Tetrahedron 69 (2013) 5495–5500

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

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

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ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 14 April 2013
Accepted 18 April 2013
Available online 25 April 2013

Keywords:
Activated alkyne
Candida antarctica
Aminolysis
Lipase

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

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http://dx.doi.org/10.1016/j.tet.2013.04.093

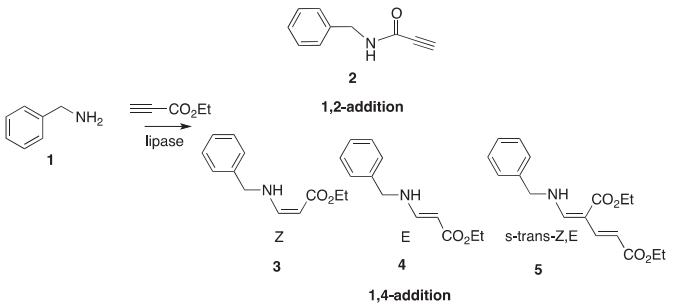
pancreatic lipase or papain. Later, the authors published the aminolysis with ammonia in dioxane catalysed by *C. antarctica* lipase.[17] When the current study was on-going, an example of transesterification of alcohols with ethyl acetylene dicarboxylate and propiolate was published by Deloisy and collaborators.[18] 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

amide formation albeit with lower selectivity compared to CALB (63% in *t*BME, see entry 26). Two enzymes, *Pseudomonas cepacia* and porcine pancreatic lipase (PPL), did not catalyse the 1,2-addition, as **2** was only obtained as traces in dioxane and *t*BME with PPL (entries 18 and 22), or in *t*BME with *P. cepacia* (entry 24).

In order to optimise the procedure, CALB was therefore selected for further reactions. As shown in Table 2, the reactions were performed in various conditions in the solvents selected above (*t*BME, dioxane or toluene). Rising temperature up to 60 °C had no significant effect on the chemoselectivity (compare entries 1 and 4). In an effort to rapidly test a variety of conditions the reactions were performed either in 1.5 ml microtubes and stirred in an orbital shaker (Thermoshaker) settled at the desired temperature, or magnetically stirred in an oil bath. Surprisingly, we observed that the stirring process had an effect on both conversion ratio and chemoselectivity. Gentle magnetic stirring (250 rpm) was the most efficient process with complete conversion after 15 h (overnight) reaction at 50 °C (entries 5, 8 and 10). With the orbital shaker, the reaction kinetics appeared slower, and did not always go to completion, even when reaction time was extended to 48 h (see, e.g., the entries 1–3). This result emphasises the need of efficient stirring process allowing contact of the reactants with the immobilised enzyme, taking into account that the uncatalysed reaction proceeds quickly in solution. The reactions worked well in the three solvents. Using dry dioxane only slightly influenced the course of the reaction in favour of the 1,2-addition (compare entries 9 and 11). Increasing the reaction time had a low effect on the amide formation, and confirmed the stability of the amide thus formed in these conditions, however, with time the enamides **3** and **4** slowly disappeared in favour of the amino-diene **5** (compare entries 6 and 7). As the uncatalysed 1,4-addition is a fast process, we thought that the order in which the reactants were added to the enzyme suspension might influence the product mixture. In our procedure the enzyme was suspended in the solvent and the first reactant, benzylamine, was added. The mixture was shaken for 1 min, before adding ethyl propiolate. In one experiment (entry 12), the ethyl propiolate was added at first to the lipase suspension and, indeed, slightly lower yield of amide **2** (compare entries 1 and 12) was obtained. At the opposite, increasing the time during which benzylamine was left in contact with the enzyme (5 min instead of 1 min) was in favour of amide formation (compare entries 1 and 13). From these experiments, we can hypothesise that the interaction of benzylamine with the enzyme decreases the amount of free amine, and therefore limits the non-catalysed 1,4-addition that quickly occurs in solution in the presence of ethyl propiolate.

