Tetrahedron: *Asymmetry* 23 (2012) 683–689

Contents lists available at SciVerse ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Enantioselective enzymatic desymmetrization of the prochiral pyrimidine acyclonucleoside

Renata Kołodziejska [a,*], Marcin Górecki [b], Jadwiga Frelek [b], Marcin Dramiński [a]

[a] *Department of General Chemistry, Collegium Medicum Nicolaus Copernicus University, Dębowa 3, 85-626 Bydgoszcz, Poland*
[b] *Institute of Organic Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland*

ARTICLE INFO

Article history:
Received 5 April 2012
Accepted 3 May 2012
Available online 8 June 2012

ABSTRACT

The effect of the solvent and the acyl group donor on the selectivity of the transesterification reaction of 1-{[(1,3-dihydroxypropan-2-yl)oxy]methyl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione was examined. Lipase (EC 3.1.1.3) Amano PS from *Burkholderia cepacia* (BCL) enabled desymmetrization of prochiral hydroxyl groups and gave the (*R*)-monoester in high enantiomeric excess (ee 88–99%). The best selectivity was obtained for the transesterification reaction with vinyl benzoate as the acylating agent (only monoester, 99% ee). The absolute configuration of the newly formed stereogenic center was determined with a high degree of confidence on the basis of the combined experimental and theoretical electronic circular dichroic (ECD) studies. The hydrolysis of prochiral diesters formed during the transesterification reaction in the presence of BCL provided the opposite enantiomer, that is, the (*S*)-monoester.

1. Introduction

The use of enzymatic reactions in synthetic organic chemistry is constantly growing and has become increasingly popular. This is undoubtedly related to the ecological aspect of this type of reaction and therefore syntheses carried out using the catalytic properties of enzymes have earned the status of green chemistry. Another equally important characteristic of enzymatic reactions is that the enzymatic proteins often allow us to obtain chiral products with high enantiomeric purity. This feature is particularly significant for biologically active compounds because of the dependence of their therapeutic properties on their enantiomeric purity. In this context, it is not surprising that the synthetic potential of catalytic lipases has been the subject of intensive explorations over recent years. As a result, it turned out that the lipases are attractive biocatalysts both in the kinetic resolution of racemic mixtures[1,2] and in the generation of asymmetry by the selection of an enantiotopic group of prochiral compounds.[3–5]

The interest in acyclonucleosides arises from their biological activity. The compound 1-{[(1,3-dihydroxypropan-2-yl)oxy]-methyl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione {common name: 1-[(1′,3′-dihydroxy-2′-propoxy)methyl]-5-methyluracil} is known as a micromole uridine phosphorylase inhibitor from *Escherichia coli* and compounds with a similar structure are reported to inhibit in vitro growth of cancer cells.[6,7]

As part of our interest in the development of enzymatic stereoselective methods for the preparation of optically active acyclonucleosides, we examined both the acylation (transesterification) of the prochiral 1-{[(1,3-dihydroxypropan-2-yl)oxy]methyl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **1** and the hydrolysis of the corresponding diesters in the presence of a lipase under various conditions (Scheme 1).

2. Results and discussion

2.1. Optimization of the reaction conditions using BCL (Amano PS from *Burkholderia cepacia*)

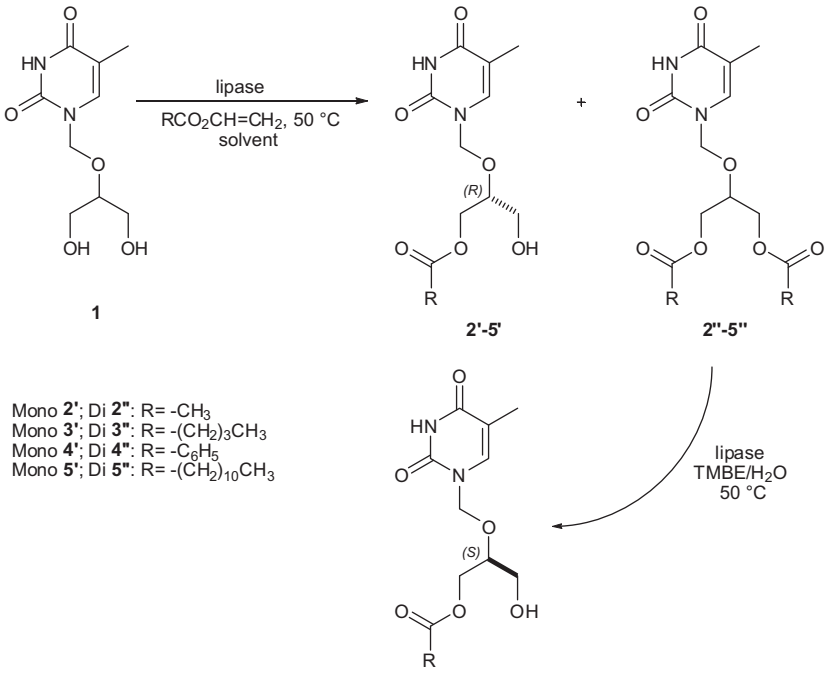
2.1.1. The influence of the solvent on the enantioselectivity of the enzymatic reaction

While planning an enzymatic synthesis, the choice of the solvent used as the reaction medium is often very significant. The solvent properties influence the activity, stability, and selectivity of the enzyme. In a polar organic solvent, a decrease in the enzymatic activity and the selectivity of the enzymatic protein was observed. This is probably caused by the interaction of a solvent molecule with a monomolecular layer of water that is essential for the activation of enzyme. In a hydrophobic organic solvent, an improvement of enzymatic enantioselectivity is usually observed.[8–10] In the transesterification reaction of compound **1**, eight different solvents were tested (Table 1). The solvents were put in order according to the increase of their hydrophobic properties (log*P*). The reactions were carried out at 50 °C for 24 h in the presence of lipase Amano PS from *Burkholderia cepacia* (BCL). Vinyl acetate was used as the acylating agent.

