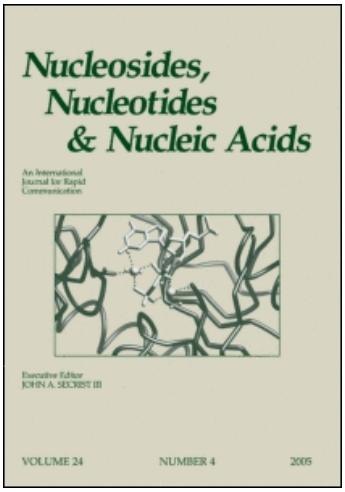

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Synthesis of Nucleotide Lipophilic Prodrugs Containing Two Inhibitors Targeted Against Different Phases of the HIV Replication Cycle

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SYNTHESIS OF NUCLEOTIDE LIPOPHILIC PRODRUGS CONTAINING TWO INHIBITORS TARGETED AGAINST DIFFERENT PHASES OF THE HIV REPLICATION CYCLE.

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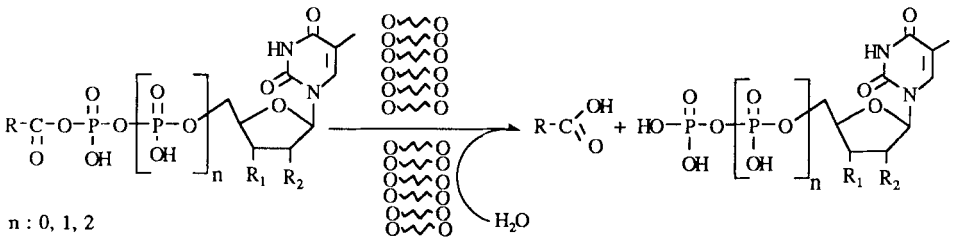
Abstract : We describe the preparation of nucleoside acyl 5'-di or 5'-triphosphates, containing a nucleoside analog moiety and 13-oxa-myristic acid as lipophilic chain. At physiological pH these products liberated exclusively the corresponding nucleotides.

Nucleoside analogues are the only drugs widely used to prevent the replication of the human immunodeficiency virus (HIV) *in vivo*. The metabolism of nucleoside analogues is a three-step process, each phosphate unit being added separately by cellular kinases[1,2]. It has been established that nucleoside analogs 5'-triphosphates (NTPs analogs) are the HIV reverse transcriptase (RT) inhibitors[1,3]. In the case of AZT, the rate-limiting step is the formation of the nucleoside analog 5'-diphosphate (NDP analog) catalysed by thymydilate kinase[1,3]. Side effects, often observed on people treated with AZT, result from the accumulation of the nucleoside analog 5'-monophosphate (NMP analog) in cells[1]. We have recently described the preparation of nucleoside analog acyl phosphates[4] which may be interesting nucleotide lipophilic prodrugs.



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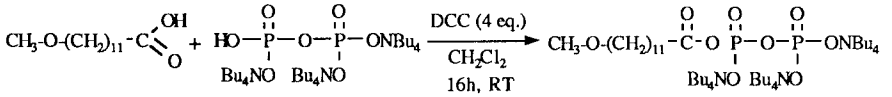
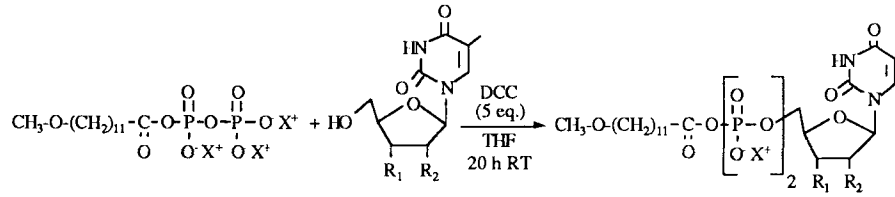
The lipophilic fatty acid moiety should allow passive diffusion of the charged nucleotide through cell membranes. Then an active metabolite should be liberated in the cell, since the mixed carboxylic phosphoric anhydride is expected to be cleaved before the symmetrical phosphoric anhydride[5].

Although nucleoside analogues are clinically used for the treatment of AIDS, they only prevent irreversible contamination of uninfected cells, but not virus production by infected cells. A fatty acid analog, 13-oxa-myristic acid **1** (13-OMA), has been described as an efficient HIV replication inhibitor, which acts by perturbing N-myristoylation of GAG polyprotein precursor, thus preventing virus assembly[6]. Using 13-OMA as lipophilic moiety in an acyl NDP or NTP analog seems very attractive : the resulting acyl nucleotide should protect uninfected cells from irreversible contamination and prevent the production of infectious virus by infected cells.

We synthesized tris-tetrabutylammonium 13-OMA pyrophosphate **3** using a previously described[4] protocol : 13-OMA was activated using dicyclohexylcarbodiimide (DCC) and condensed with tris-tetrabutylammoniumpyrophosphate[7]. After an extractive workup[4] 13-OMA pyrophosphate **3** was isolated in a 74% yield. This product was checked by ^{1}H and ^{31}P NMR (respectively 100 and 94% purity). **4** was obtained after exchange of **3** on Dowex AG50W X8.



AZT **5** and d4T **6** were condensed with **4**, in presence of DCC, giving 13-OMA myristoyl NDPs **7-8** in 7% isolated yield after C18 reverse phase flash chromatography and HPLC.



