Study of the Chemoselectivity in the Aminolysis Reaction of Methyl Acrylate Catalysed by Lipase B from *Candida antarctica*

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Abstract: The aminolysis reaction of methyl acrylate (MA) with *N*,*N*-dimethyl-1,3-propanediamine (DMAPA) in organic solvents has been optimised using lipase B from *Candida antarctica* (CAL-B) as biocatalyst. A kinetic study about the influence of the reactant concentrations, organic solvent, temperature and enzyme form has been developed focused on minimising the formation of the Michael addition products. The economic efficiency of this process has been finally investigated by reusing the enzyme in the best reaction conditions, thereby observing no significant loss of activity after three reaction cycles.

Keywords: amides; aminolysis; *Candida antarctica* lipase B; green chemistry; Michael addition

Introduction

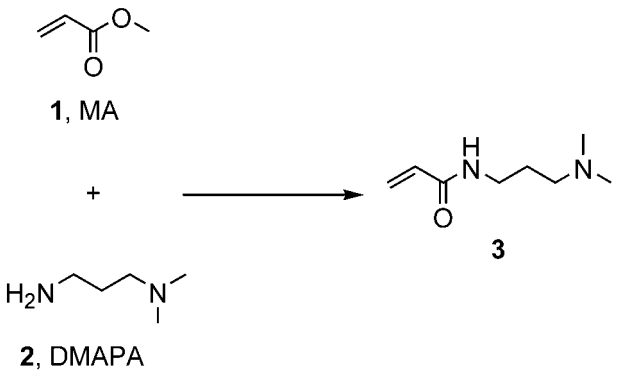
Compounds containing amides frequently show important biological activities[1] and industrial applications[2] and, consequently, their syntheses have been extensively studied over the years.[3] Although the conversion of esters to amides is a useful synthetic operation, it usually presents some disadvantages in terms of drastic reaction conditions, long reaction times or strong alkali metal as catalyst, which usually are not compatible with sensitive functionalities. Biocatalysis offers a clean and ecological way to perform chemical processes under mild reaction conditions and with high degrees of selectivity and thus, it could be an interesting alternative to provide this type of substrates.

The use of lipases to generate amide bonds in organic solvents has been extensively studied over the last decades since Klibanov[4] and Wong[5] applied the aminolysis reaction in peptide synthesis.[6] However, the first example of a lipase-catalysed aminolysis reaction was reported by our group in the reaction of racemic ethyl 2-chloropropionate with different aliphatic and aromatic amines.[7] From that moment, the aminolysis reaction has been recognised as a useful tool for the synthesis of interesting nitrogenated compounds.[8]

We report here a comprehensive study of all possible parameters affecting the reaction between methyl acrylate (MA, **1**) and *N*,*N*-dimethyl-1,3-propanediamine (DMAPA, **2**) focused on the enzymatic synthesis of *N*-(4-methyl-4-azapentyl)acrylamide (**3**), an important building block in the preparation of polymers (Scheme 1). Currently, the chemical synthesis of **3** requires the use of copper catalysts and high temperatures (over 210 °C) so the application of an enzyme could dramatically decrease both the contamination factor and the waste of energy.[9] Consequently, biocatalysis applied to this process could provide a sustainable alternative to a chemical reaction of industrial interest.[10]

Results and Discussion

In the reaction between **1** and **2**, four reaction products can be observed depending on the reaction conditions,



Scheme 1. Synthesis of *N*-(4-methyl-4-azapentyl)acrylamide (**3**).

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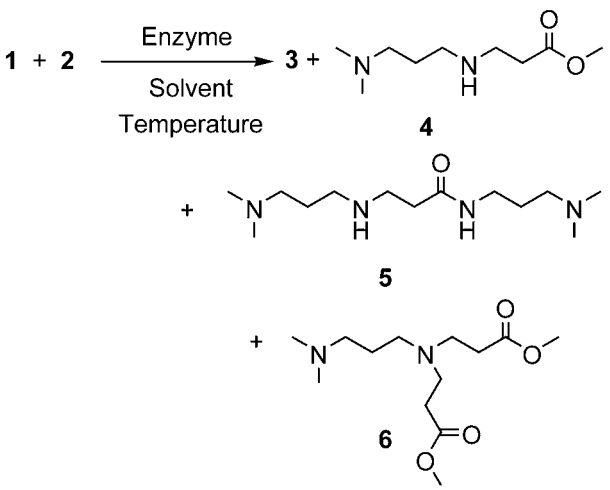
acrylamide **3** for the aminolysis reaction between **1** and **2**, Michael adduct **4** due to Michael addition of **2** to **1**, Michael-amide compound **5** for firstly Michael reaction of **1** and **2** and subsequent aminolysis reaction of **2** and **4**, and di-Michael product **6** by a double Michael reaction, between **1** and **2** to form **4** and a subsequent Michael addition of **2** to **4** (Scheme 2). The formation of Michael adducts in enzymatic processes catalysed by *Candida antarctica* lipase B has been previously reported by our group using secondary amines and acrylonitrile[11] and other authors using thiols and α,β-unsaturated carbonyl compounds.[12] Different parameters have been studied in order to optimise the formation of **3**, such as enzyme, organic solvent, ester and amine concentrations, amine addition form, temperature, amount of biocatalyst and enzyme recycling.



