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Synthesis and anti-HIV activities of phosphate triester derivatives of 3′-fluoro-2′,3′-dideoxythymidine and 3′-azido-2′,3′-dideoxythymidine

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ABSTRACT

Fatty acyl-glycol phosphate triester conjugates of 3′-fluoro-2′,3′-dideoxythymidine (FLT) were prepared in three steps from the reaction of diisopropylphoramidous dichloride with fatty acyl-substituted glycols, followed by a coupling reaction with FLT and oxidation with *tert*-butyl hydroperoxide (*t*-BuOOH). Additionally, a number of fatty alcohols were reacted with diisopropylphoramidous dichloride to produce the phosphitylating intermediates, which underwent coupling reactions with 3′-azido-2′,3′-dideoxythymidine (AZT) and FLT followed by oxidation with *t*-BuOOH to yield fatty alcohol phosphate triester derivatives of AZT and FLT.

2′,3′-Dideoxynucleoside analogs are used clinically against the human immunodeficiency virus (HIV). There are numerous reasons to utilize a nucleotide prodrug strategy in order to make anti-HIV nucleosides more effective against the virus. Bypassing the first rate-limiting phosphorylation step,[1] increasing the lipophilicity, and enhancing the cellular uptake and half-life in blood are some of them.[2]

On entering the cell, the majority of anti-HIV nucleoside analogs, such as 3′-fluoro-2′,3′-dideoxythymidine (FLT, alovudine) and 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine), are phosphorylated to monophosphates, diphosphates, and triphosphates forms by host cellular kinases before they show antiviral activity. Negatively-charged nucleoside monophosphates cannot be directly used because of their high polarity and poor cellular uptake. Furthermore, they are readily dephosphorylated on cell surfaces and in extracellular fluids by non-specific phosphohydrolases. In order to bypass the first rate-limiting phosphorylation step in the metabolic conversion of nucleoside analogs, numerous prodrugs of 5′-monophosphate types such as neutral species of phosphotriester derivatives of nucleosides have been proposed[2–9] with the hope that these prodrugs would release the corresponding nucleoside-monophosphates intracellularly. The phosphotriesters must have acceptable stability prior to cellular uptake and selective intracellular biotransformation of the active species. Furthermore, extensive efforts have been carried out to synthesize lipophilic prodrugs of anti-HIV nucleosides by an esterification approach.[2,10–12] Both strategies have yet to provide an anti-HIV prodrug agent with a clear-cut therapeutic advantage for clinical use. The major challenge of developing nucleotide prodrugs has been in the selection of a suitable phosphate-masking group. By judicious choice of the alcohols used in triester formation, it may be possible to improve cellular uptake and to direct intracellular hydrolysis to nucleoside monophosphates. Thus, further research to identify prodrugs containing both phosphotriester and lipophilic groups with distinct advantages, relative to parent anti-HIV nucleosides, is warranted.

Herein, we report the synthesis of uncharged fatty acyl and fatty alcohol phosphotriester derivatives of AZT and FLT (Fig. 1). The lipophilic moieties, fatty acyls or fatty alcohols, were incorporated into the structure with the aim of improving interaction with membrane bilayers and cellular uptake of anti-HIV nucleoside phosphotriester derivatives and to release nucleoside monophosphates intracellularly, bypassing the first phosphorylation step.

In the first class of compounds, two identical fatty acids were linked through a glycol linker to a phosphate group, which was attached to 5′-O-position of FLT to afford bis(fatty acyl-glycol)-phosphate triester derivatives. The selection of fatty acids was based on the previously reported moderate anti-HIV activities of 12-bromododecanoic acid, 12-azidododecanoic acid, and 12-thioethydodecanoic acid.[13] In the second class of compounds, fatty

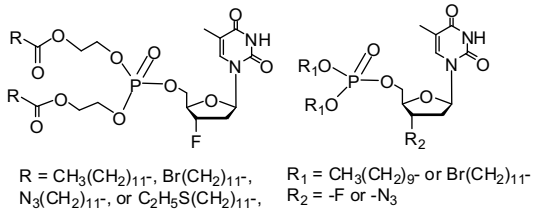

Figure 1. Fatty acyl and fatty alcohol phosphotriester derivatives of AZT and FLT.

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alcohols and nucleosides, FLT and AZT, were directly attached to a phosphotriester group.

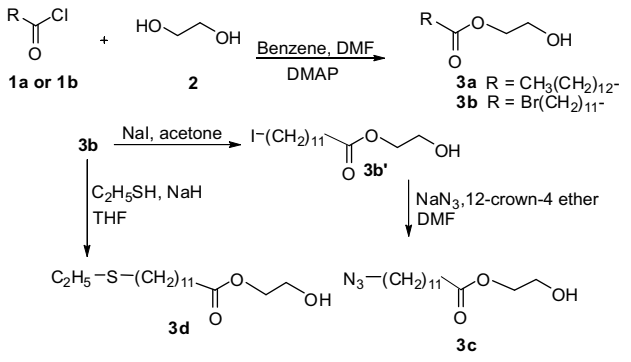
The synthetic procedures used for the synthesis of phosphotriester derivatives of nucleosides were based on P(III) chemistry using phosphoramidite approach and consisted of three steps: (i) derivatization of diisopropylphosphoramidous dichloride with fatty-acyl-glycols or fatty alcohols to afford phosphoramidites, (ii) reaction of resulting phophoramidates with FLT or AZT in the presence of ethyl-1*H*-tetrazole, and (iii) oxidation of P(III) to P(V).