The optimised procedure for propiolamide formation was therefore the CALB catalysed reaction in *t*BME or dioxane at 50 °C overnight (15 h), under magnetic stirring.

The optimised conditions were applied to the scale-up of *N*-benzyl propionamide **2** synthesis. The CALB catalysed reaction of 8.16 mmol of benzylamine with 2 equiv of ethyl propiolate in *t*BME gave the desired propionamide **2** in 67% yield as a pale yellow oil and the *s-trans-Z*,*E* adduct **5** in 15% yield as a solid. To evaluate the scope and limits of this process, the reaction was also performed at 1.5 mmolar scale with various amines (Table 3). Dioxane was chosen as solvent for its higher solubility properties.

Table 2
CALB catalysed reaction of benzylamine with ethyl propiolate: process optimisation

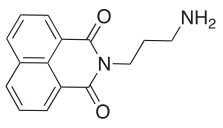
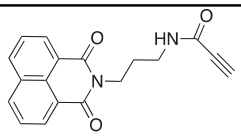
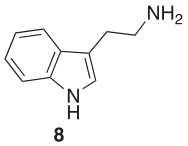
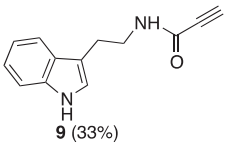
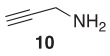
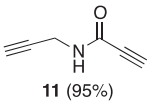
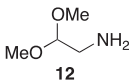
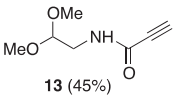
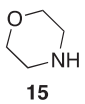
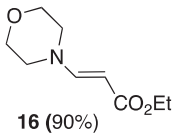
Entry	Solvent	*T* (°C)	Time (conversion)	**2**	**3**	**4**	**5**
1	*t*BME	50 °C	5 h (90%)	74	14	8	3
2	*t*BME	50 °C	24 h (89%)	83	0	0	16
3	*t*BME	50 °C	48 h (90%)	83	0	0	16
4	*t*BME	60 °C	5 h (100%)	75	11	7	5
5	*t*BME	50 °C/MS	15 h (100%)	98	0	0	2
6	Toluene	50 °C	24 h (95%)	58	17	9	nd
7	Toluene	50 °C	48 h (94%)	62	7	0	30
8	Toluene	50 °C/MS	15 h (100%)	93	7	0	0
9	Diox.	50 °C	24 h ((90%)	33	22	6	37
10	Diox.	50 °C/MS	15 h (100%)	93	2	0	4
11	Dry diox.	50 °C	24 h (100%)	53	25	11	11
12	*t*BME[a]	50 °C	5 h (90%)	66	20	12	8
13	*t*BME[b]	50 °C	5 h (90%)	90	6	3	0

Unless otherwise mentioned the reactions were performed by adding benzylamine (0.2 mmol) to the suspension of CALB (80 mg) in the solvent, the mixture was shaken for 1 min before adding ethyl propiolate (0.6 mmol). The mixtures were then kept at the chosen temperature in a thermoshaker unless otherwise mentioned (MS for magnetic stirring). The ratios in the different compounds were determined from the ^{1}H NMR integrations.

[a] Ethyl propiolate added at first to the enzyme suspension, the mixture was shaken for 1 min and then benzylamine was added.

[b] The mixture of benzylamine and CALB was shaken 5 min before adding ethyl propiolate. *t*BME=*tert*-butyl methyl ether.

Table 3
Scope of the process

Entry	Starting amines	Main reaction products (isolated yields)
1	 **6**	 **7** (65%)
2	 **8**	 **9** (33%)
3	 **10**	 **11** (95%)
4	 **12**	 **13** (45%)
5	$H_2NCH_2CO_2H$ **14**	No reaction
6	 **15**	 **16** (90%)

The reactions with CALB were performed at 1.5 mmol scale of amine in dioxane, at 50 °C overnight using 3 equivalents of ethyl propiolate.

