J. Med. Chem. 1999, 42, 4122−4128

Design and Synthesis of Lipophilic Phosphoramidate d4T-MP Prodrugs Expressing High Potency Against HIV in Cell Culture: Structural Determinants for in Vitro Activity and QSAR

Adam Q. Siddiqui, Christopher McGuigan,* Carlo Ballatore, Fabio Zuccotto, Ian H. Gilbert, Erik De Clercq,[†] and Jan Balzarini[†]

Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF1 3XF, U.K., and Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Received December 18, 1998

A series of new substituted-aryl phosphoramidate derivatives of the anti-HIV drug d4T were synthesized as membrane-soluble nucleotide prodrugs, to extend and quantify the SAR observed for an earlier series of related derivatives. All of the compounds were found to be significantly more potent against HIV in cell culture than the nucleoside analogue d4T, and most were also found to be significantly more potent than the parent phosphoramidate. A Hansch type QSAR analysis was applied to the combined series of 21 compounds. The results of this analysis revealed anti-HIV activity to be principally dependent on lipophilicity in a quadratic manner, with terms representing substituent steric bulk and electronic effects having a minimal significance.

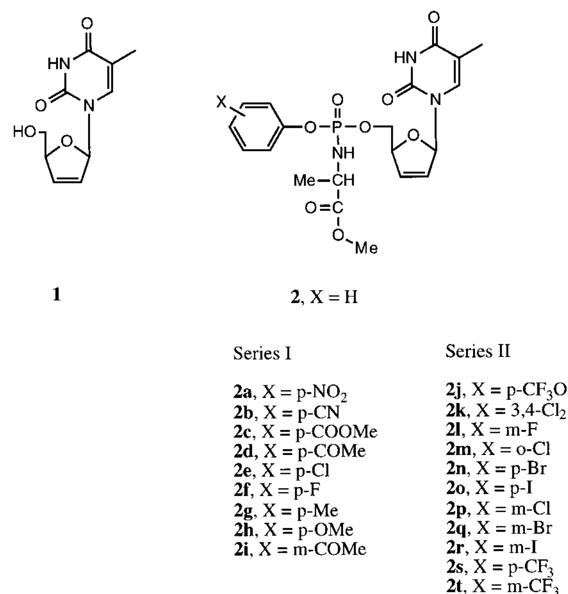
Introduction

Various 2′,3′-dideoxy-2′,3′-didehydro nucleosides are known to be potent inhibitors of HIV, the causative agent of AIDS.[1−6] In particular, the 2′,3′-dideoxy-2′,3′-didehydro analogue of thymidine, d4T (**1**, Chart 1), has been shown to exhibit a selective anti-HIV activity in cell culture and also a reduced toxicity in certain cell types (e.g. bone marrow progenitor cells) compared to the antiretroviral agent 3′-azido-2′,3′-dideoxythymidine (AZT).[4,7,8] In common with other dideoxy-didehydro nucleosides, d4T depends on host cell kinase activity to produce its active triphosphorylated form. Specifically, it is the monophosphorylation step which is rate-limiting for d4T, in contrast to AZT where phosphorylation to the nucleoside 5′-diphosphate is the slowest step in the phosphorylation pathway.[9,10] Several of these types of nucleoside analogues are known to be poor substrates for nucleoside kinases and 5′-nucleotidases.[11−15] These findings have prompted the development of prodrug strategies that bypass initial kinase dependency by the intracellular delivery of the monophosphorylated nucleoside analogue (reviewed by Jones and Bischofberger[16] and recently by Meier[17]).

Previously we have demonstrated the success of an approach using the aryloxy phosphoramidate derivatives of d4A, ddA, and d4T (**2**, Chart 1).[18−20] These compounds were shown to exhibit greatly enhanced activity against HIV compared to the parent nucleoside analogues in vitro, and in contrast to the nucleoside analogues, full activity was retained in kinase-deficient cell lines.

In recent work, we were able to demonstrate a clear dependence of antiviral activity on lipophilicity for a series of aryl-substituted phosphoramidate prodrugs of d4T.[21] The most active compound from series I (**2e**, Chart 1), with a *p*-chloro substituent on the aryl moiety, was found to express a 14-fold greater activity compared to the underivatized parent structure (**2**) against HIV-1, in cell culture (EC_{50}[**2e**] = 0.005 μM, EC_{50}[**2**] = 0.075 μM). Correspondingly, **2e** was approximately 10-fold more lipophilic than **2** based on the 1-octanol/water partition coefficient (P). It was evident that highly electron-withdrawing substituents such as *p*-cyano or *p*-nitro had a deleterious effect on antiviral activity. The observed instability of these compounds in aqueous buffer (pH 7.6, $t_{1/2} \sim 150$ h) suggested that one possible explanation was an appreciable decomposition of the prodrug to less lipophilic derivatives prior to membrane penetration, with a subsequent reduction in rate of diffusion.[21] A carboxy esterase model was introduced to

Chart 1. Anti-HIV Nucleoside Analogue d4T and Various Phosphoramidate Prodrugs



* Author for correspondence: tel/fax, +44 1222 874537; e-mail, mcguigan@cardiff.ac.uk.
† Katholieke Universiteit Leuven.

10.1021/jm9807104 CCC: $18.00 © 1999 American Chemical Society
Published on Web 09/21/1999

Table 1. Substituent Parameters, log *P*, Anti-HIV-1 and Anti-HIV-2 Activity, and Cytotoxicity of Compounds **2a**–**2t**, **2**, and d4T (**1**) in Vitro

				EC_{50}[a] (μM)			CC_{50}[b] (μM)	SI[c]
compd	*MR*	σ	log *P*	CEM/0 (HIV-1)	CEM/0 (HIV-2)	CEM/TK⁻ (HIV-2)	CEM/0	CEM/0 (HIV-1)
2a	7.36	0.78	0.90	0.13	0.19	0.09	73	562
2b	6.33	0.66	0.91	0.15	0.13	0.09	60	400
2c	12.87	0.45	1.01	0.025	0.022	0.025	31	1240
2d	11.18	0.50	0.90	0.11	0.1	0.05	40	363
2e	6.03	0.23	1.43	0.005	0.007	0.006	11	2200
2f	0.92	0.06	1.10	0.053	0.06	0.022	26	490
2g	6.65	−0.17	1.19	0.04	0.05	0.025	34	850
2h	7.87	−0.27	1.09	0.057	0.053	0.047	35	614
2i	11.18	0.38	0.92	0.08	0.09	0.045	50	625
2j	7.86	0.35	1.86	0.014	0.03	0.03	34	2429
2k	12.06	0.52	2.15	0.016	0.03	0.03	21	1313
2l	0.92	0.34	1.08	0.018	0.035	0.065	30	1667
2m	6.03	0.23	1.37	0.018	0.029	0.065	47	2611
2n	8.88	0.23	1.60	0.009	0.016	0.018	17	1889
2o	13.94	0.18	1.93	0.013	0.015	0.029	15	1154
2p	6.03	0.37	1.42	0.025	0.057	0.04	26	1040
2q	8.88	0.39	1.64	0.015	0.016	0.014	13	867
2r	2.097	0.35	1.78	0.008	0.007	0.016	25	3125
2s	5.02	0.54	1.77	0.01	0.01	0.018	13	1300
2t	5.02	0.43	1.57	0.013	0.013	0.0054	14	1077
2	1.03	0.0	1.04	0.075	0.075	0.075	>100	>1333
1			−0.59	0.651	0.770	33	174	267

