the formation of the phosphate diester metabolite **7**. Alternatively, and particularly in the case of the amino acid variations, some of the evident preference for certain amino acids may also relate either to the relative efficiency of formation of the analogues of (**7**) or to the efficiency of their subsequent activation to d4TTP. This is currently under active investigation in our laboratory.

In conclusion, the phosphoramidate derivatives of d4T herein described show advantage over d4T itself, particularly in thymidine kinase-deficient cells. This leads to enhanced antiviral selectivity relative to the parent drug. Metabolic studies indicate that the phosphoramidates lead to the intracellular generation of bioactive d4T triphosphate by a mechanism which is entirely independent of thymidine kinase, with full retention of d4TTP levels in TK^- cells. We also note the novel discovery of a new metabolite, a phosphate diester retaining the amino acid. We thus report on a new mechanism of inhibition of HIV; the d4T phosphoramidates can act via a d4T or d4TMP depot form, which may be entirely independent of thymidine kinase. We suggest that this new discovery offers great promise for the development of new improved therapies for HIV infection and AIDS.

Experimental Section

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Triethylamine and dichloromethane were dried by heating under reflux over calcium hydride for several hours followed by distillation. Dichloromethane was further dried over activated 4 Å molecular sieves. Tetrahydrofuran was dried by heating under reflux over sodium and benzophenone followed by distillation. *N*-Methylimidazole was purified by distillation. Nucleosides were dried by storage at elevated temperature *in vacuo* over P2O5. Proton, carbon, and phosphorus Nuclear Magnetic Resonance (^{1}H, ^{13}C, ^{31}P NMR) spectra were recorded on a Bruker Avance DPX spectrometer operating at 300, 75.5, and 121.5 MHz, respectively, with ^{13}C and ^{31}P spectra being recorded proton-decoupled. All NMR spectra were recorded in $CDCl_3$ at room temperature (20 °C ± 3 °C). ^{1}H and ^{13}C chemical shifts are quoted in parts per million downfield from tetramethylsilane. *J* values refer to coupling constants, and signal splitting patterns are described as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), multiplet (m), or combinations thereof. ^{31}P chemical shifts are quoted in parts per million relative to an external phosphoric acid standard. Many proton and carbon NMR signals were split due to the presence of (phosphate) diastereoisomers in the samples. The mode of ionization for mass spectroscopy unless stated was fast atom bombardment (FAB) with MNOBA as matrix. Chromatography refers to flash column chromatography and was carried out using Merck silica gel 60 (40−60 μM) as stationary phase. Thin layer chromatography was performed using Alugram SIL G/UV254 aluminum-backed silica gel plates. HPLC was conducted on an ACS quaternary system, using an ODS5 column and an eluant of water/acetonitrile, with 82% water 0−10 min, then a linear gradient to 20% water at 30 min, with a flow rate of 1 mL/min and detection by UV at 265 nm. Final products showed purities exceeding 99% with undetectable levels (<0.02) of parent nucleosides in every case. (Aryloxy)phosphorodichloridates, and (aryloxy)aminophosphorochloridates were prepared entirely as previously noted.[15]

General Procedure. Phenyl methoxyalaninyl phosphorochoridate (250 mg, 0.9 mmol, 2.0 equiv) was added to a stirred solution of d4T (**3**) (100 mg, 0.45 mmol) and *N*-methylimidazole (143.5 μL, 1.8 mmol, 4 equiv) in tetrahydrofuran (THF) (2 mL) at ambient temperature. After 4 h, the solvent was removed under reduced pressure. The gum was dissolved in chloroform (10 mL) and washed with 1 M HCl (8 mL), sodium bicarbonate solution (10 mL), and water (15 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed in *vacuo*. The residue was purified by column chromatography on silica with elution by chloroform−methanol (97:3). Pooling and evaporation of appropriate fractions gave the product as a white foam.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyalaninyl phosphate) (4a): yield 88%; δ_P 3.20, 3.86; δ_H 1.32, 1.34 (d, 3H, J = 6.8Hz, Ala-Me), 1.81, 1.84 (d, 3H, 5-Me), 3.69, 3.70 (s, 3H, OMe), 3.84−4.00 (m, 2H, Ala-CH, Ala-NH), 4.32 (m, 2H, H5′), 5.02 (m, 1H, H4′), 5.88 (m, 1H, H2′), 6.33 (m, 1H, H3′), 7.03 (m, 1H, H1′), 7.15−7.35 (m, 6H, Ph, H6), 9.22, 9.26 (bs, 1H, NH); δ_C 12.52 (5-Me), 21.02 (Ala-Me), 50.22−50.35 (Ala-CH), 52.74 (OMe), 66.62−67.29 (C5′), 84.80−84.88 (C4′), 89.69−89.93 (C1′), 111.44−111.57 (C5), 120.13−120.31 (Ph ortho), 125.30 (Ph para), 127.49−127.65 (C2′), 129.87−129.93 (Ph meta), 133.19-133.50 (C3′), 135.77−136.06 (C6), 150.51 (Ph ipso), 151.16 (C2), 164.14 (C4), 174.12 (Ala-CO); MS *m/e* FAB 466 (MH^+, 7), 340 (MH^+ − base), 200 (17), 136 (47), 89 (25), 81 (C_5H_5O, 100), HPLC t_R 22.48, 22.87 min (see experimental methods for HPLC conditions).

