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Lipase-Catalyzed Aza-Michael Reaction on Acrylate Derivatives

Peter Steunenberg,[†] Maarten Sijm,[†] Han Zuilhof,[†,§] Johan P. M. Sanders,[‡] Elinor L. Scott,[‡] and Maurice C. R. Franssen*[,†]

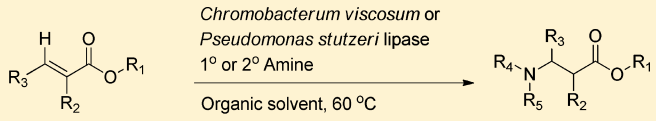
[†]Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

[‡]Biobased Commodity Chemicals, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands

[§]Department of Chemical and Materials Engineering, King Abdulaziz University, Jeddah, Saudi Arabia

Supporting Information

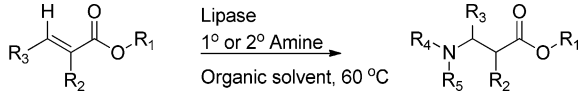
ABSTRACT: A methodology has been developed for an efficient and selective lipase-catalyzed aza-Michael reaction of various amines (primary and secondary) with a series of acrylates and alkylacrylates. Reaction parameters were tuned, and under the optimal conditions it was found that *Pseudomonas stutzeri* lipase and *Chromobacterium viscosum* lipase showed the highest selectivity for the aza-Michael addition to substituted alkyl acrylates. For the first time also, some CLEAs were examined that showed a comparable or higher selectivity and yield than the free enzymes and other formulations.



INTRODUCTION

Since the pioneering work of Klibanov in the 1980s, enzymatic reactions in organic media have attracted a lot of attention because of their enhanced space−time yields, lack of side reactions, and enhanced enantioselectivities.[1−5] Lipase B from *Candida antarctica* (Cal B) is usually taken as the enzyme for use in organic media. This is due to its high stability, activity, and wide range of substrates and media that can be applied.[6−8] This high tolerance for organic media is not uncommon, as some enzymes, like native lipases, are active at aqueous−organic interfaces to hydrolyze fats.[9,10] Several reactions have been reported to be catalyzed by Cal B,[11−13] including the Michael addition, an unexpected reaction for hydrolytic enzymes. The Michael reaction or Michael addition (1,4-addition) is a nucleophilic addition of a nucleophile to an α,β-unsaturated carbonyl compound. In the case of a nitrogen nucleophile, the reaction is called an aza-Michael addition (see Scheme 1).

Scheme 1. General Reaction Scheme for Lipase-Catalyzed Aza-Michael Reactions[a]



[a]R1: Me, Et, Bu, t-Bu; R2: H, Me; R3: H, Me, Ph; R4/R5: aromatic, aliphatic, etc.

The first example of a Michael addition in organic media catalyzed by enzymes was reported in 1988 by Kitazume et al.[14] Later, lipase-catalyzed Michael-type addition of various primary and secondary amines to acrylonitrile were reported.[15,16] Different preparations of CalB led to the Michael adduct, with Chirazyme L-2 showing the highest reaction rate,[15] while Novozyme 435 gave the best results for primary amines.[16] For the Cal B-catalyzed aza-Michael addition of imidazoles to acrylic monomers, the most efficient hydrolase was found to be lipase M.[17] The influence of solvents was examined, and it was found that solvents with a higher log *P* value led to higher conversions.

Since lipases are hydrolytic enzymes, Michael addition can compete with aminolysis of the ester. The chemoselectivity of these types of enzyme-catalyzed reactions was studied in more detail by Gotor et al.,[18] focusing on minimizing the amount of formation of the Michael addition products. Conversely, other studies tried to optimize the formation of the 1,4-addition product.[19−21] It was demonstrated that the polarity of the reaction media has an influence on the chemoselectivity between Michael addition and aminolysis.[19]

Recently, Baldessari et al.[22] have shown that Lipozyme RM IM catalyzes the formation of the Michael adduct of benzylamine to ethyl and butyl acrylate. However, lipases like Cal B and the ones from *Pseudomonas cepacia* and *Candida rugosa* formed considerable amounts of the aminolysis product.[23] Lipozyme RM IM was found to catalyze the addition of alkylamines and alkanolamines to unsubstituted acrylates chemoselectively. These *N*-substituted β-amino acid esters containing a free hydroxyl or amine group are potential monomers in the synthesis of polyesters.

