Potential Lipophilic Nucleotide Prodrugs: Synthesis, Hydrolysis, and Antiretroviral Activity of AZT and d4T Acyl Nucleotides

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Three general methods for the synthesis of acyl nucleotides (mono-, di-, and triphosphates) have been developed and applied to different HIV inhibitors. These new types of compounds, where a fatty acid moiety is linked to the nucleotide phosphate chain by an acyl phosphate bond, were designed as lipophilic prodrugs of HIV inhibitors metabolites. Acyl nucleoside monophosphates **1a,b** were prepared by acylation of the corresponding nucleoside monophosphates. Acyl nucleoside diphosphates **2a**−**c** and **3a,b** were synthesized directly from the free nucleosides using DCC activation of acyl pyrophosphates. Acyl nucleoside triphosphates **4a**−**c** and **5a** were obtained using phosphoromorpholidate chemistry and acyl pyrophosphates as nucleophiles. Hydrolysis of acyl nucleotides liberated the corresponding nucleotides by selective cleavage of the acyl phosphate bond, with half lives ranging from 51 to 185 h at 37 °C in triethylammonium acetate buffer pH 7.0. Their antiretroviral activity, measured by the inhibition of cytopathogenicity and reverse transcriptase activity in the cultures supernatants, did not reveal any differences between an acyl nucleotide and its corresponding nucleotide. These results are explained in term of rapid aminolysis of the acyl phosphate bond in culture media.

Although new therapies, such as antisense thiooligonucleotides, protease inhibitors, or cytokine based immune system restoration, are currently under clinical trials,[1] nucleoside analogs that inhibit the human immunodeficency virus reverse transcriptase (HIV RT) are the only drugs widely used to inhibit HIV replication *in vivo*. There is still an urgent need for new compounds which would be active from the beginning of the infection, since recent findings have shown that HIV is far from being inactive during the asymptomatic part of the infection but replicate at a fast rate in lymph nodes and peripheral blood cells.[2] It has been shown that the activity of some nucleoside analogs could be enhanced by preparing prodrugs able to deliver nucleoside analogs 5′-monophosphates (NMPs) in infected cells.[3] However, the metabolism of a nucleosidic HIV RT inhibitor involves, in fact, three kinase steps leading to the nucleoside analog 5′-triphosphate which is the active HIV RT inhibitor.[4] Prodrugs of nucleoside 5′-di or 5′-triphosphates (NDPs or NTPs) would be more interesting since they would bypass more metabolic steps. Moreover, severe side effects, such as peripheral neuropathies and bone marrow toxicity, which result from the accumulation of the NMP analog[4,5] may be avoided.

To our knowledge no NDP nor NTP lipophilic prodrug has ever been described. Cationic DABCO derived carriers, able to complex nucleotides, have been reported by Li et al.,[6] and are effective for liquid membrane nucleotide transport. But, when used with liposomes, these compounds act like detergents and break the bilayer structure. We have recently introduced a new type of compound: acyl nucleotides **1**−**5**, as potential nucleotide lipophilic prodrugs.[7] In such compounds the lipophilic acyl moiety should allow passive diffusion of the charged nucleotide through cells membranes, while the mixed carboxylic phosphoric anhydride is expected to be hydrolyzed before the symmetrical phosphoric anhydride,[8] thus allowing the liberation of the corresponding free nucleotides **6**−**8** (Scheme 1). In order to test these hypothesis we have developed three different synthetic approaches leading to the preparation of myristoyl NMPs **1a,b**, acyl NDPs **2**, **3** and acyl NTPs **4**, **5** (Table 1). We took advantage of the bivalent properties of the phosphate group which is a good nucleophile but which can also easily be activated to become a good electrophile: in this work, DCC was alternatively used to activate the acyl or the phosphate moiety, whereas acyl pyrophosphates were alternatively used as nucleophiles or as electrophiles. Acyl NMPs were prepared using DCC activation of the acyl moiety, followed by displacement of dicyclohexylurea by the NMPs bis(tetrabutylammonium) salts (Scheme 2). Acyl NDPs were directly prepared from acyl pyrophosphates and free nucleosides. In this reaction

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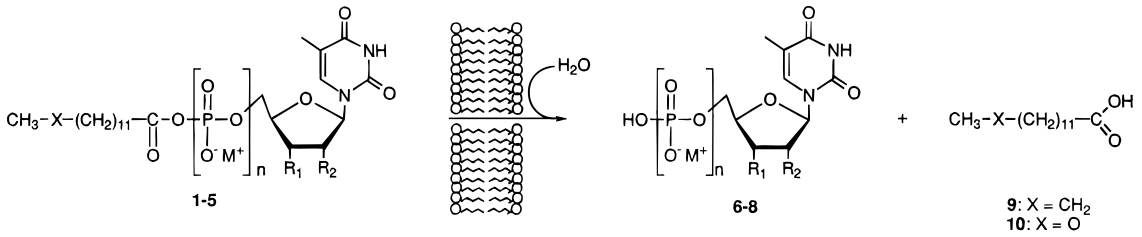
Scheme 1[a]



a) Acyl nucleotides were designed as nucleotide lipophilic prodrugs, they should: 1) enhance passive diffusion through cell membranes. 2) liberate the free nucleotide by selective hydrolysis of the acyl phosphate bond.

Table 1: Substituents and Phosphate Content for Acyl Nucleotides 1−5, Nucleotides 6−8, and Phosphoromorpholidates 17

compd	X	*n*	R_1	R_2
1a	CH_2	1	N_3	H
1b	CH_2	1	C=C	C=C
2a	CH_2	2	N_3	H
2b	CH_2	2	C=C	C=C
2c	CH_2	2	OAc	H
3a	O	2	N_3	H
3b	O	2	C=C	C=C
4a	CH_2	3	N_3	H
4b	CH_2	3	C=C	C=C
4c	CH_2	3	OH	H
5a	O	3	N_3	H
6a	−	1	N_3	H
6b	−	1	C=C	C=C
7a	−	2	N_3	H
7b	−	2	C=C	C=C
8a	−	3	N_3	H
8b	−	3	C=C	C=C
17a	−	1	N_3	H
17b	−	1	C=C	C=C
17c	−	1	OH	H

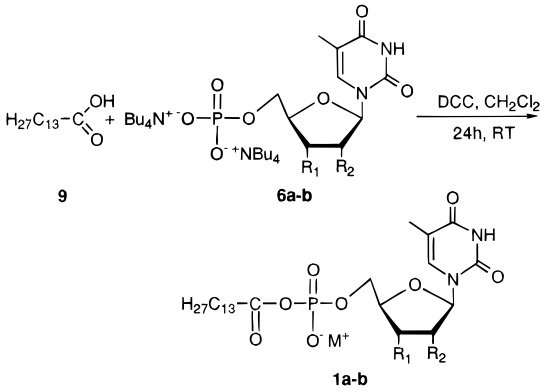
DCC was used to activate acyl pyrophosphates, generating an electrophilic phosphorus. Formation of the awaited acyl NDP bond resulted from a nucleophilic attack, at the phosphatidylurea phosphorus, by the nucleoside 5′-hydroxyl (Scheme 3). In the preparation of acyl NTPs, acyl pyrophosphates were used as nucleophiles in order to displace morpholine from various phosphoromorpholidates[9] electrophilic phosphorus (Scheme 4).

We chose to study the effect of two different lipophilic fatty acyl moieties: myristic acid (**9**) was used as a model, while 13-oxamyristic acid (**10**) (13-OMA) was used not only for its lipophilicity, but also for its ability to inhibit HIV replication by perturbing N-myristoylation of GAG polyprotein precursor, thus preventing virus assembly.[10] Hydrolyses kinetics and *in vitro* antiretroviral activities of acyl nucleotides **1**−5 are also examined in this paper.