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyglycinyl phosphate) (4b): yield 90%; δ_P 4.89, 5.52; δ_H 1.79, 1.83 (s, 3H, 5-Me), 3.69 (s, 3H, OMe), 3.70−4.05 (m, 3H, Gly-CH_2, Gly-NH), 4.32 (m, 2H, H5′), 4.99 (m, 1H, H4′), 5.92 (m, 1H, H2′), 6.38 (m, 1H, H3′), 6.98 (m, 1H, H1′), 7.05−7.38 (m, Ph, H6), 9.44, 9.46 (s, 1H, NH); δ_C 12.75 (5-Me), 43.15 (Gly-CH_2), 52.94 (OMe), 66.78−67.52 (C5′), 84.98−85.10 (C4′), 89.68−90.16 (C1′), 111.69−111.80 (C5), 120.46−120.59 (Ph ortho), 125.66 (Ph para), 127.66−127.91 (C2′), 130.22 (Ph meta), 133.48−133.87 (C3′), 136.11−136.40 (C6), 150.65 (Ph ipso), 151.45 (C2), 164.46 (C4), 171.41−171.51 (Gly-CO); MS *m/e* FAB 452 (MH^+, 74), 474 (M + Na, 46), HPLC t_R 27.21 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyvalinyl phosphate) (4c): yield 86%; δ_P 4.85, 5.40; δ_H 0.92 (m, 6H, Val-Me), 1.82 (m, 1H, Val-iPrCH), 1.89, 1.91 (s, 3H, 5-Me), 3.76 (s, 3H, OMe), 3.82 (m, 2H, Val-CH, Val-NH), 4.30−4.48 (m, 2H, H5′), 5.07 (m, 1H, H4′), 5.96 (m, 1H, H2′), 6.38 (m, 1H, H3′), 7.10 (m, 1H, H1′), 7.18−7.35 (m, 6H, Ph, H6), 9.31 (s, 1H, NH); δ_C 12.80 (5-Me), 17.77−19.24 (Val-Me), 32.43−32.62 (Val-iPrCH), 52.67 (OMe), 60.32−60.38 (Val-CH), 66.92−67.65 (C5′), 85.04 (C4′), 89.98−90.24 (C1′), 111.76−111.87 (C5), 120.45−120.56 (Ph ortho), 125.54−125.59 (Ph para), 127.81−127.86 (C2′), 130.13−130.17 (Ph meta), 133.51−133.72 (C3′), 136.01−136.28 (C6), 150.83 (Ph ipso), 150.87−151.34 (C2), 164.30−164.37 (C4), 173.56−173.65 (Val-CO); MS *m/e* FAB 493.6 (MH^+, 100), HPLC t_R 28.50 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyleucinyl phosphate) (4d): yield 87%; δ_P 4.18, 4.83; δ_H 0.91 (m, 6H, Leu-Me), 1.42−1.70 (m, 3H, Leu-CH_2CH), 1.91, 1.93 (s, 3H, 5-Me), 3.73 (s, 3H, OMe), 3.76−3.98 (m, 2H, Leu-CHm Leu-NH), 4.28−4.46 (m, 2H, H5′), 5.08 (m, 1H, H4′), 5.96 (m, 1H, H2′), 6.36 (m, 1H, H3′), 7.09 (m, 1H, H1′), 7.18−7.35 (m, 6H, Ph, H6), 9.35 (s, 1H, NH); δ_C 12.76 (5-Me), 22.23−23.01 (Leu-Me), 24.75 ($CH(CH_3)_2$), 43.86−44.11 (CH_2CH), 52.75 (OMe), 53.42−53.60 (Leu-CH), 66.92−67.55 (C5′), 85.62 (C4′), 89.92-90.19 (C1′), 111.69−111.83 (C5), 120.37−120.62 (Ph ortho), 125.55−125.58 (Ph para), 127.79 (C2′), 130.12 (Ph meta), 133.51−133.70 (C3′), 136.00−136.36 (C6), 151.05 (Ph ipso), 151.38 (C2), 164.39−164.50 (C4), 174.55−174.88 (Leu-CO); MS *m/e* FAB 508 (MH^+, 62), 530 (M + Na, 59); HPLC t_R 30.17 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxyphenylalaninyl phosphate) (4e): yield 89%; δ_P 3.96, 4.35; δ_H 1.89 (s, 3H, 5-Me), 3.00 (m, 2H, CH_2Ph), 3.74 (s, 3H, OMe), 3.80−4.28 (m, 4H, Phe-CH, Phe-NH, H5′), 4.94 (m, 1H, H4′), 5.91 (m, 1H, H2′), 6.21−6.30 (m, 1H, H3′), 7.04−7.32 (m, 12H, Ph, H1′, H6), 9.35 (s, 1H, NH); δ_C 12.54 (5-Me), 40.55 (CH_2Ph), 52.63 (OMe), 55.72−56.01 (Phe-CH) 66.50−67.10 (C5′), 84.78 (C4′), 89.71−89.95 (C1′), 111.53−111.64 (C5), 120.28 (OPh ortho), 125.40 (OPh para), 127.52 (C2′), 128.86, 129.65, 129.98 (CH_2*Ph*), 129.86−129.92 (OPh meta), 133.18−133.50 (C3′), 135.72 (CH_2*Ph* ipso), 135.79−136.06 (C6), 150.46 (OPh ipso), 151.13−151.17 (C2), 164.12−164.18 (C4), 173.00 (Phe-CO); MS *m/e* FAB 542 (MH^+, 77), 564 (M + Na, 29); HPLC t_R 29.88 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(phenyl methoxymethioninyl phosphate) (4f): yield 81%; δ_P 4.09, 4.86; δ_H 1.74, 1.79 (s, 3H, MeS), 1.94, 1.97 (s, 3H, 5-Me), 1.80−2.40 (m, 5H, C*H*CH_2CH_2S), 3.72, 3.74 (s, 3H, OMe), 3.98−4.32 (m, 4H, H5′, Met-CH, Met-NH), 4.96 (m, 1H, H4′), 5.84 (m, 1H, H2′), 6.26 (m, 1H, H3′), 6.96 (m, 1H, H1′), 7.05−7.25 (m, 6H, Ph, H6), 9.58 (bs, 1H, NH); δ_C 12.80 (5-Me), 15.68 (CH_3S), 29.95 (CH_2SCH_3), 33.73−33.85 (CH_2CH_2S), 53.06 (OMe), 53.81−54.07 (Met-CH), 67.05−67.70 (C5′), 84.90−85.03 (C4′), 89.98−90.23 (C1′), 111.66−111.86 (C5), 120.39−120.66 (Ph ortho), 125.63 (Ph para), 127.81−127.91 (C2′), 130.18 (Ph meta), 133.44−133.69 (C3′), 136.00−136.38 (C6), 150.72−150.80 (Ph ipso), 151.41 (C2), 164.52 (C4), 173.61−173.94 (Met-CO); MS *m/e* FAB 526 (MH^+, 46), 548 (M + Na, 21); HPLC t_R 29.92 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(2,4-dibromophenyl methoxy alaninyl phosphate) (5a): yield 88%; δ_P 3.07, 3.62; δ_H 1.26, 1.28 (d, 3H, J = 6.8 Hz, Ala-Me), 1.75, 1.80 (s, 3H, 5-Me), 2.11 (s, 1H, NH), 3.64 (s, 3H, OMe), 3.92−4.30 (m, 3H, Ala-CH, H5′), 4.98 (m, 1H, H4′), 5.87 (m, 1H, H2′), 6.26 (m, 1H, H3′), 6.96 (m, 1H, H1′), 7.30−7.60 (m, 4H, Ph, H6), 9.41(bs, 1H, NH); δ_C 12.51 (5-Me), 21.00 (Ala-Me), 50.24 (Ala-CH), 52.80 (OMe), 67.37−67.83 (C5′), 84.49−84.61 (C4′), 89.80−89.92 (C1′), 111.60 (C5), 115.49 (Ph), 118.26 (Ph), 122.61−122.89 (Ph), 127.70 (C2′), 131.86 (Ph), 133.06−133.21 (C3′), 135.64 (Ph), 135.75−135.88 (C6), 147.01 (Ph), 151.07 (C2), 164.03 (C4), 173.71−173.82 (Ala-CO); MS *m/e* FAB 626 (MH^+, 2 × ^{81}Br, 3), 624 (MH^+, ^{81}Br, 6), 621.9507 (MH^+, $C_{20}H_{22}O_8N_3PBr_2$ requires 621.9516, 3), 500, 498, 496 (MH^+ − base, 5,9,5), 81 (100); HPLC t_R 41.17, 41.30 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(3-trifluoromethylphenyl methoxyalaninyl phosphate) (5b): yield 80%; δ_P 2.49, 3.16; δ_H 1.36 (3H, m, Ala-Me), 1.80, 1.86 (3H, d, 5-Me), 3.70, 3.71 (3H, s, OMe) 3.97 (2H, m, Ala-NH, Ala-CH), 4.32 (2H, m, H-5′), 5.03 (1H, m, H-4′), 5.92 (1H, m, H-2′), 6.31 (1H, m, H-3′), 7.03 (1H, m, H-1′), 7.45 (5H, m, Ph, H-6), 9.06 (1H, s, NH); δ_C 12.55, 12.47 (5-Me,) 21.11, 20.99 (d, Ala-Me, J = 4.9 Hz), 50.32, 50.26 (d, Ala-CH, J = 4.8 Hz), 52.87 (OMe), 67.60, 66.89 (d, C-5′, J = 4.9 Hz), 84.61 (d, C-4′, J = 7.8 Hz), 90.04, 89.77 (C-1′), 111.61, 111.44 (C-5), 117.54 (d, Ph, J = 3.9 Hz), 122.14 (Ph), 123.98, 123.79 (Ph), 123.84 (q, CF3, J = 272.0 Hz), 127.84, 127.74 (C-2′), 130.66 (Ph), 132.00 (q, Ph, J = 32.0 Hz), 133.30, 133.02 (C-3′), 135.86, 135.66 (C-6), 150.71 (d, Ph, J = 5.9 Hz), 150.96 (C-2), 163.91, 163.86 (C-4), 174.06, 173.89 (d, Ala-CO, J = 6.8 Hz); MS *m/e* FAB 534.1201(MH^+, $C_{21}H_{24}N_3O_8PF_3$ requires 534.1253, 6), 408 (MH^+ − thymine, 8), 268 (10), 149 (10), 81 (C_5H_5O, 100); HPLC t_R 30.56 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(pentafluorophenyl methoxyalaninyl phosphate) (5c): yield 76%; δ_P 4.74, 5.66; δ_H 1.34, 1.36 (d, 3H, Ala-Me, J = 6.7Hz), 1.75, 1.81 (s, 3H, 5-Me), 3.69 (s, 3H, OMe), 3.92−4.40 (m, 4H, Ala-CH, Ala-NH, H5′), 4.97 (m, 1H, H4′), 5.85 (m, 1H, H2′), 6.29 (m, 1H, H3′), 6.93 (m, 1H, H1′), 7.19 (m, 1H, H6), 9.38 (bs, 1H, NH); δ_C 12.23−12.43 (5-Me), 20.83 (Ala-Me), 50.22−50.34 (Ala-CH), 52.99 (OMe), 67.75−68.37 (C5′), 84.42−84.52 (C4′), 89.87−90.17 (C1′), 111.75 (C5), 127.69−127.93 (C2′), 132.86−133.13 (C3′), 132−143 (m, Ph), 135.74−135.96 (C6), 151.11 (C2), 164.15 (C4), 173.64−173.76 (Ala-CO); MS *m/e* FAB 556 (MH^+, 31), 578 (M + Na, 100); HPLC t_R 35.90 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(3, 5-dichlorophenyl methoxyalaninyl phosphate) (5d): yield 70%; δ_P 2.83, 3.42; δ_H 1.48 (3H, m, Ala-Me), 1.92, 1.97 (3H, s, 5-Me), 3.84 (3H, s, OMe), 4.07 - 4.48 (4H, m, Ala-NH, Ala-CH, H-5′), 5.14 (1H, m, H-4′), 6.04 (1H, m, H-2′), 6.44 (1H, m, H-3′), 7.14 (1H, m, H-1′), 7.29 (3H, m, Ph), 7.40 (1H, s, H-6), 9.74 (1H, s, NH); δ_C 12.51 (5-Me), 20.93 (Ala-Me), 50.26 (Ala-CH), 52.85 (OMe), 66.98, 67.68 (C-5′), 84.60 (C-4′), 89.74, 90.03 (C-1′), 111.40, 111.54 (C-5), 119.40 (Ph), 125.69 (Ph), 127.83 (C-2′), 132.89, 133.14 (C-3′), 135.60 (C-6), 136.01 (Ph), 151.06 (C-2) 151.27 (Ph), 164.09 (C-4), 173.93 (Ala-CO); MS *m/e* FAB 534.0589 (MH^+, $C_{20}H_{23}N_3O_8PCl_2$ requires 534.0600, 8), 408 (MH^+ − thymine, 12), 391 (10), 149 (12), 127 (thymine H^+), 12), 81 (C_5H_5O, 100); HPLC t_R 32.19 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(4-ethylphenyl methoxyalaninyl phosphate) (5e): yield 79%; δ_P 3.43 ; δ_H 1.19 (3H, m, Ala-Me), 1.31 (3H, m, CH_2CH_3), 1.80, 1.84 (3H, d, 5-Me, J = 1.2 Hz), 2.60 (2H, q, CH_2CH$_3$, J = 7.5 Hz), 3.67, 3.70 (3H, s, OMe), 3.93 (2H, m, Ala-NH, Ala-CH), 4.38−4.25 (2H, m, H-5′), 5.00 (1H, m, H-4′), 5.88 (1H, m, H-2′), 6.28 (1H, m, H-3′), 7.00 - 7.14 (5H, m, Ph, H-1′), 7.33, 7.34 (1H, s, H-6), 9.23, 9.25 (1H, s, NH); δ_C 12.41, 12.45 (5-Me), 15.69 (CH_2CH_3), 20.90, 20.97 (d, Ala-Me, J = 4.9 Hz), 28.19 (Ph-CH_2), 50.13, 50.26 (Ala-CH), 52.65 (OMe), 66.48, 67.11 (d, C-5′, J = 4.9 Hz), 84.70, 84.88 (d, C-4′,), 111.40, 111.51 (C-5), 119.90, 120.08 (d, Ph, J = 3.9, 4.9 Hz), 127.36, 127.54 (C-2′), 129.05, 129.11 (Ph), 133.15, 133.50 (C-3′), 135.76, 136.06 (C-6), 141.19, 141.24 (Ph), 148.16, 148.29 (Ph), 151.12, 151.15 (C-2), 164.17, 164.22 (C-4), 174.12, 174.25 (Ala-CO); MS *m/e* FAB 494.1693 (MH^+, $C_{22}H_{29}N_3O_8P$ requires 494.1692, 5), 368 (MH^+ − thymine, 25), 228 (15), 81 (C_5H_5O, 100); HPLC t_R 27.23, 27.48 min.

