Synthesis and in Vitro Activity of Long-Chain 5′-*O*-[(Alkoxycarbonyl)phosphinyl]-3′-azido-3′-deoxythymidines against Wild-Type and AZT- and Foscarnet-Resistant Strains of HIV-1

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Lipophilic esters of 3′-azido-3′-deoxy-5′-*O*-(carboxyphosphinyl)thymidine (PFA-AZT) were synthesized and tested for antiretroviral activity in $CD4^+$ HT4-6C cells infected with either wild-type HIV-1_{LAI}, a PFA-resistant strain encoding a single-point mutation in reverse transcriptase (E89K), or an AZT-resistant clinical isolate (A018-post). Arbuzov condensation of 1-octadecyl, 1-eicosanyl, and 1-docosanyl chloroformate with trimethyl phosphite yielded the corresponding dimethyl long-chain alkyl triesters of PFA. Selective removal of one methyl group from the triesters with sodium iodide yielded monosodium salts, whereas treatment with bromotrimethylsilane cleaved both methyl groups while leaving the long-chain alkyl group intact. Neutralization of the resulting [(alkyloxy)carbonyl]phosphonic acids with 2 equiv of sodium methoxide afforded disodium salts of the phosphonic acid moiety. Similar chemistry was used to obtain the mono- and disodium salts of the cholesterol ester of PFA. Reaction of the triesters with phosphorous pentachloride, followed by coupling with AZT and O-demethylation with sodium iodide, afforded 3′-azido-3′-deoxy-5′-*O*-[[(1-octadecyloxy)carbonyl]phosphinyl]thymidine (**9a**), 3′-azido-3′-deoxy-5′-*O*-[[(1-eicosanyloxy)carbonyl]phosphinyl]thymidine (**9b**), 3′-azido-3′-deoxy-5′-*O*-[[(1-docosanyloxy)carbonyl]phosphinyl]thymidine (**9c**), and 3′-azido-3′-deoxy-5′-*O*-[[(3β-cholest-5-enyloxy)carbonyl]phosphinyl]thymidine (**9d**). Concentrations of **9a**−**d** found to inhibit replication of wild-type HIV-1_{LAI} by 50% (EC_{50} values) as measured in a plaque reduction assay were in the 0.1−0.3 μM range as compared with 0.013 μM for AZT and 133 μM for PFA. The concentration at which toxicity was observed in 50% of the host cells (TC_{50} values) as measured by a visual grading scale of cellular morphology was 10 μM for **9a** and **9d**, 32 μM for **9b**, and 320 μM for **9c**. Thus, the TC_{50}/EC_{50} ratio or selectivity index (SI) was 100 for **9a**, 230 for **9b**, and 1000 for **9c** but only 33 for **9d**, suggesting that the straight-chained fatty alcohol esters were more therapeutically selective. Similar TC_{50} and SI values were obtained for rapidly dividing CEM lymphoblasts as for HT4-6C cells. In assays against E89K, **9a**−**c** had mean EC_{50} values of 0.13, 0.009, and 0.17 μM, whereas the EC_{50} of PFA was >1000 μM and that of AZT was 0.009 μM; thus, E89K was highly resistant to PFA but not cross-resistant to either AZT or the lipophilic PFA-AZT conjugates. In viral replication assays against the A018C-post isolate, the mean EC_{50} values of **9a**−**c** were 0.30, 0.53, and 0.77 μM as compared with 2.9 μM for AZT and 65 μM for PFA; thus, the virus recovered from a patient pretreated with AZT was not cross-resistant to either PFA or **9a**−**c**. A notable feature of these results was that, in addition to being >1000-fold more potent than PFA against the PFA-resistant mutant, the lipophilic PFA-AZT conjugates were more potent than PFA, as well as AZT, against AZT-resistant HIV-1.

Foscarnet, the trisodium salt of phosphonoformic acid (**1**, PFA),[1,2] is a potent inhibitor of reverse transcriptase (RT) from human immunodeficiency virus type 1 (HIV-1).[3] PFA inhibits replication of HIV-1 in the laboratory[4−6] and reduces viremia in patients with AIDS.[7−9] It also inhibits DNA polymerase from cytomegalovirus (CMV), herpes simplex virus (HSV), and other DNA viruses[10,11] and thus has been useful in the treatment of secondary viral infections in AIDS patients.[12−17] Because PFA bears a structural resemblance to pyrophosphate, it can be viewed as a product analogue as opposed to a substrate analogue.[10,11] Moreover, since there appears to be only partial overlap between the binding sites of PFA and nucleoside triphosphates on reverse transcriptase (RT), additive or perhaps greater than additive antiviral activity might be achieved by combining PFA with antiretroviral dideoxynucleosides such as zidovudine (AZT, **2**). Two groups have studied different combinations of PFA and AZT on HIV-1 replication and have observed the interaction of the two drugs to be additive.[18,19] However, combinations of PFA with dideoxynucleosides other than AZT, or for that matter with non-nucleoside RT inhibitors, are yet to be reported.

As with nucleoside and non-nucleoside RT inhibitors, clinical resistance to PFA is known to occur in some patients after prolonged treatment.[20] Moreover, PFA-resistant HIV-1 strains have been produced in the laboratory by random[21,22] as well as site-specific[23,24]

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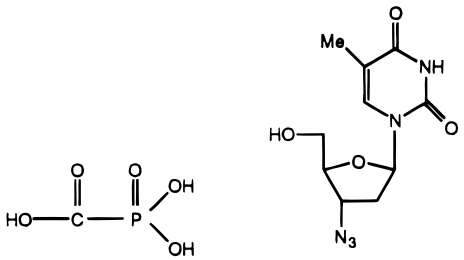


mutagenesis, and the pattern of cross-resistance of such mutants to other RT inhibitors has been extensively analyzed.[25,26] A notable finding of these studies has been that resistance to PFA in some of these mutants may be accompanied by increased sensitivity to some, though not all, nucleoside and non-nucleoside inhibitors, lending support to the view that only partial overlap exists between the RT binding sites for PFA and dideoxynucleoside 5′-triphosphates.

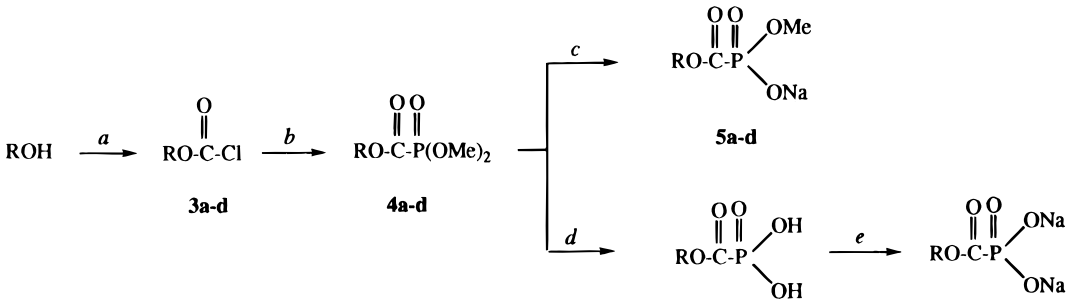
While PFA is an excellent inhibitor of RT and other DNA polymerases, its triple negative charge at physiological pH is an impediment to cellular uptake. As a result, the PFA concentration needed to block viral replication in an intact cell or in vivo is orders of magnitude greater than the concentration needed to inhibit the enzyme in a cell-free assay. In addition, its in vivo clearance is very rapid, which makes it difficult to achieve long-lasting control of viral infection. Thus there has been a longstanding interest in developing PFA prodrugs that can generate sustained levels of the parent drug in tissues and more specifically in virally infected cells. Although large numbers of simple alkyl and aryl esters of the carboxyl and/or phosphonyl moiety of PFA have been synthesized and tested as potential prodrugs, the results have been largely negative, mainly because the kinetics of bioconversion of the esters to free PFA were unfavorable.[27] Acyloxymethyl esters of the phosphonyl group of PFA have likewise been reported,[28,29] as well as ester derivatives in which the carboxyl or phosphonyl group was joined to a nucleoside[30,31] or, in particular, a dideoxynucleoside analogue,[32−34] but here again substantial gains were not achieved in terms of either potency or therapeutic selectivity. More recent work has shown that the antiviral efficacy of PFA against both HIV-1 and CMV can be increased by linking the phosphonate moiety to 1-*O*-octadecyl-*sn*-glycerol.[35] The resulting lipophilic adduct was cleaved intracellularly to PFA and displayed substantially enhanced selectivity and potency.

