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Enzymatic regioselective production of chloramphenicol esters

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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

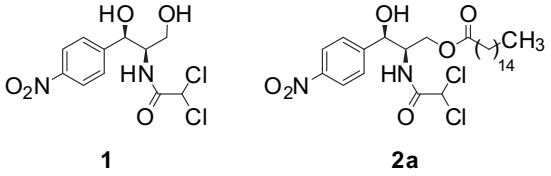

Fig. 1. Structures of chloramphenicol (**1**) and chloramphenicol palmitate ester (**2a**).

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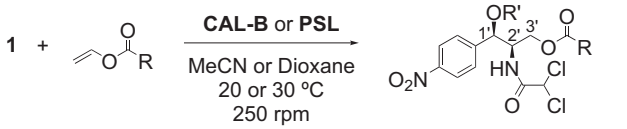
exploring the influence of different trifluoroethyl esters. *Chromobacterium viscosum* lipase (CVL) or lipase G were the best biocatalytic agents, obtaining the corresponding esters after 24–72 h of reaction at 45 °C in moderate to excellent yields.[8] Later, Lin et al. demonstrated the versatility of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

Table 4
Enzymatic acylation of **1** with 5 equiv of the vinyl ester **3a**,**c**–**f** at different temperatures in a 15 mM substrate concentration

Entry	Enzyme	T (°C)	Solvent	Final product	t (h)	Conv[a] (%)
1	CAL-B	20	MeCN	**2a**	24	>99
2	PSL-C I	30	1,4-Dioxane	**2a**	24	95
3	CAL-B	20	MeCN	**2c**	24	>99
4	PSL-C I	30	1,4-Dioxane	**2c**	24	91
5	CAL-B	20	MeCN	**2d**	24	>99
6	PSL-C Amano	30	1,4-Dioxane	**2d**	24	99
7	PSL-C I	30	1,4-Dioxane	**2d**	24	95
8	CAL-B	20	MeCN	**2e**	40	>99
9	PSL-C Amano	30	1,4-Dioxane	**2e**	40	97
10	CAL-B	20	MeCN	**2f**	40	4
11	PSL-C Amano	30	1,4-Dioxane	**2f**	40	17

[a] Percentage of compounds determined by HPLC.

Next, vinyl laurate (**3c**) was reacted with **1** also observing an excellent reactivity (entries 3 and 4), especially in the case of CAL-B, which led to quantitative conversion of the monoester **2c**. This trend was also observed when the chain length of the vinyl ester decreased, obtaining excellent results when vinyl propanoate (**3d**) was employed as acyl donor with all the biocatalysts tested (entries 5–7). Surprisingly, longer reaction times were needed to reach high conversion values using vinyl acetate (**3e**, entries 8 and 9). Vinyl benzoate (**3f**) led to the lowest conversion values (entries 10 and 11) due to the poorer reactivity of this acyl donor. These results present lipases as ideal catalysts for the regioselective acylation of chloramphenicol, showing an excellent selectivity towards the acylation of the primary alcohol. It is important to remark that in no case was the secondary hydroxyl group acylated to form the corresponding 1′-monoester or 1′,3′-diester derivatives, even after longer reaction times (data not shown).

From a preparative point of view, both higher substrate concentrations and recycling of the catalyst lead to more economical and green processes.[14] Therefore, after testing the solubility of all products involved in these processes, the reactions to achieve **2a**–**f** were carried out at a higher concentration of chloramphenicol (0.15 M), as summarised in Table 5.

Table 5
Lipase-mediated reaction of **1** with 5 equiv of the vinyl ester **3a**–**f** at different temperatures and 250 rpm at 0.15 M substrate concentration

Entry	Enzyme	T (°C)	Solvent	Final product	t (h)	Conv[a] (%)	Isolated yield[b] (%)
1	CAL-B	20	MeCN	**2a**	3	>99	91
2	PSL-C I	30	1,4-Dioxane	**2a**	3	>99	75
3	PSL-C Amano	30	1,4-Dioxane	**2a**	48	79	78
4	CAL-B	20	MeCN	**2b**	3	>99	92
5	PSL-C I	30	1,4-Dioxane	**2b**	4	>99	84
6	PSL-C Amano	30	1,4-Dioxane	**2b**	22	>99	91
7	CAL-B	20	MeCN	**2c**	4	>99	91
8	PSL-C I	30	1,4-Dioxane	**2c**	7	>99	88
9	PSL-C Amano	30	1,4-Dioxane	**2c**	10	>99	88
10	CAL-B	20	MeCN	**2d**	3	>99	89
11	PSL-C I	30	1,4-Dioxane	**2d**	6	98	90
12	PSL-C Amano	30	1,4-Dioxane	**2d**	9	>99	82
13	CAL-B	20	MeCN	**2e**	3	>99	90
14	PSL-C I	30	1,4-Dioxane	**2e**	6	99	90
15	PSL-C Amano	30	1,4-Dioxane	**2e**	9	98	79
16	CAL-B	20	MeCN	**2f**	48	31	25
17	PSL-C I	30	1,4-Dioxane	**2f**	48	53	45
18	PSL-C Amano	30	1,4-Dioxane	**2f**	48	13	8