For the synthesis of compounds in the first class, we first prepared fatty acid-glycol ester conjugates **3a–d** (Scheme 1). 2-Hydroxyethyl tetradecanoate (**3a**) and 2-hydroxyethyl 12-bromododecanoate (**3b**) were synthesized (70% yield) from the treatment of the corresponding fatty acyl chloride (**1a** or **1b**, 4 mmol) and ethylene glycol (**2**, 18 mmol) in the presence of dimethylaminopyridine (DMAP) (5 mmol) in benzene and DMF (Scheme 1).

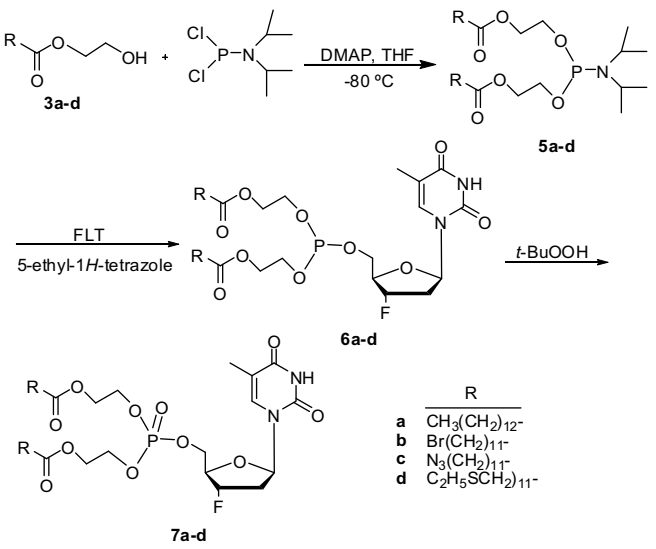
2-Hydroxyethyl 12-bromododecanoate (**3b**) was used for the synthesis of 2-hydroxyethyl 12-azidododecanoate (**3c**) and 2-hydroxyethyl 13-thiapenatadecanoate (**3d**). Bromosubstituted ester conjugate **3b** (5.3 mmol) was treated first with sodium iodide (10.7 mmol) in acetone to yield the corresponding iodosubstituted glycol ester **3b′** (95%). Compound **3b′** (4.9 mmol) was reacted with sodium azide (15.4 mmol) in the presence of 12-crown-4 ether (15.9 mmol) in DMF to yield **3c** in 55% yield. Similarly, nucleophilic reaction of ethanethiol (7.6 mmol) with **3b** (6.2 mmol) in the presence of sodium hydride (8.3 mmol) afforded 12-thioethyl substituted analog **3d** in 60% yield (Scheme 1).

Scheme 2 outlines the synthesis of bis(fatty acyl-glycol)phosphotriester derivatives of FLT (**7a–d**) using P(III) chemistry. In general, fatty acyl-glycol ester conjugates **3a–d** (3 mmol) were treated in THF with diisopropylphosphoramidous dichloride (1.5 mmol) in the presence of DMAP (3 mmol) at −80 °C to afford intermediate bis(fatty acyl-glycol) diisopropyl phosphoramidite conjugates (**5a–d**). Low temperature proved to be important for the success of this coupling reaction as shown by the failure of the reaction of **3a** with diisopropylphosphoramidous dichloride in the presence of pyridine at room temperature. The intermediates **5a–d** should be used immediately in the next reaction without purification because of the activity of the phosphorous in trivalent form in these compounds. Subsequent conversion of phosphoramidite intermediates to phosphotriesters was accomplished by treatment with FLT (1.5 mmol) in presence of 5-ethyl-1*H*-tetrazole (4.5 mmol) followed by in situ oxidation with *tert*-butyl hydroperoxide (*t*-BuOOH, 4.5 mmol) to obtain bis(fatty acyl-glycol)phosphotriester derivatives of FLT (**7a–d**). The chemical structures of **7a–d** were determined by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high-resolution ESI mass spectrometry (Table 1).