This paper reports the synthesis of a different type of PFA lipid conjugate in which the carboxyl group of PFA is linked via a C−ester bond to 1-octadecyl (stearyl, C_{18}), 1-eicosanyl (arachidyl, C_{20}), or 1-docosanyl (behenyl, C_{22}) alcohol and the phosphonyl moiety is linked via a P−ester bond to the 5′-hydroxyl group of AZT.[36] The resulting diesters, which have the potential to act as either PFA prodrugs, AZT prodrugs, or both, were found to inhibit the replication of wild-type HIV-1_{LAI} in human HT4-6C cells, as well as that of a PFA-resistant strain produced by site-specific mutagenesis (E89K) and of an AZT-resistant strain originating from a patient treated with AZT (A018-post). To our knowledge there is no precedent for the use of such PFA-AZT conjugates to overcome both PFA and AZT resistance.

Chemistry

Ester derivatives of PFA suitable for coupling to AZT were prepared as shown in Scheme 1. 1-Octadecanol, 1-eicosanol, and 1-docosanol were converted to the previously unreported chloroformates **3a**−**c** in nearly quantitative yield by reaction with 20% phosgene in toluene or triphosgene in pyridine. The products were low-melting compounds with IR spectra showing the expected acid chloride peak at 1780 cm^{-1}. Arbuzov reaction of **3a**−**c** with $P(OMe)_3$ at 80 °C led to the PFA triesters **4a**−**c** in 85−95% yield after recrystallization from hexane. The same reaction with cholesterol chloroformate afforded the known PFA triester **4d**.[33] Because of the lower reactivity of the chloroformate ester of cholesterol relative to chloroformate esters of straight-chained fatty alcohols, the Arbuzov condensation was carried out at slightly elevated temperature. Selective removal of one methyl group from **4a**−**d** was accomplished by overnight treatment at room temperature with NaI (0.5−3 mol equiv) in DMF, with or without THF or acetone as a cosolvent. The selectivity of this novel methyl displacement reaction in a phosphonate dimethyl ester was predicated on the idea that, as a soft base, the iodide ion would be nucleophilic enough to react with one methyl group in **4a**−**d** but would be prevented from reacting with the second methyl group in **5a**−**d** because of the proximity of a negative charge on the phosphorus oxygen. Yields of **5a**−**d** were 85−95%, with little or no purification other than washing with cold hexane to remove unreacted starting material. Treatment of **4a**−**d** with Me_3SiBr in CH_2Cl_2 at room temperature for 4 h afforded the phosphonic acids **6a**−**d** in 70−80% yield as hygroscopic white solids that had

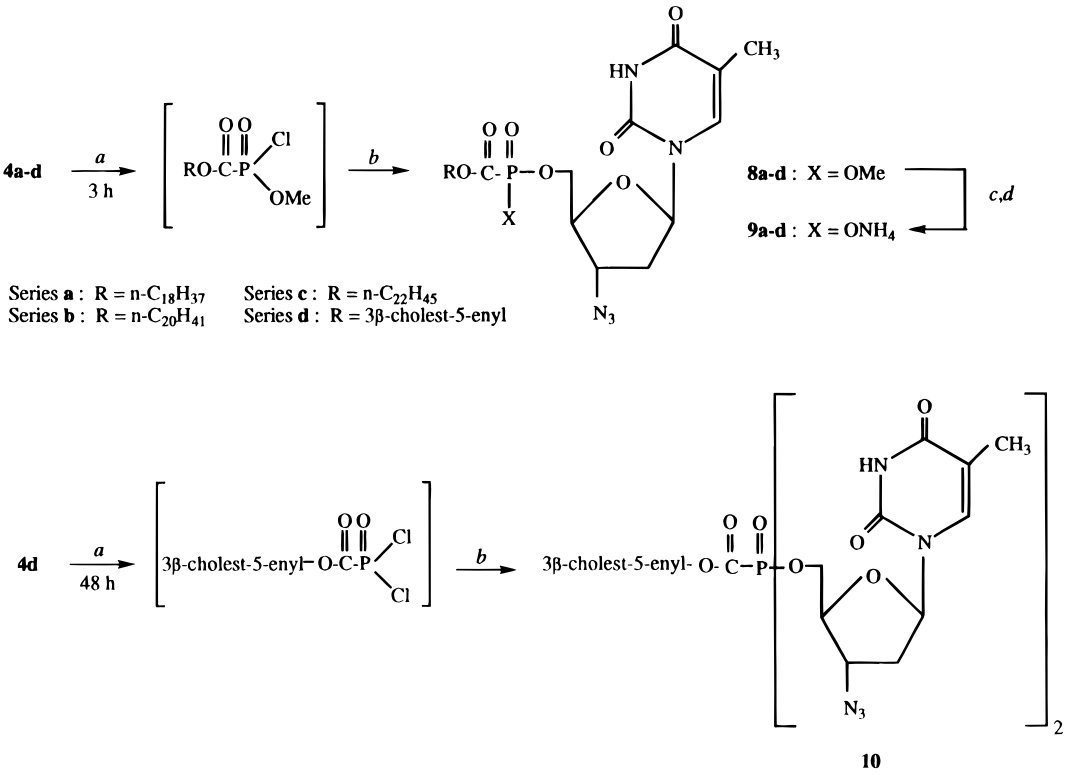
Scheme 1[a]



Series **a**: R = n-$C_{18}H_{37}$ Series **c**: R = n-$C_{22}H_{45}$
Series **b**: R = n-$C_{20}H_{41}$ Series **d**: R = 3β-cholest-5-enyl

[a] (a) Cl_2CO or $(Cl_3CO)_2CO$; (b) $P(OMe)_3$; (c) NaI/THF; (d) Me_3SiBr; (e) NaOH (2 equiv).

Scheme 2[a]



[a] (a) PCl_5/CCl_4; (b) AZT (excess)/DMF; (c) NaI/THF; (d) DEAE-cellulose, 0.05 M NH_4HCO_3.