It has been shown previously that depending on the substrate concentration, solvent, and choice of enzyme applied, either an aminolysis reaction or an aza-Michael addition between ethyl acrylate or *N*-methyl-1,3-diaminopropane occurs.[24] These acrylamides or aminoesters were later converted by enzymatic polymerization into polyamidoamines (PAMAMS), an interesting class of polymers for biomedical applications.[24] The enzymes have a dual behavior, because the enzymatic reaction

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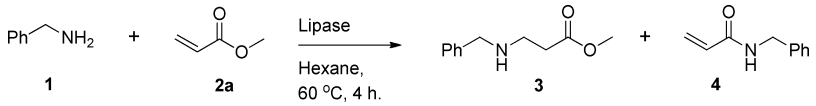
Table 1. Screening of Lipases for the Chemoselective Addition of Benzylamine 1 to Acrylate 3



entry	enzyme[a]	conversion (%)[b,c]	conversion vs blank (%)[b,d]	activity (kU/g)	1,4-addition **3** (%)	aminolysis **4** (%)
1	Chirazyme L-1 carrier-fixed lyophilized	77	35	235	96	4
2	Chirazyme L-5 carrier-fixed lyophilized	63	21	13	97	3
3	Chirazyme L-9 c.f. C2 lyophilized	62	21	80	98	2
4	Chirazyme L-9 c.f. lyophilized	83	42	66	91	9
5	*Candida antarctica* lipase on acrylic resin/Novozyme 435	>99	59	14	87	13
6, 7	Lipase N	61	20	80	98	2
8	Lipase AP6	61	20	60	96	4
9	Lipase G	52	11	50	100	0
10	Lipase PS	68	27	30	97	3
11	Lipase AH	54	13	12	100	0
12	Lipase R-10	58	17	4	100	0
13	Lipase PS-800	48	7	840	100	0
14	Lipase AK	76	35	20	96	4
15	Lipozyme TL IM	87	46	22	90	10
16	Lipozyme RM IM	72	31	3	97	3
17	Lipoprotein Lipase Amano 100s	62	21	30	100	0
18	*Aspergillus niger* lipase	53	13	3	100	0
19	*Chromobacterium viscosum* lipase (CvL)	78	37	42	96	4
20	*Pseudomonas stutzeri* Lipase (PSL)	81	40	17	97	3
21	CLEA *Candida antarctica* lipaseB	99	58	17	90	10
22	CLEA *Pseudomonas stutzeri* lipase (PSL)	85	44	17	100	0
23	CLEA *Thermomyces lanuginosa* lipase (TL) ST	77	36	510	97	3
24	CLEA *Thermomyces lanuginosa* lipase (TL) OM1	68	27	580	96	4
25	CLEA *Thermomyces lanuginosa* lipase (TL) OM2	95	54	530	97	3
26	CLEA *Rhizomucor miehei* lipase (RM) ST	58[e]	17	67	99	1
27	blank	41			100	0

[a]Names as bought from supplier, literature name can vary. [b]Based on GC−MS analysis. [c]Reaction conditions: 0.1 mmol of benzylamine, 0.1 mmol of methyl acrylate, 5 mg of enzyme, and 0.3 mL of hexane were added to a sealed vessel and stirred at 60 °C for 4 h. [d]Blank: conversion under same conditions without enzyme, conversion vs blank shows the conversion realized by the addition of the enzyme. [e]Conversion after 4 h.

of ethyl acrylate and several alkanolamines gives solely the product of aminolysis.[25]

A series of interesting β-amino acids can be produced by the enzymatic aza-Michael addition of various aliphatic and aromatic amines to acrylates and methyl-substituted derivatives. β-Amino acids are a useful class of compounds that can serve as synthetic precursors for many bioactive compounds. β-Amino acid esters are used as versatile building blocks in the production of polyamides and for the synthesis of linear cationic polymers used as gene delivery vectors. In addition, an enzymatic process has been used for the synthesis of enantiomerically pure (S)-3-aminobutanoic acid.[26,27] The reaction steps in this process comprise an initial enzyme (Novozyme 435) catalyzed aza-Michael addition, followed by enzymatic resolution via aminolysis. After hydrolysis and removal of the *N*-benzyl moiety, the desired (S)-3-amino-butanoic acid was obtained.