Scheme 2. Study of the enzymatic process between MA (**1**) and DMAPA (**2**): considered parameters and possible products of the reaction.

Influence of the Enzyme and its Preparation

In our experience, lipase B from *Candida antarctica* (CAL-B) is by far the best biocatalyst for the preparation of acrylamides,[13] so an inspection of the effect of polymer support was studied using 1,4-dioxane as solvent. An immobilised catalyst (Novozyme 435), supported (Chirazyme), lyophilised, denatured from Novozyme species, and *Pseudomonas cepacia* lipase (PSL) were considered in this study (Table 1). CAL-B Novozyme (entry 2) presented the highest reaction rate in the formation of **3** while other enzymatic preparations favoured the Michael addition, forming preferably **4** (entries 3–9); in fact, **3** was not observed in some cases. The use of other forms of the biocatalyst such as CAL-B (Chirazyme) did not result in a better selectivity (entry 11) and even other biocatalysts like PSL also favoured the formation of **4** (entries 12 and 13).

Lyophilised CAL-B (entry 3) catalysed 7 times faster the Michael addition than in the absence of catalyst (entry 1). Moreover, the support seemed not to have any in-

Table 1. Kinetics of **3** and **4** after 1 h of reaction between 10% (v/v) of **1** and 2.5% (v/v) of **2** using 50 mg of enzyme in 1,4-dioxane.

Entry	Enzyme	T [°C]	mmol of **3**	mmol of **4**	Ratio of **3/4**	V^0 (**3**)[a]	V^0 (**4**)[a]
1	–	30	0.000	0.012	0.000	0.0	0.2
2	CAL-B (Novozyme)	30	0.229	0.043	5.381	5.3	0.7
3	CAL-B (lyophilised)	30	0.002	0.089	0.020	0.0	1.4
4	CAL-B (denatured)[b]	30	0.000	0.028	0.000	0.0	0.5
5	CAL-B 1[c]	30	0.009	0.160	0.058	0.2	4.9
6	CAL-B 2[d]	30	0.000	0.048	0.000	0.0	0.6
7	CAL-B 3[e]	30	0.053	0.080	0.658	1.5	2.8
8	CAL-B 4[f]	30	0.040	0.139	0.289	0.6	4.0
9	BSA[g]	30	0.000	0.020	0.000	0.0	0.2
10	–	40	0.000	0.009	0.032	0.0	0.2
11	CAL-B (Chirazyme)	40	0.200	0.059	3.416	4.3	0.9
12	PSL[h]	40	0.001	0.038	0.015	0.0	0.6
13	PSL-C[i]	40	0.034	0.109	0.309	0.6	1.8

[a] Initial rate; units: mM min^{-1}.
[b] CAL-B denatured in refluxing water during 10 h.
[c] CAL-B supported over glyoxyl-Agarose polymer.
[d] CAL-B supported over glyoxyl-Sepabeads polymer.
[e] CAL-B supported over C18-Sepabeads polymer.
[f] CAL-B supported over glutaraldehyde-Sepabeads polymer.
[g] Bovine serum albumin.
[h] *Pseudomonas cepacia* lipase (lyophilised).
[i] *Pseudomonas cepacia* lipase (supported over polymer).

fluence on the formation of **4** as a hydrophobic support such as C18-Sepabeads (entry 7) does not diminish its rate of formation, so the main influence on the reaction rate was the presence of enzyme in the reaction media. To clearly show if catalysis of the Michael addition occurs in the lipase active site or on the enzyme surface, CAL-B was substituted by bovine serum albumin (BSA), a protein which lacks active sites but has a similar structure to the lipase. Assays with this protein (entry 9) showed similar results to that obtained without biocatalyst. Moreover, performing the reaction with lyophilised CAL-B also supported our proposal, as the Michael addition is faster than without any catalyst (compare entries 1 and 3) or in the presence of BSA (compare entries 3 and 9).

Taking all this into account, we concluded that the lipase is able to catalyse the Michael addition reaction and we suggest that the polymer support can affect the enzymatic activity, as very usually observed in biotransformations. Besides, it is clear that the best preparation of CAL-B for aminolysis reaction is Novozyme 435.

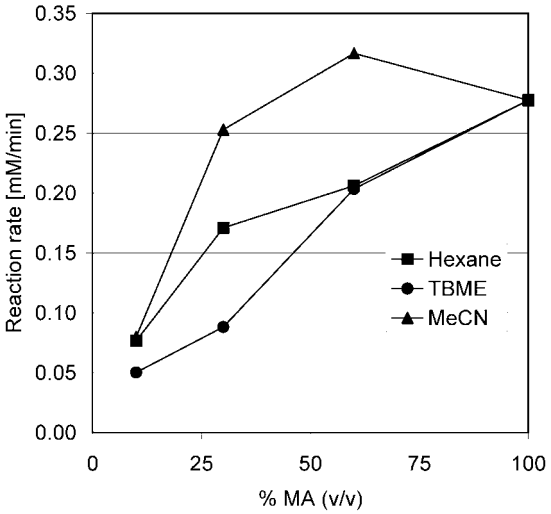


Figure 1. Reaction rates in the Michael addition catalysed by CAL-B at different concentrations of MA in different organic solvents at 30 °C.

