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Journal of Molecular Catalysis B: Enzymatic 40 (2006) 81–85
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Selective lipase-catalyzed preparation of diol monobenzoates by transesterification and alcoholysis reactions in organic solvents

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Available online 4 April 2006

Abstract

Lipases from *Mucor miehei* (MML) and *Candida antarctica* (CAL) are able to catalyze the monobenzoylation of the primary hydroxy group of 1,2- 1,4- or 1,5-diols with vinyl benzoate in an organic solvent, the reaction proceeding with high regioselectivity and moderate enantioselectivity. The lipase-catalyzed debenzoylation of 1,2-propanediol dibenzoate by alcoholysis with 1-octanol most satisfactorily occurred with *Pseudomonas cepacia* lipase absorbed onto celite that allowed also to prepare (*R*)-1-benzoyloxy-2-methylpropan-3-ol from 2-methyl-1,3-propanediol dibenzoate, a result complementary to MML-catalyzed benzoylation of 2-methyl-1,3-propanediol that affords the (*S*)-monobenzoate.

Keywords: Lipases; Benzoylation; Transesterification; Alcoholysis; Diols

1. Introduction

The selective protection of polyhydroxy compounds still remains a challenge in organic synthesis and, considering an ester as the protecting group of an alcohol, various biocatalytic approaches can be adopted for selective introduction/removal of acyl groups [1]. Among synthetically useful lipase-catalyzed methods for ester synthesis, the transesterification procedure that relies upon vinyl or propenyl esters as acylating reagents [2–4] has enjoyed widespread application in organic synthesis [5,6]. Vinyl acetate (VA) is by far the enol ester more frequently used for introduction of an acetate, although this protecting group is not sufficiently stable for synthetic manipulations and suffers of the disadvantage that migration towards a vicinal hydroxy group frequently occurs under a variety of experimental conditions, as clearly shown for 1,2-diols [7–9]. In this respect, although a benzoic ester is more resistant and less prone to migrate [10], relatively few examples of selective enzymatic benzoylation of polyhydroxylated compounds are currently available [11–14]. On the other hand, such a biocatalytic procedure could constitute a useful alternative to existing chemical approaches that often require special reagents and experimental conditions to achieve an adequate regioselection [15]. Recently, we have published a few papers dealing with the enzymatic monobenzoylation of diols catalyzed by suitable lipases in an organic solvent under transesterification conditions using vinyl benzoate (VB) as acyl transfer [16–18]. Furthermore, a preliminary report on the enzymatic debenzoylation of 1,2- and 1,3-diol diesters [19] has added alcoholysis to the biocatalytic procedures for the selective preparation of monobenzoates of polyhydroxylated compounds.

2. Results and discussion

2.1. Lipase-catalyzed benzoylation of 1,2-propanediol (*1a*)

For the enzymatic benzoylation procedure, 1,2-propanediol (**1a**) was selected as a model substrate to set up experimental conditions such as choice of the most active lipase, suitable organic solvent and correct lipase/VB ratio. Microbial lipases from *Pseudomonas cepacia* (PCL), *Muchor miehei* (MML), *Candida antarctica* (CAL), *Candida cylindracea* (CCL) and the porcine pancreas lipase (pPL) were selected as biocatalysts, CAL and MML being available in an immobilized form. The enzyme/substrate ratio was fixed as 0.1 g of the enzymatic preparation per millimole of **1a**, independently from the hydrolytic activity of the lipase. Blank reactions, carried without enzyme, showed no products in the conditions of lipase-catalyzed benzoylation. The equivalent amount of VB was 1.5 $mmol^{-1}$ of

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doi:10.1016/j.molcatb.2006.02.014

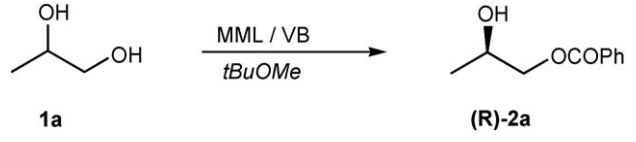
Table 1
Lipase-catalyzed benzoylation of 1,2-propanediol (**1a**)

Lipase[a]	Convn (%)	Time (h)	ee (%)	*E*
MML	100	1	–	–
MML	30	0.10	60[b]	5.3
MML	60	0.25	70[c]	5.5
CAL	100	4.5	–	–
CAL	37	1.5	54[b]	4.5
CAL	63	2.1	64[c]	4.6
PCL	52	72	–	–
CCL	21	72	–	–
pPL	33	72	–	–