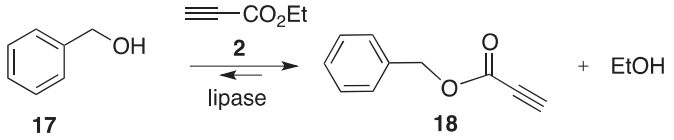
The naphthalimide derivative **7** is of interest for us, and the propiolamides **9**, **11** and **13** have been described as intermediates in the synthesis of more complex molecules.

The reaction worked well with primary amines and was compatible with the presence of larger aromatic substituent (entries 1 and 2). In the case of tryptamine (entry 2), the lower ratio of conversion was probably due to limited solubility of the starting amine that was recovered in 25% yield after treatment. Compound **11** (entry 3) that was formed in excellent yield, is of major interest as bifunctional reagent for orthogonal click chemistry. Surprisingly, no reaction occurred with the amino acid **14** (entry 5). The starting amine was recovered after washing of the supported enzyme with methanol. As exemplified by entry 6, the reaction of secondary amine only gave the 1,4-addition with formation of the *E* isomer as the major product.[26]

The chemoselectivity of CALB catalysed reactions with ethyl propiolate was further investigated by comparing the reactivity with

O- and *S*-nucleophiles, using benzyl alcohol and benzyl mercaptan, under similar conditions. A first example of enzyme-catalysed transesterification with ethyl propiolate has been recently published[18] using *C. rugosa* lipase (CRL) in petroleum ether. The present study showed that CALB also efficiently catalysed the reaction. The reaction of benzyl alcohol with ethyl propiolate was performed at 50 °C for 6 h in various solvents. As shown in Table 4, gentle magnetic stirring clearly emerged as the most effective process, with toluene as organic solvent. The alcohol-ester equilibrium may be displaced using 5 Å molecular sieves to trap ethanol,[18] but we observed that using open glassware (round-bottom flask or test tube), allowed evaporation of the ethanol released during the reaction and displaced the equilibrium in favour of ester **18**.

Table 4
CALB catalysed reaction of benzyl alcohol **17** with ethyl propiolate: influence of the reaction conditions on the ester formation



Entry	Solvent	Stirring process	**18**
1	*t*BME	TS	27
2	Toluene	Magnetic	84
3	Toluene	TS	66
4	Dioxane	Magnetic	57
5	Dioxane	TS	42
6	Acetonitrile	Magnetic	54
7	2-Me–Bu–2-OH	Magnetic	31

Reaction of benzyl alcohol **17** (0.2 mmol) with ethyl propiolate (0.6 mmol) at 50 °C for 6 h. The reactions were either performed in open vessels magnetically stirred at 250 rpm or in capped microtubes orbitally shaken at 1000 rpm in a thermoshaker (TS). The conversion ratio in ester **18** was determined from the ^{1}H NMR integrations. *t*BME=*tert*-butyl methyl ether.

The reaction was then performed on *N*-(3-hydroxypropyl) naphthalimide **19**, the hydroxy analogue of **6**, to prepare in 79% yield the ester **20** (Fig. 1).

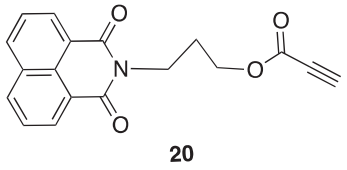
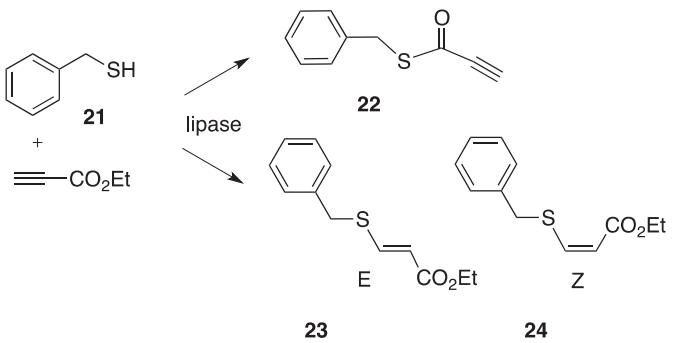


Fig. 1. Ester resulting from the reaction with *N*-(3-hydroxypropyl)naphthalimide.