Influence of the Organic Solvent

Even in the absence of biocatalyst, polar solvents favoured the formation of the Michael adduct as observed in the comparative study between hexane, *tert*-butyl methyl ether (TBME) and acetonitrile (MeCN) represented in the Figure 1. Small differences were observed in the reactions between hexane and TBME; however, a more polar solvent like MeCN increased the reaction rate in the addition of DMAPA to methyl acrylate.[14]

To extend the analysis to a wide range of solvents in the presence of enzyme, the amounts of **3** and **4** formed were quantified in the reaction using **1** and **2**, both at a 2.5% (v/v) concentration (Table 2). After usual work-up, an extra wash of the solid support with MeOH was done to assure that no products remained trapped on the catalyst surface. Best ratios corresponded to the process occurring in 1,4-dioxane which completely dissolved the reactants and the products of the reaction, as none of them were observed after an additional wash with MeOH (entry 1). Non-polar solvents as Et_2O (entry 5) or hexane (entry 8) presented problems due to the low solubility of the reactants, so high concentrations of products were obtained after washing with MeOH.

Influence of the Ester and Amine Concentrations

As mentioned before, CAL-B is able to promote both Michael addition and aminolysis reaction, with the li-

Table 2. Influence of the organic solvent in the addition of MA to DMAPA (2.5% v/v) at 30 °C using 50 mg of CAL-B and 1 mL of solvent.

Entry	Solvent	Initial rate[a]			Conversion [%] after 1 h[b]				After wash with MeOH[c]	
		V^0 **3**	V^0 **4**	V^0 **3**/V^0 **4**	**2**	**3**	**4**	**3/4**	**3** [%]	**4** [%]
1	MeCN	0.9	1.0	0.9	51.24	25.26	21.50	1.17	1	1
2	1,4-Dioxane	2.2	0.3	7.3	38.95	53.57	6.20	8.64	–	0.8
3	THF	1.4	0.4	3.5	41.84	36.57	11.59	3.16	8	2
4	CH_2Cl_2	0.5	0.4	1.3	68.93	15.65	14.42	1.09	0.6	0.4
5	Et_2O	2.0	0.4	5.0	2.54	74.85	13.61	5.50	8	1
6	TBME	1.5	0.5	3.0	47.94	36.60	10.46	3.50	5	–
7	Toluene	1.7	0.6	2.8	39.88	44.92	13.20	3.40	2	–
8	Hexane	1.2	0.5	2.4	15.13	29.86	8.03	3.72	47	–
9	1,4-Dioxane-toluene (1:1)	2.4	0.5	4.8	36.88	51.45	8.67	5.93	3	–

[a] Units: mM min^{-1}.
[b] Measured by GC and referred to initial amine concentration (0.2 M).
[c] Measured by GC considering that all amine reacted as 100%.

pase being more efficient for the aminolysis process. Additionally, Michael addition also occurs without the participation of the biocatalyst. Each process has a different rate constant, depending on many parameters. In a first approach, we decided to minimise the non-catalysed chemical reaction in order to obtain higher yields of **3**. With this in mind, the effect on the reaction rate was studied at different concentrations of ester and amine.

Ester Concentration

Firstly, the kinetics of the formation of **4** was studied in the chemical reaction at different concentrations of **1** using limonene as internal standard (Figure 2). As expected, reactions at high concentrations favoured the formation of the Michael adduct in the absence of enzyme.

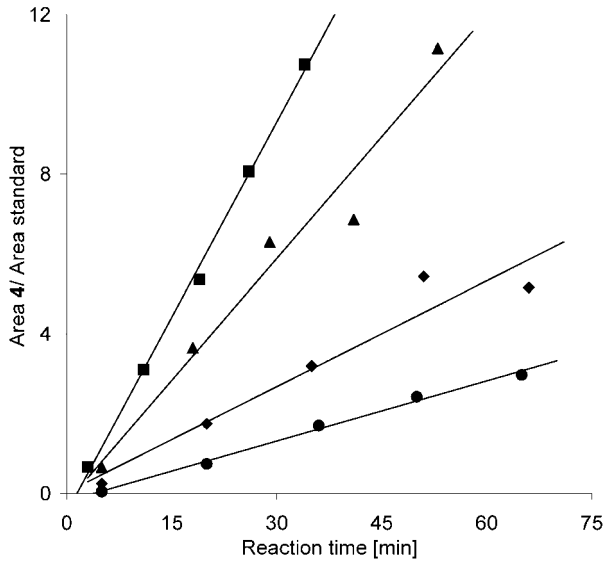


Figure 2. Michael adduct formation *versus* time course at different concentrations of MA in TBME at 30 °C (■, 100%; ▲, 60%; ◆, 30%; ●, 10%).

Using 1,4-dioxane, the most suitable solvent for the enzymatic reaction, the kinetics in the formation of all possible products were studied modifying the amount of CAL-B (Table 3). Reaction with 10% of MA led to the best results as the amide was obtained as the major product (entries 1, 5, and 7) and high concentrations of MA led to the increase of the Michael adduct obtaining high proportions of **4** when 100% of MA was used (entry 4). Low concentrations of amine led to a complete reaction with MA, which made it impossible to calculate the kinetics at short reaction times. This high reactivity also meant that the formation of the Michael amide product **5** was appreciable. An increase in the amount of CAL-B allowed the formation of slightly higher concentrations of the desired product (entries 5 and 7).