[a] EC_{50} is the 50% effective compound concentration required to protect CEM cells against the cytopathicity of HIV by 50%. Data are the mean of 2−4 independent experiments. [b] CC_{50} is the 50% cytostatic compound concentration required to inhibit CEM cell proliferation by 50%. [c] SI is the selectivity index or ratio CC_{50}/EC_{50} for HIV-1 in CEM/0 cells. EC_{50} and CC_{50} data for **1** are taken from McGuigan et al.[19]

mimic the putative initial bioactivation step for these phosphoramidates but was rejected as a quantitative model for biological activity.[21] Furthermore, regioisomeric substitution with the acetyl group suggested an independence of antiviral activity on aromatic substituent position.

Here we present the synthesis and biological activity of a range of novel aryl-substituted analogues of **2**, designed to probe the relationships previously observed and to provide a range of highly active phosphoramidate prodrugs. In addition to the data previously published, the current data provided the basis for QSAR and thus a powerful tool to predict the antiviral activities of further prodrugs, designed to satisfy particular pharmacological requirements.

Results and Discussion

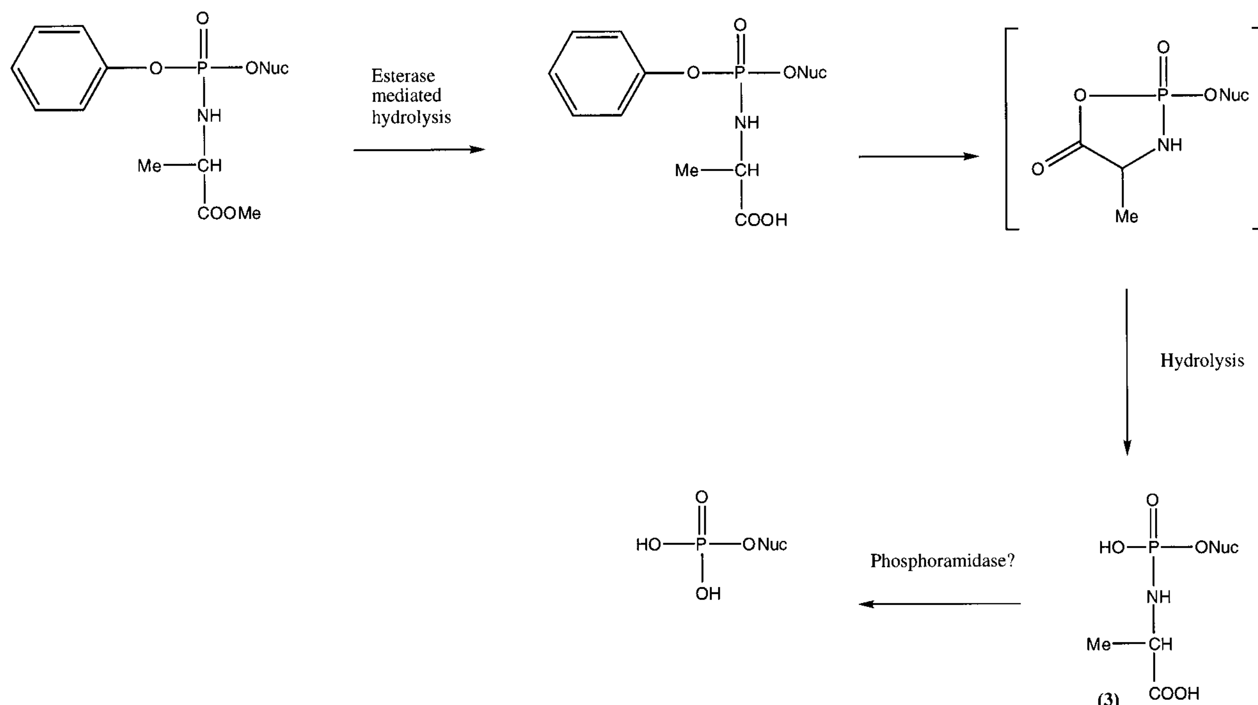
The synthesis of d4T from thymidine was conducted essentially by the method of Horwitz[22] taking into account the later comments of Mansuri.[23] Aryloxyphosphorodichloridates and aryloxyaminophosphorochloridates were prepared entirely as previously noted.[24,25] Novel phosphoramidates **2j**−**2t** (series II, Chart 1) were synthesized according to the method described previously.[19] The structure and purity of compounds **2j**−**2t** were determined by extensive multinuclear NMR and analytical HPLC using two distinct eluent gradients.

Compound Partition Coefficients. The partition coefficients (*P*) of compounds **2j**−**2t** were determined in 1-octanol/aqueous buffer (pH 7.0) (results shown in Table 1). Each compound was partitioned between 1-octanol and aqueous buffer for 5 min. After separation of the two layers the relative proportion of compound in each layer was determined by comparing the UV absorption of the octanol phase with that of a control solution. This method of determining the partition coefficients allows for a quantitative comparison between the compounds in this series. The substituents chosen were those whose Hansch values were predominantly equal to or greater than that of the chlorine atom ($\pi = 0.71$). The order of the measured values of *P* was in good agreement with the lipophilic order predicted by the Hansch values (π) of the aryl substituents. For the *para*-halogenated series, the lipophilicity values varied by approximately 1 order of magnitude (log *P*[**2l**] = 1.08, log *P*[**2o**] = 1.93), and as expected the various *meta*-substituted compounds were found to have similar log *P* values to those observed for their regioisomers. Comparative data for d4T indicates significantly increased lipophilicity (ca. 50−500-fold) for all the phosphoramidates in series II relative to the nucleoside analogue.