For the synthesis of fatty alcohol phosphotriester derivatives of the nucleosides, AZT was first attached to bis(diisopropyl-

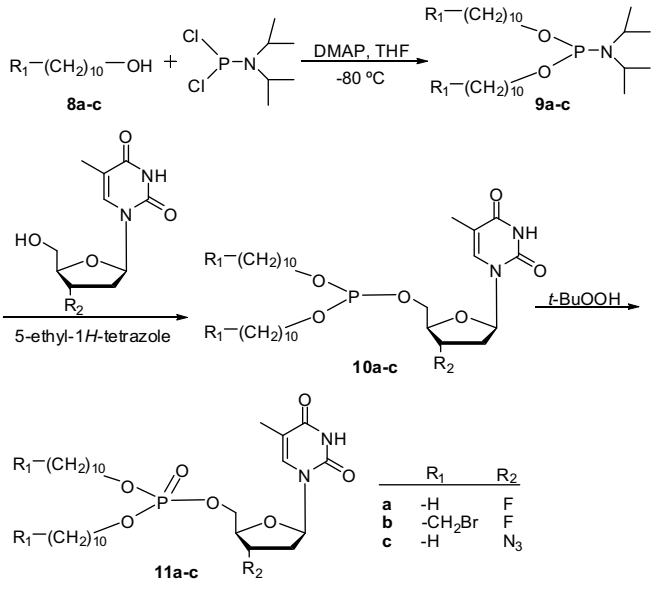


Scheme 1. Synthesis of fatty acid-glycol ester conjugates **3a–d**.



Scheme 2. Synthesis of fatty acyl-glycol ester conjugates **7a–d**.

amino)chlorophosphine in the presence of pyridine. However, the intermediate was not stable during purification by silica gel column chromatography. Alternatively, the synthesis of fatty alcohol phosphotriester derivatives of AZT and FLT (**11a–c**) was accomplished by the reaction of dialkoxy substituted phosphitylating reagents, diisopropylamino dialkoxyphosphine, with AZT and FLT in the presence of 5-ethyl-1*H*-tetrazole in THF (Scheme 3). First dialkoxy substituted phosphitylating reagents were synthesized using diisopropylphosphoramidous dichloride and different fatty alcohols (i.e., decanol and 11-bromoundecanol). Solution of alcohols **8a–c** (3 mmol) in THF (20 mL) was added to a mixture of diisopropylphosphoramidous dichloride (1.5 mmol) and DMAP (3 mmol) in THF (100 mL) at −80 °C to afford **9a–c**. FLT or AZT (1.5 mmol) and 5-ethyl-1*H*-tetrazole (4.5 mmol) were added to the reaction mixture to yield **10a–c**. Oxidation of phosphite triesters **10a–c** to phosphate triesters was accomplished with *t*-BuOOH (4.5 mmol) to afford **11a–c**. The chemical structures of **11a–c** were



Scheme 3. Synthesis of fatty alcohol phosphotriester derivatives of AZT and FLT (**11a–c**).

Table 1
The physicochemical characteristics and anti-HIV activities of compounds **7a–d** and **11a–c**

Compd. no.	^{31}P NMR[a] (δ, ppm)	HR-MS (ESI-TOF)	Anti-HIV IC_{50}[b] (μM)	Overall yield (%)
7a	4.79	855.4673 $[M+Na]^+$	>100	6.3
7b	4.78	955.4436 $[M+Na]^+$, 976.3518, $[M+2Na]^+$	63	7.7
7c	2.81	859.3552 $[M+H]^+$, 881.3245 $[M+Na]^+$, 897.2941 $[M+K]^+$	76	6.5
7d	2.82	897.3566 $[M+H]^+$	87	12.5
11a	5.09	605.0470 $[M+H]^+$, 642.9381 $[M+K]^+$	>100	14.1
11b	5.03	789.9278 $[M+H]^+$	33	7.0
11c	5.08	628.3108 $[M+H]^+$	>100	19.8

[a] The spectra were measured on a 400 MHz spectrometer using $CDCl_3$ as the solvent (H_3PO_4 85% in water as external standard).
[b] IC_{50}: 50% inhibitory concentration.

determined by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high-resolution ESI mass spectrometry (Table 1).

Using a single-round infection assay[14] with HIV-1 IIIB and transformed HeLa cells expressing HIV receptors (CD4) and coreceptors (CXCR4 and CCR5), the newly synthesized triester derivatives showed only modest antiviral activity, significantly lower than that of their parent nucleosides, AZT and FLT (IC_{50} = 10 and 0.8 μM, respectively).

In summary, the results presented herein show that the synthesis of different classes of lipophilic phosphate triesters of FLT and AZT can be successfully accomplished by using P(III) chemistry. The extension of this methodology should prove to be useful for the development of lipophilic phosphotriester prodrugs of other nucleosides. The premature hydrolysis of the phosphate-masking group bond in the extracellular medium, however, may have yielded a negatively-charged diester with low cellular uptake and reduced antiviral potency. The phosphotriesters must have acceptable stability in cell culture prior to cellular uptake and selective intracellular transformation of the active species. We were not able to determine the stability of compounds because of their extremely low water solubility. The extracellular hydrolysis of phosphotriester derivatives of nucleosides has been previously reported. For example, McGuigan et al.[15,16] reported that some of dialkyl and diaryl phosphotriester derivatives of AZT were inactive because of the rapid in vitro hydrolysis to release the nucleotide extracellularly. The utility of phosphotriester derivatives of nucleosides will be enhanced by a clearer understanding of the mechanisms pertaining to their bioconversion, uptake, and cellular incorporation.

Acknowledgments

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Supplementary data

Detailed synthetic procedure, ^{1}H NMR, ^{13}C NMR, and/or ^{31}P NMR spectra of compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.05.149.

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