Results and Discussion

Acylation of Nucleosides 5′-Mono-, Di- and Triphosphate 6−8. We have recently described a new protocol for the acylation of inorganic pyrophosphate **11** which allows the preparation of acyl pyrophosphates **12** and **13** with good yields and purity (Scheme 5).[7] In order to prepare acyl nucleotides **1**−**5**, we investigated the possibility to extend this type of reaction to the acylation

Scheme 2



of nucleotides. Although we were mostly interested in the preparation of acyl NDPs or NTPs, we first studied the acylation of 5′-monophosphate nucleoside analogs and then tried to apply it to nucleosides 5′-di- or 5′-triphosphates. Some acyl AMPs have already been prepared in the early 1960s, using DCC[11 a,b] or ethyl chloroformate[11c] activation of the carboxylic acids moiety, but their characterization mainly relied on chemical, biochemical, and electrophoretical properties, and no NMR data are available for such compounds. We chose to test this reaction on thymine-containing nucleoside analogs such as zidovudine (**14a)** (AZT) and stavudine (**16b)** (d4T), in order to avoid problem that could arise from base acylation. Nucleoside 5′-monophosphate analogs, AZTMP (**6a**) and d4TMP (**6b**), were prepared according to Tener procedure,[12] their spectroscopic data being consistent with those published in the literature.[13] Nucleotide analogs 5′-monophosphates **6a,b** were used as tetrabutylammonium salts and acylated with myristic acid (2 equiv) in methylene chloride, using DCC (2 eq) as activator (Scheme 3). In order to obtain reasonable reaction rates catalytic 4-(dimethylamino)pyridine (DMAP) was added to the reaction mixture. Longer reaction time, compared with the acylation of inorganic pyrophosphate,[7] are needed for these reactions to go to completion, this being mainly explained by lower reagent concentrations. Although these reactions were nearly quantitative by TLC analysis, isolated yields are relatively low: Myr-AZTMP (**1a**) and Myr-d4TMP (**1b**) were obtained, respectively, in 26 and 13% isolated yields.[14] It should be noted that, whatever quenching was used, great extent

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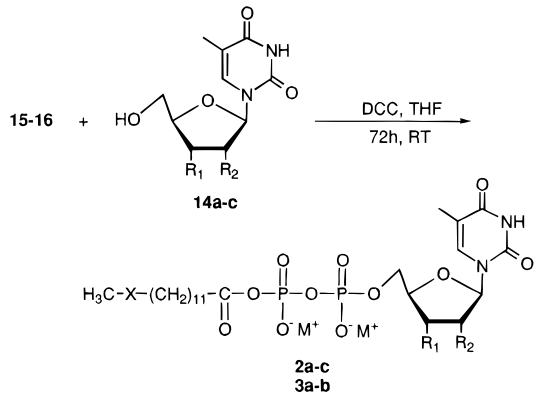
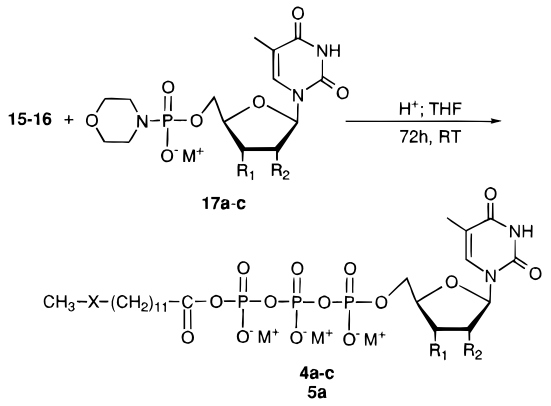
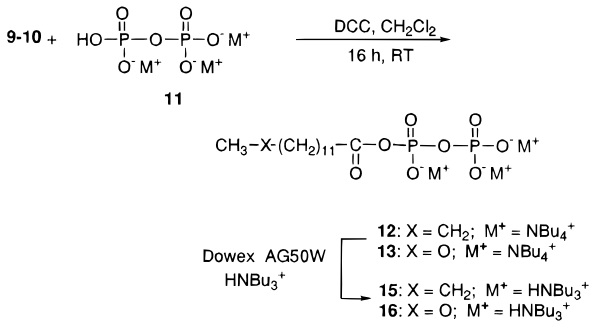
Scheme 3



Scheme 4



Scheme 5



of degradation occurred at this step. When trying to extend this reaction to NDPs **7** or NTPs **8**, degradation on quenching occurred to such an extent that only traces of acyl NDPs or NTPs could be recovered after purification.

These new acyl NMP **1a,b** were fully characterized, as sodium or triethylammonium salts, by FAB^+ mass spectra, elemental analysis,[14] and ^1H, ^{31}P, and ^{13}C NMR. We will focus here on some characteristic NMR features of acyl NMP. H_5-$H_{5'}$ signals are deshielded approximately 0.1−0.3 ppm, in respect to the parent nucleoside, this being in agreement with a 5′-phosphorylated nucleotide. In the course of our studies on pyrophosphate acylation,[7] we have already observed that an acylated phosphorus is generally 8−10 ppm more shielded than a free pyrophosphate phosphorus. ^{31}P NMR signals of acyl NMP **1a,b** are in accordance with these findings: phosphorus in **1a,b** resonate 8 ppm higher field than the parent NMPs **6a,b** signals.[15] The unambiguous proof for the presence of an acyl-phosphate bond in **1a,b** is given by the occurrence of a 9.0 Hz coupling constant between the acyl carbonyl carbon and nucleotide phosphorus.

Preparation of Acyl NDPs 2 and 3. It has been reported that NDPs may be prepared by a S_N2 displacement of a 5′-iodo or 5′-tosyl nucleoside by a pyrophosphate salt,[16] but, when using acyl pyrophosphate as nucleophile, this protocol failed leading only to degradation products. Carbodiimide activation of cyanoethyl phosphate is a well known method to prepare NMPs from the corresponding nucleoside:[12] a phosphatidylurea intermediate is generated in situ by the reaction of DCC with cyanoethylphosphate, while the nucleoside 5′-hydroxyl group acts as a nucleophile which attacks the electrophilic phosphorus. This procedure is not used to prepare NDPs, since symmetrical dinucleotides are awaited. With acyl pyrophosphates, we thought that the acyl moiety could be considered as a protective group for one of the pyrophosphate phosphorus and that it should be possible to prepare acyl NDPs directly from a nucleoside and an acyl pyrophosphate using DCC activation. This reaction was tested on 3′-acetyl thymidine **14c**[12] and then applied to different HIV inhibitors: 3′-azido-3′-deoxythymidine (AZT) (**14a**) and 3′-deoxy-2′,3′-didehydrothymidine (d4T) (**14b**) (Scheme 3). In preliminary experiments, we established that the reaction rate was much higher when 1 equiv of (±)-camphorsulfonic acid was added to the reaction mixture. When a proton source was omitted, for example when tris(tetrabutylammonium) acyl pyrophosphate was used alone, dicyclohexylurea anion accumulated and prevented protonation of the phosphatidylurea intermediate, thus lowering the reaction rate. In order to avoid the addition of a strong acid we found it more convenient to exchange the tetrabutylammonium coutercations of **12** and **13** and to use tris(tributylammonium) acyl pyrophosphates **15** and **16** as phosphorylating agents. In a typical experiment the free nucleosides were dissolved in anhydrous THF, **15** and **16** (4 equiv) and DCC (4 equiv) were added over 72 h. The new compounds acyl NDPs **2a−c** and **3a,b** were obtained in moderate 16−24% isolated yields.[14] Acylation of the 5′ free hydroxyl of the nucleosides was observed as a side reaction; this was not surprising since acyl pyrophosphates are mixed carboxylic phosphoric anhydrides. Depending on the nucleoside, 20−40% of the starting material could be recovered after Zemplen deacylation.

The characteristic NMR data of these new compounds are in accordance with the proposed structures. For acyl AZTDPs **2a,3a**, H_5-$H_{5'}$ signals are deshielded from 0.3 ppm in respect to AZT; this deshielding effect of phosphorylation is only 0.07 ppm for acyl d4TDPs **2b,3b**. The presence of an acyl phosphate bond is inferred from a

(14) The purity of acyl nucleotides **1**−5 was checked by reverse phase HPLC and typically ranged between 98−100% for acyl nucleotide in the triethylammonium form, and 90−95% for products in the sodium form, after exchange on Dowex AG50 WX8. Recently we found that precipitation of the acyl nucleotide triethylammonium salt, dissolved in the minimum methanol at 0 °C, by addition of a 1 M NaI acetone solution followed by extensive washing with cold acetone, allowed the isolation of the acyl nucleotide sodium salt without detectable decomposition. However, these products slowly decomposed even at −18 °C.