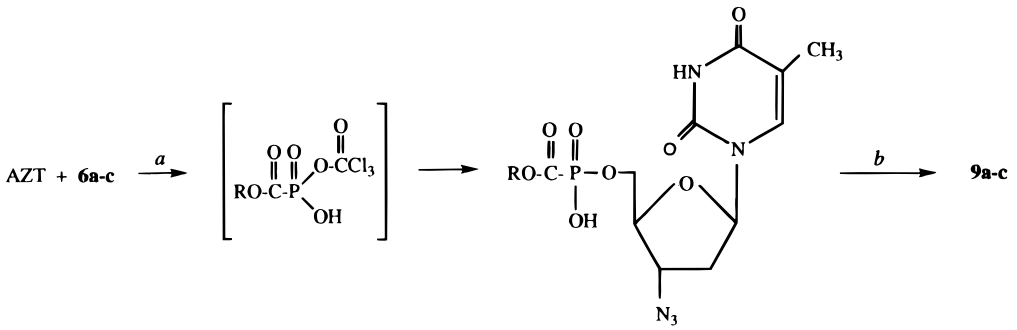
to be stored at −20 °C in tightly sealed containers to prevent them from becoming waxy. Treatment of the acids with exactly 2 mol equiv of NaOMe in MeOH converted them to their disodium salts (**7a**−**d**). Characteristic differences were noted in the IR spectra of the four types of PFA derivatives with regard to the C=O stretching frequency (1705−1710 cm^{-1} in **4a**−**d**, 1730−1735 cm^{-1} in **5a**−**d** and **6a**−**d**, and 1670−1690 cm^{-1} in **7a**−**d**). Subsequent reactions leading to esterified AZT-PFA conjugates utilized the diester monosodium salts **5a**−**d** and the monoester diacids **6a**−**d**.

Two methods were used to prepare the AZT-PFA conjugates (Scheme 2). In the first, the triesters **4a**−**d** were allowed to react with excess PCl_5 in refluxing CCl_4 for 3 h under dry argon. Excess PCl_5 was destroyed by bubbling SO_2 gas through the solution, and the reaction mixture, now containing $POCl_3$ and $SOCl_2$, was evaporated to dryness under reduced pressure with rigorous exclusion of moisture. The residue was taken up directly in dry DMF and cooled to −50 °C, a precooled solution of recrystallized, thoroughly dried AZT was introduced via syringe, and the reaction was allowed to proceed at room temperature for 24 h. The resulting P−OMe esters **8a**−**d** were purified by flash chromatography and treated at room temperature for 24 h with 1.4−1.5 equiv of NaI in THF under argon in the absence of light, and the O-demethylated products were passed through a DEAE-cellulose column with 0.05 M NH_4HCO_3 as the eluent to obtain ammonium salts **9a**−**d**. Non-optimized yields of the intermediate AZT-PFA triesters ranged from 22% (**8a**) to 61% (**8d**), and those of the final products ranged from 28% (**9c**) to 67% (**9d**). Although the reason for this divergence in yields at these two steps was not studied in detail, it is important to emphasize that, because the reaction of **4a**−**d** with PCl_5 and the subsequent coupling of **8a**−**d** to AZT are both exceedingly moisture-sensitive, the entire process has to be performed with rigorous care to obtain good results.

An unexpected feature of the straight-chained esters **8a**−**c** was that the elemental analysis for nitrogen (but not for the other elements, including phosphorus) was consistently very low despite the following incontrovertible structural evidence: (i) the C/P ratio in all three compounds was correct; (ii) their IR spectra contained a strong azide peak at 2100 cm^{-1}; and (iii) 500 MHz ^1H NMR signals could all be properly assigned and integrated. The $C_{1'}$ and 6-H protons of the AZT moiety were clearly visible at δ 6.15 (triplet) and 7.80 (singlet), and the relative areas of these peaks and the δ 4.0−4.5 multiplet for the CH_2O protons of the alkyl ester and the $C_{3'}$, $C_{4'}$, and $C_{5'}$ protons of the sugar were in the expected 6:1:1 ratio, confirming that there was one AZT moiety per alkyl side chain. Since the evidence ruled out all alternative structures that could account for a low nitrogen analysis, there was most likely incomplete conversion of the azido group to nitrogen oxides during combustion.

It was found in some experiments that sequential reaction of PFA triesters with PCl_5 and AZT produced a UV-absorbing byproduct whose TLC indicated it to be much less polar than the main product. NMR evidence also revealed that the reaction of PCl_5 with dimethyl (alkoxycarbonyl)phosphonates could replace not just one but both methoxy groups with chlorine, thereby creating the opportunity for an adduct with one PFA and two AZT moieties. To demonstrate that the latter could indeed occur, we altered the reaction conditions for the cholesterol derivative **4d** so as to favor the formation of a 2:1 adduct. Thus, in the first step **4d** was allowed to react with PCl_5 for 48 h instead of 3 h, and in the second step AZT was used at a mole ratio

Scheme 3[a]



[a] (a) Cl_3CN/pyridine; (b) silica gel, 28:15:1 $CHCl_3$−MeOH−28% NH_4OH.

Table 1. Anti-HIV Activity of PFA, AZT, and PFA-AZT Conjugates **9a**−**c** in HT4-6C Cells

	EC_{50} (μM)[a]				
virus	PFA	AZT	**9a**	**9b**	**9c**
HIV-1_{LAI}	133 ± 54	0.013 ± 0.006	0.10 ± 0.15	0.14 ± 0.16	0.32 ± 0.20
E89K[b]	>1000	0.009 ± 0.005	0.13 ± 0.14	0.009 ± 0.007	0.17 ± 0.11
A018-post	65 ± 13	2.9 ± 1.5	0.30 ± 0.32	0.53 ± 0.68	0.77 ± 1.1

[a] EC_{50} = 50% effective concentration (mean ± standard deviation). Results are the averages of three separate experiments on different days. [b] This strain is also referrred to in the literature as 89LAI-Lys.[26]

of 3.0 instead of 1.5 and the length of time was extended from 24 to 48 h. Under these conditions, a product formed in modest yield whose analysis and NMR spectrum were consistent with the predicted 2:1 adduct **10**. As expected for this structure, the ratio of peak areas for the $C_{1'}$ and 6-H protons on the AZT moiety and the vinylic proton on the cholesterol moiety was 2:2:1 rather than 1:1:1. Moreover, whereas the spectrum of **8d** showed the P-OMe protons as a doublet at δ 3.90, this peak was absent in the spectrum of **10**.

Because of the capricious course of the PCl_5 reaction with dimethyl (alkoxycarbonyl)phosphonates, we investigated a second, more direct route to AZT-PFA conjugates from AZT and (alkoxycarbonyl)phosphonic acids. After considering a number of options to activate the phosphonyl group (e.g., with 2,4,6-triisopropylbenzenesulfonyl chloride in the presence of pyridine or *N*-methylimidazole), we found that coupling could be accomplished very satisfactorily by heating **6a**−**c** with excess AZT and Cl_3CCN in pyridine under argon at 50−60 °C overnight, again with rigorous exclusion of moisture (Scheme 3).[37] After routine workup, flash chromatography on silica gel with 85:15:1 $CHCl_3$−MeOH−28% NH_4OH as the eluent, followed by freeze-drying, afforded **9a** (34%), **9b** (49%), and **9c** (30%). The products were indistinguishable from those obtained with PCl_5. However, because the Cl_3CCN method was more direct and easier to carry out, and afforded a higher overall yield (e.g., 34% versus 8.8% for **9a**), we found it to be a better route to the amphipathic PFA-AZT conjugates of the type described here.