The class of enzymes used for the catalyzed aza-Michael addition is lipase. Lipases are among the most important enzymes in terms of applications in current industry/biotechnology.[28−31] Lipases can be applied in reactions in different formulations, like free enzymes or immobilized on beads, e.g., Novozyme 435, i.e., CalB absorbed to acrylic beads. Another formulation that shows interesting properties are cross-linked enzyme aggregates (CLEAs).[32−35] CLEAs are prepared by precipitation of the enzymes, which are then reacted with a bifunctional agent (such as glutaraldehyde) to form a cross-linked gel.[33] The only aza-Michael additions known to us, which were catalyzed by a CLEA, were described by Gotor-Fernandez et al.[36] They have shown that the Alcalase CLEA from *Bacillus licheniformis* can be used for an enzymatic aza-Michael addition of secondary amines to acrylonitrile.

Up to now, the best results for the aza-Michael addition of various amines to acrylates were obtained with Novozyme 435 or Lipozyme RM IM. The main drawback of these biocatalysts is that for the addition of the amines to substituted acrylates (such as crotonate or methacrylate) low chemoselectivity was observed and the product of aminolysis was obtained in considerable amounts. In this paper, we have screened a broad range of lipases for the aza-Michael addition to various acrylates. We were able to select an enzyme that even for the substituted acrylates shows high conversion and selectivity. Using a large array of primary and secondary amines we have produced the corresponding Michael adducts in good yields.

RESULTS AND DISCUSSION

An initial screening of various enzymes for the addition of various amines to acrylates was performed. The standard reaction that was used is the aza-Michael addition of benzylamine **1** to methyl acrylate **2a**. The results of this screening are reported in Table 1.

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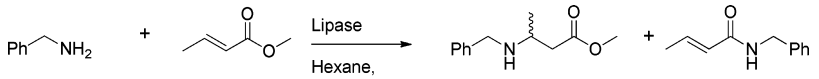
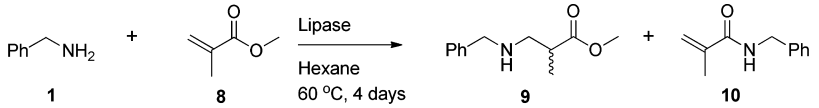
Table 2. Screening of Lipases for Chemoselective Addition of Benzylamine (1) to Methyl Crotonate (5a)



entry	enzyme	conversion (%)[a,b]	conversion vs blank (%)[a,c]	activity (kU/g)	1,4-addition **6** (%)	aminolysis **7** (%)
1	Chirazyme L-1 carrier-fixed lyophilized	69	35	235	95	5
2	Chirazyme L-9 carrier-fixed lyophilized	62	28	80	100	0
3	*Candida antarctica* lipase B on acrylic resin (Novozyme 435)	79	45	14	90	10
4	Lipase PS	48	14	30	96	4
5	Lipase PS-800	83	49	840	97	3
6	Lipase AK	68	32	20	97	3
7	Lipozyme TL IM	66	32	22	76	24
8	Lipozyme RM IM	50	14	3	95	5
9	*Chromobacterium viscosum* lipase (CvL)	69	35	42	99	1
10	*Pseudomonas stutzeri* lipase (PSL)	74	40	17	95	5
11	CLEA *Candida antarctica* lipase B	84	50	17	85	15
12	CLEA *Pseudomonas stutzeri* lipase (PSL)	76	42	17	98	2
13	CLEA *Thermomyces lanuginosa* lipase (TL) OM2	68	34	530	97	3
14	CLEA *Rhizomucor miehei* lipase (RM)	36	0	67	100	0
15	blank	34			100	0

[a]Based on GC–MS analysis. [b]Reaction conditions: 0.1 mmol of benzylamine, 0.1 mmol of methyl crotonate, 5 mg of enzyme, and 0.3 mL of hexane were added to a sealed vessel and stirred at 60 °C for 3 days. [c]Blank: conversion under same conditions without enzyme, conversion vs blank shows the conversion realized by the addition of the enzyme.