2′,3′-Dideoxy-2′,3′-didehydrothymidine 5′-(methyl methoxyalaninyl phosphate) (6a): yield 86%; δ_P 9.36, 9.70; δ_H 1.29, 1.31 (d, 3H, Ala-Me, J = 6.7Hz), 1.81, 1.83 (s, 3H, 5-Me) 3.61−3.68 (m, 6H, OMe), 3.84 (m, 2H, Ala-CH, Ala-NH), 4.16 (m, 2H, H5′), 4.95 (bs, 1H, H4′), 5.83 (bs, 1H, H2′), 6.26 (m, 1H, H3′), 6.97 (m, 1H, H1′), 7.26 (d, 1H, H6), 9.30 (bs, 1H, NH); δ_C 12.26−12.48 (5-Me), 21.11 (Ala-Me), 49.93−50.06 (Ala-CH), 52.74 (OMe), 53.19−53.54 (MeOP), 65.93−66.75 (C5′), 84.83−84.94 (C4′), 89.61−89.87 (C1′), 111.37−11.43 (C5), 127.41−127.63 (C2′), 133.22−133.64 (C3′), 135.90−136.21 (C6), 151.07 (C2), 164.10 (C4), 174.39 (Ala-CO); MS *m/e* FAB 404.1223 (MH^+, $C_{15}H_{22}O_8N_3P$ requires 404.1248, 18), 278 (MH^+ − base, 39); 81 (C_5H_5O, 100); HPLC t_R = 18.72, 22.19 min.