Bioassay

Compounds **9a**−**d** and two of the PFA esters lacking an AZT moiety, **5a** and **5c**, were tested in a plaque reduction assay with wild-type HIV-1_{LAI} in HT4-6C human $CD4^+$ lymphocytes as described earlier.[35,38] Treatment of the infected cells with **5a** or **5c** at a concentration of 1 μM, the highest achievable in the growth medium with these very hydrophobic compounds, led to a 35% reduction in the number of viral plaques. By comparison, the EC_{50} of PFA was 133 μM,

Table 2. Selectivity of PFA-AZT Conjugates **9a**−**c** in HT4-6C and CEM Cells

	HT4-6C		CEM	
compd	TC_{50} (μM)[a]	selectivity index[b]	TC_{50} (μM)[a]	selectivity index[b]
PFA	1000	>7.5	800	6
9a	10	100	28	280
9b	32	228	36	257
9c	320	1000	93	291

[a] TC_{50} = 50% toxic concentration (mean ± SD). For the HT4-6C cells, the TC_{50} was determined by using a visual grading scale of the cells after 3 days of drug treatment. The grading scale, ranging from 0 to 4, was based on the following criteria: 4 = normal appearance, 3 = slight reduction in number of normal cells, 2 = 50% reduction in number of normal cells, 1 = no normal cells, 0 = no living cells. The TC_{50} was estimated by extrapolation of grade 2. For the CEM cells, the number of viable cells after 3 days of drug treatment was determined by flow cytometry after staining with propidium iodide.[41] [b] Selectivity index (SI) = TC_{50}/EC_{50}. The EC_{50} values used to calculate SI were for HT4-C6 cells infected with wild-type HIV-1_{LAI} (cf. Table 1).

suggesting that the monosodium salt of the long-chained diester derivatives might be taken up efficiently in comparison with the trisodium salt. However the concentration of the esters that was toxic to approximately 50% of the HT4-6C cells (TC_{50}), according to a standardized grading scale of cellular morphology, was 10 μM; thus the selectivity index (SI), defined as TC_{50}/EC_{50}, was <10. On the basis of these essentially negative results, further testing of the simple PFA fatty alcohol esters **5a**−**d**, **6a**−**d**, and **7a**−**d** for activity against HIV-1_{LAI} was not pursued.

In contrast to the ordinary PFA esters, PFA-AZT conjugates **9a**−**d** all showed submicromolar activity against HIV-1_{LAI}, with EC_{50} values ranging from 0.10 μM (**9a**) to 0.32 μM (**9c**) as compared with 0.013 μM for AZT (Table 1); thus the conjugates were ca. 10−30 times less potent than AZT but 400−1400 times more potent than PFA. The TC_{50} varied from 10 μM (**9a**) to 320 μM (**9c**); thus the calculated SI varied from 100 (**9a**) to 1000 (**9c**) (Table 2). Toxicity was generally similar in rapidly dividing human T-lymphoblastic leukemia cells (CEM), and SI values ranged from 257 (**9b**) to 291 (**9c**) versus 6 for PFA (Table 2). The cholesterol conjugate **9d** had

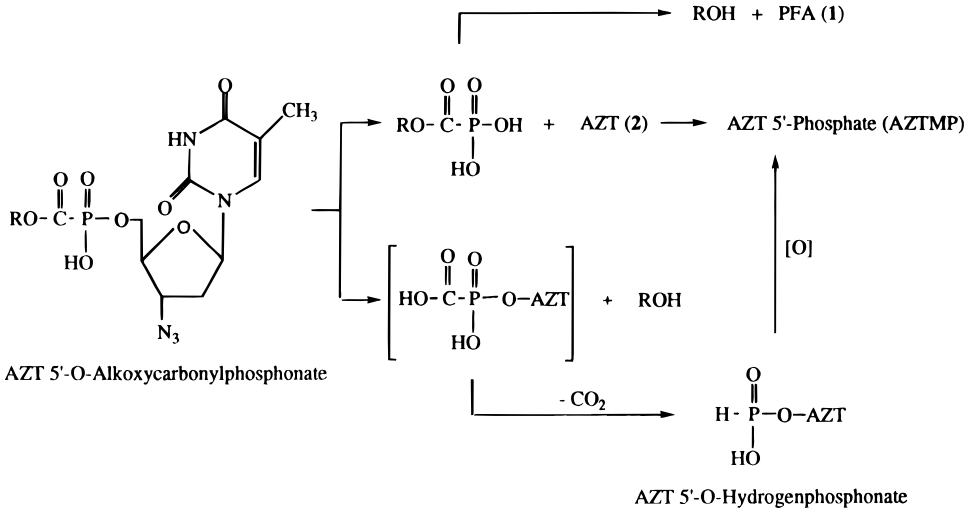


Figure 1. Possible intracellular pathways from PFA-AZT esters to AZT 5′-phosphate (AZTMP) and AZT 5′-hydrogenphosphonate.

an EC_{50} of 0.30 μM and showed cytotoxicity comparable to **9a**−**c** (data not shown). Since replacement of the fatty alcohol by cholesterol afforded no advantage in terms of either antiviral potency or selectivity, subsequent experiments were done only with **9a**−**c**.

In assays against the PFA-resistant mutant E89K (Table 1), the EC_{50} values for AZT and the most active of the PFA-AZT conjugates (**9b**) were 0.009 μM, whereas the EC_{50} of PFA was >1000 μM. Thus the PFA-resistant mutant was not cross-resistant to **9a**−**c**, and **9b** was as active as AZT itself against this mutant and approximately 10^5 times more active than PFA. Indeed, even wild-type HIV-1_{LAI} was at least 1000 times more sensitive to **9b** than to PFA. In assays against A018-post, the strain with a high degree of resistance to AZT, the EC_{50} values of PFA and the best conjugate (**9a**) were 65 and 0.30 μM, respectively. The EC_{50} of AZT against A018-post was 2.9 μM, representing >200-fold resistance relative to wild-type HIV-1_{LAI}. Thus this AZT-resistant post-treatment isolate was negligibly cross-resistant to **9a**−**c** and nearly 10 times more sensitive to **9a** than to AZT. The submicromolar activity of **9a**−**c** against the AZT-resistant as well as PFA-resistant mutants was an unanticipated feature of these compounds.

The mechanism of action of esterified PFA-AZT conjugates such as **9a**−**c** is presently unknown. The fact that **9a**−**c** are more active than either PFA or AZT against the AZT-resistant strain A018-post, as well as more active than PFA against the PFA-resistant mutant E89K, suggests that they are taken up by host cells without prior cleavage to AZT and PFA in the medium and that they are subsequently metabolized to PFA and AZT inside the cell, thus serving as dual prodrugs. As indicated in Figure 1, cleavage of the conjugates to PFA and AZT is potentially a complex process involving two or more enzymes acting independently on different parts of the molecule. Thus, the P−ester bond could be the initial cleavage site, yielding AZT and a PFA ester, or the C−ester bond could be cleaved first, e.g., by a lipase or esterase, to form 3′-azido-3′-deoxy-5′-*O*-(carboxyphosphinyl)thymidine and a fatty alcohol. Further breakdown of the PFA ester and 3′-azido-3′-deoxy-5′-*O*-(carboxyphosphinyl)thymidine would then yield PFA and AZT, respectively. However, as suggested previously,[32] there is also the possibility for 3′-azido-3′-deoxy-5′-*O*-(carboxyphosphinyl)thymidine to form AZT 5′-monophosphate (AZTMP) by decarboxylation to AZT 5′-hydrogenphosphonate followed by P-oxidation. It may be noted that AZT 5′-hydrogenphosphonate has itself been reported to inhibit HIV-1 replication,[39] but the issue of whether this compound is the active species intracellularly or is a prodrug of AZTMP was not addressed. While studies using esters of [^{13}C]PFA-[^{3}H]AZT to monitor the formation of possible metabolites such as free [^{13}C]PFA, [^{3}H]AZT, and [^{3}H]AZT phosphates were not done in this preliminary work, these experiments would obviously be worth doing in the future, inasmuch as they would reveal whether inhibition of AZT-resistant HIV-1 can be explained by the ability of the conjugates to produce higher intracellular concentrations of AZTTP than are achievable with AZT itself. A noteworthy feature of the decarboxylation−oxidation pathway is that it would bypass the requirement for enzymatic phosphorylation of the nucleoside, a potential advantage in the case of nucleosides (other than AZT) that are inefficient kinase substrates. On the downside, to the extent that it occurred, the decarboxylation−oxidation pathway would eliminate PFA as a player, i.e., the conjugates would still act as dual prodrugs but in a less than 1:1 stoichiometric ratio.