(15) The phosphorus chemical shift greatly depends on the solvent and the pH; in order to compare ^{31}P chemical shifts, acylated and unacylated nucleotide were mixed and ^{31}P NMR spectra of this mixture were recorded in D_2O.

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(17) The preparation of this compound was not optimized, nor was it fully characterized. However, ^1H, and ^{31}P NMR and FAB^+ mass data are given in the experimental part, since **4c** trilithium salt is the only acyl NTP we obtained showing a P_α−$H_{5-5'}$ coupling constant, thus allowing the unambiguous assignment of the P_α ^{31}P chemical shift.

9.0−9.5 Hz coupling constant between the acyl carbonyl carbon and P_α. By comparison of the ^{31}P chemical shifts of acyl NDPs with free NDPs[15] we concluded that the acyl chain is linked to P_β: this phosphorus resonates around −19 ppm, this being 9−10 ppm higher field than an unacylated NDP's P_β; moreover, this phosphorus presents no coupling constant with H_5-$H_{5'}$. The other phosphorus, P_α, is coupled with H_5-$H_{5'}$ ($J_{P-H} \approx 5$ Hz) and resonates around −11.5 ppm, only 0.5 ppm higher field than a nonacylated NDP's P_α. These results are in accordance with our previous studies on acyl pyrophosphates: the acylated phosphorus is generally 8−10 ppm more shielded than the nonacylated one, which resonates only 0.2−0.3 ppm higher field than free pyrophosphate phosphorus.[7]

Preparation of Acyl NTPs 4 and 5. In order to prepare acyl NTPs, we turned to classical NTP chemistry: the nucleophilic displacement of a phosphoromorpholidate by a pyrophosphate salt.[9a,b] Once more, acyl pyrophosphates proved to be valuable intermediates in the synthesis of the previously unavailable acyl NTPs **4** and **5** (Scheme 4). In this reaction, the acyl pyrophosphate was used as nucleophile to displace morpholine from the electrophilic phosphoromorpholidate phosphorus. The acyl pyrophosphates were used in the tributylammonium form, and (±)-camphorsulfonic acid (1 equiv) was added to catalyze the reaction and protonate the liberated morpholine, thus avoiding aminolysis of the labile acyl phosphate bond. It should be noted that when the proton source is omitted, only nucleoside triphosphate could be recovered at the end of the reaction. Thymidine phosphoromorpholidate[9a,b] **17c** was used as a model and the reaction was optimized with AZT and d4T phosphoromorpholidates **17a**[9c] and **17b**, leading to the acyl NTPs **4a-c** and **5a** in 7−20% isolated yields.[14]

NMR data collected for the acyl NTPs are in agreement with the proposed structure and show that P_γ, and not P_α nor P_β, was acylated. H_5-$H_{5'}$ signals for AZT derivatives are deshielded 0.3 ppm in respect to AZT, this effect being much lower for acyl d4TTP **4b**. The occurrence of an acyl phosphate bond is inferred from a 9.5 Hz J_{C-P} coupling constant determined on the carbonyl carbon signal, while P_γ resonates 9−11 ppm higher field than a nonacylated NTP P_γ.[15] The chemical shift of P_α, coupled with H_5-$H_{5'}$ ($J_{P-H} = 5.9$ Hz for **4c** in DMSO-d_6), is only slightly affected by acylation (−0.06 to −0.37 ppm).[15] The characteristic triphosphate P_β signal (triplet $J_{P-P} \approx 18$ Hz) appears around −23 ppm, only 0.5−1.5 ppm higher field than a nonacylated NTP P_β.[15] It should be noted that line broadening was observed when **4a,b** sodium salt spectra were recorded in DMSO-d_6, and this effect increased with concentration and was attributed to cluster formation. This effect also occurred with acyl NDPs disodium salts, although not to such an extent. Line broadening did not occurred when **4c** lithium salt was recorded in DMSO-d_6, or when D_2O was used as solvent.

Antiretroviral Activities of Acyl Nucleotides 1−5. The antiretroviral activities were measured on CEM 4 T cell line infected with HIV1 LAI or HIV2 Rod cell free supernatants. Two parameters were studied, at days 6 and 10 postinfection, to evaluate the antiretroviral activity of the compounds: inhibition of HIV induced cytopathic effect (ED_{50}), using the MTT cell viability assay and the inhibition of the HIV RT production in culture supernatant (RT ED_{50}).[18] We present in full details in this paper data obtained at days 6 and 10, postinfection by HIV1 (Tables 2 and 3).[19]

Table 2. Antiviral Activity on HIV1 of the Acyl Phosphate Series of AZT

		MTT ED50 (nM)		RT ED50 (nM)		SI	
	CD50 (μM)	6 days	10 days	6 days	10 days	6 days	10 days
1a	>20	10	150	<9	700	2000	133
2a	>50	30	100	9	700	1667	500
3a	>50	−	467	8	400	−	107
4a	>50	24	100	<9	500	2083	500
5a	>50	100	647	8	400	500	77
6a	>20	20	60	<9	500	1000	333
7a	>20	20	170	10	1000	1000	118
8a	>20	10	100	<9	900	2000	200
14a (AZT)	>50	20	100	8	300	2500	500

Table 3. Antiviral Activity on HIV1 of the Acyl Phosphate Series of D4T

		MTT ED50 (nM)		RT ED50 (nM)		SI	
	CD50 (μM)	6 days	10 days	6 days	10 days	6 days	10 days
1b	>20	190	540	100	1000	105	37
2b	>50	190	600	100	1500	263	83
4b	>20	220	530	100	1000	91	38
6b	>20	280	710	100	1000	71	28
7b	>20	160	600	100	1000	125	33
8b	>20	220	760	100	1000	91	26
14b (D4T)	>10	9	400	10	600	1111	25

The first parameter we examined for myristoyl nucleotides in the AZT series (**14a**, **1a**, **2a**, **4a**, **6a**, **7a**, **8a**) and the d4T series (**14b**, **1b**, **2b**, **4b**, **6b**, **7b**, **8b**) was the 50% cytotoxic dose (CD_{50} = concentration required to reduce the viability of uninfected cells by 50%). Acyl and free nucleotides were devoid of toxicity for CEM4 cells at concentration up to 50 μM, alike AZT (>50 μM) and d4T (> 10 μM). ED_{50} at day 6 postinfection for nucleotide compounds in the AZT series (Table 2) laid in the same order range as the free nucleoside (10−30 nM), whereas in the d4T series (Table 3), nucleotides and acyl nucleotides are 1 log less active than the free nucleoside. When comparing activities of acyl nucleotides with their parent nucleotide, the differences observed are in the error range of the activity determination. We concluded that no antiretroviral activity differences could be detected in these assays between acyl nucleotides and their corresponding nucleotides either 6 or 10 days after infection. These results were confirmed by the assays on the inhibition of HIV1 RT production and clearly illustrated by the selectivity index: no significant differences were detected between acyl nucleotides and their corresponding nucleotides.

In a second series of experiments, antiretroviral activity of 13-OMA-AZTDP (**3a**) and 13-OMA-AZTTP (**5a**) was compared to the activity of Myr-AZTDP (**2a**) and Myr-AZTTP (**4a**). The grafting of the anti-HIV fatty acid analog on the nucleotides did not improve their antiretroviral activity.

The same results are obtained on HIV2 (data not shown). In conclusion, myristoyl or free nucleotides are as cytotoxic as the corresponding nucleoside in this standard test. Other experiments are in progress on

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(19) It should be noted that the data obtained with cells infected by HIV2 confirm the data presented here and are not described in this paper for clarity purpose.

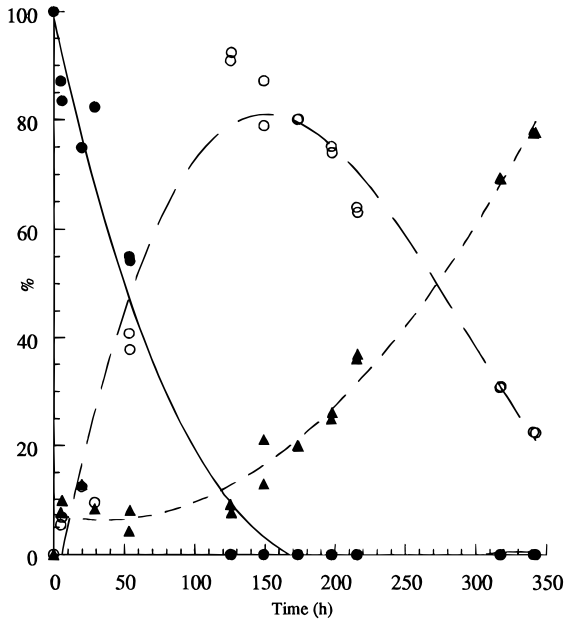

Figure 1. Degradation kinetics of Myr-AZTMP (**1a**) (●), and apparition of AZTMP (**6a**) (○), and AZT (14a) (▲) in TEAA buffer 10 mM, pH 7.0, at 37 °C.