5, 7 AZT (R_1:N_3 ; R_2 : H)
6, 8 d4T ($R_1 = R_2$: C=C)
$X^+ = HNBu_3^+$

In addition to correct FAB^+ mass spectra, the NMR spectra of these products display characteristic feature of acyl 5'-diphosphate : H_5-$H_{5'}$ signals are deshielded from 0.1 ppm in respect to the parent nucleotide ; acyl P_β signal at -19.21 ppm (d, J = 21.3 Hz) for **7**

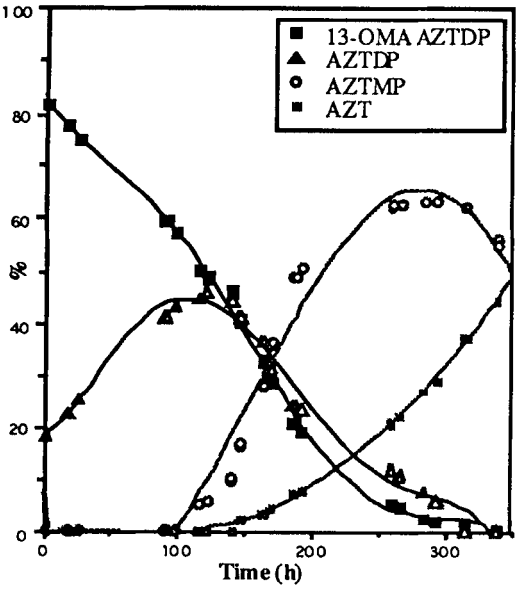


FIG 1a : Hydrolysis kinetic of 13-OMA AZTDP at 37°C, in TEAA 10 mM pH 7.

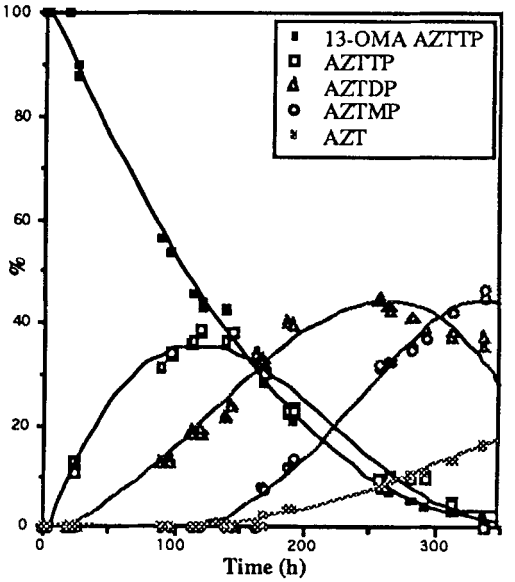
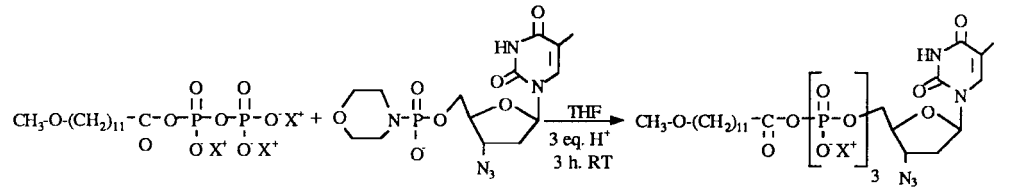


FIG 1b : Hydrolysis kinetic of 13-OMA AZTTP at 37°C, in TEAA 10 mM pH 7.

and -19.33 ppm (d, **J** = 23.0 Hz) for **8**, acyl carbonyl carbon display a 9.5 Hz coupling constant with P_β.

Acyl NTPs were synthesised using phosphoromorpholidate derivatives[8]. AZT phosphoromorpholidate **9** was mixed with 13-OMA pyrophosphate **4** ; camphorsulfonic acid was added to catalyse the reaction and protonate the liberated morpholine, in order to avoid aminolysis of the labile acyl phosphate bond. After purification, 13-OMA AZTTP **10** was recovered in low yield (4%). FAB^+ mass spectra and ^1H, ^{31}P and ^{13}C spectra are in accordance with the proposed structure. H_5-$H_{5'}$ signals are deshielded of approximately 0.1 ppm in respect to the parent nucleotide ; P_γ signal at -19.30 ppm (d, J = 19.3 Hz) and P_β signal at -23.10 ppm (br. t), while the acyl carbonyl is coupled with P_γ (J = 9.4 Hz).



$X^+ = HNBu_3^+$

In order to test our basis hypothesis, we followed the hydrolysis kinetics at 37°C of 13-OMA AZTDP **7** and 13-OMA AZTTP **10**, at a 2.5 µg/mL concentration, in a 10 mM triethylammonium acetate (TEAA) buffer at physiological pH. The disappearance of the acyl nucleotide and the occurrence of the mono-, di- and tri-phosphates of AZT were monitored by HPLC on reverse phase column, using authentic nucleotide samples[1,3] for calibration. The results are shown in FIG. 1a-b and corroborate our hypothesis : acyl nucleotides are cleanly hydrolysed into their corresponding nucleotides. Hydrolysis of **7** gave exclusively AZT 5'-diphosphate, with a half life of 145 h (FIG. 1a), this nucleotide being then further degraded into AZTMP and finally AZT. Similarly (FIG. 1b), hydrolysis of **10** liberated only AZTTP (half life 105 h), which was then further hydrolysed into AZTDP, AZTMP and finally AZT.

Antiretroviral activity of these products is under current investigation.

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