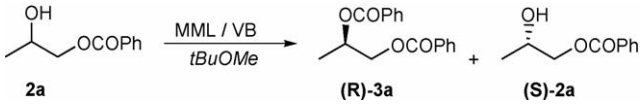
[a] 100 mg enzyme/mmol substrate.
[b] Determined by ^{1}H NMR analysis of MTPA ester of monobenzoate **2a**.
[c] Determined by ^{1}H NMR analysis of MTPA esters of unreacted **1a**.

substrate and was kept as such for all following substrates. All the reactions were stopped after 72 h and *tert*-butyl methyl ether (*t*BuOMe) was selected as solvent. The results of the enzymatic benzoylation of **1a** with all selected lipases (Table 1) show that CAL and MML are able to catalyze the regioselective acylation to the monobenzoate **2a** much faster than other enzymes, MML being the most active biocatalyst. The monobenzoate **2a** is a stable compound and ^{1}H NMR analysis of the ester obtained by reaction with (*S*)-MTPACl [20] indicated 60% enantiomeric excess (ee) at 30% conversion of diol **1a** that corresponds to a value of 5.3 of enantiomeric ratio *E* [21]. The (*R*)-configuration was assigned to enzymatically prepared monobenzoate **2a** (Scheme 1) by analysis of ^{1}H NMR data of (*R*)- and (*S*)-MTPA esters of **2a**, according to the Mosher's modified method [22]. Comparison of the optical rotation of enzymatically prepared **2a** with the value reported in literature [23] confirmed the assigned configuration. The reaction catalyzed by CAL proceeded at slower rate, but no improvement of the enantioselectivity was observed.

It has been previously reported [24] that also in the lipase-catalyzed acetylation of racemic 1,2-diols, the resulting 1-monoacetate is not optically pure and in order to achieve a stereoselective resolution the diol has to be converted into the corresponding diacetate. This so called "sequential acetylation" allows to prepare the diacetate or the unreacted diol in an enantiomerically pure form [25]. We considered the possibility that also the enantioselectivity of the lipase-catalyzed benzoylation could be enhanced by converting the chemically prepared racemic monobenzoate **2a** to the dibenzoate **3a** (Scheme 2). The



Scheme 1. MML-catalyzed benzoylation of 1,2-propanediol (**1a**) in *t*BuOMe.



Scheme 2. MML-catalyzed benzoylation of monobenzoate **2a**.

Table 2
MML-catalyzed benzoylation of monobenzoate **2a** in different solvents

Solvent	Convn (%)	Time (h)	ee[a] (%)	*E*
Hexane	61	4	85	8.7
Toluene	63	29	82	6.9
*t*BuOMe	62	40	88	9.2
$CHCl_3$	17	168	–	–
THF	26	168	–	–

[a] Determined by ^{1}H NMR analysis of MTPA esters of unreacted monobenzoate **2a**.

dibenzoylation was slow (30% in 24 h) but the enatioselectivity was improved to *E* 9.2, a value that can be considered significant for the stereochemical outcome of an enzymatic resolution. This prompted us to further study the dependence of the enantioselectivity on the nature of the solvent and results are shown in Table 2.

The reaction was faster in apolar solvents such as hexane or toluene with no improvement of *E* whereas in polar solvents such as $CHCl_3$ or THF the reaction was considerably slower and the enantioselectivity not further examined.

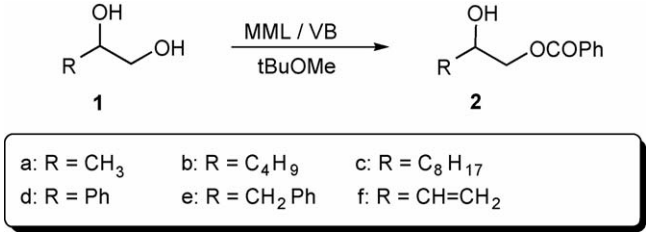
2.2. Monobenzoylation of 1,2-diols *1b–f*

We extended the above preliminary observations to a few additional 1,2-diols (Scheme 3) that were characterized by the presence of residues R that correspond to aliphatic (**1b** and **1c**), phenyl (**1d**), benzyl (**1e**) or unsaturated (**1f**) moieties.

For all substrates we examined only the MML-catalyzed benzoylation in *t*BuOMe that proceeded to a quantitative conversion in 1–3 h with high regioselectivity and low enantioselectivity (Table 3). For aliphatic diols **1b** and **1c**, fast benzoylations were more easily monitored at >50% conversion and compared with **1a** at the same extent. Furthermore, MML- and CAL-catalyzed dibenzoylation of monobenzoates **2b–f** proceeded at slow rate and enantioselectivity that was lower than the one observed for **2a**.