Amine Concentration

At this point we considered it important to increase simultaneously the amount of amine and ester (Table 4) and, as expected, high concentrations of reactants promoted a drop in the selectivity of the process. Best conditions for the formation of the amide were obtained at low concentrations of amine and ester which minimise the formation of Michael adducts (entry 1). A lack of selectivity was observed at 10% of MA and DMAPA, especially when small amounts of enzyme were used (entries 3, 5, and 7).

Form of Addition of Amine

To improve the ratio between **3** and **4** in the formation of the amide, the rate of the addition of DMAPA was considered (Table 5). Good selectivity was achieved when the addition was done by continuous flow addition compared with the reaction with the amine present since the start of the reaction (entries 1 and 2); in fact, better results were obtained if the addition was done during longer times (entry 3).

Table 3. Reactions with varying amounts of CAL-B and concentrations of **1** in 1,4-dioxane at 30 °C.

Entry	CAL-B [mg]	[MA] [%]	Conversion [%][a]				Rate [$mM \cdot min^{-1}$]	
			3	**4**	**5**	**6**	**3**	**4+5+6**
1	50	10	67.62	25.44	6.52	0.42	6.55	5
2	50	30	62.61	34.45	1.33	1.61	11.6	4.7
3	50	60	50.38	45.55	0.60	3.46	n.m.[b]	n.m.[b]
4	50	100	40.78	44.38	0.25	14.59	n.m.[b]	n.m.[b]
5	80	10	69.78	23.29	6.52	0.41	11.5	6.9
6	80	30	58.43	39.33	1.36	0.88	n.m.[b]	n.m.[b]
7	100	10	71.64	22.35	5.60	0.41	n.m.[b]	n.m.[b]
8	100	30	66.12	32.14	0.95	0.79	n.m.[b]	n.m.[b]

[a] Conversion once the amine had completely disappeared, measured by GC.
[b] n.m.: not measured.

Table 4. Reactions with varying amounts of CAL-B, **1**, and **2** in 1,4-dioxane at 30 °C.

Entry	CAL-B [mg]	[**1**] and [**2**] [%]	Conversion [%][a]				Rate [mM·min^{-1}]	
			3	**4**	**5**	**6**	**3**	**4+5+6**
1	50	2.5	90.40	1.92	7.68	0.00	2.0	0.3
2	50	5	72.01	9.18	18.42	0.38	4.2	2.3
3	50	10	40.75	42.26	15.35	1.65	8.6	18.3
4	100	5	70.03	10.05	18.80	0.29	4.4	1.9
5	100	10	45.94	37.06	15.33	1.17	14.5	19.6
6	150	5	80.92	5.47	13.47	0.15	10.3	3.5
7	150	10	50.48	31.62	16.83	1.07	24.0	31.5

[a] Conversion once that amine had completely disappeared, measured by GC.

Table 5. Study of the addition of **2** in portions in the reaction with **1** (10% v/v) using 150 mg of CAL-B in 1,4-dioxane at 30 °C.

Entry	Addition of **2** [mL/min]	Addition time [h]	Conversion [%][a]			
			3	**4**	**5**	**6**
1	–	–	50.48	31.62	16.83	1.07
2	0.0004	5	65.16	14.21	6.90	5.02
3	0.0002	9	72.68	17.53	2.96	3.88

[a] Conversion once the amine had completely disappeared, measured by GC.

Influence of the Temperature

Temperature is a key factor in all biocatalytic processes as high temperatures usually allow high conversions, generally in spite of a loss in the selectivity. The reactions were studied in the presence of CAL-B over a range of temperatures between 10 and 60 °C, and the courses of the reactions are represented against the ratio of products **3**/**4** (Figure 3). Formation of **3** was slightly favoured at higher temperatures up to 45 °C, while the reaction conducted at 60 °C showed a decrease in the selectivity of the process, probably due to thermal denaturation of the enzyme.

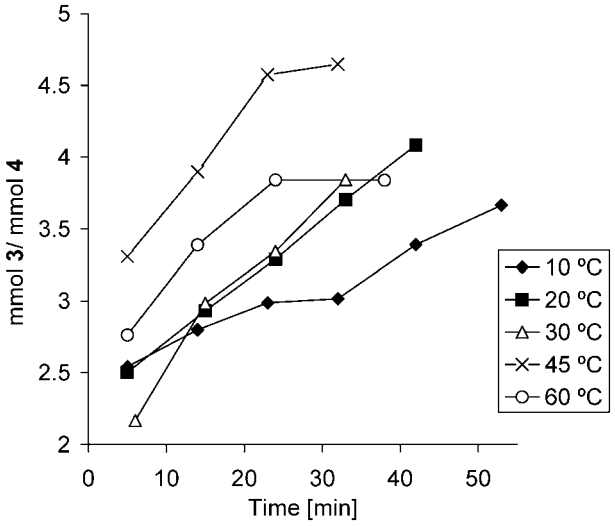


Figure 3. Ratio of acrylamide **3** and Michael product **4** during the time course of the reaction between **1** (10% v/v) and **2** (2.5% v/v) in 1,4-dioxane at different temperatures.