Concerning the reactivity with thiol, the reaction with benzyl mercaptan **21** may lead 1,2- and 1,4-addition products (formation of **22** and **23**/**24**, respectively, see Scheme 2). Thioacrylates **23** and **24** are characterised by pairs of doublets at 6.68 and 5.79 (*J* 15 Hz) for **23** and 7.1 and 5.8 (*J* 10 Hz) for **24**, and the doublet at 3.7 ppm of the benzylic protons of thiol **21** is replaced by a singlet at 4.02 ppm. The propargylic proton of the thioester **22** is expected at 3.3 ppm. The reactions were performed in 1.5 ml microtubes to limit air oxidation of the thiol into disulphide.



Scheme 2. Reaction of benzyl mercaptan with ethyl propiolate: structures of the potential reaction products.

In the chosen conditions (Table 5), no formation of thioester **22** was observed whatever conditions were applied, the starting mercaptan **21** being mainly recovered at the end of the reaction. Note that in similar conditions, the uncatalyzed reaction readily gave a mixture of **23** and **24** (entry 7).

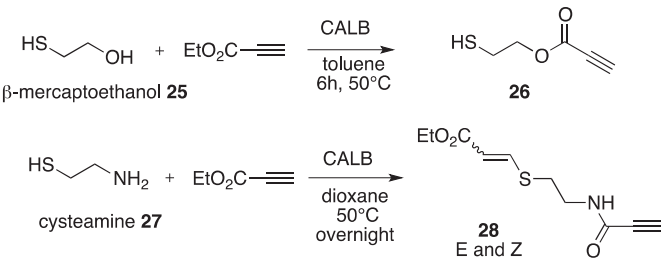
Table 5
CALB catalysed reaction of benzyl mercaptan **21** with ethyl propiolate: influence of the reaction conditions on the reaction mixture composition

Entry	Solvent	Time	**21**	**23** (*E*)	**24** (*Z*)
1	Dioxane	24 h	93	4	1
2	Dioxane	5 h	96	0	0
3	CH_3CN	24 h	94	3	2
4	CH_3CN	5 h	96	0	0
5	*t*BME	24 h	91	6	3
6	*t*BME	5 h	>99	Traces	Traces
7	*t*BME without enzyme	5 h	90	5	2

The reactions of benzyl mercaptan (0.2 mmol) with ethyl propiolate (0.6 mmol) were performed in 1.5 ml capped microtubes and the mixtures were stirred at 50 °C. The ratios in the different compounds were determined from the ^{1}H NMR integrations, traces of the corresponding disulfide (<4%) may also form.

Finally, it was appealing to try the reaction with *S*,*N*- or *S*,*O*-difunctional nucleophiles (cysteamine and β-mercaptethanol, respectively).

The reactions were achieved in round-bottom flasks in the optimised conditions selected for amidation (dioxane, 15 h) and transesterification (toluene, 6 h). The crude residues obtained after filtration of the enzyme and evaporation of the solvents, were analyzed by ^{1}H NMR. As depicted in Scheme 3, the reaction with β-mercaptoethanol **25** gave a major product that was identified as resulting from the selective O-esterification. The CH_2O of β-mercaptoethanol is characterised by a triplet at 3.75 ppm, had shifted to 4.35 ppm. The CH_2SH signals, a quintuplet at 2.73 ppm (CH_2) and a triplet at 1.45 ppm (SH) in the starting material, appeared at 2.83 ppm and 1.59 ppm. A singlet at 2.97 ppm, integrating for 1 proton, confirmed the presence of propiolic CH. Any attempt to purify the mixture only yielded to the decomposition of **26** with increased formation of by-products. Nevertheless this method allows the formation of compound **26** that cannot be prepared by usual chemical methodologies without protection of the thiol function. And indeed, the uncatalysed reaction gives a 9/1 mixture of *Z*- and *E*-ethyl 3-(2-hydroxyethylthio)acrylates resulting from the 1,4-addition of the thiol group.[22]



Scheme 3.

