

Synthesis of Haptens for the Development of a Solid-Phase Immobilized Epitope-Immunoassay (SPIE-IA) of AZT-TP

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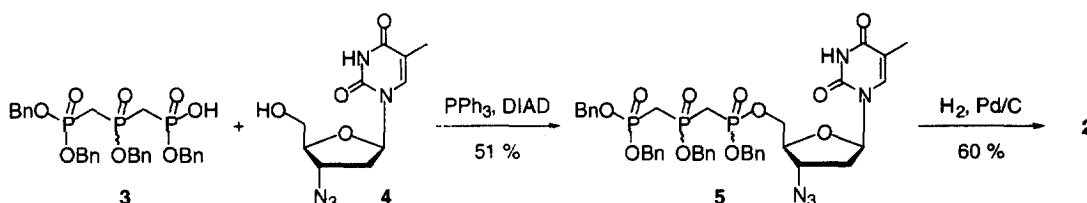
Abstract: The synthesis of the reduced form of AZT-triphosphate, 3'-amino-3'-deoxythymidine-5'-triphosphate, and of its stable analog, 3'-amino-3'-deoxythymidine-5'-(α,β,γ -bis-methylene)triphosphate, is described. AMT-TP and AMT-PCPCP were prepared to develop a specific immunometric assay for AZT-TP. © 1999 Elsevier Science Ltd. All rights reserved.

Till the discovery in the mid-1980s that AIDS results from infection by a retrovirus, human immunodeficiency virus (HIV), therapy has focused on the design of inhibitors targeted to HIV-reverse transcriptase (RT) and protease (PR), two main enzymes of the virus. Despite several classes of promising new anti-HIV agents, the clinical emergence of drug resistant variants of HIV has severely limited the long term effectiveness of these drugs¹⁻⁴. Combination of anti-HIV agents is now being explored as therapeutic modalities to prevent or delay emergence of virus-drug resistance⁵⁻¹¹. In these conditions however the emergence of other causes leading to therapy failure are observed. In 1998 a treatment escape mechanism related to the metabolism of nucleoside RT inhibitors (NRTIs) has been reported^{12,13}, supported by previous observations indicating negative metabolic interferences between antiviral nucleosides¹⁴⁻¹⁶. In order to precociously detect the emergence of such resistance phenomena, clinicians need efficient tools to monitor the intracellular concentration of NRTIs and of their metabolites (NRTI-MPs, NRTI-DPs, and NRTI-TPs).

Herein we describe our strategy for the production of specific antibodies directed against AZT-triphosphate (AZT-TP) and the elaboration of an enzyme immunometric assay called SPIE-IA (solid-phase immobilized epitope-immunoassay)¹⁷⁻²¹. In the SPIE-IA technique, a specific monoclonal antibody is immobilized on a 96-well titerplate to ensure full capture of hapten during the immunological reaction (Sch. 1). The trapped molecule is then covalently linked to the plate by means of either a bifunctional cross-linking

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AMT-TP has been prepared by direct careful hydrogenolysis of AZT-TP to avoid thymine reduction²⁵. That procedure proved to be more convenient than that described in two earlier reports and involving the azide reduction by triphenylphosphine^{26,27}. Immunizations of mice by injection after coupling to keyhole limpet hemocyanin (KLH) however did not trigger the synthesis of specific antibodies in the recipient animals. This is likely due to the rapid hydrolysis of the pyrophosphate bridges of the hapten *in vivo*²⁸. In order to alleviate the chemical and enzymatic instability of the pyrophosphate, the stable analog AMT-PCPCP **2** has been synthesized. It was prepared *via* direct Mitsunobu coupling of phosphonic acid **3**²⁹ and AZT **4**³⁰ to produce the intermediate compound **5**³¹. Careful hydrogenolysis of the four benzyl esters and of the azido group yielded AMT-PCPCP³².



- Scheme 2 -

Compound **2** has been coupled to KLH and the conjugate has been injected into mice. The production and selection of monoclonal antibodies are currently underway and results will be reported in due course.

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25. Preparation of **1**. AZT-TP (14 mg, 27.6 μ mol) and Pd/C 10% (7 mg) are stirred under 1 atm H₂ in methanol/water 1:1 (0.6 ml) for 2.5 h at room temperature. The catalyst is removed by filtration, the filtrate is reduced under vacuum and lyophilized to yield a white hygroscopic powder (10 mg, 74 %). ¹H-NMR (D₂O, 300 MHz) δ 7.52 (s, H6); 6.16 (t, *J* = 6.0 Hz, H1'); 4.27-4.23 (m, H3', H4'); 4.15-4.11 (m, 2H5'); 2.52-2.46 (m, 2H2'); 1.76 (s, 3H5).
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31. Preparation of **5**. Compound **3** (170 mg, 0.28 mmol), **4** (74 mg, 0.28 mmol), and DIAD (220 μ l, 1.10 mmol) in anhydrous THF (3 ml) are treated dropwise at room temperature with PPh₃ (250 mg, 0.95 mmol). The reaction mixture is stirred for 30 min before the solvent is removed under vacuum. The residue is purified by chromatography over silica gel (AcOEt/EtOH 100:0 to 85:15) to yield **5** as a glassy solid (mixture of 4 diastereomers, 122 mg, 51 %). ¹H-NMR (CDCl₃, 200 MHz) δ 7.36-7.27 (m, 20HAr, H6); 6.21-6.01 (m, H1'); 5.11-4.95 (m, 8HCAr); 4.40-3.83 (m, H3', H4', 2H5'); 3.17-2.65 (m, 4PCHP); 2.28-2.18 (m, 2H2'); 2.07 (s, 3H5). ³¹P-NMR (CDCl₃, 80 MHz) δ 40.9-39.1 (m, P β); 22.7-20.4 (m, P α , P γ). IR (neat, cm⁻¹) ν 3173; 3066; 2955; 2107; 1699; 1456; 1374; 1251; 998.
32. Preparation of **2**. Compound **5** (82 mg, 95 μ mol) and Pd/C 10% (7 mg) in EtOH/THF 7:3 (10 ml) are stirred for 1 h at room temperature under 1 atm H₂. The suspension is half reduced under vacuum and water (5 ml) is added before the resulting mixture is stirred under hydrogen for an additional 2 h period. The catalyst is removed by filtration and the filtrate is washed twice with AcOEt. The aqueous layer is lyophilized to yield **2** as a white hygroscopic powder (27 mg, 60 %). ¹H-NMR (D₂O, 200 MHz) δ 7.43 (s, H6); 6.09 (m, H1'); 4.24-4.14 (m, H4'); 4.07-3.90 (m, 2H5'); 4.87-3.76 (m, H3'); 2.65-2.27 (m, 2H2', 4PCHP); 1.69 (s, 3H5). ³¹P-NMR (D₂O, 80 MHz) δ 38.2 (m, P β); 17.1 (m, P α , P γ). IR (neat, cm⁻¹) ν 2962(b); 1696; 1475; 1373; 1270; 1202. Anal HPLC (Zorbax SB C18, 4.6x250 mm, 5 μ , Et₃NH⁺ HCO₃⁻: 0.062 M, CH₃CN: 0.1 %, pH 7.63) *t*_R 45.1 min.