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Investigation of the lipase-catalysed reaction of aliphatic amines with ethyl propiolate as a route to *N*-substituted propiolamides

Simon Bonte [a], Ioana Otilia Ghinea [b], Isabelle Baussanne [a], Jean-Paul Xuereb [a,†], Rodica Dinica [c], Martine Demeunynck [a,*]

[a] Department of Pharmacomolecular Chemistry, UMR 5063 & FR 2607, CNRS, Joseph Fourier University, BP 53, 38041 Grenoble cedex 9, France
[b] Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, Department of Food Science, Food Engineering and Applied Biotechnology, 111 Domneasca Street, Galati, Romania
[c] Dunarea de Jos University of Galati, Faculty of Science and Environment, Department of Chemistry, Physics and Environment, 111 Domneasca Street, 800201 Galati, Romania

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ABSTRACT

The lipase-catalysed reaction of aliphatic amine with ethyl propiolate was investigated using benzylamine as reference amine. The conditions were optimised to favour the 1,2-addition, i.e., formation of *N*-benzylprop-2-ynamide, versus the 1,4-addition. Immobilised *Candida antarctica* lipase (CALB) was found to be the most efficient enzyme, and the reactions were performed in solvents, such as *t*BME, dioxane or toluene. The methods were used to prepare propiolamides from aliphatic amines in good to excellent yields. The reactivity of *O*- and *S*-nucleophiles was compared in the same conditions.

1. Introduction

With the development of the biocompatible click reactions, molecules containing activated electron-deficient or strain alkynes are becoming very attractive synthons to functionalise biomolecules. Propiolic acid derivatives, both esters and amides, have gained interest because of their good reactivity as dipolarophiles for metal free click chemistry[1–3] and in other cycloaddition reactions.[4] These electron-deficient alkynes are useful reactants for the Huisgen cycloaddition with azide, performed at room temperature in water and in absence of metal catalyst.[5]

Preparation of propiolic esters,[6,7] thioesters and amides generally involves the use of coupling reagents (DCC or EDC carbodiimides for examples) and the reactions proceed with low to good yields. There are only few examples of the use of propiolyl chloride, prepared from propiolic acid and PCl_5 and immediately engaged in the next reaction[8] with alcohol or amine. Propiolic anhydride, generated in situ from propiolic acid and DCC, has been reported for synthesis of propiolamides, although with moderate yields. Therefore, propiolamides are generally prepared from propiolic acid with intermediate formation of activated esters.[9] Recently, an efficient synthesis of propiolamides was reported using trimethylsilyl propiolic acid as starting material.[10,11]

In an effort to design alternative methodologies for the preparation of propiolamides, we turned our attention to the lipase-catalysed reactions. The biological role of lipases is the hydrolysis of aliphatic esters in aqueous media, but they may also efficiently catalyse the reverse reaction of esterification in non-aqueous media (organic solvents, ionic liquids). Therefore lipases were optimised to catalyse numerous reactions of industrial interest. Immobilisation of lipases confers to the enzymes a good stability that enables their use in organic solvents and temperatures (up to 60 °C for *Candida antarctica* lipase B for instance). The separation steps are also simplified thus allowing the development of continuous processes.[12,13] The enzyme-catalysed reactions of acrylate esters with amines and alcohols have been studied in detail,[13,14] but so far there are only a few reports of lipase-catalysed reactions of propiolic ester. In 1989, Gotor successfully realised the aminolysis of ethyl propiolate with aniline in the presence of *Candida cylindracea* lipase (now known as *Candida rugosa* lipase or CRL) in CCl_4.[15,16] However, the authors reported an important limitation, the aliphatic amines only giving the 1,4-addition in the same conditions. It was also mentioned that the aminolysis did not occur with porcine

* Corresponding author. Tel.: +33 (0)476635314; e-mail address: martine.demeunynck@ujf-grenoble.fr (M. Demeunynck).
† Present address: Lycée franco-libanais MLF-AEFE, Verdun Beyrouth, Lebanon.