Assuming that PFA and AZT 5′-triphosphate (AZTTP) are both ultimately formed from **9a**−**c** in the cell in a ratio which would depend on precisely how the PFA-AZT conjugate is processed, one might expect synergistic inhibition of reverse transcriptase (RT), which has different binding sites for PFA and AZTTP. K_i values determined in the same laboratory with wild-type HIV-1 RT inhibition in the presence of [^{3}H]dTTP and synthetic $(rA)_n(dT)_{12-18}$ as the template are reported to be 0.4 μM for PFA[4] and 0.006 μM for AZTTP.[40] Thus, assuming merely additive interaction, there would have to be ca. 1500 times as much PFA as AZTTP in the cell to produce the same degree of RT inhibition. A study using different combinations of PFA and AZTTP has found that HIV-1 RT inhibition is additive rather than synergistic and that the interaction of the two ligands is mutually exclusive.[18] According to this interpretation the two molecules cannot enter their respective binding sites at the same time because there is partial overlap between the two sites. Interestingly, when different combinations of PFA and AZT were used to block viral

replication in intact cells, moderate synergy was observed.[18] Although the PFA/AZT ratios in the viral replication assay were higher than the PFA/AZTTP ratios in the RT assays, this synergy was interpreted to mean that, at high PFA/AZT ratios, the antiviral effect may involve more than just RT inhibition (e.g., interference with attachment of the virion to the cell by trianionic PFA). While we do not know at this point how much PFA and AZTTP are produced from **9a**−**c** inside the cell, the maximum achievable PFA/AZTTP ratio *cannot be greater than 1:1* and could even be lower if there were significant loss of PFA by decarboxylation as shown in Figure 1. The fact that **9a**−**c**, with EC_{50} values in the 0.1−0.5 μM range, were 10−100 times more active than AZT against AZT-resistant HIV-1 suggests that the observed antiviral effect was not due to AZT alone and that at least some intracellular PFA had to have formed. The presence of a hydrophobic C_{18-22} side chain together with a single, weakly acidic OH group on the phosphorus atom would be expected to favor transport of the prodrug across the cell membrane, ultimately yielding an intracellular PFA level not normally attainable except at extracellular PFA concentrations of 100 μM or more.

Further studies on conjugates of long-chained esters of PFA with dideoxynucleosides other than AZT would be of potential interest and could in principle be extended to viruses other than HIV-1.

Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer; ^{1}H NMR spectra were obtained on Varian Model EM360L and Bruker AM-500 instruments at 60 and 500 MHz, respectively. TLC was on Whatman MK6F and Baker 250F silica gel plates with a fluorescent indicator dye. Spots were visualized under a 254-nm UV lamp, in an iodine chamber, or by spraying with H_2SO_4/H_2O/EtOH or molybdic acid spray reagent. Melting points were obtained on a Fisher-Johns hot-stage apparatus and are not corrected. Chemicals were purchased from Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), and Fisher (Boston, MA). Solvents were routinely stored over Linde 4A molecular sieves. Microchemical analyses were done by Quantitative Technologies, Inc., Whitehouse, NJ.

1-Octadecyl Chloroformate (3a). A. A solution of 1-octadecanol (6.0 g, 22 mmol) in a mixture of toluene (240 mL) and Et_2O (120 mL) was added dropwise to a stirred, ice-cold solution of 20% phosgene in toluene (26.7 mL, 50 mmol) over 30 min. The reaction mixture was allowed to come to room temperature, and stirring was continued under an argon atmosphere for 2 h. Evaporation at water aspirator pressure followed by trituration with Et_2O, suction filtration, and drying yielded a waxy white solid (7.12 g, 97%): mp 30−32 °C; IR (KBr) ν 2950−2900, 2850, 1780, 1455, 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.7−1.9 (m, 35H, $CH_3(CH_2)_{16}$), 4.3 (t, 2H, CH_2O). Anal. ($C_{19}H_{37}ClO_2$) C, H, Cl.

B. Solid triphosgene (2.7 g, 9.1 mmol) was added to a stirred solution of 1-octadecanol (5.0 g, 1.8 mmol) and dry pyridine (0.15 g, 1.9 mmol) in dry CCl_4 (20 mL) cooled in an ice−salt mixture. After 5 min at −15 °C, the mixture was allowed to come to room temperature, stirred for 1.5 h, and finally warmed to 40 °C in a water bath and stirred overnight at room temperature. After filtration of the pyridinium chloride salt, the solvent was evaporated to a colorless gum indistinguishable from the triester obtained by procedure A; yield 6 g (99%).

1-Eicosanyl Chloroformate (3b). The same methods as described for **3a** gave a waxy white solid from which traces of toluene were not fully removed (95% yield, used directly for the next step): mp 33−34 °C; TLC R_f 0.49 (silica gel, 98:2 $CHCl_3$−MeOH); IR (KBr) ν 2980−2890, 2850, 1780, 1470, 1380, 1300, 1215 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.8−1.6 (m, 39H, $CH_3(CH_2)_{18}$), 4.3 (t, 2H, CH_2O). Anal. ($C_{21}H_{41}ClO_2$·0.1$C_6H_5CH_3$) C, H, Cl.

1-Docosanyl Chloroformate (3c). A mixture of 1-docosanol (3.3 g, 10 mmol) in a mixture of toluene (25 mL), CH_2Cl_2 (25 mL), and Et_2O (25 mL) was warmed to 30−35 °C to obtain a clear solution which was then added dropwise to an ice-cold solution of 20% phosgene (15.0 mL, 28 mmol) in toluene over 10 min. Workup as in the synthesis of **3a** yielded a waxy white solid (3.82 g, 98%): mp 39−40 °C; TLC R_f 0.50 (silica gel, 98:2 $CHCl_3$−MeOH); IR (KBr) ν 2960−2900, 2860, 1780, 1470, 1360, 1300, 1265 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.8−1.8 (m, 43H, $CH_3(CH_2)_{20}$), 4.3 (t, 2H, CH_2O). Anal. ($C_{23}H_{45}ClO_2$) C, H, Cl.

Dimethyl [(1-Octadecyloxy)carbonyl]phosphonate (4a). A mixture of **3a** (7.0 g, 21 mmol) and $P(OMe)_3$ (37.2 mL) was stirred at 80 °C for 2 h and allowed to cool overnight. The white solid which formed was collected, washed with hexane, and dried overnight in a vacuum oven; yield 6.0 g. A second crop was obtained from the filtrate upon cooling (2.1 g); total yield 8.1 g (95%): mp 41−42 °C (hexane); IR (KBr) ν 2950−2880, 2830, 1710, 1470, 1375, 1350, 1280, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.8−1.8 (m, 35H, $CH_3(CH_2)_{16}$), 3.9 (d, 6H, $P(OCH_3)_2$), 4.3 (t, 2H, CH_2O). Anal. ($C_{21}H_{43}O_5P$) C, H, P.