Antiviral Activity. All of the phosphoramidates **2j**−**2t** were evaluated for their ability to inhibit the replication of HIV, as previously described.[25] The results obtained using HIV-1- or HIV-2-infected CEM cells are displayed in Table 1. The tests were also conducted against HIV-2 in thymidine kinase-deficient (TK⁻) CEM cells. Previously obtained data[19] for d4T, **2**, and **2a**−**2i** are also displayed.

All of the synthesized derivatives were significantly more potent than d4T against HIV in cell culture, with several compounds showing a 10-fold superior selectivity index (SI) to the nucleoside analogue. The majority of the derivatives were also significantly more potent compared to the underivatized parent compound **2**, and all the derivatives expressed a similar selectivity index to the lead compound (SI[**2**] > 1333). The elevations in activity observed for the *p*-bromo and *p*-iodo derivatives compared to the underivatized parent structure (6- and 9-fold, respectively) were of a similar magnitude to that observed previously for the *p*-chloro derivative (14-fold). It is notable that the increased compound lipophilicity, conferred by bromo and iodo substituents, did not enhance antiviral activity beyond that observed for the *p*-chloro derivative, indicating a possible second-order

Scheme 1. Proposed Activation Pathway for Phosphoramidate Prodrugs



polynomial relationship between lipophilicity and antiviral activity. In common with the derivatives from series I, all the compounds from series II retained full antiviral activity in thymidine kinase-deficient cells, which is indicative of an effective kinase bypass, resulting from efficient intracellular delivery of the free nucleotide.

The release of variously substituted phenols upon prodrug bioactivation may lead to differences in specific phenol toxicty, for example, via the uncoupling of oxidative phosphorylation.[26,27] The lack of a consistent trend between the constituent properties of the released phenol (i.e. lipophilicity and acidity) and the selectivity index values for the corresponding prodrug suggests that toxicity is relatively independent of the chemical nature of the released phenol, within the scope of the substituents used in the series.

The increased potency of the more lipophilic prodrugs offers the possibility of reducing the observed clinical side effects of d4T (peripheral neuropathy and liver damage/inflammation) by using significantly reduced concentrations of the prodrug to produce an equivalent clinical response. This is a desirable goal given the indefinite time periods over which highly active antiretroviral therapies (HAART) for HIV are currently administered.

In the putative bioactivation pathway for these phosphoramidates (Scheme 1), the initial step is believed to be the carboxy esterase-mediated hydrolysis of the amino ester moiety.[28] The extent to which the aryl group may be derivatized without affecting the ability of the compound to behave as a substrate is currently unknown. To further test for constraints in the position of substitution, the *meta*-substituted regioisomers of various *para*-substituted derivatives were synthesized. For the majority of these regioisomeric compounds, no appreciable differences in antiviral potency were observed, indicating an apparent tolerance to specific aryl substituent position by the putative enzyme(s) responsible for bioactivation. Exceptions to this general observation are the *m*-fluoro (**2l**) and *m*-chloro (**2p**) derivatives which respectively displayed a greater and lesser antiviral potency than might have been predicted from the activities of their *para*-counterparts (**2f** and **2e**, respectively). The *o*-chloro derivative **2m** displayed a similar antiviral activity to the *p*-chloro derivative **2e**—within the limits of experimental accuracy—indicating no apparent *o*-effect for this particular substituent.

The high in vitro potency of the *p*-chloro derivative **2e**, suggested the synthesis of the 3,4-dichloro derivative **2k** according to the SAR methodology outlined by Topliss.[29] This was found to be highly active compared to **2** but certainly no more active than the *p*-chloro derivative **2e**. This suggests that disubstitution on the aryl ring is not restrictive in terms of in vitro activity and opens the way to further manipulate the properties of the aromatic ring by using combinations of substituents. The superior lipophilicity of the disubstituted compound compared to **2e** ($\log P[\mathbf{2k}] = 2.15$, $\log P[\mathbf{2e}] = 1.42$) further supports the suggestion of a nonlinear relationship between antiviral activity and lipophilicity.

QSAR. A Hansch type analysis was conducted on all 21 compounds from series I and II, using physicochemical constants taken from Hansch and Leo (Table 1).[30,31] Correlations were sought between antiviral activity in cell culture (as measured by EC_{50}[HIV-1]) and various physicochemical parameters associated with each substituent or its effects: $\log P$ and $(\log P)^2$ to represent compound lipophilicity, Hammet σ values to represent the substituent electronic effects, and *MR*, the molar refractivity, as an approximate measure of substituent steric bulk, taking into account the comments of Hansch et al.[31] The two most significant equations generated are given below. Partial least-squares (PLS) computational methods were applied using cross-validation to determine the optimum model composition and 'boot-

strapping' iterative techniques to calculate the standard errors of the coefficients (given in parentheses). Also given for each equation are the squared correlation coefficients (r^2), the standard error of estimate (s), the F statistic, and Q^2, the cross-validated, squared correlation coefficient.

$$\log 1/\mathrm{EC}_{50}[\text{HIV-1}] = -2.793 + 0.075(0.187)\sigma + 0.009(0.016)MR + 5.588(1.548)\log P - 1.661(0.561)(\log P)^2 \quad (1)$$

$$n = 21,\ s = 0.208,\ r^2 = 0.814,\ F_{4,16} = 17.519,\ F_{4,16}\alpha\ 0.001 = 7.94,\ Q^2 = 0.685$$

$$\log 1/\mathrm{EC}_{50}[\text{HIV-1}] = -2.440 + 5.157(1.117)\log P - 1.499(0.395)(\log P)^2 \quad (2)$$

$$n = 21,\ s = 0.200,\ r^2 = 0.807,\ F_{2,18} = 37.735,\ F_{2,18}\alpha\ 0.001 = 10.40,\ Q^2 = 0.745$$

By calculating the t statistic for each of the descriptors in eq 1, it can be shown that the lipophilic terms log P and (log P)^2 are both significant at the 1% level, whereas the terms MR and σ are both rejected at the 50% level. Additionally, a comparison between the values of Q^2 reveals eq 2, presenting a simple quadratic relationship between lipophilicity and antiviral activity, to be the more robust of the two and thus the most likely to give accurate predictions. Using this equation, a reasonable estimate of optimum prodrug lipophilicity can be made (log $P_{\text{optimum}} = 1.72$).

These results suggest that lipophilicity is the major determinant for in vitro activity. The low significance of the terms σ and MR in eq 1 demonstrates the relatively minor contribution to antiviral activity made by substituent electronic or steric properties, within the parameters of the analysis. An identical treatment of the antiviral data for HIV-2 revealed similar quantitative and qualitative relationships to those observed for HIV-1 (data not shown).