Table 3. Screening of Lipases for Chemoselective Addition of Benzylamine 1 to Methyl Methacrylate 8



entry	enzymes	conversion (%)[a,b]	conversion vs blank (%)[a,c]	activity (kU/g)	1,4-addition **9** (%)	aminolysis **10** (%)
1	Chirazyme L-1 carrier fixed lyophilized	47	39	235	100	0
2	Chirazyme L-9 c.f. lyophilized	49	40	13	100	0
3	*Candida antarctica* lipase on acrylic resin/Novozyme 435	80	71	14	82	18
4	Lipase PS-800	69	61	840	100	0
5	Lipase AK	46	37	20	94	6
6	Lipozyme TL IM	26	17	22	100	0
7	*Lipozyme* RM IM	32	23	3	100	0
8	*Chromobacterium viscosum* lipase (CvL)	61	0	42	100	0
9	*Pseudomonas stutzeri* lipase (PSL)	80	71	17	100	0
10	CLEA *Candida antarctica* lipase B	81	72	17	80	20
11	*CLEA Pseudomonas stutzeri* lipase (PSL)	84	75	17	100	0
12	CLEA *Thermomyces lanuginosa* lipase (TL) OM2	81	72	530	100	0
13	CLEA *Rhizomucor miehei* lipase (RM)	10	0	67	100	0
14	blank	9			100	0

[a]Based on GC–MS analysis. [b]Reaction conditions: 0.1 mmol of benzylamine, 0.1 mmol of methyl methacrylate, 5 mg of enzyme, and 0.3 mL of hexane were added to a sealed vessel and stirred at 60 °C for 4 days. [c]Blank: conversion under same conditions without enzyme, conversion vs blank shows the conversion realized by the addition of the enzyme.

It was observed that indeed all lipases do catalyze this reaction, although their catalytic effect can vary. The chemoselectivity of most enzymes is strongly toward the 1,4-addition product, indicating that a fully chemoselective reaction might be feasible. As can be seen from Table 1, *Candida antarctica* lipase B (Cal B), formulated as Novozyme 435, shows the highest conversion after 4 h (>99%). However, a drawback of Novozyme 435 as a catalyst for these aza-Michael reactions is that the selectivity is relatively low, 87:13 for 1,4-addition/aminolysis (entry 5). A higher selectivity was obtained for lipases from *Chromobacterium viscosum* (CvL) (96:4; entry 18) and *Pseudomonas stutzeri* (PSL) (97:3; entry 19), but the conversion is lower, 85% and 77%, respectively. It can also be concluded from Table 1 that the enzymes with the highest activity do not give the highest conversion or selectivity. Lipozyme TL IM (*Thermomyces lanuginosa,* TL) shows a high conversion with low selectivity (entry 14), and Lipozyme RM IM, like all other *Rhizomucor miehei* formulations, shows a higher selectivity for the 1,4-addition, but lower conversion (entry 15). This is in accordance with the recent report of

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Baldessari et al.,[22] although they did not report on aminolysis. For the first time we here also show that a CLEA of various lipases can be used for the aza-Michael addition of benzylamine and methyl acrylate. From Table 1, it can be concluded that a CLEA gives a higher conversion than other formulations while retaining a high selectivity. This makes CLEAs comparable with Novozyme 435. CLEAs of Cal B, PSL, RM, and TL were tested on their conversion and selectivity for the aza-Michael addition reaction. Especially CLEA TL OM2, a CLEA of *Thermomyces lanuginosa* developed for organic media, showed a higher selectivity for the Michael addition than the lipozyme RM IM and CLEA ST (standard) or CLEA OM1 (organic media/old version). Remarkably, *Pseudomonas stutzeri* lipase gave a very good conversion and selectivity, although the enzyme has a much lower activity per gram than other enzymes (based on triglyceride conversion). The enzymes and formulations that gave the best results with the unsubstituted acrylates were also tested for crotonate and methacrylate (Table 2).

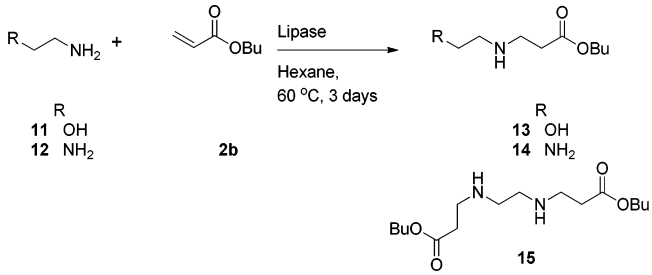
The data obtained for the aza-Michael addition of benzylamine **1** with methyl crotonate **5a** were compared to the results of Priego et al.,[19] who optimized this reaction to give high conversion and selectivity for the product of 1,4-addition. Their best results were obtained for lipase-PS 800, but this formulation is no longer commercially available. Here it is also shown that CvL and PSL give a higher selectivity for the desired aza-Michael adduct (Table 2, entries 9 and 10), though in lower yield than Novozyme 435 (entry 3). This can be circumvented by the use of CLEA PSL, which improved the conversion (76%) and retained the high selectivity (entry 12).