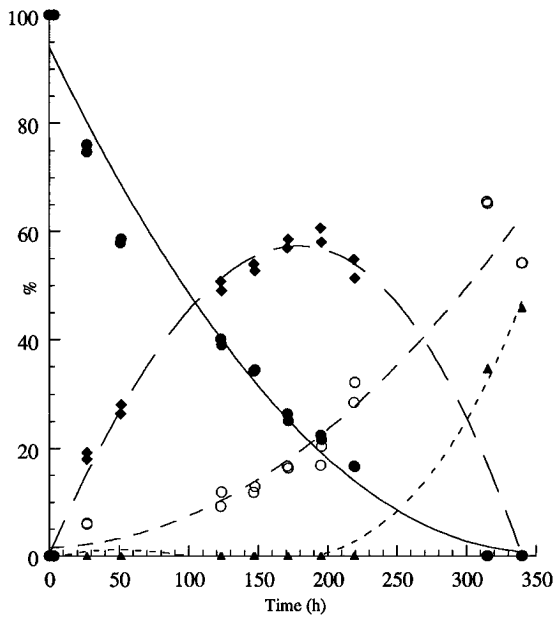

Figure 2. Degradation kinetics of Myr-AZTDP (**2a**) (●), and apparition of AZTDP (**7a**) (◆), AZTMP (**6a**) (○), and AZT (**14a**) (▲) in TEAA buffer 10 mM, pH 7.0, at 37 °C.

peripheral blood lymphocytes with measurements after longer periods of infection.

Hydrolysis of Acyl Nucleotides 1−5. The basic hypothesis on which lies this work is the preferential hydrolysis of the acylphosphate bond over the polyphosphate bond in acyl nucleotides[8] (Scheme 1). In order to test this hypothesis we followed the hydrolysis kinetics of acyl nucleotides **1**−**5** at 37 °C in a 10 mM triethylammonium acetate (TEAA) buffer at physiological pH. The disappearance of the acyl nucleotide and the apparition of the nucleoside mono-, di-, and triphosphates of AZT (**14a**) or d4T (**14b**) were monitored by HPLC, using authentic nucleotide samples for calibration.[13,20] As predicted from the hydrolysis free enthalpy of acetyl phosphate, and ATP (into ADP and phosphate),[8] the results corroborated our hypothesis: acyl nucleotides are cleanly hydrolyzed into their corresponding nucleotides. Hydrolysis of Myr-AZTMP (**1a**) gave the corresponding nucleotide AZTMP (**6a**) with a half-life of 51 h (Figure 1), the latter being further degraded into AZT. The same experiment performed on Myr-AZTDP (**2a**) showed that this product was exclusively hydrolyzed into AZTDP (**7a**), with a half-life of 100 h (Figure 2), this nucleotide being then further degraded into AZTMP and finally AZT. Similarly (Figure 3), hydrolysis of Myr-AZTTP (**4a**) liberated only AZTTP (**8a**) (half-life 150 h), which is then further hydrolyzed into AZTDP, AZTMP, and finally AZT. Similar results were also obtained in the d4T series **1b**, **2b**, **4b**, and with the 13-OMA AZT acyl nucleotides **3a** and **5a**.[21]

On the basis of these hydrolysis experiments, we were rather confident in the ability of acyl nucleotides to enhance nucleotide transmembrane diffusion, since half-lives of 2 to 7 days should be sufficient to allow diffusion

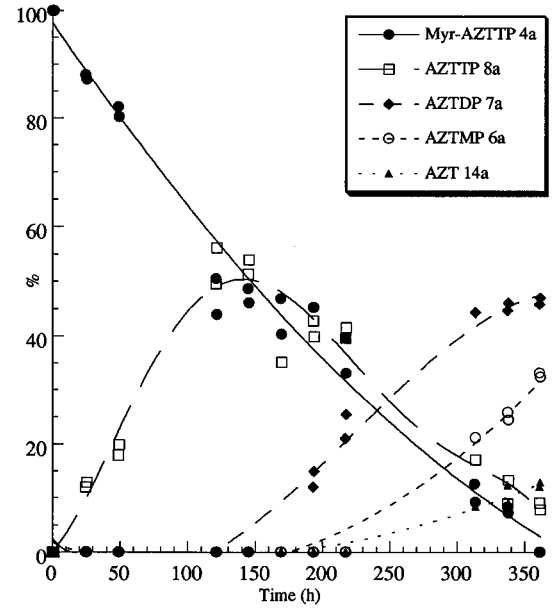

Figure 3. Degradation kinetics of Myr-AZTTP (**4a**) (●), and apparition of AZTTP (**8a**) (□), AZTDP (**7a**) (◆), AZTMP (**6a**) (○) and AZT (**14a**) (▲) in TEAA buffer 10 mM, pH 7.0, at 37 °C.

of acyl nucleotides into the cells during the 6−10 days cell culture. Unfortunately, the antiviral activity did not reveal any differences between acyl nucleotides **1**−**5** and their corresponding free nucleotides **6**−**8**. As already mentioned, acyl nucleotides are mixed anhydrides, and some of us have shown that acyl pyrophosphates may be used as water soluble fatty acyl donors for the acylation of lysine ϵ-amino groups in proteins.[22] These experiments suggested that acyl nucleotides may be prone to rapid aminolysis in RPMI culture media containing aminated compounds and proteins. We undertook comparative hydrolysis kinetics on acyl nucleotides **2a** and **4a**, in

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(21) Bonnaffé, D.; Dupraz, B.; Ughetto-Monfrin, J.; Namane, A.; Huynh Dinh, T. *Proceedings of the 11th Round Table on Nucleosides, Nucleotides and their Biological Applications;* Leuven, Belgium, 1994. *Nucleosides Nucleotides* **1995**, *14*, 783.

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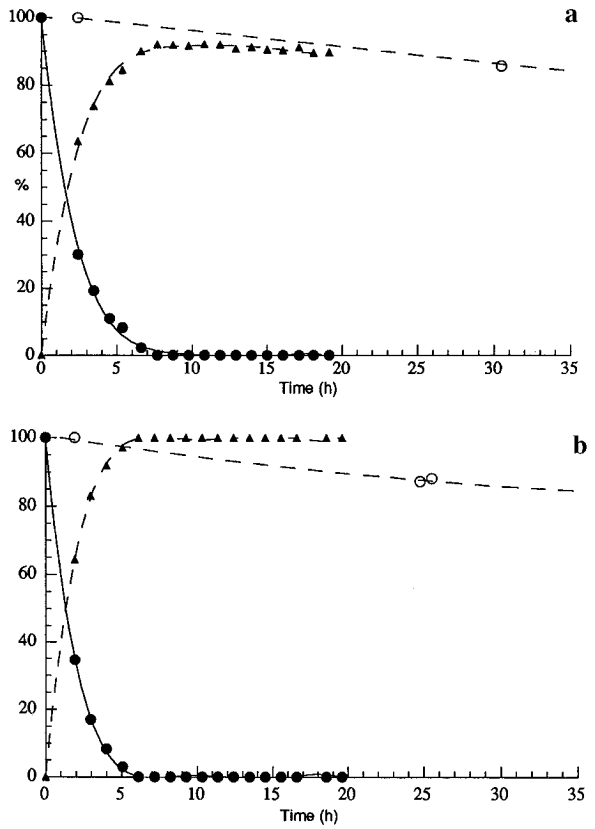

Figure 4. (a) Hydrolysis at RT of Myr-AZTDP (**2a**) in RPMI culture medium (●) or TEAA buffer (○). Apparition of AZTDP (**7a**) by hydrolysis of Myr-AZTDP (**2a**) in RPMI medium (▲). (b) Hydrolysis at RT of Myr-AZTTP (**4a**) in RPMI culture medium (●) or TEAA buffer (○). Apparition of AZTTP (**8a**) by hydrolysis of Myr-AZTTP (**4a**) in RPMI medium (▲).