The QSAR analysis is consistent with cellular transport by passive diffusion being a major determinant of in vitro activity. The absence of a deleterious effect on in vitro activity when the aryl substituent is relatively bulky or when the substituent position is varied or when the aromatic ring is disubstituted suggests that any interactions between the phenoxy moiety of the prodrug and the esterase(s) proposed for the first step in intracellular activation are not sufficiently restrictive to significantly decrease the rate of hydrolysis. Furthermore, the absence of a marked influence on in vitro activity exerted by the electron-withdrawing/donating characteristics of the aryl substituent suggests that although the leaving group ability of the phenoxy group is important (to allow selectivity between the cleavage of the P−OAr bond in preference to the P−O nucleoside bond or the P−N bond) it is not sensitive, unless the substituent exerts an electron-withdrawing effect of sufficient magnitude to enable substantial phosphotriester hydrolysis prior to diffusion through cellular membranes. On the basis of the current study, an efficient leaving group would be one with a conjugate acid pK_a value within the range 10.2−7.2 (defined by the pK_a values for p-methoxyphenol and p-nitrophenol, respectively). In previous work, the replacement of the phenoxy moiety with inferior leaving groups such as methoxy and ethoxy (methanol pK_a = 15, ethanol pK_a = 16) produced inactive compounds.[19] The full range of conjugate acid pK_a values (particularly the upper limit) that would characterize a biologically efficient leaving group remains to be determined, as does the possibility of replacing the phenoxy group with alternative chemical structures with acceptable conjugate acid pK_a values.

The high dependence of biological activity on lipophilicity alone suggests that the scope of this analysis is sufficiently broad to allow us to predict the antiviral activities of a wide variety of substituted-aryl derivatives of **2**. In the production of a medicinally viable drug it is often important to improve the pharmacological profile of a lead compound without diminishing its in vitro potency. Stability in vivo, bioavailability, and water solubility are example areas for potential optimization. The apparent insensitivity to substituents of modest bulk with variable electron-donating/withdrawing characteristics at the aryl moiety suggests the potential for optimizing prodrug pharmacological parameters by simple derivatization at this site. Several of the compounds from series I were found to have an enhanced aqueous solubility compared to the lead compound **2** (data not shown). Of these only the p-ester-substituted derivative **2c** displayed an elevated antiviral potency compared to **2**. Significantly, although **2c** is approximately 12 times more water-soluble than **2** (saturation concentrations [water]: **2** = 5.4 mmol/L, **2c** = 67.0 mmol/L) the lipophilicity of the two compounds is approximately equal.

It should be noted that the inclusion in the QSAR of data from a range of compounds bearing halogeno substituents necessarily introduces an element of collinearity between the terms used in the analysis, for example, between MR and log P. The relatively small contribution made by MR to eq 1 suggests that this is not a significant source of error. A further series of compounds bearing aryl substituents with larger values of MR than those used in this analysis but similar values of log P and σ would be useful for identifying trends for substituents of high steric bulk. Similarly, compounds with comparable values of MR and log P but varying values of σ would help to examine the influence of electronic effects.

In conclusion, we have synthesized a range of new, highly potent anti-HIV phosphoramidate prodrugs, the majority of which exhibited significantly higher anti-HIV activity than our lead compound **2** and all of which were significantly more potent and selective compared to the parent nucleoside analogue d4T. We have demonstrated antiviral activity to be principally dependent on lipophilicity and in a quadratic manner, with steric and electronic effects adding relatively minor contributions. Additionally, the position of the monosubstituent had no consistently obvious effect on in vitro antiviral activity.

By the appropriate use of the developed QSAR, it is our intention to design and synthesize a range of pharmacologically optimized prodrugs based on the phosphoramidate pronucleotide concept, by simple derivatization at the aryl moiety. Work to determine the

extent to which these findings can be applied to the delivery of other therapeutic nucleoside analogues is currently in progress.

Experimental Section

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Triethylamine was dried by refluxing over calcium hydride. Anhydrous tetrahydrofuran and dichloromethane were purchased from Aldrich. *N*-Methylimidazole was purified by distillation. Nucleosides were dried by storage at elevated temperature over P_2O_5 in vacuo. 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. All ^{13}C and ^{31}P spectra were recorded proton-decoupled. All NMR spectra were recorded in $CDCl_3$ at room temperature (20 ± 3 °C). Chemical shifts for ^{1}H and ^{13}C spectra are quoted in parts per million downfield from tetramethylsilane. Coupling constants are referred to as *J* values. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). Chemical shifts for ^{31}P spectra 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 spectrometry was fast atom bombardment (FAB) using MNOBA as matrix. Column chromatography refers to flash column chromatography carried out using Merck silica gel 60 (40−60 μm) as stationary phase. HPLC (Shimadzu) was conducted on an SSODS2 reverse-phase column using a water/acetonitrile (Fisher, HPLC grade) eluent; gradient I (standard gradient): 0−80% CH_3CN (0−60 min), 80−0% CH_3CN (60−65 min), flow rate 1 mL/min, UV detection at 265 nm; gradient II: 0−10% CH_3CN (0−5 min), 10−70% CH_3CN (5−55 min), 70−0% CH_3CN (55−60 min), flow rate 1 mL/min, UV detection at 265 nm. Final products showed purities exceeding 99% with undetectable levels (<0.02) of parent nucleosides in every case. Partition coefficients were determined using 1-octanol and phosphate buffer, pH 7.0 (Fisons). UV absorptions were determined using a Unicam Helios α UV spectrometer.

General Procedure. Aryl methoxyalaninylphosphorochloridate (5 mmol) was added to a stirred solution of d4T (1.7 mmol) and *N*-methylimidazole (5 mmol) in tetrahydrofuran (THF) (20 mL) at ambient temperature. After 16 h, the solvent was removed under reduced pressure. The residual gum was dissolved in chloroform (50 mL) and washed with 1 M HCl (50 mL), sodium bicarbonate solution (50 mL), and water (50 mL). The organic phase was dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica with elution by dichloromethane−methanol (97:3). Pooling of appropriate fractions, followed by removal of solvent under reduced pressure, gave the product as a brittle white foam.

