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# On the design of polymeric 5′-*O*-ester prodrugs of 3′-azido-2′,3′-dideoxythymidine (AZT)

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ABSTRACT

A new 5′-*O*-AZT prodrug was synthesized by conjugating 3′-azido-2′,3′-dideoxythymidine (AZT) with poly(oxyethylene *H*-phosphonate) at room temperature under Atherton-Todd reaction conditions. The acute toxicity of poly(5′-*O*-AZT-oxyethylene phosphate) was reduced significantly in comparison with non-immobilized AZT.

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The development of an effective antiviral therapy for the treatment of individuals infected with human immunodeficiency virus (HIV) presents a unique challenge.[1] Nucleoside analogues have demonstrated widespread utility as antiviral and anticancer therapeutics.[2] They are currently being investigated as anti-HIV agents. Among the available drugs for HIV therapy, 3′-azido-2′,3′-dideoxythymidine (AZT) is one of the most effective. AZT-based treatment delays HIV progression and improves survival rates in patients with advanced-stage disease. The major therapeutic limitations of AZT drugs are caused by their inherent clinical toxicities which include bone marrow suppression, hepatic abnormalities, and myopathy.[3] These limitations have prompted the development of strategies for designing prodrugs of AZT.[1,4] One of the promising strategies to suppress the toxicity of drugs involves their conjugation with polymers.[5] The most common methods for using polymers as drug delivery platforms involve physical blending or chemical bonding to a polymer. Poly(oxyalkylene *H*-phosphonate)s present a family of biodegradable, hydrophilic, and nontoxic polymers, which mimic biomacromolecules. In addition these polymers contain poly(ethylene glycol) (PEG) moieties as building blocks and can be easily converted into poly(oxyalkylene phosphate)s. All these valuable features make poly(oxyalkylene *H*-phosphonate)s promising candidates for an immobilization template.[6] Encouraged by the significant reduction of cysteamine toxicity after its immobilization onto polyphosphoesters[5d] we decided to synthesize a polymeric prodrug of AZT. Poly(5′-*O*-AZT oxyethylene phosphate) (Poly-AZT) (Fig. 1) was the main target of this study. Its structure was designed to meet several functional requirements: reduced toxicity, increased anti-HIV activity and improved pharmacokinetic properties, drug localization, and patient acceptability.

The presence of the highly reactive P–H group[7] in the repeating unit of the poly(oxyethylene *H*-phosphonate) **1** (derived from PEG 200, molecular weight 4700 Da, polydispersity 1.18) obtained as described in Ref. 6a, predetermines the employment of Atherton-Todd reaction conditions for the immobilization of 3′-azido-2′,3′-dideoxythymidine (AZT) (Scheme 1).

The extent of conversion of **1** into the corresponding phosphate structure **2** was monitored by NMR spectroscopy. The absence of any resonance with a $^{1}J$(P,H) coupling constant larger than

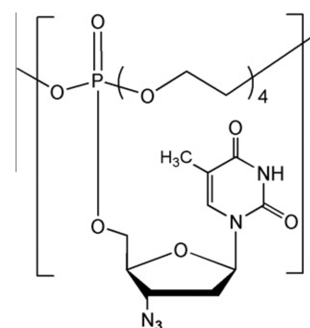


**Figure 1.** The repeating unit of poly(5′-*O*-AZT-oxyethylene phosphate).

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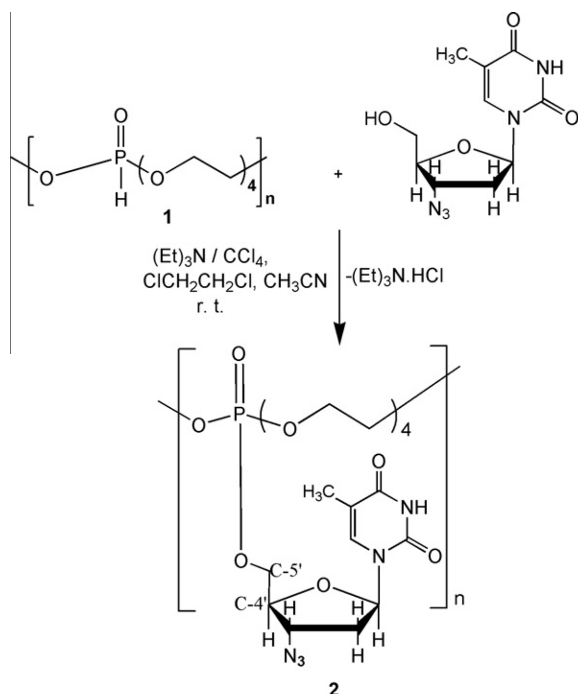