RPMI and TEAA media at room temperature. As shown by the apparition curves of AZTDP (Figure 4a) and AZTTP (Figure 4b), acyl nucleotides are also selectively cleaved in RPMI medium into their corresponding nucleotide. However, their half lives are dramatically lower than in TEAA (Figure 4a,b): 1.7 h versus 114 h for Myr-AZTDP (**2a**) and 1.4 h versus 156 h for Myr-AZTTP (**4a**). This rapid aminolysis could explain the lack of antiretroviral activity enhancement of nucleotide acylation: the half-lives of acyl nucleotides in RPMI medium is too low to allow transmembrane diffusion, and thus it is impossible to detect any antiretroviral activity differences between the nucleotide and the acylated prodrug.

In conclusion, we have described three general methods allowing the preparation of acyl nucleosides mono-, di-, and triphosphates. As awaited, the hydrolysis of such compounds liberated the corresponding nucleotide by selective cleavage of the acyl phosphate bond. Hydrolysis experiments in TEAA showed that the half-lifes of acyl nucleotides were compatible with their use in aqueous media. However, preliminary *in vitro* anti HIV activity data did not allowed us to ascertain the hypothesis of a nucleotide transmembrane diffusion acceleration by the lipophilic acyl chain, since our compounds are readily aminolyzed in RPMI culture media.

Experimental Section

All moisture sensitive reactions were performed under nitrogen using oven-dried glassware. Solvents were dried and distilled prior to use: THF from sodium/benzophenone and CH_2Cl_2 from phosphorus pentoxide. Reactions were monitored on silica gel 60 F_{254}, eluting with acetonitrile/200 mM ammonium bicarbonate/AcOEt (3/1/1) ternary mixture. Detection was performed using UV light, anisaldehyde−sulfuric acid for the nucleoside moiety, and a modified Ditmer and Lester reagent for phosphate revelation.[23] Flash column chromatographies were performed at 4 °C on reverse phase C18 silica gel, using a 0−70% water−acetonitrile gradient. Preparative HPLC were performed using a C18 reverse phase radial pack cartridge and a 10−60% 10 mM TEAA pH 7.0 acetonitrile gradient. NMR spectra were recorded at 300 MHz for ^{1}H, 75 MHz for ^{13}C, and 121.5 MHz for ^{31}P. ^{1}H and ^{13}C chemical shifts are given in ppm (δ) relative to TMS as internal standard in $CDCl_3$ and external standard in D_2O. ^{31}P chemical shifts are given in ppm (δ) relative to 85% H_3PO_4 as external standard. Quint and sext are the abbreviations, respectively, used for quintuplet and sextuplet. DEPT 135 sequence was used for the attribution of ^{13}C signals, and 2D ^{1}H−^{13}C heteronuclear COSY for the attribution of d4T ^{1}H and ^{13}C signals. FAB mass spectra were recorded using a glycerol or glycerol/thioglycerol (50/50) matrix. 3′-Azido-3′-deoxy-5′-phosphoromorpholidate-thymidine **17a** and 3′-deoxy-2′,3′-didehydro-5′-phosphoromorpholidate-thymidine **17b** were prepared using Moffat procedure[9a-c] and used without further purification. Hydrolysis kinetics were performed in duplicate at a 1 mg/mL acyl nucleotide concentration in 10 mM TEAA, pH 7.0, or RPMI culture medium (RPMI 1640, Life Technologies, with 10% foetal calf serum and 1% glutamine). HPLC was carried out using a 5 μm C18 column (150 × 4.6 mm) and eluting with TEAA 10 mM pH 7.0/acetonitrile gradients: 6 min isocratic elution with 4.8% acetonitrile, followed by a linear gradient from 4.8 to 95% over 14 min. The detection was performed at 265 nm using a diode-array detector.

Tetradecanoyl 3′-azido-3′-deoxy-5′-thymidylphosphate (1a): 200 mg of AZTMP (**6a**)[12,13a] disodium salt (0.51 mmol) was exchanged on Dowex AG 50WX8 200 (NBu_4^+) and lyophilized. The resulting powder was dissolved in 10 mL of anhydrous methylene chloride and 232 mg of myristic acid (1.02 mmol), 210 mg of DCC (1.02 mmol), and 5 mg of DMAP were added. The mixture was kept for 72 h at room temperature under magnetic stirring, time after which no more starting material could be detected by TLC. The mixture was diluted with 5 mL of toluene and extracted three times with 10 mL of H_2O at 0 °C, using centrifugation to facilitate decantation. The aqueous phases were pooled together and evaporated under oil pump vacuum. Flash chromatography, lyophilization, and exchange on Dowex AG 50 WX8 200 (Na^+)[14] gave 68 mg of **1a** sodium salt (23%): ^{1}H NMR for **1a** tetrabutylammonium salt ($CDCl_3$) δ 7.91 (br s, 1H), 7.76 (br s, 1 H), 6.15 (t, 1 H, J = 8 Hz), 4.39−4.30 (m, 1 H), 4.07−3.98 (m, 2 H), 3.91−3.83 (m, 1 H), 3.25−3.03 (m, 8 H), 2.31−2.09 (m, 4 H), 1.81 (s, 3 H), 1.56−1.19 (m, 10 H), 1.25 (sext, 8 H, J = 7.3 Hz), 1.06 (br s, 20 H), 0.81 (t, 12 H, J = 7.3 Hz), 0.70 (t, 3 H, J = 6.7 Hz); ^{13}C NMR **1a** sodium salt (D_2O) δ 172.1 (d, J_{C-P} = 9.1 Hz), 166.7, 151.7, 137.9, 112.5, 85.6, 84.0 (d, J_{C-P} = 8.9 Hz), 65.1 (d, J_{C-P} = 4.4 Hz), 61.5, 37.0, 36.0 (d, J_{C-P} = 6.1 Hz), 32.7, 30.6−29.9, 25.3, 23.4, 14.6, 12.4; ^{31}P NMR ($CDCl_3$) δ −10.14 (br t, J_{P-H} = 4.3 Hz); MS FAB$^+$ for $C_{24}H_{39}N_5O_8PNa$ m/z 602.6 (M + Na)$^+$. Anal. Calcd for $C_{24}H_{39}N_5O_8PNa\cdot H_2O$: C, 48.24; H, 6.92; N, 11.72. Found: C, 48.52; H, 6.56; N, 11.72.

Tetradecanoyl 3′-deoxy-2′,3′-didehydro-5′-thymidinylphosphate (1b): d4TMP (**6b**)[12,13b] disodium salt (178 mg, 0.51 mmol) was treated as described for the preparation of **1a**, giving 70 mg of **1b** sodium salt (26%): ^{1}H NMR for **1b** tetrabutylammonium salt ($CDCl_3$) δ 8.12 (br s, 1 H), 7.82 (br s, 1 H), 7.00 (quint, 1 H, J = 1.6 Hz), 6.39 (dt, 1 H, J = 1.6, 6.1 Hz), 5.72 (dt, 1 H, J = 1.6, 5.8 Hz), 4.95 (br s, 1 H), 4.32−4.21 (m, 1 H), 4.21−4.09 (m, 1 H), 3.42−3.20 (m, 8 H), 2.29 (t, 2 H, J = 7.5 Hz), 1.97 (d, 3 H, J = 1.0 Hz), 1.72−1.49 (m, 10 H), 1.41 (sext, 8 H, J = 7.5 Hz), 1.42 (br s, 20 H), 0.97 (t, 12 H, J = 7.5), 0.86 (t, 3 H, J = 6.7 Hz); ^{1}H NMR for **1b** sodium salt (DMSO-d_6) δ 11.28 (br s, 1 H), 7.73 (br s, 1 H), 6.94 (quint, 1 H, J = 1.5 Hz), 6.36 (dt, 1 H, J = 1.5, 6.1 Hz), 5.91 (dt, 1 H, J = 1.5, 5.8 Hz), 4.87 (br s, 1 H), 4.09−3.98 (m, 1 H), 3.93−

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3.69 (m, 1 H), 2.29 (t, 2 H, J = 7.3 Hz), 1.82 (s, 3 H), 1.56−1.39 (m, 2 H, CH_2 β), 1.26 (br s, 20 H), 0.89 (t, 3 H, J = 6.5 Hz); ^{13}C NMR (D_2O) δ 172.0 (d, J_{C-P} = 9.4 Hz), 166.8, 152.5, 138.7, 135.0, 126.6, 111.9, 90.5, 86.7 (d, J_{C-P} = 8.7 Hz), 67.5 (d, J_{C-P} = 4.0 Hz), 35.9 (d, J_{C-P} = 5.6 Hz), 32.8, 30.7−29.8, 25.3, 23.5, 14.7, 12.5; ^{31}P NMR ($CDCl_3$) δ −10.00 (t, J_{P-H} = 5.3 Hz); ^{31}P NMR (DMSO-d_6) δ −6.00 (t, J_{P-H} = 6.7 Hz). MS FAB$^-$ for $C_{24}H_{38}O_8N_2PNa$ m/z 513 (M − Na)$^-$.