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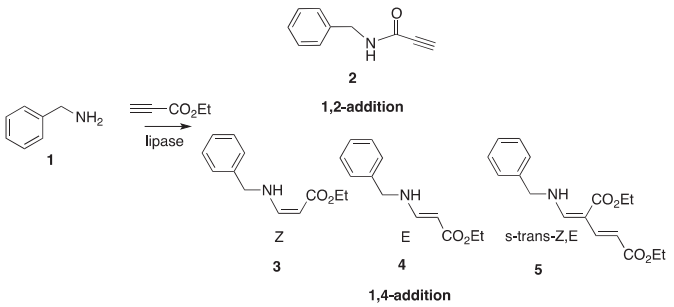
pancreatic lipase or papain. Later, the authors published the aminolysis with ammonia in dioxane catalysed by *C. antarctica* lipase.[17] When the current study was on-going, an example of transesterification of alcohols with ethyl acetylene dicarboxylate and propiolate was published by Deloisy and collaborators.[18] These reactions were catalysed by *C. rugosa* lipase (CRL) using petroleum ether as solvent.

In this paper, we report the results of our study of the lipase-catalysed reactivity of ethyl propiolate with aliphatic amines. Benzylamine was used as a typical amine. We first screened the ability of a panel of lipases to catalyse the chemoselective 1,2-addition. The reactions were performed in various organic solvents in order to select the best conditions (enzyme/solvent couple) that were then applied to the preparation in larger scale of propiolamides that will be further used as dipolarophiles in coupling reactions in our group. The chemoselectivity was also checked by comparing the reactivity with *O*- and *S*-nucleophiles.

2. Results and discussion

Using enzymes in organic solvent is a large field of research to develop alternative procedures to known organic reactions. The difficulty is the numbers of parameters that influence the chemo-, regio- and stereoselectivities, such as enzyme nature, temperature, dilution, solvent or stoichiometry. We took as starting conditions, the data reported by Gotor's[13,14] and Castillo's[19] groups for the lipase-catalysed reaction of nucleophiles (alcohols or amines) with acrylic esters. We focused our study on the influence of the nature of the solvent and of the enzyme, which clearly appeared as key factors for the selectivity.

As shown in Scheme 1, controlling the chemoselectivity of the addition of amine to ethyl propiolate is challenging as this reaction may lead to four main compounds: propiolamide **2** resulting from the 1,2-addition, *Z*- or *E*-aminoacrylates **3** and **4** resulting from the 1,4-addition, and *s-trans-Z,E*-dienamino ester **5** issued from two successive 1,4-additions.



Scheme 1. Reactivity of benzylamine **1** with ethyl propiolate **2**: structures of major reaction products.

The reactions were monitored by TLC and the product mixtures were identified by ^{1}H NMR by comparison with literature data. Relative yields in the different products were determined using the following typical ^{1}H NMR signals in $CDCl_3$ of each product: CH singlet at 2.85 ppm (4.17 ppm in DMSO-d_6) and the CH_2 doublet at 4.25 ppm for **2**; CH multiplet at 6.92–6.97 ppm, the NH multiplet at 8.13 ppm and CH_2 doublet at 4.25 ppm for **3**; CH multiplet at 7.61–7.66 ppm for **4** and CH doublet at 6.00 ppm (1H, d) and broad NH multiplet at 9.3 ppm for **5**.

In the absence of catalyst, amines are known to quickly react with ethyl propiolate in a 1,4-Michael addition.[20–22] The enaminoesters **3**/**4** have thus been prepared in excellent yields in polar solvents (H_2O, CH_3CN, MeOH, DMF) in less than 1 h at room temperature, with a *Z*/*E* ratio=2/1. At higher temperatures (over 60 °C), the *s-trans-Z,E*-dienamine **5** was isolated as the sole product.[23–25] We checked the uncatalysed reactivity of benzylamine with ethyl propiolate in the conditions that were later used in our study (stoichiometry, solvent, temperature). As shown in Table 1 (entry 27), we obtained a mixture of the enaminoesters **3** and **4** in a 67/23 *Z*/*E* ratio.