**Scheme 1.** Synthesis of poly(5′-*O*-AZT-oxyethylene phosphate) (**2**).

700 Hz, characteristic of PH protons in the $^{1}$H NMR spectra,[8] confirmed the quantitative progression of the Atherton-Todd reaction. There were no signals at $\delta$ 11.23, 10.53, and 8.40 in the $^{31}$P{H} NMR spectrum, typical of the phosphonate structure **1**. On the other hand, a new signal appeared at $\delta$ 1.02 in the $^{31}$P{H} NMR spectrum assigned to the phosphorus in phosphate **2**. Further evidence for the conjugation of AZT to the poly(oxyethylene phosphate) at the 5′ position was the appearance of a new doublet at $\delta$ 86.47 [$^{3}J$(P,C) = 6.3 Hz] in the DEPT 135 $^{13}$C{H} NMR spectrum of **2** due to the C-4′ carbon. The doublet for the C-5′ atom overlapped with the doublets for the PO*C*H$_2$– and POCH$_2$*C*H$_2$– carbons. The prodrug **2** was obtained in quantitative yield[8] based on the $^{31}$P{H}NMR spectroscopic data.

The solution behavior in aqueous media is an important evaluation factor in any drug screening. In water, the prodrug **2** underwent a well pronounced self-assembly as shown by size-exclusion chromatography (SEC) (Fig. 2).

Most of the substance (90% w/w) is organized in micelles with an apparent molecular mass of 94,000 Da, which elutes at

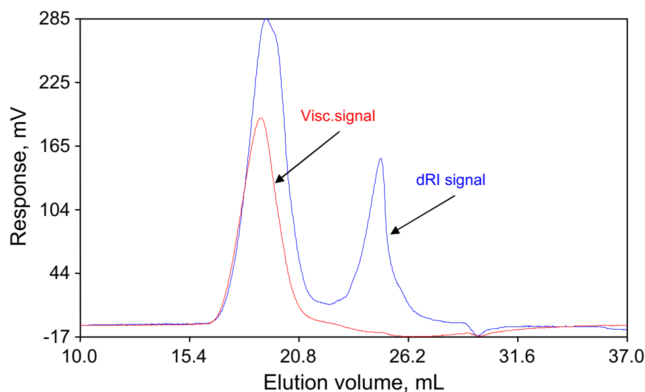


**Figure 2.** Aqueous SEC of **2**. Differential refractive index detector (dRI) and differential viscometric detector (Visc.) traces are shown in blue and red, respectively.

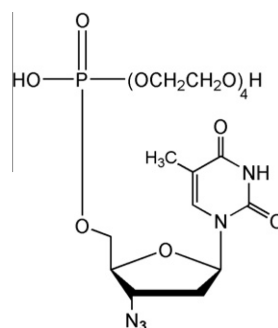


**Figure 3.** AZT tetraethylene glycol monophosphate, one of the possible products from the acidic hydrolysis of **2**.

19.17 mL, while the remainder exists in a monomolecular form (peak at 24.84 mL).

A preliminary hydrolytic screening was performed at 37.5 °C in buffered aqueous solution at pH 3.5. Aliquots from the incubated solution were taken at 3 h intervals over a 24 h period and subjected to SEC analysis in THF. The data obtained indicated that the prodrug **2** degraded gradually into PEG oligomers eluting between 24.8 and 28.3 mL, and AZT conjugates (retention volume = 29.7 mL). The apparent molecular mass of the final peak (510 Da, PEG calibration) was rather close to the calculated molecular mass of an AZT monophosphate (523.17 Da) which was expected to be released after the hydrolytic degradation of **2**. The hypothetical structure is shown in Figure 3.

A cytotoxicity evaluation was performed with CHO-Chinese hamster ovary and BALB/c 3T3 clone A31 mouse embryo cells using standard procedures.[9,10] The results from the comparative study showed that the IC$_{50}$ value of the original AZT was 1.63 and 1.35 mg/mL, for the CHO and BALB/c 3T3 lines, respectively. Remarkably, the IC$_{50}$ value of Poly-AZT was 4.74 mg/mL for CHO and 3.40 mg/mL when tested in BALB/c 3T3 clone 31 cell cultures.