The reaction with cysteamine gave a mixture of two major products that could not be separated by chromatography. They were identified as the *E* and *Z* isomers of compound **28** resulting from the reaction of cysteamine with two molecules of ethyl propiolate, i.e., 1,2-addition of the amino group to one ethyl propiolate and 1,4-addition of the thiol group to a second molecule of ethyl propiolate. The ^{1}H NMR data were compared to those of similar compounds.[27,28] The cysteamine SH (1.5 ppm) is not present and the CH_2N signal (triplet at 2.69 ppm for the starting cysteamine) now appeared downfield as a multiplet centred at 3.60 ppm. Two CH_2S triplets can be found at 2.98 and 3.05 ppm (with a 1/0.7 ratio), the ethyl ester groups are observed as multiplets centred at 1.33 and 4.22 ppm. The presence of characteristic doublets of 1,4-addition of the thiol to the triple bond are observed at 5.89 and 7.64 ppm (*E* isomer, *J* 15 Hz) and 5.95 and 7.07 ppm (*Z* isomer, *J* 10 Hz), with a *E*/*Z* ratio=1/0.7. The formation of the propiolamide bond is indicated by the presence of a large signal at 6.56 ppm and two singlets at 2.88 and 2.89 ppm with a 1/0.7 ratio for the *E* and *Z* isomers. The difference in reactivity of the thiol group can be attributed to the existence of the cysteamine as internal salt ($^-SCH_2CH_2NH_3^+$) that greatly increases the nucleophilic properties against triple bond.[27]

3. Conclusion

In the search of efficient route to variously *N*-substituted propiolamides as activated alkynes, we have optimised the lipase biocatalysed addition of benzylamine to ethyl propiolate. Immobilised *C. antarctica* lipase (CALB) emerged as the enzyme of choice to favour the chemoselective 1,2-addition, using *t*BME, dioxane or toluene under overnight (15 h) gentle magnetic stirring at 50 °C. In these conditions, propiolamide **2** was prepared in gram scale in good yield, *s-trans-Z*,*E*-dienamine **5** being formed as by-products. The reactions worked well with other primary amines but not with secondary amines that only gave the corresponding aminoacrylates.

The chemoselectivity of CALB with *N*- and *S*-nucleophiles was also checked. The transesterification, preparation of propiolic ester, also worked in good yields in *t*BME, toluene or dioxane. The best yields (near quantitative) were observed when the reactions were carried out in open vessels under gentle magnetic stirring at 50 °C for 6 h. The transesterification was chemoselective in presence of thiols, are indicated by the formation of 2-sulfanylethyl prop-2-ynoate **18** from β-mercaptoethanol.

4. Experimental section

4.1. General

Melting points were determined using a Reicher Thermovar apparatus and are uncorrected. NMR spectra were recorded on Bruker Avance 400 spectrometer using the solvent as the internal reference ($CDCl_3$ at 7.24 ppm); the chemical shifts are reported in parts per million, in δ units.

Ethyl propiolate, benzylamine, benzyl alcohol and benzyl mercaptan purchased from Sigma Aldrich were used without further purifications. Ionic liquids, $EOPipNTf_2$ (EOP), $MOPyrroNTf_2$ (MOP) and $MOImNTf_2$ (MOI) were prepared by Nathalie Kardos from Université de Savoie (France).