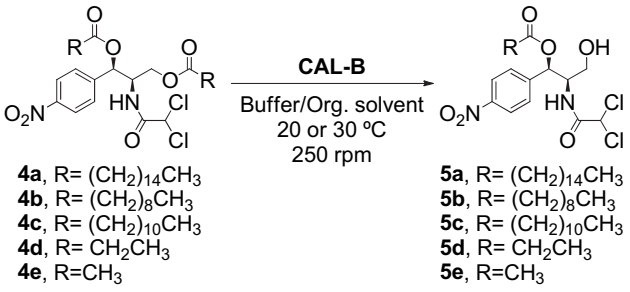
[a] Percentage of compounds determined by HPLC.
[b] Isolated yields after purification by *flash* chromatography.

In all cases the reaction times diminished in comparison with the more diluted one (see Table 4), finding that for CAL-B only 3 or 4 h were enough to afford total conversions into the corresponding monoesters **2a**–**e**. Among all the enzymatic processes carried out with PSL, PSL-C I showed better reaction rates than PSL-C Amano. Finally, the biotransformations with vinyl benzoate (entries 16–18) led also to higher conversions at 150 mM concentration rather than 15 mM, obtaining 45% isolated yield of monoester **2f** with PSL-C I (entry 17).

The next step was to study the recycling of the enzyme by performing the acylation at 0.15 M concentration of **1** and vinyl palmitate with CAL-B in MeCN at 20 °C. The biocatalyst performed the regioselective synthesis of **2a** with total conversion after 3 h during 10 cycles of reutilisation.

After having found excellent conversion in the biocatalysed regioselective acylation of diol **1**, we set up a preparative experiment at 1 g-scale. For that, the biocatalytic processes to obtain derivatives **2a**–**e** were performed using the best conditions previously found for the acylation at 0.15 M concentration of **1**: CAL-B as biocatalyst, 5 equiv of the corresponding ester and MeCN as solvent at 20 °C. All the reactions proceeded until complete conversion to isolate **2a**,**b**,**d**–**e** in 3 h, requiring 4 h for the laurate ester **2c**. Additionally, the palmitate derivative **2a** was obtained in 98% conversion when the reaction was repeated at 0.25 M concentration of chloramphenicol. Further increase of substrate concentration led to solubility problems.

Previous experiments have demonstrated that the acylated derivatives of chloramphenicol at the primary alcohol can be obtained with high yields, but we were also interested in the obtaining of analogues acylated at the more hindered secondary position. The chemical modification of a secondary alcohol in the presence of a primary one is a complicated feature, which has scarcely been observed.[15] Herein, taking advantage of the chemical complementarity shown by lipases in stereo- and regioselective processes,[16] we focused on the preparation of monoesters **5a**–**e** by firstly synthesising the diacylated derivatives **4a**–**e**, and later performing the regioselective lipase-catalysed hydrolysis at the primary position (Scheme 2).



Scheme 2. Lipase-catalysed hydrolysis of diacylated chloramphenicol derivatives **4a**–**e** in aqueous media.

Enzymatic hydrolyses were initially performed with the diacetylated compound **4e** by employing a mixture of phosphate buffer 100 mM pH 7.0 and an organic co-solvent (80:20 v v^{-1}), such as MeCN, 1,4-dioxane or THF with the previous lipases, but only CAL-B showed activity (Table 6). Using this biocatalyst the 3′-acylated derivative **2e** was not observed in any case and the desired product **5e** was mainly achieved in MeCN at 20 °C with high conversion (78%), giving substrate **4e** and chloramphenicol **1** as minor compounds (entry 3). Percentage of MeCN in the reaction medium (5–80%) and the pH of the reaction (range 5.5–8.5) were also studied (see Supplementary data for more information), but no further improvement of the conversion value was achieved.