2′,3′-Didehydro2′,3′-dideoxythymidine 5′-(ethyl methoxyalaninyl phosphate) (6b): yield 74%; δ_P 7.66, 7.69 ; δ_H 1.26 (6H, m, Ala-Me, CH_2CH_3), 1.83, 1.85 (3H, d, 5-Me, J = 1.2 Hz), 3.67, 3.68 (3H, s, OMe), 3.82−4.16 (6H, m, Ala-NH, Ala-CH, CH_2OP, H-5′), 4.94 (1H, m, H-4′), 5.88 (1H, m, H-2′), 6.28 (1H, m, H-3′), 6.95 (1H, m, H-1′), 7.22, 7.31 (1H, d, H-6, J = 1.2, 1.3 Hz) 10.01 (1H, s, NH); δ_C 12.28, 12.41(5-Me), 16.18 (d, CH_2CH_3, J = 6.8 Hz), 20.84, 20.92 (d, Ala-Me, J = 5.2 Hz), 49.83, 49.90 (Ala-CH), 52.51 (OMe), 62.90, 63.07 (d, CH_2OP, J = 4.9 Hz), 66.63, 66.93 (C-5′), 84.85 (d, C-4′, J = 8.8 Hz), 89.48, 89.75 (C-1′), 111.25, 111.31 (C-5), 127.28, 127.39 (C-2′), 133.20, 133.51 (C-3), 135.80, 136.03 (C-6), 151.15, 151.19 (C-2), 164.30 (C-4), 174.26, 174.32 (d, Ala-CO, J = 6.9 Hz); HPLC t_R = 38.90, 40.82 min.