Dimethyl 1-[(Eicosanyloxy)carbonyl]phosphonate (4b). Compound **3b** (6.0 g, 16.6 mmol) was added slowly to refluxing $P(OMe)_3$ (30 mL), and after 2 h the mixture was left at room temperature overnight. Excess $P(OMe)_3$ was removed by vacuum distillation, and the remaining white solid was collected, washed with cold hexane, and recrystallized from hexane to obtain colorless crystals from which traces of hexane were not fully removed (7.0 g, 97% yield): mp 53−54 °C; TLC R_f 0.39 (silica gel, 98:2 $CHCl_3$−MeOH); IR (KBr) ν 2950, 2915, 1705, 1540, 1280, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.8−1.8 (m, >39H, $CH_3(CH_2)_{18}$, hexane), 3.9 (d, 6H, $P(OCH_3)_2$), 4.3 (t, 2H, CH_2O). Anal. ($C_{23}H_{47}O_5P$·0.15C_6H_{14}) C, H, P.

Dimethyl [(1-docosanyloxy)carbonyl]phosphonate (4c): prepared from **3c** and worked up as in the reaction of **3b**; 85% yield (including a trace of residual hexane); mp 60−61 °C; TLC R_f 0.49 (silica gel, 98:2 $CHCl_3$−MeOH); IR (KBr) ν 2960, 2910, 2850, 1710, 1470, 1285, 1225 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.8−2.8 (m, >43H, $CH_3(CH_2)_{20}$, hexane), 4.1 (d, 6H, $P(OCH_3)_2$), 4.4 (t, 2H, CH_2O). Anal. ($C_{25}H_{51}O_5P$·0.15C_6H_{14}) C, H, P.

Dimethyl [(3β-Cholest-5-enyloxy)carbonyl]phosphonate (4d). A mixture of cholesteryl chloroformate (4.6 g, 10 mmol; Aldrich, Milwaukee, WI) and $P(OMe)_3$ (15 mL) was heated to 114 °C, maintained under reflux for 2 h, allowed to cool to room temperature, and diluted with hexane (20 mL). The precipitate was filtered, washed with cold hexane, and recrystallized from hexane. Drying in vacuo yielded colorless plates (4.7 g, 90% yield): mp 176−177 °C; IR (KBr) ν 3400, 2940, 2910, 2840, 1705, 1460, 1370, 1325, 1280, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.30−2.70 (m, 44H, cholestenyl CH_3, CH_2, CH), 3.90 (d, 6H, $P(OCH_3)_2$), 4.60 (m, 1H, cholestenyl 3α-H), 5.35 (1H, cholestenyl CH=). The product obtained by this method was indistinguishable from the previously analyzed reference specimen (note: in a previous publication from our laboratory[33] the melting point of **4d** was given as 100 °C as a result of a proofreading error).

Sodium Methyl [(1-Octadecyloxy)carbonyl]phosphonate (5a). A mixture of NaI (553 mg, 3.69 mmol) and **4a** (1.0 g, 2.46 mmol) in a mixture of DMF (3 mL) and THF (10 mL) was stirred under argon in a flask protected from light. After 20 h, a second portion of NaI (277 mg, 1.85 mmol) was added, and stirring was continued fo a total of 44 h, at which time TLC showed all the starting material to be gone. The solvents were removed on a rotary evaporator, and the residue was triturated with acetone, filtered, and dried in vacuo over P_2O_5 to a white solid (0.96 g, 94% yield): mp 92−93 °C; IR (KBr) ν 2980, 2965, 2930, 2860, 1720−1690 br, 1470, 1270 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.9−1.8 (m, 35H, $CH_3(CH_2)_{16}$), 3.7 (d, 3H, P(ONa)(OCH_3)), 4.2 (t, 2H, CH_2O). Anal. ($C_{20}H_{40}O_5NaP$) C, H, Na, P.

Sodium Methyl [(1-Eicosanyloxy)carbonyl]phosphonate (5b). Demethylation of **4b** with NaI was carried out as in the reaction of **4a** except for the solvent, which was a mixture of DMF (3 mL), THF (8 mL), and acetone (2 mL); yield

0.86 g (84%): mp 95−96 °C; IR (KBr) ν 2960, 2930, 2860, 1735, 1470, 1235, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.8−1.8 (m, 39H, $CH_3(CH_2)_{18}$), 3.8 (d, 3H, P(ONa)(OCH_3)), 4.2 (t, 2H, CH_2O). Anal. ($C_{22}H_{44}O_5NaP$) C, H, Na, P.

Sodium methyl [(1-docosanyloxy)carbonyl]phosphonate (5c): from **4c**, 88% yield; mp 99−100 °C; IR (KBr) ν 2960, 2920, 2860, 1735, 1470, 1250 br cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.8−1.8 (m, 43H, $CH_3(CH_2)_{20}$), 3.7 (d, 3H, P(ONa)-(OMe)), 4.1 (t, 2H, CH_2O). Anal. ($C_{24}H_{48}O_5NaP$) C, H, Na, P.

Sodium Methyl [(3β-Cholest-5-enyloxy)carbonyl]phosphonate (5d). Cholesteryl chloroformate (9.0 g, 20 mmol) was added slowly to refluxing $P(OMe)_3$ (30 mL), and refluxing was continued for 1 h. The reaction mixture was then allowed to cool to room temperature, hexane (25 mL) was added, and the white solid was filtered, washed with hexane (2 × 25 mL), and dried in vacuo to obtain triester **4d** (8.99 g, 86%); mp 171−176 °C. A portion of **4d** (1.0 g, 1.0 mmol) was dissolved in a mixture of DMF (10 mL), THF (15 mL), and acetone (10 mL), and the solution was treated with NaI (431 mg, 2.88 mmol) with protection from light. After 24 h of stirring under argon, the solvents were evaporated and the oily residue was triturated with acetone. Filtration and drying afforded a white solid (0.95 g, 94%): mp 275−276 °C; IR (KBr) ν 2950−2850, 1685 br, 1470, 1440, 1380, 1365, 1270, 1230 cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.6−2.5 (m, 43H, cholestenyl CH_3, CH_2, CH), 3.7 (d, 3H, P(ONa)(OCH_3)), 4.5 (m, 1H, cholestenyl 3α-H), 5.4 (m, 1H, cholestenyl =CH). Anal. ($C_{29}H_{48}O_5NaP\cdot0.2H_2O$) C, H, Na, P.

[(1-Octadecyloxy)carbonyl]phosphonic Acid (6a). Me_3-SiBr (0.812 mL, 6.15 mmol) was added to a solution of **4a** (1.0 g, 2.46 mmol) in CH_2Cl_2 (20 mL) under argon, and the yellow solution was stirred at room temperature for 4 h and evaporated under reduced pressure. Trituration of the oily residue with hexane, followed by filtration and drying, afforded a hygroscopic white solid (0.757 g, 81%): mp 81−82 °C; IR (KBr) ν 2960, 2920, 2860, 1730, 1720, 1475, 1465, 1265, 1240, 1225 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.8−1.8 (m, 35H, $CH_3(CH_2)_{16}$), 4.25 (t, 2H, CH_2O). Anal. ($C_{19}H_{39}O_5P\cdot0.3H_2O$) C, H, P.