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(4-(trifluoromethoxy) phenyl methoxyalaninylphosphate) (2j): yield 65%; δ_P 4.18, 4.81; δ_H 1.34 (3H, m, Ala-Me), 1.84 (3H, d, 5-Me), 3.70 (3H, s, OMe), 3.97 (2H, m, Ala-NH, Ala-CH), 4.31 (2H, m, H-5′), 5.10 (1H, m, H-4′), 5.89 (1H, m, H-3′), 6.32 (1H, m, H-2′), 7.20 (1H, m, H-1′), 7.27 (5H, m, H-6, Ar−H), 9.83 (1H, d, NH); δ_C 12.72 (5-Me), 21.16 (Ala-Me), 50.56 (Ala-CH), 52.99 (OMe), 53.85 (CF_3), 66.97, 67.72 (C-5′), 84.95 (C-4′), 89.94, 90.21 (C-1′), 111.63, 111.78 (C-5), 121.81, 121.98 (Ph), 122.64, 122.82 (Ph), 127.90, 127.95 (C-2′), 133.28, 133.61 (C-3′), 135.96, 136.22 (C-6), 146.28 (Ph), 149.02 (Ph), 151.44 (C-2), 164.00, 164.53 (C-4), 174.20, 174.29 (d, *J* 6.9, Ala-CO); MS *m*/*e* FAB 550.2110 (MH$^+$, $C_{21}H_{24}F_3N_3O_9P$ requires 550.1202); HPLC t_R 36.42, 36.85 min; t_R 39.36, 39.87 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(3,4-dichlorophenyl methoxyalaninylphosphate) (2k): yield 35%; δ_P 4.45, 3.87; δ_H 1.35 (3H, m, Ala-Me), 1.82 (3H, d, 5-Me), 3.70 (3H, s, OMe), 3.86 (1H, m, Ala-CH), 4.26 (2H, m, 2H-5′), 5.05 (1H, m, H-4′), 5.92 (1H, m, H-3′), 6.32 (1H, m, H-2′), 7.05 (2H, m, Ph, H-6), 7.17 (1H, m, H-1′), 7.34 (2H, m, Ph), 8.38 (1H, d, NH); δ_C 12.80, 12.84 (5-Me), 21.27, 21.41 (d, *J* 5.66, *J* 5.64, Ala-Me), 50.54, 50.62 (Ala-CH), 53.17 (OMe), 67.17, 67.87 (d, *J* 0, *J* 5.0, C-5′), 84.85, 84.95 (C-4′), 90.06, 90.34 (C-1′), 111.68, 111.84 (C-5), 120.22, 120.39 (d, *J* 5.1, Ph), 122.75, 122.89 (d, *J* 5.0, Ph), 126.27 (Ph), 128.03, 128.13 (C-2′), 129.50 (Ph), 131.44 (Ph), 133.22, 133.53 (C-3′), 135.89, 136.10 (C-6), 149.45, 149.56 (d, *J* 6.0, *J* 6.2, Ph), 151.21 (C-2), 164.13, 164.16 (C-4), 174.14, 174.32 (d, *J* 7.2, *J* 13.4, Ala-CO); MS *m*/*e* FAB 534.0631 (MH$^+$, $C_{20}H_{22}Cl_2N_3O_8P$ requires 534.0600); HPLC t_R 35.66, 36.00 min; t_R 38.56, 38.97 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(3-fluorophenyl methoxyalaninylphosphate) (2l): yield 26%; δ_P 4.31, 3.66; δ_H 1.31 (3H, m, Ala-Me), 1.84 (3H, d, 5-Me), 3.67 (3H, s, OMe), 3.89 (2H, m, Ala-NH, Ala-CH), 4.27 (2H, m, H-5′), 4.99 (1H, m, H-4′), 5.87 (1H, m, H-3′), 6.28 (1H, m, H2′), 6.91 (4H, m, 2 Ar−H, H-1′, H-6), 7.22 (3H, m, Ar−H), 8.37 (1H, d, NH); δ_C 11.32, 11.37 (5-Me), 19.83, 19.90 (Ala-Me), 51.66 (OMe), 65.61, 66.26 (C-5), 83.55, 83.61 (C-4′), 88.58, 88.84 (C-1′), 107.14, 107.47 (Ph), 110.27, 110.42 (Ph), 111.15, 111.44 (C-5), 115 (Ph), 126.47, 126.61 (C-2′), 129.47, 129.60 (Ph), 131.91, 132.22 (C-3′), 134.52, 134.76 (C-6), 149.80 (C-2), 159.53 (Ph), 100.32 (Ph), 162.69, 162.75 (C-4), 172.80, 172.96 (d, *J* 6.9, *J* 6.2, Ala-CO); MS *m*/*e* FAB 484.1285 (MH$^+$, $C_{20}H_{24}N_3O_8FP$ requires 484.1285); HPLC t_R 30.37, 30.69 min; t_R 32.34, 32.72 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(2-chlorophenyl methoxyalaninylphosphate) (2m): yield 45%; δ_P 3.59, 4.34; δ_H 1.38 (3H, m, Ala-Me), 1.90 (3H, d, 5-Me), 3.76 (3H, d, OMe), 4.05 (2H, m, Ala-NH, Ala-CH), 4.42 (2H, m, H5′), 5.09 (1H, m, H4′), 5.96 (1H, m, H3′), 6.38 (1H, m, H2′), 7.07 (1H, m, H-1′), 7.26 (3H, m, Ph, 1H-6), 7.48 (2H, m, Ph), 8.44 (1H, s, NH); δ_C 15.04, 15.09 (5-Me), 23.64, 23.82 (d, *J* 13.1, Ala-Me), 52.88, 52.96 (Ala-CH), 55.43 (OMe), 69.80, 70.19 (C-5′), 87.21, 87.32 (C-4′), 92.42, 92.55 (C-1′), 114.21, 114.24 (d, *J* 2.7, Ph), 124.35, 124.72 (C-2′), 128.80, 128.90 (Ph), 130.15, 130.23 (C-3′), 130.80 (Ph), 133.34 (Ph), 135.90, 136.05 (C-6), 138.40, 138.55 (d, *J* 6.8, *J* 6.0, Ph), 149.33 (Ph), 153.44 (C-2), 166.22, 166.27 (C-4), 176.35, 176.67 (d, *J* 7.7, *J* 11.9, Ala-CO); MS *m*/*e* FAB 500.0750 (MH$^+$, $C_{20}H_{23}ClN_3O_8P$ requires 500.0990); HPLC t_R 32.33, 32.88 min; t_R 34.78, 35.42 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(4-bromophenyl methoxyalaninylphosphate) (2n): yield 46%; δ_P 4.57, 3.93; δ_H 1.39 (3H, m, Ala-Me), 1.90 (3H, d, 5-Me), 3.71 (3H, s, OMe), 4.02 (2H, m, Ala-NH, Ala-CH), 4.36 (2H, m, H-5′), 5.08 (1H, m, H-4′), 5.93 (1H, m, H-3′), 6.36 (1H, m, H-2′), 7.07 (1H, m, H-1′), 7.12 (2H, m, meta-Ar), 7.29 (1H, m, H-6), 7.48 (2H, m, ortho-Ar), 9.31 (1H, d, NH); δ_C 12.80, 12.84 (5-Me), 21.34 (t, *J* 4.5, Ala-Me), 50.48, 50.60 (Ala-CH), 53.11 (OMe), 67.01, 67.73 (d, *J* 4.6, *J* 5.2, C-5′), 84.90, 85.02 (d, *J* 1.9, *J* 2.0, C-4′), 90.00, 90.26 (C-1′), 111.68, 111.83 (C-5), 118.51, 118.52, 118.59, 118.61 (Para-Ar), 122.29, 122.35, 122.45, 122.52 (Ar), 127.87, 128.03 (C-2′), 133.15, 133.19 (Ph), 133.34, 133.68 (C-3′), 135.98, 136.26 (C-6), 149.72 149.79, 149.86, 149.95 (ipso-Ar), 151.32 (C-2), 164.27, 164.32 (C-4), 174.18, 174.28, 174.38, 174.47 (Ala-CO); MS *m*/*e* FAB 544.0476 (MH$^+$, $C_{20}H_{24}N_3O_8PBr$ requires 500.0484); HPLC t_R 34.22, 34.65 min; t_R 37.54, 39.07 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(4-iodophenyl methoxyalaninylphosphate) (2o): yield 50%; δ_P 4.47, 3.83; δ_H 1.39 (3H, m, Ala-Me), 1.88 (3H, d, 5-Me), 3.77 (3H, s, OMe), 3.99 (2H, m, Ala-NH, Ala-CH), 4.35 (2H, m, H-5′), 5.06 (1H, m, H-4′), 5.95 (1H, m, H-3′), 6.36 (1H, m, H-2′), 7.01 (2H, m, meta-Ar), 7.07 (1H, m, H-1′), 7.28 (1H, m, H-6), 7.66 (2H, m, ortho-Ar), 9.08 (1H, d, NH); δ_C 12.81, 12.86 (5-Me), 21.37 (t, *J* 5.0, Ala-Me), 50.48, 50.62 (Ala-CH), 53.13 (OMe), 67.00, 67.76 (C-5′), 85.00, 85.03 (C-4′), 90.01, 90.28 (C-1′), 111.68, 111.84 (C-5), 122.67, 122.74, 122.83, 122.89 (Ar), 127.88, 128.04 (C-2′), 133.35, 133.69 (C-3′), 135.96, 136.24 (C-6), 139.17, 139.20 (Ar), 150.56, 150.63, 150.70, 150.79 (ipso-Ar), 151.19, 151.22 (C-2), 164.09, 164.15 (C-4), 174.14, 174.24, 174.34, 174.43 (Ala-CO); MS *m*/*e* FAB 592.0330 (MH$^+$, $C_{20}H_{24}IN_3O_8P$ requires 592.0346); HPLC t_R 34.76, 35.25 min; t_R 36.89, 37.49 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(3-chlorophenyl methoxyalaninylphosphate) (2p): yield 59%; δ_P 4.38, 3.75; δ_H 1.43 (3H, m, Ala-Me), 1.99 (3H, d, 5-Me), 3.81 (3H, s, OMe), 4.05 (2H, m, Ala-NH, Ala-CH), 4.41 (2H, m, H-5′), 5.10 (1H, m, H-4′), 5.98 (1H, m, H-3′), 6.39 (1H, m, H-2′), 7.10 (1H, m, H-1′), 7.21 (5H, m, H-6, Ar−H), 8.68 (1H, d, NH); δ_C 12.74, 12.79 (5-Me), 21.37 (t, *J* 5.1, Ala-Me), 50.54, 50.63 (Ala-CH), 53.09 (OMe), 67.19, 67.78 (d, *J* 0, *J* 5.3, C-5′), 84.90, 85.01 (C-4′), 90.08, 90.33 (C-1′), 111.69, 111.83 (C-5), 121.12, 122.23 (d, *J* 5.3, Ar), 125.93, 125.96, 125.98 (Ar), 127.94, 128.06 (C-2′), 130.92, 130.95 (Ar), 133.33, 133.60 (C-3′), 135.44, 136.91 (C-6), 151.07, 151.08 (C-2), 163.89, 163.94 (C-4), 174.15 (t, *J* 9.0, Ala-CO); MS *m*/*e* FAB 500.0995 (MH^+, $C_{20}H_{24}ClN_3O_8P$ requires 500.0990); HPLC t_R 32.72, 32.99 min; t_R 34.88, 35.21 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(3-bromophenyl methoxyalaninylphosphate) (2q): yield 61%; δ_P 4.35, 3.75; δ_H 1.40 (3H, m, Ala-Me), 1.93 (3H, d, 5-Me), 3.81 (4H, m, OMe, Ala-NH), 4.08 (1H, m, Ala-CH), 4.41 (2H, m, H-5′), 5.10 (1H, m, H-4′), 5.98 (1H, m, H-3′), 6.38 (1H, m, H-2′), 7.08 (1H, m, H-1′), 7.30 (5H, m, H-6, Ar−H), 8.60 (1H, d, NH); δ_C 12.75, 12.79 (5-Me), 21.38 (t, *J* 5.1, Ala-Me), 50.53, 50.62 (Ala-CH), 53.12 (OMe), 67.19, 67.79 (d, *J* 0, *J* 5.3, C-5′), 84.97, 85.05 (C-4′), 90.09, 90.34 (C-1′), 111.69, 111.83 (C-5), 119.29, 119.42, 119.49 (Ar), 124.01 (Ar), 127.97, 128.07 (C-2′), 128.87 (Ar), 131.25 (Ar), 133.32, 133.58 (C-3′), 135.90, 136.10 (C-6), 151.08 (C-2), 163.93 (C-4), 174.17 (Ala-CO); MS *m*/*e* FAB 546.0461 (MH^+, $C_{20}H_{24}BrN_3O_8P$ requires 546.0464); HPLC t_R 33.21, 33.51 min; t_R 35.45, 35.83 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(3-iodophenyl methoxyalaninylphosphate) (2r): yield 53%; δ_P 4.33, 3.70; δ_H 1.43 (3H, m, Ala-Me), 1.90 (3H, d, 5-Me), 3.80 (4H, m, OMe, Ala-NH), 4.03 (1H, m, Ala-CH), 4.40 (2H, m, H-5′), 5.09 (1H, m, H-4′), 5.98 (1H, m, H-3′), 6.40 (1H, m, H-2′), 7.16 (4H, m, Ar−H, H-6, H-1′), 7.60 (2H, m, Ar−H), 8.58 (1H, d, NH); δ_C 12.41, 12.58 (5-Me), 21.02 (t, *J* 5.8, Ala-Me), 50.14, 50.23 (Ala-CH), 53.11 (OMe), 66.71, 67.34 (d, *J* 0, *J* 5.1, C-5′), 84.56, 84.67 (C-4′), 89.65, 89.90 (C-1′), 111.32, 111.47 (C-5), 119.63, 119.78 (Ar), 125.88 (Ar), 127.59, 127.68 (C-2′), 129.23, 129.36 (Ar), 132.97, 133.22 (C-3′), 135.56, 135.76 (C-6), 150.58, 150.71 (C-2), 163.58, 163.53 (C-4), 173.70, 173.92 (Ala-CO); MS *m*/*e* FAB 592.0342 (MH^+, $C_{20}H_{24}IN_3O_8P$ requires 592.0346); HPLC t_R 34.31, 34.63 min; t_R 36.61, 36.99 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(4-(trifluoromethyl)phenyl methoxyalaninylphosphate) (2s): yield 54%; δ_P 4.33, 3.72; δ_H 1.40 (3H, m, Ala-Me), 1.90 (3H, d, 5-Me), 3.74 (3H, s, OMe), 3.89 (1H, m, Ala-NH), 4.03 (1H, m, Ala-CH), 4.37 (2H, m, H-5′), 5.09 (1H, m, H-4′), 5.99 (1H, m, H-3′), 6.40 (1H, m, H-2′), 7.09 (1H, m, H-1′), 7.36 (3H, m, H-6, Ar−H), 7.68 (2H, m, Ar−H), 8.80 (1H, d, NH); δ_C 12.81, 12.86 (5-Me), 21.44 (t, *J* 4.3, Ala-Me), 50.54, 50.63 (Ala-CH), 53.15 (OMe), 67.15, 67.88 (d, *J* 4.3, *J* 5.8, C-5′), 84.86, 84.97 (C-4′), 90.05, 90.32 (C-1′), 111.69, 111.85 (C-5), 120.85, 120.99 (d, *J* 5.0, Ph), 127.62, 127.95 (C-2′), 128.10 (Ar), 133.30, 133.63 (C-3′), 135.93, 136.16 (C-6), 151.14, 151.16 (C-2), 164.00, 164.05 (C-4), 174.06, 174.16, 174.23 (Ala-CO); MS *m*/*e* FAB 534.1236 (MH^+, $C_{21}H_{24}F_3N_3O_8P$ requires 534.1253); HPLC t_R 35.57, 35.92 min; t_R 37.89, 38.34 min (gradient II).