2′,3′-Didehydro-2′,3′-dideoxythymidine 5′-(Alaninyl phosphate) (7). Compound **4a** (0.116 g, 0.25 mmol) was dissolved in a 1:1 mixture of triethylamine and water (8 mL). After 3 h at room temperature the triethylamine phase was removed and the aqueous phase evaporated under high vacuum at ambient temperature. The resulting crude product was purified on silica using the chromatotron with the mixture $CHCl_3$/MeOH/H_2O/NH_4OH: 120/70/10/1 as eluent. Pooling and freeze-drying of appropriate fractions gave the pure compound: yield 0.051 g, 54); δ_P (D_2O) 7.63; δ_H 1.12 (d, 3H, J = 6.9Hz, Ala-Me), 1.73 (s, 3H, 5-Me), 3.42 (m, 1H, Ala-CH), 3.83 (m, 2H, H5′), 4.93 (m, 1H, H4′), 5.80 (m, 1H, H2′), 6.34 (m, 1H, H3′), 6.78 (m, 1H, H1′), 7.45 (s, 1H, H6); δ_C (D_2O) 11.80 (5-Me), 19.65 (Ala-Me), 50.21 (Ala-CH), 65.32 (C5′), 86.26 (C4′), 90.38 (C1′), 111.57 (C5), 125.19 (C2′), 134.86 (C3′), 138.67 (C6), 152.57 (C2), 166.92 (C4), 179.30 (Ala-CO_2H); MS *m/e* FAB 398 (MNa+, 17), 376 (MH^+, 6); HPLC t_R 3.15 min.