Amount of Biocatalyst

It is known that CAL-B catalyses both aminolysis and the Michael addition reactions, however, which is the more favoured enzymatic process? This study was done in 1,4-dioxane as solvent and using 2.5% (v/v) of MA and DMAPA with different amounts of enzyme (5, 10, 20, 35, 50, 80, and 150 mg) by measuring the conversion and reaction rate of all possible products at 75 min (Table 6). More catalyst implied a major selectivity in the process directed towards the formation of the amide product.

The ratio acrylamide/all possible Michael products was measured along the time course, and it remained constant with respect to the conversion except in the 150 mg reaction (entry 7) where the ratio significantly increased (Figure 4). An increase in the amount of enzyme directed the process towards the faster formation of acrylamide **3**, the rate of formation of compound **3** increasing from 0.1 to 2.2 (Table 6), in this manner lower amounts of starting materials are present in the media and the Michael addition is less favoured along the time course. Another key point is that *Candida antarctica* lipase B is not able to catalyse the formation of compound **5** from **3** through an enzymatic Michael reaction, rather compound **4** reacts with **2** to form **5** through an aminolysis process. This has been checked in independent experiments (data not shown).

Table 6. Influence of the amount of CAL-B used in the reaction between **1** and **2** (2.5% v/v) in 1,4-dioxane at 30 °C after 75 min.

Entry	CAL-B [mg]	Conversion after 75 min [%][a]				Rate [mM·min^{-1}]		
		2	**3**	**4**	**5**	**3**	**4**	**5**
1	5	89.54	5.03	5.35	0.08	0.1	0.2	0.0
2	10	73.20	15.70	10.15	0.95	0.5	0.3	0.0
3	20	50.13	33.27	12.50	4.10	1.0	0.3	0.2
4	35	43.77	45.83	7.32	3.08	1.2	0.2	0.1
5	50	41.73	50.37	5.37	2.55	1.3	0.1	0.1
6	80	21.28	70.39	4.41	3.92	1.7	0.1	0.1
7	150	8.74	87.01	1.40	2.85	2.2	0.0	0.1

[a] Measured by GC and referred to the initial amount of amine (0.2 M).

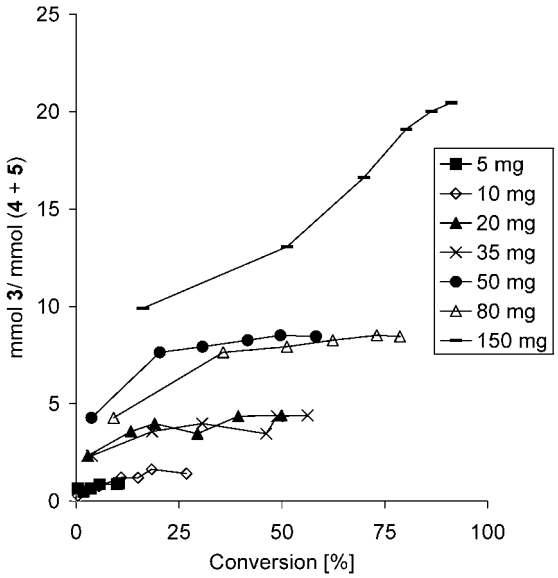


Figure 4. Ratio of acrylamide **3** and Michael products (**4** and **5**) versus the percent conversion in the reaction between **1** (2.5% v/v) and **2** (2.5% v/v) in 1,4-dioxane at 30 °C using different amounts of CAL-B.

Study of Enzyme Recycling

One of the main advantages of supported lipases is their easy recovery by simple filtration, thus they can be reused without any significant loss of activity. To take advantage of this, the reaction of MA and DMAPA catalysed by CAL-B was repeated four times until complete disappearance of the amine, while quantifying the amount of acrylamide obtained by simple work-up (Table 7). Under these conditions CAL-B maintained its activity during three cycles without any significant decrease.

Table 7. Study of enzyme recycling in the reaction between **1** and **2** (10% v/v) using 150 mg of CAL-B in 1,4-dioxane at 30 °C.

Entry	Cycle	*t* [min]	Conversion of **3** [%][a]
1	1	60	47.56
2	2	60	47.30
3	3	80	46.39
4	4	80	37.42

[a] Calculated by GC.

Conclusion

The reaction between methyl acrylate and *N*,*N*-dimethyl-1,3-propanediamine catalysed by CAL-B has been studied in order to optimise the formation of the corresponding acrylamide. Non-polar solvents, low concentrations of reactants, and temperatures around 45 °C favoured the formation of the amide with respect to the Michael addition product. Also, slow addition of the amine increased dramatically the formation of the acrylamide. The potential of CAL-B has been demonstrated for the synthesis of acrylamides avoiding the use of extreme temperatures and the copper catalyst required for the conventional chemical synthesis.