C. rugosa (also called *cylindracea*) lipase (CRL) (700 U/mg solid) and type II lipase crude from porcine pancreas (PPL) (190 U/mg protein) were purchased from Sigma Chemical Co.; *C. antarctica* lipase B (CALB) Novozyme 435 (7400 PLU/g) and lipase immobilised from *Rhizomucor miehei* (Lipozyme RM IM) (LIP) (7800 U/g) were generous gifts of Novozymes A/S. Biochemika lipase immobilised in Sol-Gel-AK from *P. cepacia* lipase (40 U/g).

4.2. Enzyme screening procedure

The reactions were performed in triplicate in 1.5 ml microtubes or in 5-ml test tubes containing a small stirring bar. The nucleophile (benzyl alcohol, thiol or amine, 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 (ultrasound stirring) before addition of ethyl propiolate (11 μl, 0.6 mmol). The capped microtubes were orbitally shaken at 1000 rpm in a thermoshaker (TS) at the chosen temperature for the desired time. The open test tubes were magnetically stirred at 250 rpm at the chosen temperature for the desired time. The suspension were then filtered off to remove the enzyme, the solid was washed twice with EtOH and Et_2O and the solvents were evaporated under reduce pressure. The residues were then diluted with DMSO-d_6 or $CDCl_3$ for NMR analysis.

4.3. Applications to the preparation of *N*-substituted propiolamides

*4.3.1. N-Benzylprop-2-ynamide **2**.* Benzylamine (1 ml, 9.16 mmol) was added to a suspension of the CALB (2 g) in *t*BME (10 ml) in a round-bottom flask. The mixture was mixed for 1 min (ultrasound stirring) before addition of ethyl propiolate (1.85 ml, 18.3 mmol). The mixture was gently stirred (250 rpm) overnight at 50 °C. The suspension was then filtered to remove the enzyme, the solid was washed twice with CH_2Cl_2 and the solvents were evaporated under reduced pressure. Methanol was added to the oily residue. The white powder was filtered and dried to give *s-trans-Z*,*E* compound **5** (422 mg, 1.39 mmol) in 15% yield. The filtrate was evaporated to dryness to afford an oily residue that was triturated in diethyl ether to give **2** (980 mg, 6.1 mmol) as a white powder in 67% yield.

The ^{1}H NMR spectra of **2**[9] and **5**[24] were conformed to the literature data:

Compound **2**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.30–7.44 (m, 5H); 6.33 (br s, 1H); 4.18 (d, 2H, *J* 8 Hz); 2.85 (s, 1H).

Compound **5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 9.25 (br s, 1H); 7.28–7.44 (m, 7H); 6.09 (d, 1H, *J* 15.7 Hz); 4.41 (d, 2H, *J* 5.9 Hz), 4.30 (q, 2H, *J* 7.1 Hz); 4.23 (q, 2H, *J* 7.1 Hz); 1.40 (t, 3H, *J* 7.1 Hz); 1.32 (t, 3H, *J* 7.1 Hz).

*4.3.2. N-[(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-propyl]-propen-2-ynamide **7**.* 3-(1,3-Dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-propyl amine **6**[29] (400 mg, 1.57 mmol) was dissolved in dioxane (15 ml) in a round-bottom flask. CALB (1.6 g) was added, and the mixture was shaken 1 min before adding ethyl propiolate **2** (443 μl, 4.37 mmol). The mixture was gently stirred (250 rpm) at the 50 °C overnight. The suspension was then filtered to remove the enzyme, the solid was washed twice with CH_2Cl_2 and the solvents were evaporated under reduced pressure. A methanol–1 N HCl mixture (1/1) was added to the residue. The solid part was filtered, washed with water and dried. Crystallisation from methanol afforded compound **7** (611 mg, 1.02 mmol) as a pale yellow solid in 65% yield.