Antiretroviral Evaluation. HIV-1 (HTLV-IIIB) was obtained from persistently HIV-infected H9 cells as described previously.[25] Virus stocks were prepared from the supernatants of HIV-1 (IIIB)-infected MT4 cells. HIV-2 (ROD) was provided by Dr. L. Montagnier (Pasteur Institute, Paris, France). MT-4 cells were provided by Dr. N. Yamamoto (Tokyo

Medical and Dental University School of Medicine, Tokyo, Japan). CEM/0 cells were obtained from the American Tissue Culture Collection (Rockville, MD), and CEM/TK$^-$ cells were a gift from Prof. S. Eriksson and Dr. A. Karlsson (Karolinska Institute, Stockholm, Sweden).

MT-4 and CEM cells were infected with HIV-1 as previously described.[23] Briefly, 5×10^5 MT-4 or CEM cells/mL were infected with HIV-1 or HIV-2 at approximately 100 CCID50 (50% cell culture infective dose)/mL of cell suspension. Then, 100 μL of the infected cell suspension were transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the test compounds. After 4 days, giant cell formation was recorded microscopically in the HIV-infected CEM cell cultures, and after 5 days, the number of viable cells was determined by trypan blue staining of the HIV-infected MT-4 cell cultures. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were defined as the compound concentrations required to reduce by 50% the number of viable cells (MT-4) or giant cells (CEM) in the virus-infected and mock-infected cell cultures, respectively.