Finally, the aza-Michael addition of benzylamine **1** and methyl methacrylate **8** was studied. The most promising lipases and formulations were screened under conditions that were found to be optimal for the previous described additions. Selectivity and conversion were compared to Novozyme 435. Here again, CvL and PSL showed higher selectivity (Table 3, entries 8 and 9), this time for PSL even conversion is higher. This is also the case for Lipase PS-800 (entry 4). Additionally some CLEAs were screened and here CLEA PSL gave higher yield and higher selectivity for the 1,4-addition of benzylamine to methyl methacrylate (entry 11).

Baldessari et al. showed recently that *N*-substituted β-amino acids could be formed catalyzed by Lipozyme RM IM starting from various unsubstituted acrylates and ethanolamine and 1,3-propanediamine.[22] However, no reaction was observed when substituted acrylates were used. In the current work, PSL and CvL were found to catalyze the aza-Michael addition of ethanolamine and various diamines to substituted acrylates. These enzymes were less selective for the addition of the amines to unsubstituted acrylates because both amine functionalities of the diamines underwent an aza-Michael addition leading to diester **15** (see Scheme 2). When 3 equiv of the acrylate **2b** was added, full conversion was observed and the diester **15** was obtained in a 4:1 ratio. Diester **15** can be seen as a promising building block for application in polymer chemistry. The 1,4-addition of ethanolamine to butyl acrylate selectively gave the mono adduct, with the amine as the nucleophile, at a conversion of 78% for PSL and 69% for CvL.

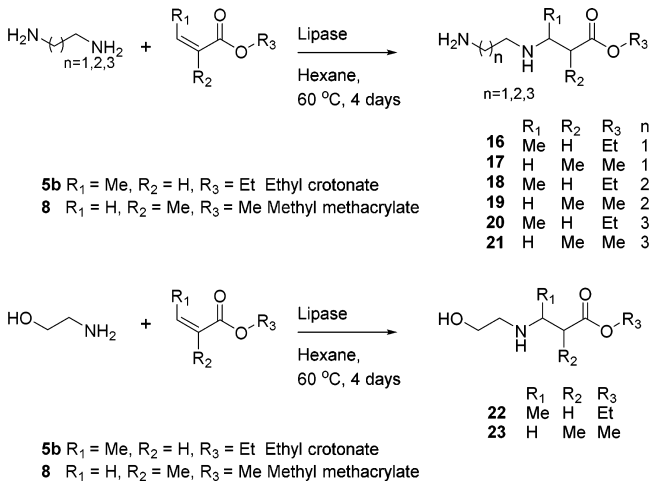
When diamines such as 1,3-propanediamine were added to ethyl acrylate, again a double addition was observed. It appears that chain length or type of ester did not have any effect on the outcome of the reaction in terms of chemoselectivity. Finally, instead of unsubstituted acrylates, a lipase-catalyzed (PSL or CvL) aza-Michael addition of 1,3-propanediamine and methyl

Scheme 2. Aza-Michael Reaction of Diamines and Ethanolamine to Butyl Acrylate Catalyzed by PSL and CvL



methacrylate or ethyl crononate was carried out (Scheme 3), and now the addition reaction was completely selective and

Scheme 3. Aza-Michael Reaction of Diamines and Ethanolamine to Substituted Acrylates Catalyzed by PSL and CvL



methyl substituted *N*-substituted β-amino acids with a free amine function could be prepared (see Table 4). Afterward, the addition reaction was also carried out with ethanolamine and 1,4-diaminobutane. In all examples, only monoaddition took place without transesterification or aminolysis. All products were isolated and purified, except for compound **23**, which polymerized during bulb-to-bulb distillation.

Table 4. Aza-Michael Addition of Substituted Acrylates with Diamines and Ethanolamine Catalyzed by CvL and PSL

entry	Amine $X(CH_2)_nCH_2NH_2$	Michael acceptor	product	conversion (%) PSL[a,b]	conversion (%) CvL[a,b]
1	n = 1, X = NH_2	**5b**	**16**	79	72
2	n = 1, X = NH_2	**8**	**17**	69	63
3	n = 2, X = NH_2	**5b**	**18**	81	75
4	n = 2, X = NH_2	**8**	**19**	68	61
5	n = 3, X = NH_2	**5b**	**20**	75	70
6	n = 3, X = NH_2	**8**	**21**	64	54
7	n = 1, X = OH	**5b**	**22**	82	74
8	n = 1, X = OH	**8**	**23**	78	72

[a]Based on GC–MS analysis. [b]Reaction conditions: 0.1 mmol of benzylamine, 0.1 mmol of methyl methacrylate, 5 mg of enzyme, and 0.3 mL of hexane were added to a sealed vessel and stirred at 60 °C for 4 days.