Experimental Section

General remarks

Candida antarctica lipase B, CAL-B Novozyme 435 (7300 U/g), was a gift from Novo Nordisk co. CAL-B Chirazyme L-2 ($\geq$200 U/g) is commercially available from Roche Diagnostics, lyophilised CAL-B (590 U/g) was purchased from Fluka. *Pseudomonas cepacia* lipase in lyophilised (PSL, 30000 U/g) and immobilised (PSL-C, 783 U/g) forms are marketed by Amano Pharmaceuticals. CAL-B preparations 1–4 were a gift from Professor Guisán (CSIC Biocatalysis Institute, Madrid). Chemical reagents obtained from Aldrich, Fluka, Lancaster or Prolabo. Solvents were distilled over an appropriate desiccant under nitrogen. Gas chromatography (GC) was car-

ried out with flame ionisation detection (FID) and an HP-1 capillary column (25 m × 0.2 mm × 0.2 μm) coated with methylsilicone gum using nitrogen as carrier gas. In this method the injector and the detector were set at 150 °C and 280 °C, respectively, initial column temperature was 60 °C increasing the temperature by 20 °C/min until 260 °C; retention times: **2** at 1.4 min, **3** at 4.4 min, **4** at 4.6 min, **5** at 8.9 min, and **6** at 7.4 min. ^{1}H NMR, ^{13}C NMR and DEPT spectra were recorded in Bruker AC-200, Bruker AC-300 or Bruker AC-300 DPX spectrometers using $CDCl_3$ as solvent. The chemical shift values (δ) are given in ppm and the coupling constants (*J*) are given in Hz. The positive electrospray ionisation (ESI^+) mode was used to record mass spectra on a Hewlett-Packard 110 Series spectrometer. Microanalyses were performed on a Perkin-Elmer model 2400 instrument.

Influence of the Enzyme and its Polymer Support (Table 1)

0.9 mL of 1,4-dioxane and 0.1 mL of MA were mixed in the presence of 50 mg of enzyme (if necessary) and shaken at 30 °C and 250 rpm. After 5 min, 25 μL of an internal standard solution formed by 203.9 mg of limonene and 5 mL of DMAPA were added. Aliquots were taken every 10 min and injected in the GC.

Influence of the Organic Solvent (Figure 1)

Solutions of MA (concentrations 10, 30, 60, or 100% v/v) in 1 mL of solvent (hexane, TBME, and MeCN) were shaken at 250 rpm and 30 °C during 5 min. After that time, 50 μL of an internal standard solution formed from 202.3 mg of limonene and 5 mL of DMAPA were added. Aliquots were taken every 5 or 10 min and injected in the GC.

Influence of the Organic Solvent (Table 2)

To 50 mg of CAL-B (Novozyme 435) 25 μL of MA and 1 mL of solvent were added. The mixture was shaken at 30 °C and 250 rpm during 5 min, then 25 μL of an internal standard solution formed from 203.9 mg of limonene and 5 mL of DMAPA were added, and every 10 min aliquots were taken and injected in the GC. After 1 h, the enzyme was filtered off and washed with 2 × 0.5 mL of MeOH. One μL of the filtered solution was also injected in the GC.

Influence of the Ester Concentration (Figure 2)

Solutions of MA at concentrations 10, 30, 60, and 100% (v/v) in 1 mL of TBME, were shaken at 250 rpm and 30 °C during 5 min. After that time, 50 μL of an internal standard solution formed from 202.3 mg of limonene and 5 mL of DMAPA were added, and every 5 or 10 min aliquots were taken and injected in the GC.

Influence of the Ester Concentration (Table 3)

Solutions of MA at concentrations 10, 30, 60, and 100% (v/v) in 1,4-dioxane were shaken at 250 rpm and 30 °C during 5 min in the presence of CAL-B (Novozyme 435). After that time 50 μL of an internal standard solution formed from 203.6 mg of limonene and 5 mL of DMAPA were added, and every 10 min aliquots were taken and injected in the GC until complete consumption of the amine.

Influence of the Amine Concentration (Table 4)

Mixtures with the same volumes (25, 50 or 100 μL) of MA and an internal standard solution forming from 203.6 mg of limonene and 5 mL of DMAPA were added to a total volume of 1 mL and different amounts of CAL-B (Novozyme 435). Mixtures were shaken at 250 rpm and 30 °C until complete consumption of the amine, and aliquots were taken every 10 min and injected in the GC.

Influence of the Form of Addition of Amine (Table 5)

A mixture of 150 mg of CAL-B (Novozyme 435), 0.1 mL of MA and 0.8 mL of an internal standard solution formed from 19.8 mg of limonene in 10 mL of dioxane was magnetically stirred in a round-bottom flask and heated at 30 °C. After 10 min, the addition of DMAPA (100 μL at 0.0004 mL/min during 5 h or 0.0002 mL/min during 9 h) was started. Once the amine was completely added, aliquots were taken and injected in the GC until complete consumption of the amine.

Influence of the Temperature (Figure 3)

A mixture containing 50 mg of CAL-B (Novozyme 435) and 25 μL of MA dissolved in 1,4-dioxane was shaken at 250 rpm during 5 min. Then, 25 μL of an internal standard solution formed from 203.9 mg of limonene and 5 mL of DMAPA were added. Aliquots were taken every 10 min and injected in the GC.

Influence of the Amount of Biocatalyst (Table 6 and Figure 4)

A mixture of 25 μL of MA, different amounts of CAL-B (Novozyme 435) in 1 mL of 1,4-dioxane was shaken at 30 °C and 250 rpm during 5 min. Then 25 μL of an internal standard solution formed from 203.6 mg of limonene and 5 mL of DMAPA were added and aliquots were taken every 10 min and injected in the GC.