2′,3′-Didehydro-2′,3′-dideoxythymidine-5′-(3-(trifluoromethyl)phenyl methoxyalaninylphosphate) (2t): yield 67%; δ_P 4.39, 3.83; δ_H 1.45 (3H, m, Ala-Me), 1.90 (3H, d, 5-Me), 3.78 (4H, s, OMe), 4.07 (1H, m, Ala-CH), 4.40 (2H, m, H-5′), 5.07 (1H, m, H-4′), 5.98 (1H, m, H-3′), 6.39 (1H, m, H-2′), 7.08 (1H, m, H-1′), 7.30 (1H, m, H-6), 7.51 (4H, m, Ar), 8.40 (1H, d, NH); δ_C 12.74, 12.83 (5-Me), 21.47 (t, *J* 4.3, Ala-Me), 50.64, 50.61, 50.64 (Ala-CH), 53.12 (OMe), 67.17, 67.85 (d, *J* 5.1, *J* 5.1, C-5′), 84.83, 84.88, 84.95 (C-4′), 90.06, 90.32 (C-1′), 111.69, 111.86 (C-5), 117.74 (d, *J* 5.0, Ph), 127.98, 128.09 (C-2′), 128.10 (Ar), 130.92 (Ar) 133.30, 133.58 (C-3′), 135.92, 136.11 (C-6), 150.87, 151.01, 151.05 (C-2), 163.87, 163.91 (C-4), 174.03, 174.144 (Ala-CO); MS *m*/*e* FAB 534.1241 (MH^+, $C_{21}H_{24}F_3N_3O_8P$ requires 534.1253); HPLC t_R 35.77, 35.95 min; t_R 37.96, 38.41 min (gradient II).