2.3. Lipase-catalyzed benzoylation of 1,4- and 1,5-diols

The selective monoprotection of two chemically equivalent primary hydroxy groups in 1,4-diols constitutes a challenge in organic synthesis and chemical methods usually lead to a mixture of unreacted, mono- and diprotected diols, unless special experimental conditions are developed. When the MML-catalyzed benzoylation procedure was applied to 1,4-diols **9–12a** (Fig. 1), selective monobenzoylation was observed [16].



Scheme 3. MML-catalyzed benzoylation of diols **1a–f**.

Table 3
MML-catalyzed benzoylation of diols **1a–f** in *t*BuOMe

Substrate	Convn (%)	Time (h)	ee (%)	*E*
1a	100	1	–	–
1a	60	0.25	61[a]	5.5
1b	100	2	–	–
1b	72	0.5	31[a]	1.6
1c	100	2	–	–
1c	70	0.5	30[a]	1.7
1d	100	3	–	–
1d	36	0.25	51[b]	4.0
1e	100	5	–	–
1e	39	1.1	15[b]	1.5
1f	100	1	–	–
1f	23	0.4	27[b]	1.9

[a] Determined by ^{1}H NMR analysis of MTPA esters of unreacted diols.
[b] Determined by ^{1}H NMR analysis of MTPA esters of monobenzoates.

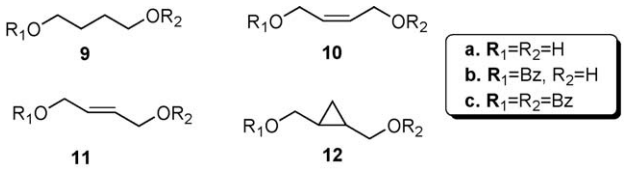


Fig. 1. Structure of 1,4-diols and their benzoates (compounds **9a–c** to **12a–c**).

The highest regioselectivity was evidenced for unsaturated diols **10a** and **11a**, (*E*)-diol **11a** reacting faster than the (*Z*)-isomer **10a**. The monobenzoylation of the (*E*)-cyclopropyl diol **12a** proceeds with lower regioselectivity (Table 4).

Comparing 2-methyl-1,4-butanediol (**13a**) and 2-methyl-1,5-pentanediol (**14a**), the hydroxy group more distant from the methyl group was preferentially benzoylated and this was more evident for the 1,5-diol **14a** (**14b/14c** ratio, 85:15 versus **13b/13c** ratio, 6:4 in Table 5; Fig. 2).

Interestingly, 2-methylene diol (**15a**) was benzoylated exclusively at the C-1 hydroxy group with a regioselectivity outcome opposite to that observed for diol **13a**, but similar to that reported for the PCL-catalyzed acetylation of the same substrate **15a** [26]. The above regioselective outcome suggests a specific role for the π electrons of the methylene moiety. All the above results of the enzymatic benzoylation of diols **13–15a** are collected in Table 5.

Table 4
MML-catalyzed esterification of 1,4-diols (**9–12a**) in *t*BuOMe by means of VB

Substrate	Time (h)	Products	Ratio[a]	Yield[b] (%)
9a	4.0	**9b/9c**	82/18	72
10a	5.0	**10b/10c**	93/7	82
11a	0.8	**11b/11c**	92/8	80
12a	1.5	**12b/12c**	67/33	58

[a] At 90% conversion, relative ratio established by ^{1}H NMR.
[b] Yields of regioisomeric monobenzoates isolated as mixture after flash chromatography.

Table 5
Enzymatic esterification of diols (**13–15a**) in *t*BuOMe by means of VB

Substrate	Time[a] (h)	Products	Ratio	Yield (%)
13a	2.0	**13b/13c**	60/40	80[b]
14a	6.5	**14b/14c**	85/15	90[b]
15a	0.5	**15b/15c**	0/100	92[b]

[a] Time for 100% conversion.
[b] Yields of purified regioisomeric monobenzoates isolated as mixture after flash chromatography.

2.4. Lipase-catalyzed debenzoylation of 1,2-propanediol dibenzoate *16a* by alcoholysis

We have recently studied also the enzymatic deacylation of 1,2-diol dibenzoates by alcoholysis [19], a reaction that should preferably occur at the primary benzoate moiety, thus allowing the preparation of 2-monobenzoates, compounds that can hardly be obtained by selective chemical procedures [27]. The dibenzoate of 1,2-propanediol **16a** was selected as substrate to set up the experimental conditions of MML-catalyzed debenzoylation that was carried out most conveniently in diisopropyl ether (DIPE).