Study of Enzyme Recycling (Table 7)

A mixture of 150 mg of CAL-B, 0.1 mL of MA, 0.8 mL of 1,4-dioxane and 100 μL of an internal standard solution formed from 412.9 mg of limonene and 10 mL of DMAPA was shaken at 30 °C and 250 rpm. Consumption of amine was followed by injecting aliquots in the GC. Once the amine had completely reacted, the enzyme was filtered and washed with MeOH.

This enzyme was recovered for further use under identical reaction conditions.

Characterisation of Products

***N*-(4-Methyl-4-azapentyl)acrylamide (3):** Colourless liquid; IR (film): $\nu = 1653$ (C=O), 3076 (C=C), 3277 cm^{-1} (NH); ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 7.41$ (br s, 1H, NH), 6.20 (dd, 1H, $H1_{trans}$, $J = 17.1$ Hz, 1.8 Hz), 6.06 (dd, 1H, H2, $J = 17.1$ Hz, 10.0 Hz), 5.58 (dd, 1H, $H1_{cis}$, $J = 10.0$ Hz, 1.8 Hz), 3.41 (m, 2H, H3), 2.39 (t, 2H, H5, $J = 6.4$ Hz), 2.23 (s, 6H, $2NCH_3$), 1.68 (m, 2H, H4); ^{13}C NMR ($CDCl_3$, 75.5 MHz): $\delta = 165.4$ (C=O), 131.4 (C2), 125.2 (C1), 58.7 (C3), 45.3 ($2NCH_3$), 39.5 (C5), 25.7 (C4); MS (ESI^+): $m/z = 179$ [$(M+Na)^+$, 100%]; anal. calcd. for $C_8H_{16}N_2O$: C 61.51; H 10.32, N 17.93; found: C 61.4, H 10.4, N 17.9.

8-Methyl-4,8-diazamethyl nonanoate (4): Colourless liquid; IR (film): $\nu = 1734$ (C=O), 3318 cm^{-1} (NH); ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 3.58$ (s, 3H, OCH_3), 2.76 (t, 2H, H4, $J = 6.5$ Hz), 2.53 (t, 2H, H3, $J = 7.1$ Hz), 2.40 (t, 2H, H6, $J = 6.6$ Hz), 2.19 (t, 2H, H2, $J = 7.1$ Hz), 2.09 (s, 6H, $2NCH_3$), 1.53 (m, 2H, H5); ^{13}C NMR ($CDCl_3$, 75.5 MHz): $\delta = 172.9$ (C=O), 57.6 (OCH_3), 51.2 (C4), 47.8 (C3), 45.2 ($2NCH_3$), 44.8 (C6), 34.2 (C2), 27.7 (C5); MS (ESI^+): $m/z = 189$ [$(M+H)^+$, 100%], 211 [$(M+Na)^+$, 30%]; anal. calcd. for $C_9H_{20}N_2O_2$: C 57.42; H 10.71, N 14.88; found: C 57.5, H 10.8, N 14.7.

***N*-(4-Methyl-4-azapentyl)-8-methyl-4,8-diazanonanamide (5):** Green oil; IR (film): $\nu = 1653$ (C=O), 3282 cm^{-1} (NH); ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 7.90$ (br s, 1H, NH), 3.32 (m, 2H, H6), 2.90 (t, 2H, H4, $J = 6.1$ Hz), 2.71 (t, 2H, H3, $J = 6.9$ Hz), 2.32–2.41 (m, 6H, H1 + H5 + H8), 2.24 (s, 6H, $2NCH_3$), 2.23 (s, 6H, $2NCH_3$), 1.62–1.74 (m, 4H, H2 + H7); ^{13}C NMR ($CDCl_3$, 75.5 MHz): $\delta = 172.1$ (2C=O), 58.1 (C6), 58.0 (C4), 48.1 (C3), 45.6 (C5), 45.5 (2C, $2NCH_3$), 45.4 (2C, $2NCH_3$), 38.3 (C8), 35.6 (C1), 27.4 (C2), 26.8 (C7); MS (ESI^+): $m/z = 259$ [$(M+H)^+$, 100%], 281 [$(M+Na)^+$, 25%]; anal. calcd. for $C_{13}H_{30}N_4O$: C 60.41; H 11.71, N 21.69; found: C 60.3, H 11.9, N 21.6.

***N*-4-(4-Methyl-4-azapentyl)iminodimethyl dipropanoate (6):** Orange liquid; IR (film): $\nu = 1734$ cm^{-1} (C=O); ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 3.64$ (s, 6H, $2OCH_3$), 2.72 (t, 4H, H4 + H4′, $J = 7.0$ Hz), 2.43 (m, 8H, H1 + H3 + H5 + H5′), 2.36 (s, 6H, $2NCH_3$), 1.68 (m, 2H, H2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): $\delta = 172.9$ (2C=O), 57.1 ($2OCH_3$), 51.4 (C4 + C4′), 49.0 (C1 + C3), 44.7 (2C, $2NCH_3$), 32.3 (C5 + C5′), 24.3 (C2); MS (ESI^+): $m/z = 275$ [$(M+H)^+$, 100%], 297 [$(M+Na)^+$, 90%]; anal. calcd. for $C_{13}H_{26}N_2O_4$: C 56.90; H 9.56, N 10.21; found: C 56.8, H 9.6, N 10.1.