Determination of Compound Partition Coefficients. The following method was used to determine the *P* values for compounds **2j**−**2t**. A sample of each compound (2 μmol) was dissolved in 1-octanol (10 mL) by rapid magnetic stirring for 10 min. The UV absorption of the octanol solution was determined at 265 nm. Aqueous buffer (pH 7.0) (Fisons) (5 mL) was added to an aliquot of the octanol solution (5 mL), and the two phases were mixed by rapid magnetic stirring for 5 min at 25 °C. The two layers were separated by standing for a further 5 min. An aliquot of the octanol layer was removed and the UV absorption determined at 265 nm. The *P* values were calculated from the ratio of the absorptions of the octanol aliquot and the original octanol solution. Each experiment was repeated at least three times.

Determination of Compound Aqueous Solubility. The following method was used to assess the aqueous solubility of compounds **2** and **2c**. The extinction coefficient (ϵ) of each compound was calculated by dissolving a portion of each compound (ca. 1 mg) in water (15 mL, pH 6.2) and measuring the UV_{max} absorption of the resulting solution. The process was repeated twice more and an average value for ϵ calculated. A portion of each compound (10−40 mg) was added to water (500 μL) and the mixture stirred rapidly for 1 h at 25 °C. Following filtration through glass wool and appropriate dilution, the UV_{max} absorption was measured and the extinction coefficient used to calculate the concentration of the saturated solution. The results shown are the mean of 3 independent experiments [ϵ ($l{\cdot}mol^{-1}{\cdot}cm^{-1}$): **2** = 7640, **2c** = 6208; saturation concentration (mmol/L): **2** = 5.4, **2c** = 67.0].

Antiviral Activity Determinations. Viruses: HIV-1(strain III_B) was kindly provided by Dr. R. C. Gallo (at that time at the National Institutes of Health, Bethesda, MD). Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells. HIV-2(strain ROD) was a gift from Dr. L. Montagnier (Pasteur Institute, Paris, France), and virus stocks were prepared from the supernatants of HIV-2-infected MT-4 cells.

Antiretrovirus assays: CEM/0 and CEM/TK^- cells were suspended at 250, 000cells/mL of culture medium and infected with HIV-1 or HIV-2 at ∼20 and ∼100 $CCID_{50}$ (50% cell culture infective dose)/mL, respectively. Then, 100 μL of the infected cell suspensions were added to 200-μL microtiter plate wells containing 100 μL of an appropriate dilution of the test compound. After 4 days incubation at 37 °C, cell cultures were examined for giant cell formation.