Tetradecanoyl (3′-azido-3′-deoxy-5′-thymidinyldiphosphate (2a): 2.5 mL of a 0.4 M methylene chloride stock solution of tris(tributylammonium) myristoyl pyrophosphate (**15**) (1 mmol) was added to 267 mg of AZT (**14a**) (1 mmol). The mixture was evaporated in vacuo and redissolved in 5 mL of anhydrous THF, and then 1.03 g of DCC (5 mmol) was added. The mixture was kept 24 h at rt. Myristoyl pyrophosphate **15** (0.4 M solution) (2.5 mL, 1 mmol) and 500 mg of DCC (2.5 mmol) were mixed, evaporated, redissolved in 2.5 mL of THF and added to the reaction mixture. This reagent addition was repeated every 24 h until no more AZT could be detected by TLC (generally 72 h reaction, 3 equiv **15** and 10 equiv of DCC). In order to destroy excess DCC, the reaction mixture was diluted with 50 mL of anhydrous THF and 900 mg of oxalic acid (10 mmol), dissolved in 50 mL of THF, were added dropwise over 4 h, checking regularly that the pH did not drop under 3. THF was evaporated in vacuo and the residue dissolved in 20 mL of CH_2Cl_2. The solution was kept overnight at −10 °C and then filtered. The solvent was evaporated in vacuo, and toluene and 100 mM carbonate buffer, pH 7.0 (10 mL each), were added at 0 °C. The mixture was decanted by centrifugation and the organic phase[24] extracted two times with 5 mL of water. The aqueous phases were filtered and directly purified using reverse phase flash chromatography and preparative HPLC, as described in the general methods section, and finally desalted on Sephadex G10 at 4 °C. The fractions containing the product were lyophilized and conditioned in the sodium form on Dowex AG50 WX8 200 (Na^+),[14] giving 153 mg **2a** disodium salt (23%): ^{1}H NMR (DMSO-d_6) δ 11.12 (br s, 1 H), 7.87 (br d, 1 H, J = 1.0 Hz), 6.16 (dd, 1 H, J = 6.2, 7.9 Hz), 4.69−4.61 (m, 1 H), 4.01−3.95 (br s, 1H), 3.96−3.80 (m, 2H), 2.50−2.35 (m, partly in DMSO signal, J = 14.0 Hz), 2.31 (t, 2H, J = 7.3 Hz), 2.22 (ddd, 1H, J = 3.0, 6.2, 14.0 Hz), 1.82 (s, 3 H), 1.54−1.38 (m, 2 H), 1.24 (br s, 20 H), 0.86 (t, 3H, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6) δ 170.7 (d, J_{C-P} = 9.0 Hz), 164.0, 150.8, 136.4, 110.3, 83.7, 83.2 (d, J_{C-P} = 8.5 Hz), 64.7 (d, J_{C-P} = 7.5 Hz), 61.3, 35.9, 34.9, 33.6, 29.0−28.4, 24.4, 24.1, 22.0, 13.9, 12.1; ^{31}P NMR (DMSO-d_6) δ −8.38 (dt, 1 P, J_{P-P} = 20.6 Hz, J_{P-H} = 6.0 Hz), −16.10 (d, 1 P, J_{P-P} = 20.6 Hz); ^{31}P NMR (D_2O) δ −11.02 (br s 50 Hz, 1 P), −18.88 (br s, 50 Hz, 1 P); MS FAB$^+$ for $C_{24}H_{39}N_5O_{11}P_2Na_2$ m/z 704 (M + Na)$^+$. Anal. Calcd for $C_{24}H_{39}N_5O_{11}P_2Na_2 \cdot H_2O$: C, 41.21; H, 5.91; N, 10.01; P, 8.86. Found: C, 40.92; H, 5.11; N, 9.50; P, 8.13.

Tetradecanoyl 3′-deoxy-2′,3′-didehydro-5′-thymidinyldiphosphate (2b): d4T (**14b**) (224 mg, 1 mmol) was treated with **15** and DCC as described for the preparation of **2a**, giving 102 mg of **2b** disodium salt (16%);[24] ^{1}H NMR (DMSO-d_6) δ 11.13 (br s, 1H), 7.57 (d, 1 H, J = 1.0 Hz), 6.70 (m, 1 H, J = 1.6 Hz), 6.31 (br d, 1 H, J = 5.9 Hz), 5.74 (br d, 1 H, J = 5.6 Hz), 4.74 (br s, 1H), 3.94−3.82 (m, 1 H), 3.77−3.65 (m, 1 H), 2.18 (t, 2 H, J = 7.4 Hz), 1.69 (br s, 3 H), 1.42−1.28 (m, 2 H), 1.25 (br s, 20 H), 0.73 (t, 3 H, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6) δ 170.7 (d, J_{C-P} = 9.0 Hz), 164.0, 150.8, 136.8, 134.8, 125.9, 109.8, 88.7, 85.7 (d, J_{C-P} = 9.0 Hz), 65.4 (d, J_{C-P} = 4.5 Hz), 35.0 (d, J_{C-P} = 4.5 Hz), 31.4, 29.1−28.5, 24.3, 22.2, 14.0, 12.0; ^{31}P NMR (DMSO-d_6) δ −8.31 (dt, 1 P, J_{P-P} = 20.7 Hz, J_{C-P} = 4.8 Hz), −16.10 (d, 1 P, J_{P-P} = 20.7 Hz); ^{31}P NMR (D_2O) δ −11.99 (dt, 1 P, J_{P-P} = 20.5 Hz, J_{C-P} = 4.8 Hz), −19.02 (d, 1 P, J_{P-P} = 20.5 Hz); MS FAB$^+$ for $C_{24}H_{38}N_2O_{11}P_2Na_2$ m/z 662 (M + Na)$^+$, 639 (M + H)$^+$. Anal. Calcd for $C_{24}H_{38}N_2O_{11}P_2Na_2 \cdot 2H_2O$: C, 42.72; H, 6.28; N, 4.15; P, 9.19. Found: C, 42.33; H, 5.89; N, 3.85; P, 10.31.

(24) The organic layer contains mainly 5′-acyl nucleoside. After evaporation, Zemplen deacylation, and silica gel flash chromatography, 20−40% starting nucleoside could be recovered.

Tetradecanoyl 3′-*O*-acetyl-5′-thymidinyldiphosphate (2c): 3′-*O*-acetyl-5′-thymidine (**14c**) (284 mg, 1 mmol) was treated with **15** and DCC as described for the preparation of **2a**, giving 170 mg of **2c** disodium salt (24%);[24] ^{1}H NMR (DMSO-d_6) δ 11.29 (br s, 1 H), 7.87 (br s), 6.21 (dd, 1 H, J = 5.7, 9.3 Hz), 5.21 (d, 1 H, J = 5.5 Hz), 4.11−4.03 (br s, 1H), 4.0,1−3.83 (m, 2H), 2.31 (ddd, 1 H, J = 5.5, 9.3, 13.7 Hz), 2.28 (t, 2H, J = 7.3 Hz), 2.16 (dd, 1H, J = 5.7, 13.7 Hz), 2.05 (s, 3 H), 1.81 (s, 3 H), 1.52−1.36 (m, 2 H), 1.21 (br s, 20 H), 0.84 (t, 3H, J = 7.6 Hz); ^{13}C NMR (DMSO-d_6) δ 170.8 (d, J_{C-P} = 9.0 Hz), 170.1, 164.0, 150.8, 136.4, 110.5, 83.8, 83.4 (d, J_{C-P} = 8.5 Hz), 75.9, 65.0 (d, J_{C-P} = 5.0 Hz), 36.4, 35.1 (d, J_{C-P} = 4.5 Hz), 31.5, 29.3−28.6, 24.4, 22.3, 21.0, 14.1 , 12.3; ^{31}P NMR (DMSO-d_6) δ −8.11 (br dt, 1 P, J_{P-P} = 19.1 Hz, J_{C-P} = 7.5 Hz), −15.98 (d, 1 P, J_{P-P} = 19.1 Hz); MS FAB$^+$ for $C_{26}H_{42}N_2O_{13}P_2Na_2$ m/z 722 (M + Na)$^+$, 699.9 (M + H)$^+$. Anal. Calcd for $C_{26}H_{42}N_2O_{13}P_2Na_2 \cdot 2H_2O$: C, 42.51; H, 6.31; N, 3.81; P, 8.43. Found: C, 42.61; H, 5.93; N, 3.85; P, 7.71.