A typical procedure for the preparation of polymeric 5′-*O*-ester prodrugs of 3′-azido-2′,3′-dideoxythymidine is presented below:

General procedure for conjugation of 3′-azido-2,3′-dideoxythymidine with **1**.

1,2-Dichloroethane (5 mL), CCl$_4$ (15 mL), Et$_3$N (2 mL), CH$_3$CN (7 mL), and 3′-azido-2,3′-dideoxythymidine (250 mg, 0.94 mmol) were placed in a three-necked flask equipped with a magnetic stirrer, reflux condenser, dropping funnel, and inert gas outlet. A solution of **1** (232 mg, 0.94 mmol of repeating units) in 1,2-dichloroethane (5 mL) was added dropwise at ambient temperature with continuous stirring. The reaction was allowed to proceed for 24 h. After filtration of the precipitated triethylamine hydrochloride, the filtrate was concentrated and the polymer conjugate **2** was precipitated by the addition of Et$_2$O. The isolated product was dried at 35–40 °C under reduced pressure (10 mbar). Elemental analysis for chlorine showed a trace of Cl$_2$, yield 485 mg (100%).

In conclusion, the results of this study indicate strongly that the immobilization of AZT onto poly(oxyethylene *H*-phosphonate) enables the formation of a potent prodrug substance that has high water solubility. The polymeric product can be hydrolyzed in a controlled manner in a medium mimicking the stomach, and demonstrates significantly decreased cytotoxicity in both the cell lines investigated.

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8. NMR data of compound **2**: $^{1}$H NMR ($CD_3OD$): $\delta$ (ppm) 1.88 (s, 3H, C$H_3$-5), 2.44 (t, 2H, $^{3}J$(H,H) = 6.3Hz, H-2′), 3.70 (t, 8H, $^{3}J$(H,H) = 4.6 Hz –OC$H_2$C$H_2$O–), 3.66–3.92 (m, 4H, P-OC$H_2$–, H-5′), 4.37 (q, 1H, $^{3}J$(H,H) = 6.6Hz, H-3′), 6.17 (t, 1H, $^{3}J$(H,H) = 6.4 Hz, H-1′, H-4′), 7.81 (s, 1H, H-6), 11.15 (br s, H-3). $^{13}$C{H} NMR ($CD_3OD$): $\delta$ (ppm) 12.9 (s, $CH_3$-5), 38.6 (s, C-2′), 62.2 (s, C-3′), 66.9 (d, $^{2}J$(P,H) = 7.3 Hz –P-O$CH_2$–, C-5′), 71.3 (d, $^{3}J$(P,C) = 4.9 Hz –POCH$_2$$CH_2$–), 71.9 (s, O$CH_2$$CH_2$O– repeating unit of PEG), 86.5 (d, $^{3}J$(P,C) = 8.8 Hz, C-4′), 112.0 (s, C-5), 138.5 (s, C-6), 152.7 (s, C-2),166.7 (s, C-4); $^{31}$P {H} NMR ($CD_3OD$): $\delta$ (ppm) 1.02.
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10. Cytotoxicity assays were performed according to the Standard Operating Procedure for the BALB/c 3T3 neutral red uptake cytotoxicity test.[9] The test systems BALB/c 3T3 clone A31 mouse embryo cells[9a] obtained from Centro Substrati Cellulari (Brescia, Italy) and CHO—Chinese hamster ovary[9d] continuous cell lines were used throughout the experiments. The cells were routinely grown as monolayers in 75 mL tissue culture flasks (Cellstar, Greiner Bio-One GmbH, Germany), at 37 °C in a humidified atmosphere (7.5% $CO_2$/air). BALB/c 3T3 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), (Sigma), and the *CHO* cells in Nutrient Mixture Ham's F 12 (Sigma), both supplemented with 5% FBS (BioWhittaker Europe) and with the antibiotics Penicillin (100 UI/mL) and Streptomycin (100 μg/mL). The cells were routinely passaged at a cell density of $\sim 1 \times 10^6$ cells in 75 mL flasks every 3–4 days (average doubling time is 20–24 h).