After preliminary experiments, an enzyme/substrate ratio of 1 g/mmol was selected, since higher amount of lipase only slightly influenced reaction rate and resulted less practical from a preparative point of view. Among alcohols tested as deacylating agents, 1-octanol carried out the fastest alcoholysis affording in 7 days the expected monobenzoate **17a** (75% yield). Differently from the unstable 2-acetate of 1,2-diols [23,24], the monobenzoate **17a** is a stable product that did not show attitude to migrate during the purification by silica gel chromatography or on standing at room temperature. Low enantioselectivity (20% ee) and (*R*)-configuration was established for the monobenzoate **17a** at 30% conversion. Other lipases such as pPL, CCL and CAL were then examined, but no improvement in activity or enantioselectivity was observed. Interestingly, lipase from *Pseudomonas* sp. (PSL) showed a good enantioselectivity (78% ee), although the (*R*)-monobenzoate **17a** was obtained in only 20% yield after 14 days at room temperature. This observation prompted us to study experimental conditions to accelerate the process and it turned out that PSL absorbed onto celite (PSL-C) [28] (30%, w/w, enzyme/substrate ratio 1.2 g/mmol, corresponding to 0.36 g of PSL/mmol) catalyzed, at room temperature, the debenzoylation at an extent of 33% in 2 days, the enantioselectivity (82% ee) remaining substantially unaffected (Scheme 4).

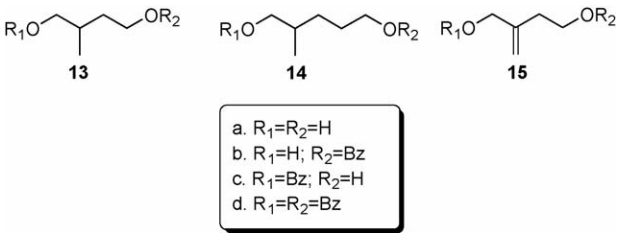
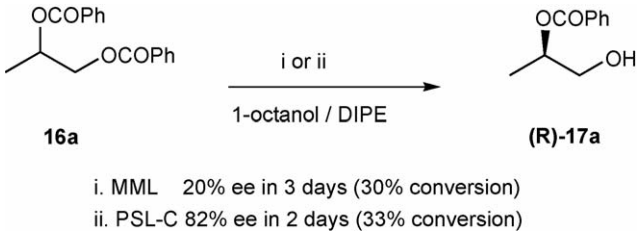
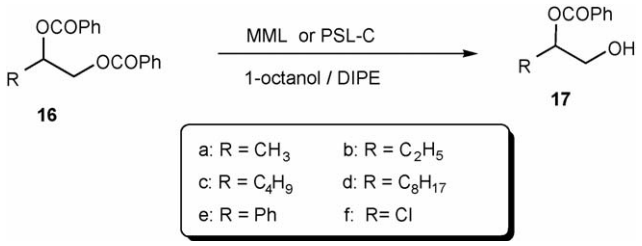


Fig. 2. Structure of 2-substituted 1,4- or 1,5-diols and their benzoates (compounds **13a–d** to **15a–d**).



Scheme 4. Alcoholysis of dibenzoate **16a** in DIPE catalyzed by MML and PSL-C.



Scheme 5. Alcoholysis of dibenzoate **16a–f** in DIPE catalyzed by MML or PSL-C.

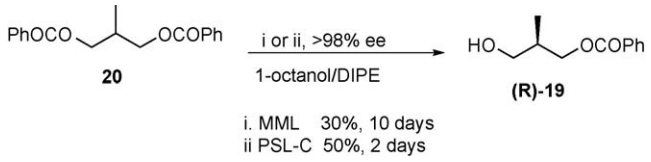
Table 6
PCL-catalyzed alcoholysis of dibenzoates **16a–f** in DIPE

Substrate	Convn (%)	Time (h)	ee[a] (%)	*E*
16a	33	48	82	14.0
16b	29	19	71	7.8
16c	24	72	70	7.0
16d	36	24	51	4.0
16e[b]	–	–	–	–
16f	45	16	20	1.7

[a] Determined by ^{1}H NMR analysis of MTPA esters of monobenzoates.
[b] No reaction.