13-Oxatetradecanoyl 3′-azido-3′-deoxy-5′-thymidinyldiphosphate (3a): AZT (**14a**) (134 mg, 0.5 mmol), 13-oxatetradecanoyl pyrophosphate (**16**) and DCC were mixed in 2.5 mL of THF as described for **2a**, giving 68 mg of **3a** disodium salt (20%);[24] ^{1}H NMR (D_2O) δ 7.80 (s, 1 H), 6.29 (t, 1 H, J = 6.7 Hz), 4.63−4.50 (m, 1 H), 4.30−4.10 (m, 3 H), 3.48 (t, 2 H, J = 6.7 Hz), 3.34 (s, 3 H), 2.48 (t, 2 H, J = 5.9 Hz), 2.42 (t, 2 H, J = 7.4 Hz), 1.94 (s, 3 H), 1.65−1.50 (m, 4 H), 1.35−1.15 (m, 14 H); ^{13}C NMR (D_2O) δ 172.8 (d, J_{C-P} = 9.4 Hz), 167.0, 152.1, 137.9, 112.4, 85.4, 83.6 (d, J_{C-P} = 9.2 Hz), 73.4, 66.2 (d, J_{C-P} = 5.6 Hz), 61.5, 58.2, 37.1, 35.5 (d, J_{C-P} = 6.2 Hz), 33.5, 29.2−28.7, 25.8, 24.5, 12.3; ^{31}P NMR (D_2O) δ −11.48 (br d, 1P, J_{P-P} = 21.3 Hz), −19.21 (d, 1P, J_{P-P} = 21.3 Hz); MS FAB$^+$ for $C_{23}H_{37}N_5O_{12}P_2Na_2$ m/z 706 (M + Na)$^+$, 684 (M + H)$^+$.

13-Oxatetradecanoyl 3′-deoxy-2′,3′-didehydro-5′-thymidinyldiphosphate (3b): d4T (**14b**) (112 mg, 0.5 mmol) was treated with **16** and DCC as described for **3a**, giving 60 mg of **3b** disodium salt (15%);[24] ^{1}H NMR for **3b** bis(triethylammonium) salt (D_2O) δ 7.69 (d, 1 H, J = 1 Hz), 6.94 (q, 1 H, J = 1.6 Hz), 6.51 (dt, 1 H, J = 1.6, 6.1 Hz), 5.93 (dt, 1 H, J = 1.6, 5.8 Hz), 5.09 (br s, 1 H), 4.20−4.07 (m, 2 H), 3.47 (t, 2 H, J = 6.7 Hz), 3.33 (s, 3 H), 3.19 (q, 18 H, J = 7.5 Hz), 2.40 (t, 2 H, J = 7.4 Hz), 1.90 (d, 3 H, J = 1.0 Hz), 1.22−1.52 (m, 4 H), 1.27 (t, 27 H, J = 7.5 Hz), 1.23 (br s, 14 H); ^{13}C NMR (D_2O) δ 172.5 (d, J = 9.6 Hz), 167.3, 152.7, 139.0, 134.9, 126.1, 112.2, 90.5, 86.6 (d, J_{C-P} = 8.4 Hz), 73.5, 67.2 (d, J_{C-P} = 6.1 Hz), 58.6, 47.3, 35.6 (d, J_{C-P} = 6.2 Hz), 29.6−28.8, 25.9, 24.6, 12.6, 8.9; ^{31}P NMR (D_2O) δ −11.41 (br d, 1P, J = 23.0 Hz), −19.33 (d, 1P, J = 23.0 Hz); MS FAB$^+$ for $C_{23}H_{36}N_2O_{12}P_2$ $2HNEt_3^+$ m/z 799.9 (M + H)$^+$.

Tetradecanoyl 3′-azido-3′-deoxy-5′-thymidinyltriphosphate (4a): 212 mg of AZT phosphoromorpholidate (**17a**)[9c] (0.3 mmol) and 1.5 mL of 0.4 M methylene chloride solution of tris(tributylammonium) myristoyl pyrophosphate (**15**) (0.6 mmol) were evaporated and redissolved in 5 mL of anhydrous THF; 209 mg of camphorsulfonic acid was then added (0.9 mmol, 1.5 equiv in respect to pyrophosphate **15**). The mixture was kept 4 h at rt under magnetic stirring. The solution was then cooled to 0 °C, diluted with 10 mL of toluene, and extracted with three times 5 mL of TEAA, 1 M, pH 7.0. The aqueous phases were pooled together, lyophilized, and purified as described for acyl NDPs, giving 48 mg of **4a** trisodium salt (20%);[13] ^{1}H NMR (DMSO-d_6) δ 11.28 (br s, 1 H), 7.85 (br s, 1 H), 6.20 (t, 1 H, J = 7.1 Hz), 4.72−4.61 (m, 1 H), 4.12−3.92 (br s, 3H), 2.38 (t, 2H, J = 7.0 Hz), 2.35−2.30 (m, 1H), 2.30−2.17 (m, 1 H), 1.87 (s, 3 H), 1.60−1.45 (m, 2 H), 1.28 (br s, 20 H), 0.90 (t, 3H, J = 6.8 Hz); ^{13}C NMR (D_2O) δ 173.1 (d, J_{C-P} = 9.9 Hz), 167.3, 152.4, 138.2, 112.7, 85.9, 84.0 (d, J_{C-P} = 8.3 Hz), 66.7 (d, J_{C-P} = 7.4 Hz), 62.1, 37.4, 35.8, 32.3, 31.2, 29.8−29.2, 24.8, 23.1, 14.4, 12.6; ^{31}P NMR (DMSO-d_6) δ −6 to −7 (m, 1P), −14.8 to −15.8 (m, 1P), −15.8 to −16.8 (m, 1P); ^{31}P NMR (D_2O, proton decoupled) δ −11.03 (d, 1 P, J = 18.6 Hz), −19.04 (d, 1 P, J = 18.6 Hz), −22.69 (t, 1P, J = 18.6 Hz); MS FAB$^+$ for $C_{24}H_{39}N_5O_{14}P_3Na_3$ m/z 806.6 (M + Na)$^+$, 784.8 (M + H)$^+$. Anal. Calcd for $C_{24}H_{39}N_5O_{14}P_3Na_3 \cdot 2.5H_2O$: C, 34.79; H, 5.35; N, 8.45; P, 11.22. Found: C, 34.71; H, 5.15; N, 7.49; P, 10.83.

Tetradecanoyl 3′-deoxy-2′,3′-didehydro-5′-thymidinyl-triphosphate (4b): d4T phosphoromorpholidate (**17b**) (179 mg, 0.3 mmol), acyl pyrophosphate **15**, and camphorsulfonic acid were mixed as described for **4a**, giving 25 mg of **4b** trisodium salt (11%); ^{1}H NMR (DMSO-d_6) δ 11.13 (s, 1H), 7.59 (d, 1 H, J = 1.0 Hz), 6.78 (s, 1 H), 6.46 (d, 1 H, J = 5.5 Hz), 5.81 (br d, 1 H, J = 5.5 Hz), 4.44 (br s, 1H), 4.05 (br s, 1 H), 3.88 (br s, 1 H), 2.29 (t, 2 H, J = 6.8 Hz), 1.76 (br s, 3 H), 1.40−1.24 (m, 2 H), 1.19 (br s, 20 H), 0.81 (t, 3 H, J = 6.8 Hz); ^{31}P NMR (DMSO-d_6) δ −6.10 (br s, 1 P), −15.03 (br s, 1 P), −16.03 (br s, 1 P); MS FAB$^+$ for $C_{24}H_{38}N_2O_{14}P_3Na_3$ m/z 741 (M + H)$^+$. Anal. Calcd for $C_{24}H_{38}N_2O_{14}P_3Na_3 \cdot 3H_2O$: C, 36.26; H, 5.58; N, 3.53. Found: C, 35.71; H, 5.05; N, 3.48.