Table 6
Lipase-mediated hydrolysis of **4e** with CAL-B in a buffer/organic solvent system (80:20 v v^{-1}) at different temperatures and 250 rpm at 0.15 M concentration (t=24 h)

Entry	T (°C)	Solvent	**1**[a] (%)	**4e**[a] (%)	**5e**[a] (%)
1	20	1,4-Dioxane	23	63	14
2	30	1,4-Dioxane	18	73	9
3	20	MeCN	14	8	78
4	30	MeCN	8	18	74
5	20	THF	12	21	67
6	30	THF	16	54	30

[a] Percentage of compounds determined by HPLC and ^{1}H NMR.

The hydrolyses of diacylated derivatives **4a**–**d** were then carried out under the optimal conditions depicted in entry 3 of Table 6, observing a correlation between the chain length of the ester moiety and the enzymatic conversion (Fig. 2). Thus, when longer ester moieties were employed, lower conversions were reached. While the dipropionate compound **4d** afforded 74% of **5d**,[17] didecanoate **4b** provided only a 10% of **5b**, dilaurate **4c** a 4% of **5c** and dipalmitate **4a** did not react. This effect can be explained because bulkier diacylated products could not correctly fit in the active site of the enzyme.

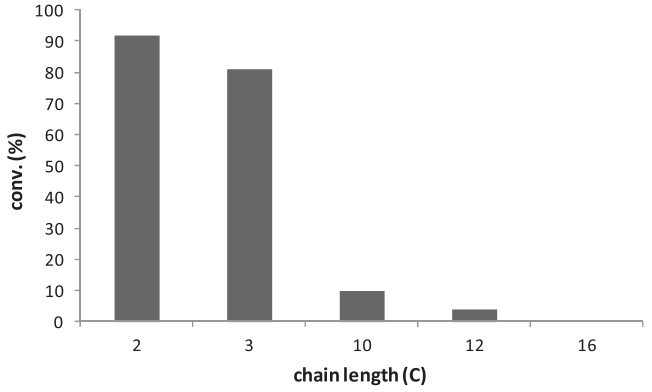


Fig. 2. Effect of the chain length in the CAL-B-catalysed hydrolysis of diacylated chloramphenicol derivatives.

3. Conclusions

In summary, we have successfully demonstrated the versatility of lipases, especially CAL-B, applied to the regioselective production of chloramphenicol esters, acylating or hydrolysing the primary position in short reaction times. Industrial application of the transesterification reaction has been considered showing the possibility to carry out the experiments at high substrate concentrations (0.25 M) and recycling the enzyme for a large number of cycles (up to 10), thus obtaining interesting compounds, such as the palmitate derivative with excellent yields. Furthermore, monoacylated compounds on the secondary alcohol group, difficult to achieve by conventional methodologies, were obtained with good to moderate yields through biocatalysed regioselective hydrolytic processes.

4. Experimental section

4.1. General

Immobilised *C. antarctica* lipase type B (CAL-B, Novozym 435, 7300 PLU/g) was a gift from Novo Nordisk Co. Immobilised *P. cepacia* lipases PSL-C Amano (1019 U/g) and PSL-C I (1638 U/g) were acquired from Amano Pharmaceutical Co. and Sigma–Aldrich, respectively. *C. antarctica* lipase A (CAL-A) was purchased from Codexis (2.6 U/mg of solid). All other reagents were purchased from different commercial sources and used without further purification. Solvents were distilled over an adequate desiccant under nitrogen. *Flash* chromatographies were performed using silica gel 60 (230–240 mesh).

4.2. General procedure for the esterification reaction of (−)-chloramphenicol at 0.25 M substrate concentration using vinyl palmitate

To a suspension of (−)-chloramphenicol (**1**, 1 g, 3.1 mmol) and CAL-B (1 g) in dry MeCN (12.5 mL) under nitrogen atmosphere, vinyl palmitate (**3a**, 4.26 g, 15.0 mmol) was added, and the reaction was shaken at 20 °C and 250 rpm. Aliquots were regularly analysed by HPLC and the reaction was stopped after complete consumption of the starting material after 1 h. Finally the enzyme was filtered off and washed with EtOAc (3×10 mL). The solvent was evaporated under reduced pressure. The reaction crude was purified by *flash* chromatography on silica gel (30% EtOAc/hexane) affording the corresponding ester **2a** (87% isolated yield). Characterizations of monoester **2a** and all novel compounds are given in the Supplementary data file.

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.02.070. These data include MOL files and InChIKeys of the most important compounds described in this article.

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17. 3′-Acylated compound **3d** (3%) and chloramphenicol (4%) were also detected.