C3H/3T3 cells were seeded into Costar Tissue Culture Cluster plates (Costar Broadway, Cambridge, MA) at 20 000 cells/mL into 1 cm^2 wells and grown to confluency. Cell cultures were then infected by 75 foci-forming units of Moloney murine sarcoma virus (MSV) during 90 min, whereafter medium was replaced by 1 mL of fresh culture medium containing different concentrations of test compound. After 6 days the transformation of the test cultures was examined microscopically. The EC_{50} was defined as the compound concentration that is required to inhibit MSV-induced transformation by 50%.

Esterase Hydrolysis. Compound **4a** was incubated with hog liver esterase (20 units/μL) at pH 7.6 in 25 mM Tris·HCl containing 10 mM magnesium dichloride at 37 °C and samples were analyzed by HPLC at appropriate time points.

Acknowledgment. We thank the AIDS Directed Programme of the MRC, the Biomedical Research Programme and the Human Capital and Mobility Programme of the European Commission, the Belgian Geconcerteerde Onderzoeksacties, and the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek for financial support and Mrs. A. Absillis for excellent technical assistance.

References

(1) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Nusinoff-Lehrman, S.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. (1985) 3′-Azido-3′-deoxythymidine (BWA509U): An agent that inhibits the infectivity and cytopathic effect of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096−7100.

(2) Mitsuya, H.; Broder, S. Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotropic virus, type III/lymphadenopathy-associated virus (HTLV III/LAV) by 2′,3′-dideoxynucleosides. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1911−1915.

(3) Balzarini, J.; Kang, G.-J.; Dalal, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; Johns, D. G. The anti-HTLV-III (anti-HIV) and cytotoxic activity of 2′,3′-didehydro-2′,3′-dideoxyribonucleosides. A comparison with their parental 2′,3′-dideoxyribonucleosides. *Mol. Pharmacol.* **1987**, *32*, 162−167.

(4) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Pauwels, R.; Baba, M.; Broder, S.; Vanderhaeghe, H. 3′-Substituted 2′,3′-dideoxynucleoside analogues as potential anti-HIV (HTLV-III/LAV) agents. *J. Med. Chem.* **1987**, *30*, 1270−1278.

(5) Balzarini, J.; Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E. Anti-retrovirus activity of 3′-fluoro- and 3′-azido-substituted pyrimidine 2′,3′-dideoxynucleoside analogues. *Biochem. Pharmacol.* **1988**, *37*, 2847−2856.

(6) Furman, P. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Nusinoff Lehrman, S.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. Phosphorylation of 3′-azido-3′-deoxythymidine and selective interaction of the 5′-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333−8337.

(7) Balzarini, J.; Herdewijn, P.; De Clercq, E. Differential patterns of intracellular metabolism of 2′,3′-didehydro-2′,3′-dideoxythymidine and 3′-azido-2′,3′-dideoxythymidine, two potent anti-human immunodeficiency virus compounds. *J. Biol. Chem.* **1989**, *264*, 6127−6133.

(8) Hao, Z.; Cooney, D. A.; Hartman, N. R.; Perno, C. F.; Fridland, A.; De Vico, A. L.; Sarngadharan, M. G.; Broder, S.; Johns, D. G. Factors determining the activity of 2′,3′-dideoxynucleosides in suppressing human immunodeficiency virus in vitro. *Mol. Pharmacol.* **1988**, *34*, 431−435.

(9) Balzarini, J.; Cooney, D. A.; Dalal, M.; Kang, G.-J.; Cupp, J. E.; De Clercq, E.; Broder, S.; Johns, D. G. 2′,3′-Dideoxycytidine: regulation of its metabolism and anti-retroviral potency by natural pyrimidine nucleosides and by inhibitors of pyrimidine nucleotide synthesis. *Mol. Pharmacol.* **1987**, *32*, 798−806.

(10) Starnes, M. C.; Cheng, Y.-C. Cellular metabolism of 2′,3′-dideoxycytidine, a compound active against human immunodeficiency virus in vitro. *J. Biol. Chem.* **1987**, *262*, 988−991.

(11) Hao, Z.; Cooney, D. A.; Farquhar, D.; Perno, C. F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N. R.; Balzarini, J.; Johns, D. G. Potent chain termination activity and selective inhibition of human immunodeficiency virus reverse transcriptase by 2′,3′-dideoxyuridine-5′-triphosphate. *Mol. Pharmacol.* **1990**, *37*, 157−163.