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Table 5. Solvent Engineering for the PSL- and CvL-Catalyzed Addition of Benzylamine 1 to Methyl Methacrylate 8

entry	solvent system	$E_{T(30)}$	conversion Cal B (%)[a]	result Cal B[b]	conversion PSL (%)[a]	result PSL[b]	conversion CvL (%)[a]	result CvL[b]
1	*n*-hexane	31.0	80	71/29/0	80	100/0/0	62	100/0/0
2	toluene	33.9	76	34/66/0	71	63/37/0	69	86/14/0
3	*i*Pr−O-*i*Pr	34.1	87	18/47/35	60	50/39/11	54	85/15/0
4	MTBE	34.7	80	17/83/0	76	72/28/0	69	71/29/0
5	Ph-O-Ph	35.3	81	20/67/13	70	50/40/10	56	89/11/0
6	dioxane	36.0	77	17/83/0	62	78/22/0	51	84/16/0
7	Me-THF	36.5	74	29/67/4	4	100/0/0	56	87/13/0
8	THF	37.4	84	13/76/11	25	73/27/0	61	70/30/0
9	3:1[c]	38.3	93	17/47/36	88	74/18/8	83	82/10/8
10	1:1[c]	39.2	93	14/43/43	84	72/21/6	83	83/11/7
11	1:3[c]	40.1	93	19/36/45	90	65/19/16	87	81/8/11
12	DCM	40.7	65	67/33/0	54	85/15/0	45	98/2/0

[a]Based on GC−MS analysis. [b]Ratio 1,4-addition/aminolysis/1,4-addition and successive aminolysis in percent. [c]Ratio of hexane/2-methyl-2-butanol. Reaction conditions: 0.1 mmol of benzylamine, 0.1 mmol of methyl methacrylate, 5 mg of enzyme, and 0.3 mL of solvent were added to a sealed vessel and stirred at 60 °C for 4 days.

As described previously, enzyme performance can be influenced by changing the solvent system. The complexity of these solvent−protein interactions makes it hard to quantify this effect. Polarity, hydrophobicity and other solvent parameters did not show much correlation in their effects on the enzymatic activity. Laane et al.[37] showed that highly apolar solvents (log P > 4) retained the highest enzymatic activity in most cases. On the basis of this report, the choice was made to try a broad spectrum of solvents, as functional group interaction of the solvent most likely plays a role along with various other interactions. However, from the screening of enzymes no clear trend could be observed based on log P.

An analogous comparison was made by Priego et al.[19] for the addition of benzylamine to crotonate based on Reichardt's $E_{T(30)}$ scale.[38,39] These authors used *n*-hexane, toluene, diisopropyl ether, THF, and 2-methyl-2-butanol (2M2B) as solvents. If we use the same solvent series for the aza-Michael addition of benzylamine **1** to methacrylate **8** catalyzed by PSL and CvL, a comparable order of chemoselectivity is observed[19] (see Table 5). *n*-Hexane gives for all enzymes the best results (entry 1), and for PSL conversion is 80% with a selectivity of 100% for the product of 1,4-addition. Although the same solvent (hexane) shows only 58% conversion in combination with CvL, it does show full selectivity toward 1,4-addition. Compared to Cal B this can be seen as an improvement: with CalB the conversion is high (80%), but the chemoselectivity is clearly lower (71:29). The 1,4-addition product and the aminolysis product are somewhat difficult to separate, which might induce that this selectivity might become the determining factor for the choice of enzyme.

Looking to some solvents specifically, it becomes visible that 2M2B indeed enhances enzyme activity. This was already proposed by Klibanov in 2001[40] and is explained by formation of hydrogen bonds with the protein. The enzyme is "loosened up" and gets activated by the organic molecules, mimicking water molecules. At the same time, either the previous mentioned activation or the possibly polarity of 2M2B decreases selectivity toward the 1,4-addition product. At the same time an increasing amount of product is seen that is formed by successive 1,4-addition and aminolysis. The enhancing effect of 2M2B on the rate of reaction, like other polar solvents, should be applicable to a lot of enzyme-catalyzed reactions, but the decrease in selectivity prevents use of this solvents.