Mp 177–178 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.65 (dd, 2H, *J* 7.4, 1.0 Hz); 8.29 (dd, 2H, *J* 8.3, 1.0 Hz); 7.82 (dd, 2H, *J* 8.3, 7.4 Hz); 7.05 (br s, 1H, NH); 4.32 (t, 2H, *J* 6.1 Hz); 3.36 (m, 2H); 2.04 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 164.7, 152.2, 134.3, 131.6, 131.5, 128.2, 127.1, 122.4, 73.0, 37.3, 36.4, 27.7.

*4.3.3. N-[2-(1H-Indol-3-yl)ethyl]prop-2-ynamide **9**.* The reaction was performed as described for **7**, starting from tryptamine **8** (240 mg, 1.5 mmol), ethyl propiolate (443 μl, 4.37 mmol), CALB (960 mg) in dioxane (9 ml). After filtration and evaporation of the solvent, CH_2Cl_2 was added to the residue. The insoluble part was filtered off and identified as the starting tryptamine (60 mg, 25%).

The propiolamide **9** was isolated in 33% yield (105 mg, 0.49 mmol) after column chromatography (elution: CH_2Cl_2/diethyl ether 5/1).

The ^{1}H NMR was conformed to the literature data:[30] ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 10.82 (br s, 1H), 8.35 (br s, 1H), 7.52 (d, 1H, *J* 7.7 Hz), 7.34 (d, 1H, *J* 7.7 Hz), 7.16 (s, 1H), 7.07 (t, 1H, *J* 7.7 Hz), 6.99 (t, 1H, *J* 7.7 Hz), 4.10 (s, 1H), 3.31–3.40 (m, 2H), 2.83–2.86 (m, 2H).

*4.3.4. N-(Prop-2-yn-1-yl)prop-2-ynamide **11**.* The reaction was performed as described for **7**, starting from propargylamine **10** (32 μl, 1.5 mmol), ethyl propiolate (443 μl, 4.37 mmol), CALB (130 mg) in dioxane (2 ml). The propiolamide **11** was obtained as an oil in 95% yield (414 mg, 1.42 mmol).

The ^{1}H NMR was conformed to the literature data:[31] ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.18 (br s, 1H), 4.09 (dd, J 5.2, 2.4 Hz), 2.84 (s, 1H), 2.28 (t, 1H, *J* 2.4 Hz).

*4.3.5. N-(2,2-Diethoxyethyl)prop-2-ynamide **13**.* The reaction was performed as described for **7**, starting from 2,2-diethoxyethylamine **12** (163 μl, 1.5 mmol), ethyl propiolate (443 μl, 4.37 mmol), CALB (600 mg) in dioxane (6 ml). The propiolamide **13** was isolated in 45% yield after column chromatography (elution: pentane/diethyl ether).

The ^{1}H NMR was conformed to the literature data:[32] ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 6.20 (br s, 1H), 4.44 (t, 1H, *J* 5.6 Hz), 3.47–3.50 (m, 2H), 3.44 (s, 6H), 2.86 (s, 1H).

*4.3.6. Ethyl (E)-3-(morpholin-4-yl)acrylate **16**.* The reaction was performed as for **7** starting from morpholine **15** (132 μl, 1.5 mmol), ethyl propiolate (443 μl, 4.37 mmol), CALB (440 mg) in dioxane (5 ml). Compound **16** was isolated as an oil (235 mg, 1.32 mmol) after removal of the enzyme and evaporation of the solvent.

The ^{1}H NMR was conformed to the literature data:[26] ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.38 (d, 1H, *J* 13.0 Hz), 4.72 (d, 1H, *J* 13.0 Hz), 4.16 (q, 2H, J 7.1 Hz), 3.72–3.74 (m, 4H), 3.20–3.25 (m, 4H), 1.28 (t, 2H, *J* 7.1 Hz).

