Tetrahedron 67 (2011) 2858–2862

Contents lists available at ScienceDirect

Tetrahedron

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

Enzymatic regioselective production of chloramphenicol esters

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ARTICLE INFO

Article history:
Received 19 January 2011
Received in revised form 21 February 2011
Accepted 22 February 2011
Available online 1 March 2011

Keywords:
Biocatalysis
Chloramphenicol
Lipases
Regioselective processes
Medium engineering

ABSTRACT

An enzymatic study has been performed in the search for synthetic routes to produce chloramphenicol derivatives through regioselective processes using lipases. Complementary transesterification and hydrolytic reactions have been carried to synthesize chloramphenicol regioisomers. Reaction parameters, such as biocatalyst, solvent, acyl donor, and temperature have been optimised in order to obtain chloramphenicol esters with high yields through acylation processes. Scale-up of the enzymatic reactions (1 g-scale at 0.25 M) and catalyst recycling (up to 10 cycles) have been successfully achieved. Furthermore, monoacylated derivatives at the more hindered secondary position could also be obtained employing hydrolysis processes.

1. Introduction

The selective modification of one alcohol group in a polyhydroxylated compound is an important issue for organic chemists since usually it requires time-consuming protection and deprotection steps that enhance costs and by-products lowering the final yields. In this sense, biocatalytic transformations have become standard procedures applied to the regioselective acylation of polyfunctionalised derivatives maximising the efficiency of these processes.[1] Furthermore, in many cases the acylated derivatives obtained present better biological or availability properties than the parent substrates.[1,2]

This is also the case of chloramphenicol, (1*R*,2*R*)-2-dichloroacetamido-1-*p*-nitrophenyl-1,3-propanediol (**1**, Fig. 1),[3] a bacteriostatic antimicrobial, which acts as an effective agent against a broad spectra of *gram*-positive and *gram*-negative bacteria. It was introduced for clinical treatment in human and animals around the mid 20th century, and its first indication was in the treatment of typhoid. Furthermore, its efficiency in the treatment of tetracycline-resistant cholera, staphylococcal brain abscesses, meningitis, influenza, pneumonia, and several infections has effectively been demonstrated over the years.[4] Unfortunately, it can also produce

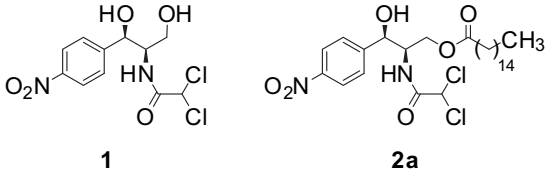

Fig. 1. Structures of chloramphenicol (**1**) and chloramphenicol palmitate ester (**2a**).

adverse effects, such as aplastic anaemia, bone marrow suppression, childhood leukaemia, and grey baby syndrome, so this compound requires medical prescription. Therefore, for the administration of this drug there are currently several ways, for instance capsules, oily or liquid form, but the bitter taste of the pharmaceutical has led to the production of different alternatives, such as chloramphenicol succinate or chloramphenicol palmitate (**2a**, Fig. 1) esters by selective modification of the primary hydroxyl group.

Originally, chloramphenicol was isolated from the bacterium *Streptomyces venezulae*,[5] but the production of this drug and its derivatives have attracted very much attention by means of regio- or stereoselective chemical[6] and enzymatic[7] methods. Among all biocatalytic processes, the use of lipases presents many advantages in comparison with other synthetic transformations due to the mild reaction conditions and the high level of selectivity displayed by this type of catalysts. In this manner, Ottolina et al. reported the lipase-mediated regioselective esterification of chloramphenicol for the synthesis of several derivatives in anhydrous acetone

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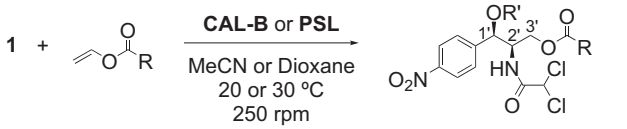
0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.02.070

exploring the influence of different trifluoroethyl esters. *Chromobacterium viscosum* lipase (CVL) or lipase G were the best biocatalytic agents, obtaining the corresponding esters after 24–72 h of reaction at 45 °C in moderate to excellent yields.[8] Later, Lin et al. demonstrated the versatility of hydrolases, such as Lipozyme or the protease from *Bactillus subtilis* in the synthesis of chloramphenicol vinyl esters.[9]

Herein we develop the synthesis of a wide set of chloramphenicol esters by using complementary synthetic approaches, such as acylation and hydrolysis processes. We have especially focused on the optimisation of the reaction conditions including the scaling-up and the enzyme recycling outcome.

2. Results and discussion

First of all, different commercially available lipases were tested[10] in order to find the highest activity in the esterification of chloramphenicol with a half-size chain activated ester, such as vinyl decanoate (**3b**). From previous studies developed in our research group, 1,4-dioxane was selected as a suitable solvent using 5 equiv of the acyl donor (Scheme 1, Table 1).