In order to determine optimum catalyst loading, a series of experiments were carried out with different loadings of the enzyme (see Figure S1, Supporting Information). The amount of catalyst is dependent on the activity; however, these activities are measured in aqueous solutions. These values of course can give an indication for amount of enzyme to be used, but in order to be efficient and generate less waste, optimal loading was determined. All samples contained an identical concentration of substrate, only the amount of enzyme was varied. It turned out that PSL is a very active enzyme, even at low loadings. It reaches maximum conversion rate with 13 U/mL (3 mg/mL) even though the enzyme has only 17 U/mg activity in aqueous systems. For CvL a higher loading of the enzyme is necessary to achieve optimum conversion rates, stabilization occurs at 65 U/mL (17 mg/mL). From these experiments it can also be seen that for reactions catalyzed by CvL hardly any product of aminolysis is observed. For PSL the product of aminolysis is observed, as well as the product of subsequently 1,4-addition and aminolysis, that are formed at higher enzyme loadings.

From Figure 1 it can be observed that for reactions catalyzed by Novozyme 435, the benchmark, the result does not vary considerably by varying the concentration of the substrate. The overall conversion stays between 70 and 80%, and with increase of the substrate concentration, the chemoselectivity decreased. For PSL we see a similar pattern, at lower concentrations chemoselectivity is high and mainly the product of 1,4-addition is formed. At higher concentrations a substantial amount of the product of both 1,4-addition and aminolysis is formed. For CvL another pattern can be observed, at higher concentrations the conversion increases, but the chemoselectivity remains.

To verify if the reaction occurs in the enzyme active site or that the enzyme catalyzes the reaction as a general acid/base catalyst, the enzyme was inactivated by heating at 120 °C for 5 days. Afterward the reaction between morpholine and methyl acrylate was studied using inactivated enzyme. As can be seen from Figure S2 (see the Supporting Information) the enzymatic activity of the inactivated enzymes of PSL and CvL is comparable to the noncatalyzed reaction (when one takes into account the final conversion), whereas the enzymes as received showed an increased conversion.

With the selected lipases from *Pseudomonas stutzeri* and *Chromobacterium viscosum* the aza-Michael addition of various amines and acrylates was catalyzed (Tables 6−8). The results

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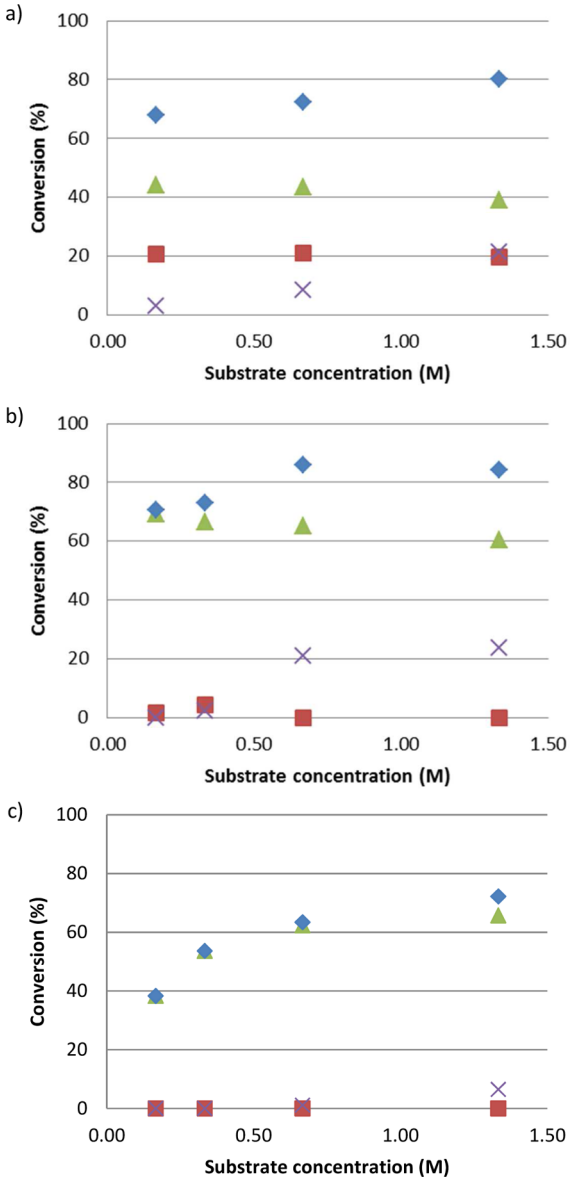

