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# Chemoenzymatic synthesis of Tenofovir

## AUTHORS

Beata ZDUN / WARSAW UNIVERSITY OF TECHNOLOGY, FACULTY OF CHEMISTRY, NOAKOWSKIEGO 3 STREET, WARSAW
Wolfgang KROUTIL / INSTITUTE OF CHEMISTRY, UNIVERSITY OF GRAZ, GRAZ, AUSTRIA, HEINRICHSTRASSE 28/II, GRAZ
Tamara REITER / INSTITUTE OF CHEMISTRY, UNIVERSITY OF GRAZ, GRAZ, AUSTRIA, HEINRICHSTRASSE 28/I, GRAZ
Paweł BOROWIECKI / WARSAW UNIVERSITY OF TECHNOLOGY, FACULTY OF CHEMISTRY, NOAKOWSKIEGO 3 STREET, WARSAW

## PURPOSE OF THE ABSTRACT

Tenofovir, and its lipophilic prodrugs [i.e., tenofovir disoproxil fumerate (TDF) and tenofovir alafenamide (TAF)] are powerful antiretrioviral agents that have already gained the status of the ‘frontline drugs' for the treatment of Human Immunodeficiency Virus (HIV) infection and chronic hepatitis B caused by HBV [1-2]. The anti-HIV activity of parent tenofovir, resulting from competitive inhibition of HIV reverse transcriptase inhibitor concerning for to dATP, is strictly related to the absolute configuration of its stereogenic center. In this context the (R)-configurated Tenofovir is ca. 100-fold more active as a nucleoside reverse transcriptase inhibitor (NRTI) than its enantiomeric counterpart [3].

In this study, optimization of the reaction conditions for lipase-catalyzed kinetic resolution of a racemic 1-(6-chloro-9H-purin-9-yl)-propan-2-ol was performed. This task allowed us to select the most efficient biocatalytic system, which consisted of immobilized lipase from Burkholderia cepacia (Amano PS-IM) suspended in toluene and vinyl acetate as an acyl donor. The optically pure (R)-ester (>99% ee) was obtained on a 500 mg-scale (60mM conc.) in 47% yield when employing preparative silica-gel column chromatography at a purification step or in 31% yield when using liquid-liquid extractive workup.

Alternatively, stereoselective bioreduction of the corresponding prochiral ketone, namely 1-(6-chloro-9H-purin-9-yl)-propan-2-one was performed. The most satisfactory result in terms of enantiomeric purity of the product was obtained in the reaction catalyzed by lyophilized E. coli cells harboring recombinantly overexpressed variant of alcohol dehydrogenase (ADH) from Lactobacillus kefir (E.coli/Lk-ADH-Prince). In this case, the desired (R)-alcohol was isolated with 86% yield and excellent optical purity (>99% ee).

The key-(R)-intermediate obtained in this way was further functionalized toward a tenofovir in a three-step reaction sequence consisting of “one-pot” aminolysis-hydrolysis or (R)-acetate in NH3-saturated methanol, alkylation of the resulting (R)-alcohol with tosylated diethyl (hydroxymethyl)phosphonate, and bromotrimethylsilane (TMSBr)-mediated cleavage of the formed phosphonate ester into the free phosphonic acid. The overall isolated yield of enantiomerically enriched tenofovir (99% ee) prepared in this manner reached 72% after four steps.

The elaborated enzymatic strategy could be applicable in the asymmetric synthesis of two other blocbuster antiretroviral tenofovir prodrug derivatives, including 5'-disoproxil fumerate (TDF, Viread ®) and 5'alafenamide (TAF, Vemlidy ®), respectively.

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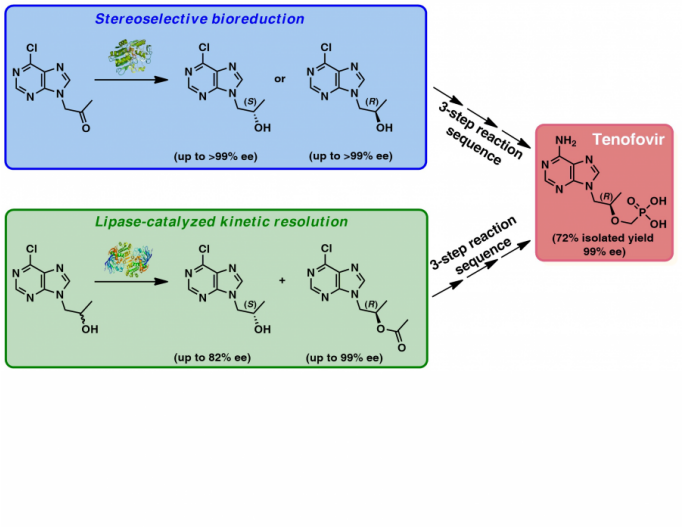
## FIGURES




## FIGURE 1

Chemoenzymatic Synthesis of Tenofovir.

## FIGURE 2


## KEYWORDS

Tenofovir | chemoenzymatic synthesis | lipase | alcohol dehydrogenase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