[(1-Eicosanyloxy)carbonyl]phosphonic acid (6b): from **4b**, 77% yield; mp 87−89 °C; IR (KBr) ν 2960, 2930, 2860, 1735, 1470, 1235, 1220 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.9−1.8 (m, 39H, $CH_3(CH_2)_{18}$), 4.2 (t, 2H, CH_2O). Anal. ($C_{21}H_{43}O_5P$) C, H, P.

[(1-Docosanyloxy)carbonyl]phosphonic acid (6c): from **4c**, 77% yield; mp 92−93 °C; IR (KBr) ν 2950, 2910, 1730, 1465, 1240−1210 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.8−1.8 (m, 43H, $CH_3(CH_2)_{20}$), 4.3 (t, 2H, CH_2O). Anal. ($C_{23}H_{47}O_5P$) C, H, P.

[(3β-Cholest-5-enyloxy)carbonyl]phosphonic acid (6d): from **4d**, 78% yield; mp 152−153 °C dec; IR (KBr) ν 3400, 2980−2950, 2870, 1720, 1470, 1440, 1385, 1260, 1225 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.5−2.5 (m, 43H, cholestenyl protons), 4.8 (m, 1H, cholestenyl 3α-H), 5.4 (m, 1H, cholestenyl =CH). Anal. ($C_{28}H_{47}O_5P\cdot H_2O$) C, H, P.

Disodium [(1-Octadecyloxy)carbonyl]phosphonate (7a). A stirred solution of **6a** (100 mg, 0.264 mmol) in a mixture MeOH (8 mL) and $CHCl_3$ (8 mL) was cooled to −5 °C under dry argon, and to it was added dropwise over 5−10 min a solution of NaOMe in MeOH, prepared by dissolving Na metal (12.1 mg, 0.528 mmol) in anhydrous MeOH (8 mL). After 1.5 h of stirring, the solvents were evaporated, fresh MeOH (10 mL) was added, and the mixture was kept in a sonication bath until a fine white solid formed. Filtration, extensive washing with MeOH to remove any mono- or dimethyl ester, and drying in vacuo over P_2O_5 afforded a white powder (70 mg, 63%): mp >350 °C; IR (KBr) ν 2960, 2920, 2925, 1690, 1470, 1380 cm^{-1}. Anal. ($C_{19}H_{37}Na_2O_5P\cdot0.3CH_3OH$) C, H, Na, P.

Disodium [(1-eicosanyloxy)carbonyl]phosphonate (7b): from **6b** in 81% yield, white powder; mp >350 °C. Anal. ($C_{21}H_{41}Na_2O_5P\cdot0.3CH_3OH$) C, H, Na, P.

Disodium [(1-docosanyloxy)carbonyl]phosphonate (7c): from **6c** in 98% yield, white powder; mp 270−272 °C, prior softening. Anal. ($C_{23}H_{45}Na_2O_5P\cdot1.3CH_3OH$) C, H, Na, P.

Disodium [(3β-cholest-5-enyloxy)carbonyl]phosphonate (7d): from **6d**, 68% yield, hygroscopic white powder; mp 295−300 °C, softening above 230 °C. Anal. ($C_{28}H_{45}O_5Na_2P\cdot0.8CH_3OH\cdot1.2H_2O$) C, H, Na, P.

3′-Azido-3′-deoxy-5′-*O*-[[(1-octadecyloxy)carbonyl]phosphinyl]thymidine (9a). A. PCl_5 (1.68 g, 8.10 mmol) was added to a solution of **4a** (3.1 g, 7.63 mmol) in dry CCl_4 (40 mL), and the reaction mixture was heated to reflux for 3 h. The unreacted PCl_5 was decomposed by passing dry SO_2 gas through the solution at room temperature for 5 min, and the CCl_4, $SOCl_2$, and $POCl_3$ were distilled off under high vacuum (0.01−0.05 Torr) at 40 °C. The residue was taken up in dry DMF (6 mL), taking extreme care to minimize exposure to moisture, and the solution was cooled to −50 °C in a dry ice−acetone bath. AZT was thoroughly dried by several cycles of addition and rotary evaporation of freshly redistilled pyridine, a sample of the anhydrous nucleoside (0.68 g, 2.55 mmol) was dissolved in dry DMF (1 mL), and the solution was precooled to −50 °C and added in a single portion under dry argon via a syringe to the stirred DMF solution of (alkoxycarbonyl)phosphonyl chloride reagent. When addition was complete, the reaction mixture was allowed to come to room temperature and stirring was continued for 24 h. The solvent was evaporated under reduced pressure, and the foamy residue was purified by flash chromatography on silica gel (98:2 $CHCl_3$−MeOH) to obtain the desired AZT-PFA triester **8a** as a colorless solid which was used directly in the next step (see below); yield 1.1 g (22%).

Solid NaI (0.18 g, 1.2 mmol) was added to a solution of **8a** (0.55 g, 0.86 mmol) in dry THF (10 mL) in a flask wrapped in aluminum foil, and stirring was continued under argon at room temperature for 24 h. After evaporation of the solvent under reduced pressure, the residue was applied onto a DEAE-cellulose column (HCO_3^- form). The column was eluted successively with distilled H_2O and 0.05 M NH_4HCO_3. Fractions of the latter eluent containing the desired product were pooled and freeze-dried to a colorless solid (220 mg, 40% yield): mp 224 °C dec; TLC R_f 0.25 (silica gel, 85:15:1 $CHCl_3$−MeOH−28% NH_4OH); HPLC 4.4 min (C_{18} silica gel, 30% MeCN in 0.01 M NH_4OAc, pH 6.7, 1 mL/min); IR (KBr) ν 3400, 3150 br, 2890, 2820, 2080 (N_3), 1710−1650 br, 1455, 1390, 1240 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.40−2.80 (m, 40H, $CH_3(CH_2)_{17}$, 5-Me, $C_{2'}$-H), 4.0−4.5 (m, 6H, CH_2O, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H), 6.15 (t, 1H, $C_{1'}$-H), 7.80 (s, 1H, C_6-H). Anal. ($C_{29}H_{50}N_5O_8P\cdot NH_3\cdot0.5H_2O$) C, H, P.

B. A mixture of **6a** (0.15 g, 0.4 mmol) and AZT (0.216 g, 0.81 mmol) was rigorously dried by repetitive addition and rotary evaporation of freshly distilled pyridine (3 × 10 mL). The dried reactants were redissolved in pyridine (5 mL), and the solution was flushed with a stream of dry argon for 15 min at room temperature. Cl_3CCN (0.54 g, 4.0 mmol) was then added and the reaction mixture kept at 50−60 °C in an oil bath overnight under dry argon. The solvent was removed by rotary evaporation and the residue redissolved in CH_2Cl_2 (40 mL). The organic layer was washed with H_2O (2 × 20 mL), dried over Na_2SO_4, and evaporated. Flash chromatography on silica gel with $CHCl_3$−MeOH−28% NH_4OH (85:15:1) as the eluent yielded a white solid (85 mg, 34%): mp 220 °C. IR and ^{1}H NMR spectra of this material and of the product obtained by procedure A were virtually indistinguishable.