The EC_{50} was defined as the compound concentration (μM) required to inhibit HIV-induced cytopathicity (giant cell formation) in CEM cell cultures by 50%. The CC_{50} was defined as the compound concentration (μM) required to inhibit CEM cell proliferation by 50%.

QSAR. Statistical analysis of the relationships between antiviral activity and substituent properties of interest was performed by partial least-squares algorithm using the QSAR module of SYBYL software.[32] The training set used for the analysis included all 21 compounds presented in Table 1. The optimal number of components for each PLS model generated was determined by cross-validation procedures using 6 random groups. This protocol was repeated 10 times and an average value of Q^2 calculated.

Acknowledgment. We thank the U.K. Medical Research Council, the Biomedical Research Programme of the European Commission, and the Belgian Geconcerteerde Onderzoeksacties (Project 95/5) for financial support. We also thank Mrs. A. Absillis for excellent technical assistance and Ms. Helen Murphy for secretarial support.

References

(1) De Clercq, E. In search of a Selective Antiviral Chemotherapy. *Clin. Microbiol. Rev.* **1997**, *10*, 674−693.

(2) 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. 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.
(3) 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′-didoxynucleosides. *Proc. Natl. Acad Sci U.S.A.* **1986**, *83*, 1911−1915.
(4) 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.
(5) 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.
(6) 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.
(7) Baba, M.; Pauwels, R.; Herdewifn, 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.
(8) 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. Properties and Mouse Toxicities of 3 Thymidine Analogues Active Against Human-Immunodeficiency-Virus. *Antimicrob. Agents Chemother.* **1990**, *17*, 637−641.
(9) Furman, P. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, J. A.; Lehrman, S. N.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. Phosphorylation of 3′-Azido-3′-dideoxythymidine and Selective Interaction of the 5′-Triphosphate with Human Immunodeficiency Virus Reverse Transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8333−8337.
(10) 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.
(11) 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.
(12) Starnes, M. G.; Cheng, Y.-C. Cellular metabolism of 2′,3′-Dideoxycytidine, a compound active against human immunodeficiency virus in vitro. *J. Biol. Chem.* **1987**, *32*, 798−806.
(13) 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.
(14) 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.
(15) Johnson, M. A.; Fridland, A. Phosphorylation of 2′,3′-dideoxyinosine by cytosolic 5′-nucleotidase of human lymphoid cells. *Mol. Pharmacol.* **1989**, *36*, 291−295.
(16) Jones, R. J.; Bischofberger, W. Minireview − Nucleotide Prodrugs. *Antiviral Res.* **1995**, *27*, 1−17.
(17) Meier, C. Pro-Nucleotides Recent Advances in the Design of Efficient Tools for the Delivery of Biologically Active Nucleosides Monophosphates. *Synlett* **1998**, 233−242.
(18) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. Phosphoramidate derivatives of d4T with improved anti-HIV efficacy retain full activity in thymidine kinase-deficient cells. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1183−1186.
(19) McGuigan, C.; Cahard, D.; Sheeka, H. M.; De Clercq, E.; Balzarini, J. Aryl phosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. *J. Med. Chem.* **1996**, *39*, 1748−1753.
(20) Balzarini, J.; Kruining, J.; Wedgwood, O.; Pannecouque, C.; Aquaro, S.; Perno, C. F.; Naesens, L.; Witvrouw, M.; Heijtink, R.; De Clercq, E.; McGuigan, C. Conversion of 2′,3′-dideoxyadenosine (ddA) and 2′,3′- didehydro-2′,3′-dideoxyadenosine (d4A) to their corresponding aryloxyphosphoramidate derivatives markedly potentiates their activity against human immunodeficiency virus and hepatitis B virus. *FEBS Lett.* **1997**, *410*, 324−328.
(21) Siddiqui, A. Q.; McGuigan, C.; Ballatore, C.; De Clercq, E.; Balzarini, J. The Presence of Substituents on the Aryl Phosphoramidate Derivative of d4T Enhances Anti-HIV Efficacy in Cell Culture: A Structure−Activity Relationship. *J. Med. Chem.* **1999**, *42*, 393−399.
(22) Horwitz, J. P.; Chua, J.; DaRooge, M. A.; Noel, M.; Klindt, I. L. Nucleosides. IX. The formation of 2′,3′-unsaturated pyrimidine nucleosides via a novel β-elimination rection. *J. Org. Chem.* **1996**, *31*, 205−211.
(23) Mansuri, M. M.; Starrett Jr., J. E.; Ghazzouli, I.; Hitchcock, M. J. M.; Sterzycki, R. Z.; Brankovan, V.; Lin, T. S.; August, E. M.; Prussoff, W. H.; Sommadossi, J. P.; Martin, J. C. 1-(2′,3′-dideoxy-β-D-glycero-pent-2-enfuranosyl)thyimine. A highly Potent and Selective Anti-HIV Agent. *J. Med. Chem.* **1989**, *32*, 462−446.
(24) McGuigan, C.; Cahard, D.; Saigado, A.; De Clercq, E.; Balzarini, J. Phosphoramidates as potent prodrugs of anti-HIV nucleotides: studies in the amino region. *Antiviral Chem. Chemother.* **1996**, *7*, 31−36.
(25) 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.
(26) Fujita, T.; Miyoshi, H. Quantitative analyses of the uncoupling activity of substituted phenols with mitochondria from muscles of house flies. *Biochim. Biophys. Acta* **1988**, *935*, 312−321.
(27) Fujita, T.; Nishioka, T.; Miyoshi, H. Quantitative Relationship Between Protonophoric and Uncoupling Activities of Substituted Phenols. *Biochim. Biophys. Acta* **1987**, *891*, 194−204.
(28) Valette, G.; Pompon, A.; Girardet, J. L.; Cappellacci, L.; Franchetti, P.; Grifantini, M.; LaColla, P.; Loi, A. G.; Perigaud, C.; Gosselin, G.; Imbach, J. L. Decomposition pathways and in vitro HIV inhibitory effects of isoddA pronucleotides − toward a rational approach for intracellular delivery of nucleoside 5′-monophosphates. *J. Med. Chem.* **1996**, *39*, 1981−1990.
(29) Topliss, J. G. Utilization of Operational Schemes for Analogue Synthesis in Drug Design. *J. Med. Chem.* **1972**, *15*, 1006−1011.
(30) Hansch, C.; Leo, A. *Substitution Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.
(31) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. “Aromatic” Substituent Constants for Structure−Activity Correlations. *J. Med. Chem.* **1973**, *16*, 1207−1216.
(32) SYBYL, version 6.5; Tripos Associates: St. Louis, MO, 1998.

JM9807104