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References and Notes

[1] For example: a) V. Gududuru, E. Hurh, G. G. Durgam, S. S. Hong, V. M. Sardar, H. Xu, J. T. Dalton, D. D. Miller, *Bioorg. Med. Chem.* **2004**, *14*, 4919–4923; b) D. X. Sun, L. Liu, B. Heinz, A. Kolykhalov, J. Lamar, R. B. Johnson, Q. M. Wang, Y. Yip, S.-H. Chen, *Bioorg. Med. Chem.* **2004**, *14*, 4333–4338; c) M. Guzmán, J. Lo Verme, J. Fu, F. Oveisi, C. Blázquez, D. Piomelli, *J. Biol. Chem.* **2004**, *279*, 27849–27854.

[2] For example: a) O. Marder, F. Albericio, *Chim. Oggi*, **2003**, *21*, 35–40; b) K. M. Hosamani, R. S. Pattanashettar, *Ind. Eng. Chem. Res.* **2004**, *43*, 4979–4999.

[3] a) G. M. Copola, H. F. Shuster, *Asymmetric Synthesis. Construction of Chiral Molecules using Amino Acids*, Wiley, New York, **1987**; b) B. Alcaide, P. Almendros, C. Aragoncillo, *Chem. Eur. J.* **2002**, *8*, 3646–3652; c) S. Tosaki, H. Yoshihiro, T. Nemoto, T. Ohsima, M. Shibasaki, *Chem. Eur. J.* **2004**, *10*, 1527–1544.

[4] A. M. Klibanov, *Trends Biochem. Sci.* **1989**, *14*, 141.

[5] C. H. Wong, *Science* **1989**, *244*, 1145.

[6] C. D. Reeve, R. A. Holt, S. R. Rigby, K. Hazell, *Chim. Oggi* **2001**, *19*, 31.

[7] V. Gotor, R. Brieva, F. Rebolledo, *Tetrahedron Lett.* **1988**, *29*, 6973.

[8] a) V. Gotor, *Bioorg. Med. Chem.* **1999**, ***7***, 2189–2197; b) F. van Rantwijk, M. A. P. J. Hacking, R. A. Sheldon, *Monatsh. Chem.* **2000**, *131*, 549–569; c) N. Aoyagi, T. Izumi, *Tetrahedron Lett.* **2002**, *43*, 5529–5531; d) C. Bozzo, M. D. Pujol, X. Solans, M. Font-Bardia, *Tetrahedron*, **2003**, *59*, 1227–1236; d) M. Fernández-Pérez, C. Otero, *Enz. Microb. Technol.* **2003**, *33*, 650–660; e) F. van Rantwijk, R. A. Sheldon, *Tetrahedron* **2004**, *60*, 501–509; f) I. Alfonso, V. Gotor, *Chem. Soc. Rev.* **2004**, *33*, 201–209.

[9] a) P. T. Anastas, J. B. Zimmerman, *Environ. Sci. Technol.* **2003**, *37*, 95A-101A; b) G. Centi, S. Perathoner, *Catal. Today* **2003**, 287–297.

[10] a) U. Kragl, M. Eckstein, N. Kaftzik, *Curr. Opin. Biotechnol.* **2002**, *13*, 565–571; b) S. Park, R. J. Kazlauskas, *Curr. Opin. Biotechnol.* **2003**, *14*, 432–437; c) R. A. Sheldon, F. van Rantwijk, *Aust. J. Chem.* **2004**, *57*, 281–289.

[11] O. Torre, I. Alfonso, V Gotor, *Chem. Commun.* **2004**, 1724–1725.

[12] P. Carlqvist, M. Svendehal, C. Branneby, K. Hult, T. Brinck, P. Berglund, *ChemBioChem* **2005**, *6*, 331–336.

[13] a) M. Quirós, V. M. Sánchez, R. Brieva, F. Rebolledo, V. Gotor, *Tetrahedron: Asymmetry* **1993**, *4*, 1105; b) S. Puertas, R. Brieva, F. Rebolledo, V. Gotor, *Tetrahedron* **1993**, *49*, 4007; c) V. Gotor, E. Menéndez, Z. Mouloungui, A. Gaset, *J. Chem. Soc. Perkin Trans. 1* **1993**, 2453.

[14] For example: a) G. Simonian, N. M. Beylerian, *Oxid. Commun.* **2003**, *26*, 485–491; b) P. Kotrusz, S. Toma, H.-G. Schmalz, A. Adler, *Eur. J. Org. Chem.* **2004**, 1577–1583; c) Y. Cai, X.-F. Sun, N. Wang, X.-F. Lin, *Synthesis* **2004**, 671–674; d) X. Zheng, Y. Zhang, *J. Chem. Res. (S)* **2004**, 412–413; e) S. B. Tsogoeva, S. B. Jagtap, Z. A. Ardemasova, V. N. Kalikhevich, *Eur. J. Org. Chem.* **2004**, 4014–4019.