3′-Azido-3′-deoxy-5′-*O*-[[(1-eicosanyloxy)carbonyl]phosphinyl]thymidine (9b). A. Treatment of **4b** (1.66 g, 3.82 mmol) with PCl_5 (0.84 g, 4.04 mmol) in CCl_4 (20 mL) as described for **4a**, followed by reaction with AZT (0.68 g, 2.55 mmol) and purification by silica gel flash chromatography (98:2 $CHCl_3$−MeOH), afforded the AZT-PFA triester **8b** as a waxy white solid (0.76 g, 30% yield). Direct reaction of **8b** (0.50 g, 0.75 mmol) with NaI (0.16 g, 1.1 mmol) in THF (80 mL) under dry argon in the absence of light, followed by purification on DEAE-cellulose (HCO_3^- form; H_2O, then 0.05 M NH_4HCO_3), afforded a colorless solid (165 mg, 33%): mp 230 °C dec; TLC R_f 0.29 (silica gel, 85:15:1 $CHCl_3$−MeOH−28% NH_4OH); HPLC 4.7 min (C_{18} silica gel, 30% MeCN in 0.01 M NH_4OAc, pH 6.7, 1.0 mL/min); IR (KBr) ν 3450, 3250 sh, 2920, 2850, 2100 (N_3), 1710−1640 br, 1470, 1400, 1370, 1250 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.60−2.60 (m, 44H, $CH_3(CH_2)_{18}$, 5-Me, $C_{2'}$-H), 4.0−4.6 (m, 6H, CH_2O, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H), 6.15 (t, 1H, $C_{1'}$-H), 7.80 (s, 1H, 6-H). Anal. ($C_{31}H_{54}N_5O_8P\cdot NH_3\cdot0.5H_2O$) C, H, P.

B. Coupling of **6b** (0.42 g, 1.02 mmol) and AZT (0.55 g, 2.05 mmol) by the CCl_3CN method (see above), followed by flash chromatography on silica gel (85:15:1 $CHCl_3$−MeOH−28% NH_4OH), gave a white solid (133 mg, 49%): mp 228 °C dec. IR and ^1H NMR spectra of this material and of the one obtained from **4b** via the PCl_5 method were virtually indistinguishable.

3′-Azido-3′-deoxy-5′-*O*-[[(1-docosanyloxy)carbonyl]phosphinyl]thymidine (9c). A. Treatment of **4c** (1.75 g, 3.82 mmol) with PCl_5 (0.84 g, 4.04 mmol) in CCl_4 (20 mL) as described for **4a**, followed by reaction with AZT (0.77 g, 2.90 mmol) and purification by silica gel flash chromatography (98:2 $CHCl_3$−MeOH), afforded the AZT-PFA triester **8c** as a waxy white solid (1.0 g, 50% yield). Direct reaction of **8c** (0.50 g, 0.75 mmol) with NaI (0.16 g, 1.1 mmol) in THF (8 mL) under dry argon, followed by purification on DEAE-cellulose (HCO_3^- form; H_2O, then 0.05 M NH_4HCO_3), afforded a colorless solid (140 mg, 28%): mp 238 °C dec; TLC R_f 0.31 (silica gel, 85:15:1 $CHCl_3$−MeOH−28% NH_4OH); HPLC 4.8 min (C_{18} silica gel, 30% MeCN in 0.01 M NH_4OAc, pH 6.7, 1.0 mL/min); IR (KBr) ν 3440, 3200, 2920, 2850, 2100 (N_3), 1710−1680 br, 1470, 1400, 1320, 1250 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 0.65−2.60 (m, 48H, $CH_3(CH_2)_{20}$, 5-Me, $C_{2'}$-H), 4.0−4.5 (m, 6H, CH_2O, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H), 6.15 (t, 1H, $C_{1'}$-H), 7.83 (s, 1H, 6-H). Anal. ($C_{33}H_{58}N_5O_8P\cdot NH_3\cdot 1.25H_2O$) C, H, P.

B. Coupling of **6c** (0.25 g, 0.58 mmol) and AZT (0.0.31 g, 1.16 mmol) by the CCl_3CN method (see above), followed by flash chromatography on silica gel (85:15:1 $CHCl_3$−MeOH−28% NH_4OH), gave a white solid (120 mg, 30%): mp 230 °C dec. IR and ^1H NMR spectra of this material and of the product obtained from **4c** via the PCl_5 method were virtually indistinguishable.

3′-Azido-3′-deoxy-5′-*O*-[[(3*β*-cholest-5-enyloxy)carbonyl]phosphinyl]thymidine (9d). Treatment of **4d** (4.0 g, 7.65 mmol) with PCl_5 (1.60 g, 7.65 mmol) in CCl_4 (40 mL) as described for **4a**, followed by reaction with AZT (0.68 g, 2.55 mmol) (**4d**/AZT molar ratio = 3.0) and purification by silica gel flash chromatography (98:2 $CHCl_3$−MeOH), afforded the intermediate triester **8d** as a colorless solid (1.18 g, 61% yield): mp 102 °C; TLC R_f 0.58 (silica gel, 98:2 CH_2Cl_2−MeOH). Further reaction of **8d** (2.42 g, 0.75 mmol) (combined from two runs) with NaI (0.51 g, 3.42 mmol) in THF (14 mL) under dry argon, followed by purification on DEAE-cellulose (HCO_3^- form; H_2O, then 0.05 M NH_4HCO_3), afforded a colorless solid (1.68 g, 67%): mp 220 °C dec; TLC R_f 0.23 (silica gel, 85:15:1 $CHCl_3$−MeOH−28% NH_4OH); HPLC 4.2 min (C_{18} silica gel, 30% MeCN in 0.01 M NH_4OAc, pH 6.7, 1.0 mL/min), with no detectable AZT; IR (KBr) ν 3420, 3160, 2940, 2105 (N_3), 1710−1670 br, 1470, 1440, 1400, 1385, 1325, 1250 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 0.4−2.6 (m, 49H, cholestenyl CH_3, CH_2, CH, 5-Me, $C_{2'}$-H), 4.0−4.5 (m, 5H, cholestenyl 3α-H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H), 5.25 (m, 1H, cholestenyl CH═), 6.15 (t, 1H, $C_{1'}$-H), 7.80 (s, 1H, 6-H). Anal. ($C_{38}H_{58}N_5O_8P\cdot NH_3\cdot H_2O$) C, H, N, P.

Bis(*O*-3′-azido-3′-deoxythymidin-5′-yl) [(3*β*-Cholest-5-enyloxy)carbonyl]phosphonate (10). A mixture of **4d** (4.0 g, 7.65 mmol) and PCl_5 (1.62 g, 7.65 mmol) in dry CCl_4 (40 mL) was refluxed for 48 h and evaporated to dryness under reduced pressure. The residue was taken up in dry DMF (10 mL), and the solution was cooled to −50 °C in a dry ice−acetone bath. A precooled (−50 °C) solution of AZT (1.36 g, 5.1 mmol) (**4d**/AZT molar ratio = 1.5) in dry DMF (1 mL) was then added under dry argon via a syringe, and the mixture was stirred at room temperature for 48 h. Solvent evaporation under high vacuum, followed by flash chromatography (silica gel, 97:3 $CHCl_3$−MeOH), gave colorless crystals (0.35 g, 14%): mp 123−124 °C; TLC R_f 0.60 (85:15:1 $CHCl_3$−MeOH−28% NH_4OH); IR (KBr) ν 3450, 3190, 3050, 2950, 2860, 2100 (N_3), 1750−1650 br, 1470, 1400, 1385, 1365, 1320, 1275, 1225 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 0.6−2.6 (m, 54H, cholestenyl CH_3, CH_2, CH, 5-Me, $C_{2'}$-H), 4.00−4.55 (m, 5H, cholestenyl 3α-H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H), 5.20 m, 1H, cholestenyl CH═), 6.1 (t, 2H, $C_{1'}$-H), 7.45 (s, 2H, 6-H). Anal. ($C_{48}H_{69}N_{10}O_{11}P\cdot 3H_2O$) C, H, N, P.

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