(12) Johnson, M. A.; Ahluwalia, G.; Connelly, M. C.; Cooney, D. A.; Broder, S.; Johns, D. G.; Fridland, A. Metabolic pathways for the activation of the antiretroviral agent 2′,3′-dideoxyadenosine in human lymphoid cells. *J. Biol. Chem.* **1988**, *263*, 15354−15357.

(13) Johnson, M. A.; Fridland, A. Phosphorylation of 2′,3′-dideoxyinosine by cytosolic 5′-nucleotidase of human lymphoid cells. *Mol. Pharmacol.* **1989**, *36*, 291−295.

(14) Perno, C. F.; Yarchoan, R.; Cooney, D. A.; Hartman, N. R.; Webb, D. S. A.; Hao, Z.; Mitsuya, H.; Johns, D. G.; Broder, S. Replication of human immunodeficiency virus in monocytes. *J. Exp. Med.* **1989**, *169*, 933−951.

(15) McGuigan, C.; Pathirana, R. N.; Balzarini, J.; De Clercq, E. Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.* **1993**, *36*, 1048−1052.

(16) Baba, M.; Pauwels, R.; Herdewijn, P.; De Clercq, E.; Desmyter, J.; Vandeputte, M. Both 2′,3′-dideoxythmidine and its 2′,3′-unsaturated derivative (2′,3′-dideoxythymidinene) are potent and selective inhibitors of human immunodeficiency virus replication in vitro. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 128−134.

(17) Mansuri, M. M.; Hitchcock, M. J. M.; Buroker, R. A.; Bregman, C. L.; Ghazzouli, I; Desiderio, J. V.; Starrett Jr, J. E.; Sterzycki, R. Z.; Martin, J. C. Comparison of in vitro biological properties and mouse toxicities of three thymidine analogs active against human immunodeficiency virus. *Antimicrob. Agents Chemother.* **1990**, *34*, 637−641.

(18) Horwitz, J. P.; Chua, J.; DaRooge, M. A.; Noel, M.; Klundt, I. L. Nucleosides. IX. The formation of 2′,3′-unsaturated pyrimidine nucleosides via a novel β-elimination reaction. *J. Org. Chem.* **1966**, *31*, 205−211.

(19) Mansuri, M. M.; Starrett Jr, J. E.; Ghazzouli, I.; Hitchcock, M. J. M.; Sterzycki, R. Z.; Brankovan, V; Lin, T.-S.; August, E. M.; Prusoff, W. H.; Sommadossi, J.-P.; Martin, J. C. 1-(2,3-dideoxy-β-D-glycero-pent-2-enfuranosyl)thymine. A Highly potent and selective anti-HIV agent. *J. Med. Chem.* **1989**, *32*, 461−466.

(20) McGuigan, C.; Pathirana, R. N.; Mahmood, N.; Devine, K. G.; Hay, A. J. Aryl phosphate derivatives of AZT retain activity against HIV1 in cell lines which are resistant to the action of AZT. *Antiviral Res.* **1992**, *17*, 311−321.

(21) Mark, V.; Dungan, C. H.; Crutchfield, M. M.; Van Wazer, J. R. In *Compilation of ^{31}P NMR data*; Grayson, M., Griffith, E. J., Ed,.; Topics in Phosphorus Chemistry, vol. 5; Wiley: New York, 1969.

(22) McGuigan, C.; Davies, M.; Pathirana, R. N.; Mahmood, N.; Hay, A. J. Synthesis and anti-HIV activity of some novel diaryl phosphate derivatives of AZT. *Antiviral Res.* **1994**, *24*, 69−77.

(23) Balzarini, J.; Karlsson, A.; Perez-Perez, M.-J.; Camarasa, M.-J.; De Clercq, E. Knocking-out concentrations of HIV-1-specific inhibitors completely suppress HIV-1 infection and prevent the emergence of drug-resistant virus. *Virology* **1993**, *196*, 576−585.

(24) Balzarini, J.; Karlsson, A; Perno, C.-F.; Cahard, D.; Naesens, L.; De Clercq, E.; McGuigan, C. Dual mechanism of action of masked alaninyl d4TMP derivatives. Manuscript submitted for publication.

(25) Popovic, M.; Sarngadharan M. G.; Read, E.; Gallo, R. C. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **1984**, *224*, 497−500.

JM950605J