Table 1
Reaction of benzylamine with ethyl propiolate. Influence of the nature of the enzyme and of the solvent on the chemoselectivity

Entry	Lipase	Solvent	**2**	**3**	**4**	**5**
1	CRL	EOP	0	63	37	0
2	CRL	Dioxane	0	59	41	0
3	CRL	Isooctane	0	37	nd	48
4	CRL	CH_3CN	0	55	38	6
5	CRL	Toluene	20	50	30	0
6	CRL	*t*BME	7	54	37	1
7	CALB	EOP	0	25	nd	59
8	CALB	MOI	0	67	33	0
9	CALB	MOP	0	18	0	63
10	CALB	Dioxane	93	2	0	4
11	CALB	Isooctane	78	4	Traces	13
12	CALB	CH_3CN[a]	39	35	21	4
13	CALB	Toluene	93	7	0	Traces
14	CALB	*t*BME	98	2	0	0
15	PPL	EOP	2	61	36	0
16	PPL	MOI	0	59	41	0
17	PPL	MOP	0	62	37	1
18	PPL	Dioxane	6	58	35	0
19	PPL	Isooctane	0	57	39	3
20	PPL	CH_3CN	0	41	29	23
21	PPL	Toluene	0	59	41	0
22	PPL	*t*BME	8	50	35	2
23	*P. cepacia*	Dioxane	0	67	33	0
24	*P. cepacia*	*t*BME	5	56	35	4
25	LIP	Dioxane	15	51	30	3
26	LIP	*t*BME	63	19	11	5
27	No enzyme	Dioxane	0	63	27	0

Benzylamine (0.2 mmol) was added to a suspension of the chosen enzyme (80 mg) in 1 ml of the chosen solvent. The mixture was shaken for 1 min before adding ethyl propiolate (0.6 mmol). The reactions were performed in a flask gently stirred at 40 °C for 15 h otherwise mentioned. The ratios in the different compounds were determined from the ^{1}H NMR integrations.

[a] Presence of by-products suggested by a series of multiplets between 6 and 8 ppm in the ^{1}H NMR spectrum. Enzymes: *Candida rugosa* lipase (CRL), porcine pancreatic type II lipase (PPL), *Candida antarctica* lipase B (CALB), lipozyme RM (LIP) and *Pseudomonas cepacia* lipase. *t*BME=*tert*-butyl methyl ether, EOP=EOPipNTf$_2$, MOP=MOPyrroNTf$_2$ and MOI=MOImNTf$_2$.

We then performed the reaction following the conditions reported by Gotor[15,16] for the lipase-catalysed amidation of aniline with ethyl propiolate, using *C. cylindracea* lipase (now known as *C. rugosa* (CRL)) in CCl_4. We found out that CRL catalysed the amidation of benzylamine in CCl_4, but with a low conversion rate (30% after 5 h of stirring at 60 °C).

In the search of more efficient catalysts, a selection of enzymes was evaluated in different solvents. The reactions were performed at 40 °C overnight (15 h) in open round-bottom flasks, and quenched by filtration of the enzyme. The reaction mixtures were then quantified by ^{1}H NMR. The results are collected in Table 1. In all the tested conditions, the conversion was quantitative. The course of the reaction was strongly dependent of both the nature of the enzyme and of the solvent. The 1,2-addition was favoured by the use of the less polar solvents (entries 10, 13, 14 or 26), the best selectivity being obtained in toluene, dioxane or *tert*-butyl methyl ether (*t*BME). Ionic liquids only favoured 1,4-addition (entries 1, 7–9 or 15–17). From the five enzymes tested in this study, CALB emerged as the enzyme of choice with near chemoselective 1,2-addition in dioxane and *t*BME (entries 10, 13 and 14). As found earlier in CCl_4, CRL gave a low ratio of 1,2-addition but only in toluene (entry 5). Lipozyme (LIP) was also able to catalyse the