3a, R= $(CH_2)_{14}CH_3$
3b, R= $(CH_2)_8CH_3$
3c, R= $(CH_2)_{10}CH_3$
3d, R= CH_2CH_3
3e, R= CH_3
3f, R= Ph

2a, R= $(CH_2)_{14}CH_3$, R'= H;
2b, R= $(CH_2)_8CH_3$, R'= H;
2c, R= $(CH_2)_{10}CH_3$, R'= H;
2d, R= CH_2CH_3, R'= H;
2e, R= CH_3, R'= H;
2f, R= Ph, R'= H

4b, R= $CO(CH_2)_8CH_3$, R'= $CO(CH_2)_8CH_3$

Scheme 1. Lipase-mediated esterification of chloramphenicol (**1**) using vinyl esters **3a**–**f** in organic solvents.

Table 1
Lipase-mediated reaction of **1** with 5 equiv of vinyl decanoate (**3b**) in 1,4-dioxane at 30 °C after 48 h

Entry	Enzyme	**2b**[a] (%)	**4b**[a] (%)
1	CAL-A	22	11
2	CAL-B	72	5
3	PSL-C Amano	87	—
4	PSL-C I	94	—

[a] Percentage of compounds determined by HPLC.

After 48 h at 30 °C and 250 rpm, *Candida antarctica* lipase A (CAL-A, entry 1) gave low conversion and poor selectivity, affording the formation of the 3′-acylated compound (**2b**) and the 1′,3′-diacylated product (**4b**). *C. antarctica* lipase B (CAL-B) catalysed the acylation reaction with good selectivity and reaction rate, yielding 72% of the desired 3′-monoacylated derivative (entry 2). Meanwhile, as shown in entries 3 and 4, two different preparations of *Pseudomonas cepacia* lipase from Amano (PSL-C Amano, also known as *Burkholderia cepacia* lipase) and type I from Aldrich (PSL-C I), produced with complete selectivity the monoester **2b** in 87% and 94% conversion, respectively, as the sole product. These achievements were ideal starting points for further optimisation of the reaction conditions.

Due to the results obtained for these two biocatalysts, a search for a suitable solvent was carried out taking CAL-B and PSL as catalysts applied to the regioselective esterification of chloramphenicol. Reactions were performed for 24 and 48 h (Table 2). The influence of tetrahydrofuran (THF), acetonitrile (MeCN), and *tert*-butyl methyl ether (TBME) was initially examined with CAL-B (entries 1–6), where the highest conversions were with MeCN (entry 4), which allowed the formation of **2b** in 80% yield after 48 h. It should be noted that the diacylated compound **4b** was not detected in any case. Different results were attained when using PSL as a biocatalyst (entries 7–18). None of the three solvents improved the high performance obtained in 1,4-dioxane (87 and 94% in entries 3 and 4 of Table 1, respectively) although with acetonitrile higher conversions than THF and TBME were achieved.

Table 2
Lipase-mediated reaction of **1** with 5 equiv of vinyl decanoate (**3b**) in different organic solvents at 30 °C

Entry	Enzyme	Solvent	*t* (h)	**2b**[a] (%)
1	CAL-B	THF	24	31
2	CAL-B	THF	48	37
3	CAL-B	MeCN	24	74
4	CAL-B	MeCN	48	80
5	CAL-B	TBME	24	27
6	CAL-B	TBME	48	29
7	PSL-C Amano	THF	24	15
8	PSL-C Amano	THF	48	21
9	PSL-C Amano	MeCN	24	51
10	PSL-C Amano	MeCN	48	62
11	PSL-C Amano	TBME	24	42
12	PSL-C Amano	TBME	48	51
13	PSL-C I	THF	24	12
14	PSL-C I	THF	48	17
15	PSL-C I	MeCN	24	33
16	PSL-C I	MeCN	48	50
17	PSL-C I	TBME	24	38
18	PSL-C I	TBME	48	43

[a] Percentage of monoester **2b** determined by HPLC.

The effect of the temperature was analysed when employing the best conditions previously found: (a) CAL-B and MeCN; or (b) PSL-C I and 1,4-dioxane (Table 3).[11] Surprisingly, CAL-B led to a higher conversion into **2b** at 20 °C rather than at 30 °C (entries 1 and 2), although this enzyme usually presents an optimum temperature between 30 and 40 °C.[12] When using PSL-C I, a remarkable decrease of the conversion occurred when going from 30 to 40 °C (entries 4 and 5), maybe caused by deactivation of the biocatalyst, meanwhile the reaction at 20 °C (entry 3) led to a similar value than the one obtained at 40 °C.

Table 3
Temperature effect in the lipase-catalysed acylation of **1** with 5 equiv of vinyl decanoate (**3b**) after 48 h

Entry	Enzyme	Solvent	*T* (°C)	**2b**[a] (%)
1	CAL-B	MeCN	20	94
2	CAL-B	MeCN	30	80
3	PSL-C I	1,4-Dioxane	20	61
4	PSL-C I	1,4-Dioxane	30	94
5	PSL-C I	1,4-Dioxane	40	58

[a] Percentage of compounds determined by HPLC.

After optimisation of the experimental conditions for the acylation reaction, we explored the possibilities to regioselectively produce several chloramphenicol ester derivatives of different length chain using CAL-B or both preparations of PSL-C as biocatalysts in MeCN or 1,4-dioxane as solvent, respectively. The best results have been summarised in Table 4. Chloramphenicol palmitate ester (**2a**) was selected as the first candidate due to its therapeutical uses[13] and results were in accordance with the ones obtained when using vinyl decanoate as acyl donor. Both CAL-B and PSL-C I led to nearly complete conversions after 24 h of reaction (entries 1 and 2).