Tetradecanoyl 5′-thymidinyl triphosphate (4c):[17] thymidine phosphoromorpholidate (**17c**)[9a,b] (70 mg, 0.1 mmol) was treated with **15** and camphorsulfonic acid as described for **4a**, excepted for the final ion exchange which was performed on Dowex AG50 WX8 200 lithium form, giving 5.4 mg of **4c** trilithium salt (8%): ^{1}H NMR (DMSO-d_6) δ 7.68 (d, 1 H, J = 0.8 Hz), 6.10 (t, 1 H, J = 7.5 Hz), 5.73 (d, 1 H, J = 4.2 Hz), 4.39−4.28 (m, 1 H), 4.03−3.84 (m, 2 H), 3.84−3.74 (m, 1 H), 2.28 (t, 1 H, J = 7.5 Hz), 2.14−1.89 (m, 2 H), 1.75 (s, 3 H), 1.50−1.31 (m, 2 H), 1,31−0.97 (m, 20 H), 0.78 (t, 3 H, J = 6.7 Hz); ^{31}P NMR (DMSO-d_6) δ −8.06 (dt, 1 P, J_{C-P} = 5.9 Hz, J_{P-P} = 18.4 Hz), −16.31 (d, 1 P, J_{P-P} = 18.4 Hz), −17.72 (t, 1 P J_{P-P} = 18.4 Hz); MS FAB$^+$ for $C_{24}H_{41}N_2O_{15}P_3Na_2$ (**4c** disodium salt) m/z 737 (M + H)$^+$.

13-Oxatetradecanoyl 3′-azido-3′-deoxy-5′-thymidinyl-triphosphate (5a): AZT phosphoromorpholidate (**17a**) (354 mg, 0.5 mmol) was treated with 13-oxamyristoyl pyrophosphate (**16**) and camphorsulfonic acid as described for **4a**, giving 25 mg of **5a** triethylammonium salt (5%): ^{1}H NMR (D_2O) δ 7.80 (d, 1 H, J = 1.0 Hz), 6.28 (t, 1 H, J = 7.2 Hz), 4.60 (m, 1 H), 4.21 (m, 3 H), 3.47 (t, 2 H, J = 7.4 Hz), 3.33 (s, 3 H), 3.19 (q, 18 H, J = 7.3 Hz), 2.47 (m, 2 H), 2.44 (t, 2 H, J = 7.9 Hz), 1.93 (br s, 3 H), 1.58 (m, 4 H), 1.26 (t, 41 H, J = 7.3 Hz); ^{13}C NMR (D_2O) δ 173.0 (d, J_{C-P} = 9.4 Hz), 167.2, 152.4, 138.1, 112.7, 85.6, 83.8 (d, J_{C-P} = 9.4 Hz), 73.6, 66.5 (d, J_{C-P} = 5.0 Hz), 61.9, 58.3, 47.4, 37.1, 35.6 (d, J = 6.1 Hz), 29.4−28.9, 25.9, 24.6, 12.5, 9.0; ^{31}P NMR (D_2O) δ −11.30 (br d, 1 P, J = 20.5 Hz) −19.30 (d, 1 P, J = 19.3 Hz), −23.21 (br t, 1 P); MS FAB$^+$ for $C_{23}H_{38}N_5O_{15}P_3(HNEt_3)_2$ (**5a** diethylammonium salt) m/z 922.9 (M + H)$^+$.

Tris(tetrabutylammonium) tetradecanoyl pyrophosphate (12): tris(tetrabutylammonium) pyrophosphate (**11**) (18 g, 20 mmol), myristic acid (**9**) (18.3 g, 80 mmol), and DCC (16.5 g, 80 mmol) were treated and purified as previously described,[7] giving 15.4−17.6 g of **12** (63−70%); ^{1}H NMR ($CDCl_3$) δ 3.45−3.23 (m, 19 H)[25] 2.53 (t, 2 H, J = 7.5 Hz), 1.64−1.53 (m, 21 H), 1.45 (sext, 19 H, J = 7.3 Hz), 1.22 (br s, 20 H), 0.97 (t, 29 H, J = 7.3 Hz), 0.86 (t, 3 H, J = 6.7 Hz); ^{13}C NMR δ 170.4 (d, J_{C-P} = 7.8 Hz), 58.4, 35.3, 31.7, 30.1−29.0, 24.5, 23.9, 22.5, 19.5, 14.0, 13.6; ^{31}P NMR δ −10.53 (d, 1P, J_{P-H} = 18.5 Hz), −18.04 (br d, 1P, J_{P-H} = 18.5 Hz); ^{31}P NMR (D_2O) δ −10.44 (br d, 1P, J_{P-H} = 15.8 Hz), −19.44 (br d, 1P, J_{P-H} = 15.8 Hz); MS FAB$^+$ for $C_{14}H_{28}O_8P_2(NBu_4)_2$ m/e 871 (M + H)$^+$. Anal. Calcd for $C_{14}H_{27}O_8P_2Na_3$: C, 37.02; H, 5.99; P, 13.64. Found: C, 36.96; H, 6.03; P, 13.63.

Tris(tetrabutylammonium) 13-oxatetradecanoyl pyrophosphate (13): tris(tetrabutylammonium) pyrophosphate (**11**) (4.78 g, 5 mmol) 13-oxamyristic acid[10] (**10**) (4.60 g, 20 mmol) and DCC (4.12 g, 20 mmol) were treated and purified as previously described,[7] giving 4.12 g **13** (74%); ^{1}H NMR ($CDCl_3$) δ 3.48−3.23 (m, 29 H[25]), 2.46 (t, 2 H, J = 7.4 Hz), 1.76−1.57 (m, 26 H), 1.47 (sext, 24 H, J = 7.3 Hz), 1.41−1.21 (br s, 18 H), 1.00 (t, 36 H, J = 7.3 Hz); ^{13}C NMR δ 170.2 (d, J_{C-P} = 8.4 Hz), 73.1, 58.7, 58.6, 35.5 (d, J_{C-P} = 5.0 Hz), 29.7−29.0, 26.2, 25.2, 24.6, 24.1, 20.2, 19.7, 13.8; ^{31}P NMR δ −11.16 (d, 1P, J_{P-H} = 17.9 Hz), −19.13 (br d, 1P, J_{P-H} = 17.9 Hz).

Tris(tributylammonium) tetradecanoyl pyrophosphate (15) and tris(tributylammonium) 13-oxatetradecanoyl pyrophosphate (16): Dowex AG50 WX8 200 was conditioned in the tributylammonium form as follows: tributylamine was added to a suspension of Dowex AG50 WX8 200 (H^+) under magnetic stirring in 95/5 MeOH/H_2O until the pH remained basic. The resin was filtered off and extensively washed with methanol and then water; before use the resin was washed with acetonitrile. Acetonitrile solutions of **12** and **13** were passed through columns of 10 mol equiv Dowex AG 50 WX8 200 (NBu_3H^+). After evaporation under vacuo, 0.4 M methylene chloride stock solution was prepared without further purification. It should be noted that **12**, **13**, **15,** and **16** methylene chloride solution are stable for months when kept at −20 °C.

Antiviral Activity. The assays were performed as described in ref 18.

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Supporting Information Available: ^{1}H and ^{31}P NMR spectra of **1a**, **1b**, **2a**, **2b**, **2c**, **3a**, **3b**, **4a**, **4b**, **4c**, **5a**, **12**, and **13** (26 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(25) The tetrabutylammonium countercation content of acyl pyrophosphates **12** and **13** may vary from two to three depending on the sample. Yields for each preparation were calculated from ^{1}H and ^{31}P NMR spectra.