The alcoholysis procedure in the presence of PSL-C has been also extended to other benzoates **16b–f** (Scheme 5) and a few preliminary results have been obtained [29]. Interestingly, going from dibenzoate **16a** to longer chain **16b** and **16c** the enantioselectivity decreased only from 82 to 70%, whereas drop of enantioselectivity was more pronounced for longer alkyl chain (**16d**, 51% ee). Introduction of a chlorine as in dibenzoate **16e** caused a dramatic effect on enantioselectivity (from 82% in **16a** to 20% for **16f**) and no activity of PSL-C was observed when an aromatic ring was present in the structure, as in dibenzoate **16e** (Table 6).

Although unsatisfactory in terms of enantioselectivity, the debenzoylation by alcoholysis with MML and PCL represents the first enzymatic access to a 1,2-diol protected as benzoate at the secondary hydroxy group. This reaction proceeds with complete regioselectivity and constitutes a good alternative to the selective chemical procedures that require organotin reagents and have to be carried out under controlled conditions [28].



Scheme 7. Alcoholysis of dibenzoate **20** in DIPE catalyzed by MML or PSL-C.

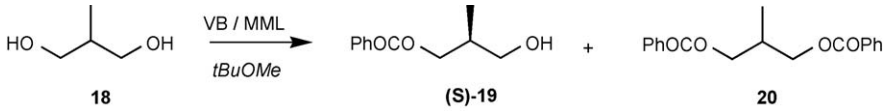
2.5. Lipase-catalyzed asymmetrization of 2-methyl-1,3-propanediol and its dibenzoate: a biocatalytic access to enantiomerically pure (R)- and (S)-2-methyl-1,3-propanediol monobenzoates

The enantiotopic asymmetrization of prochiral 1,3-diol **18** has been realized utilizing the above described MML-catalyzed benzoylation [18]. The reaction was stopped before dibenzoate **20** was formed [84% conversion to 65% ee (*S*)-monobenzoate **19** in 0.5 h] and repeated to complete disappearance of the diol **18**. In this way, 58% conversion to diester **20** was reached in 1.5 h and enantiomerically pure (*S*)-**19** was obtained in 40% yield after purification (Scheme 6).

In addition to benzoylation of 1,3-diol **18**, the deacylation of dibenzoate **20** could constitute an additional example of successful enantioselective enzymatic desymmetrization of prochiral substrates, a topic that has been exhaustively reviewed [30,31]. When the alcoholysis with 1-octanol of 2-methyl-1,3-propanediol dibenzoate **20** was carried with MML, 30% conversion was reached in 10 days and optically pure (*R*)-monobenzoate 19 (>98% ee) was obtained. This stereoisomer could be also prepared by alcoholysis of dibenzoate **20** mediated by PSL-C that afforded in 2 days (50% conversion) enantiomerically pure (*R*)-monobenzoate **19** (>98% ee, Scheme 7).

3. Conclusion

We have shown that, among various lipases, CAL and MML are the most suitable enzymes to carry out the benzoylation of 1,2-diols in organic solvents at a rate compatible with preparative applications. The enantioselectivity of the monobenzoylation is appreciable mainly for propane-1,2-diol **1a** and a further benzoylation can enhance the above stereoselection, bringing enantiomeric excess from 20 to 82%. Enzymatic benzoylation of other diols **1b–f** proceeded with low enantioselectivity that could not be improved by a further benzoylation, too slow to constitute a preparative opportunity. Nonetheless, a high degree of regioselectivity has been observed and the same observations applied to the debenzolation procedure. This alcoholysis reaction furnished most satisfactory results with PSL absorbed onto celite



Scheme 6. MML-catalyzed benzoylation of diol **18**.

that catalyzes the deacylation of propane-1,2-diol dibenzoate with good enantioselectivity (82% ee). When this debenzoylation procedure was extended to other 1,2-diol dibenzoates, stable 2-benzoates were obtained with excellent regioselectivity and low enantioselectivity. Finally, enzymatic benzoylation of 2-methyl-1,3-propanediol and the deacylation of the corresponding dibenzoate afford enantiomerically pure (*R*)- and (*S*)-monobenzoates. Both enantiomers may be important chiral building blocks derived from *meso*-2-methylpropane-1,3-diol and are now available as optically pure synthons by a new biocatalytic route. The overall picture of enzymatic benzoylation/debenzoylation procedures here outlined evidences the feasibility and limits of the biocatalytic approach. Further studies are required to investigate in more details these enzymatic reactions and to answer to questions raised by the results so far obtained.

Acknowledgement

This work has been financially supported by Università degli Studi di Milano (Fondi FIRST).

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