The transesterification reaction of diol **1** generally proceeded as expected. The BCL selectively acetylated enantiotopic hydroxyl

* Corresponding author.
E-mail address: renatakol@poczta.fm (R. Kołodziejska).

0957-4166/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetasy.2012.05.003



Scheme 1. A strategy to obtain (*R*)- and (*S*)-mono-derivatives.

Table 1
Transesterification of diol **1** with vinyl acetate in different organic solvents

Solvent	log*P*	Mono		**2**″ (%)	Σ[a]	ee[b] (%)
		(*S*)-**2**′ (%)	(*R*)-**2**′ (%)			
[BMIM][BF_4]	−2.51[11]	4.1	17.0	4.6	0.24	61
[BMIM][PF_6]	−2.39[9]	1.3	18.2	–	0.07	86
Dioxane	−1.10[12]	4.3	32.9	–	0.13	77
THF	0.49[13]	9.6	44.5	–	0.22	65
Pyridine	0.71[14]	1.9	17.8	–	0.11	81
TBME	1.30	3.3	52.1	44.0	0.06	88
Cyclohexane	3.40[12]	19.4	21.0	44.0	0.92	4
Hexane	3.50[13]	21.4	27.9	26.0	0.77	13

[a] Where Σ = [ratio of the (*S*)-enantiomer]/[ratio of the (*R*)-enantiomer].
[b] The ee was determined by HPLC.

groups to give the (*R*)-monoester. The determination of the absolute configuration will be discussed in Section 2.4.

The best results of the transesterification were obtained using *tert*-butyl methyl ether (TBME). After 24 h, monoester **2**′ was obtained with high yield (55.4%) and enantioselectivity (88% ee), as can be seen in Table 1. In addition to the monoester, the diacetyl derivative **2**″ was obtained with a 44.0% yield. In the two remaining ethers (dioxane or THF), the enantiomeric purity of monoester **2**′ was lower. In dioxane, it was 77% ee, but in the less polar THF, it was 65% ee. The BCL in pyridine and ionic liquid [Bmim][PF_6] displayed lower activity than in TBME, but the selectivity was similar. Monoacetylated derivative **2**′ was obtained with an enantiomeric excess similar to that observed in TBME. The reaction in pyridine, similar to [Bmim][PF_6], provided the monoester in 20% yield. However, pyridine was a better medium for the reaction, due to the better solubility of the substrate.

In ionic liquid [Bmim][BF_4] a decrease in enantioselectivity was observed. The solvent with a nucleophilic character of the accompanying anion probably had an influence on the lipase activity and enantioselectivity in ionic liquids. Ionic liquid [Bmim][PF_6] practically does not mix with water (slightly soluble in water—0.13%) while [Bmim][BF_4] has unlimited solubility. Moreover, BF_4^- ion is a stronger nucleophile than PF_6^- ion and thus has more strength in the active site of the enzyme contributing to the disadvantageous conformation changes of a catalyst.[9,15]

In the case of hydrophobic organic solvents, such as cyclohexane and hexane, an improvement of reaction selectivity was expected. The degree of conversion was high. Monoesters **2**′ were obtained with 40% and 49% yield, respectively. However, along with an increase of hydrophobicity of the reaction medium, a drastic decrease of enantioselectivity was observed. In hexane and cyclohexane, the monoester was obtained with a low enantiomeric excess. Under those conditions, BCL did not allow sufficient diversification of the prochiral –OH groups.

The relationship between the solvent hydrophobicity and its activity and BCL enantioselectivity was not straightforward. In standard hydrophobic organic solvents, such as cyclohexane or hexane (log*P* >3), an increase in the BCL catalytic activity was observed, with a simultaneous decrease of enantiomeric selectivity. Lowering the hydrophobic properties of the solvents led to an increase in the enantiomeric properties of BCL as well as a decrease in lipase reactivity in the majority of solvents with a low log*P* value. On the basis of these experiments, it can be concluded that TBME was the optimal solvent for the enzymatic transesterification reaction of compound **1** by vinyl acetate.

For the purpose of investigating the course of the transesterification reaction of **1** and an assessment of the enantiomeric purity of the resulting monoesters, an analysis of the reaction mixtures was conducted at specified intervals. The reaction was carried out for 24 h at 50 °C (Table 2, Fig. 1) and samples were analyzed by HPLC.

The transesterification reaction of **1** in TBME proceeded at a high rate and almost entirely stereoselectively. After 10 min, monoester **2**′ was obtained in 22% yield and with 82% ee. After one hour, the degree of conversion of **1** into the respective monoacetylated derivative reached 47.9% yield and enantiomeric purity was 88% ee. The diacetyl derivative **2**″ was detected as 5% of the reaction mixture. For the next 23 h of the reaction, no lowering of the enantioselectivity was observed. Finally, 55.4% of monoester **2**′ and 44.0% diacetyl derivative **2**″ were obtained.

On the basis of the results presented, it can be concluded that BCL in TBME allows for the enantiofacial differentiation of prochi-