*4.3.7. Benzyl prop-2-ynoate **18**.* CALB (80 mg) was added to a solution of benzyl alcohol **17** (21.6 mg, 0.2 mmol), dissolved in toluene (1 ml) in a round-bottom flask. The solution was vigorously shaken and then ethyl propiolate **2** (60 μl, 0.6 mmol) was added. The mixture was magnetically stirred at 50 °C for 6 h in an open round flask. The immobilised enzyme was filtered off, washed with ethanol and the organic solvents were evaporated under reduced pressure. The resulting oil was crystallised in methanol to afford **18** (27 mg, 0.17 mmol) as a white powder in 85% yield.

The ^{1}H NMR was conformed to the literature data:[33] ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.30–7.44 (m, 5H); 5.22 (s, 2H); 2.89 (s, 1H).

*4.3.8. 3-[(1,3-Dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)-propyl]-prop-2-ynoate **20**.* CALB (2.10 g) was added to a solution of 3-(1,3-dioxo-1*H*-benzo[*de*]isoquinolin-2(3*H*)-yl)-propan-1-ol[34] **19** (700 mg, 2.74 mmol) dissolved in toluene (15 ml) in a round-bottom flask. The solution was vigorously shaken and then ethyl propiolate **2** (557 μl, 5.5 mmol) was added. The mixture was magnetically stirred at 50 °C for 18 h in an open vessel. The immobilised enzyme was filtered off, washed with ethanol and the organic solvents were evaporated under reduced pressure. The resulting oil was crystallised in methanol to afford **20** (667 mg, 2.17 mmol) as a white powder in 79% yield.

Mp 123–124 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.63 (dd, 2H, *J* 7.4, 1.0 Hz); 8.25 (dd, 2H, *J* 8.3, 1.0 Hz); 7.79 (dd, 2H, *J* 8.3, 7.4 Hz); 4.35–4.38 (m, 4H); 2.83 (s, 1H); 2.21 (quint, 2H, *J* 7.6 Hz): ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 164.1, 152.6, 134.0, 131.5, 131.3, 128.1, 127.0, 122.4, 74.6, 64.3, 37.3, 27.1.

*4.3.9. 2-Sulfanylethyl prop-2-ynoate **26**.* The reaction was performed in toluene as described for **18**, starting from β-mercaptoethanol **25** (210 μl, 3 mmol), CALB (900 mg) (10 ml) and ethyl propiolate (912 μl, 9 mmol). The reaction was stirred at 50 °C for 6 h. After filtration of the enzyme, and evaporation, the crude residue (239 mg) was analysed by NMR.

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 4.35 (t. 2H, *J* 6.7 Hz); 2.97 (s, 1H, CH); 2.83 (m, 2H); 1.59 (t, 1H, *J* 8.6 Hz).

*4.3.10. Ethyl 3-[(2-(prop-2-ynamido)-ethyl)sulfanyl]acrylate. Mixture of E and Z isomers **28**.* The reaction was performed in dioxane (5 ml) as described for **7**, starting from cysteamine **27** (115 mg, 1.5 mmol), CALB (400 mg) and ethyl propiolate (440 μl, 3.7 mmol).

After filtration of the enzyme, and evaporation, the crude residue was analysed by NMR and correspond to a 1/0.7 mixture of *Z*/*E* isomers.

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.64 (d, 1H, *J* 15.0 Hz), 7.07 (d, 0.7H, *J* 10.0 Hz), 6.57 (br s, 1.5 H), 5.95 (d, 0.7H, *J* 10.0 Hz), 5.90 (d, 1H, *J* 15.0 Hz), 4.19–4.22 (m, 3.4H), 3.57–3.54 (m, 3.4H), 3.05 (t, 2H, *J* 6.8 Hz), 2.98 (t, 1.4H, *J* 6.8 Hz), 2.89 (s, 1H), 2.88 (s, 0.7H), 1.30–1.34 (m, 5.1H).

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCE-2011-3-0226 and IOG was financed by the Project SOP HRD – TOP ACADEMIC 76822. Dr. Luc Choisnard from our department (DPM) is gratefully acknowledged for his suggestions and discussions on biocatalysis by lipases.

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