Figure 1. Chemoselectivity of Novozyme 435 (a), PSL (b) and CvL (c) at various substrate concentrations. Reaction conditions: benzylamine: methyl methacrylate 1: 1, 5 mg enzyme and 0.3 mL of hexane in a sealed vessel stirred at 60 °C for 4 days: ♦, overall conversion; ▲, Michael adduct; ■, product of aminolysis; ×, product of aminolysis and successive Michael addition.

show that the lipase-mediated aza-Michael addition has a very broad applicability including linear and cyclic amines, primary and secondary amines, and substituted acrylate esters with various alcohol chain lengths. Only allylamine gave a low product yield. Additionally, it can be seen from Table 6−8 that cyclic secondary amines show higher conversion than primary aromatic amines. From the results of the lipase-catalyzed aza-Michael addition of unsubstituted benzylamines to various acrylates, it can also be concluded that in accordance with the results obtained by Lin et al.,[17] with increasing the length of the aliphatic chain of the ester, the conversion decreases. However, this analogy is not confirmed for the methoxy-substituted benzylamines 3-methoxybenzylamine and 3,4-methoxybenzylamine. In this case, conversion was around 70 and 80% for the respective amines, regardless of the length of the aliphatic chain of the ester. We also tested other esters like *tert*-butyl and benzyl. They showed high conversion for the aza-Michael addition, but do not undergo aminolysis. Methyl substitution on the acrylate influences the conversion clearly, giving lower yield for the products starting from crotonate or methacrylate compared to acrylate.

■ CONCLUSIONS

Lipases from *Pseudomonas stutzeri* (PSL) and *Chromobacterium viscosum* (CvL) are excellent catalysts for the aza-Michael addition of amines to substituted or unsubstituted acrylates (high product selectivity, good yields). In an extensive comparative study other lipases, including Novozyme 435, were shown to be less effective. The selective Michael addition of diamines to these substituted acrylates was also achieved. The use of CLEAs of various lipases for this aza-Michael addition was shown, and especially the lipase CLEAs of *Thermomyces lanuginosa* OM2 and PSL gave the 1,4-adduct of the acrylates and benzylamine with high selectivity and in good yields.

■ EXPERIMENTAL SECTION

Materials and Methods. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR at 100 MHz. All spectra are referenced to residual proton solvent signal. Abbreviations used include singlet (s), doublet (d), doublet of doublets (dd), quartet (q), triplet (t) doublet of triplets (dt), symmetric multiplet (sm), and unresolved multiplet (m). HR-MS analyses were obtained by an electrospray ionization (ESI) apparatus using time-of-flight (TOF). IR spectra were recorded using a spectrometer mounted with a ATR platinum diamond. GC-MS analysis was done using a DB5MS column, length 30 m, internal size 0.25 mm, film thickness 0.25 μm, split ratio 30:1, flow 0.831 mL/min, injector temperature: 250 °C, oven temperature: 6 min on 40 °C, raised by 20 °C per minute up to 250 °C, hold time 6 min. MS scan range: 50−550 amu. For enzymatic activity, data were used as obtained from supplier. Alternatively, the activity was determined using a pH-stat, 1 U catalyzes the release of 1 μmol of butyric acid per minute from tributyrin ((10% v/v) in 25 mM phosphate buffer, pH 7.5 and 40 °C), titrated with 0.1 M NaOH. Methyl acrylate, *tert*-butyl acrylate, methyl methacrylate, methyl crotonate, ethyl crotonate, hexane, toluene, diisopropyl ether, and MBTE (all anhydrous) were purchased from Sigma Aldrich. The enzymes used are lipases (EC 3.1.1.3). Novozyme 435 (14000 U/g), Lipozyme RM IM (3000 U/g) and Lipozyme TL IM (22000 U/g) were generous gifts from Novozymes, *Pseudomonas stutzeri* lipase FE 117 (17500 U/g), CLEA *Pseudomonas stutzeri* lipase 117 OM (17600 U/g), CLEA *Candida antarctica* lipase B 102-ST (17000 U/g), CLEA *Rhizomucor miehei* lipase 105 ST (67000 U/g) and CLEA *Thermomyces lanuginosa* lipase 104 ST (510000 U/g), 104 OM1 (580000 U/g) and 104 OM2 (530000 U/g) were purchased or a generous gift from CLEA technologies (Delft, The Netherlands). *Chromobacterium viscosum* lipase (42000 U/g) and *Aspergillus niger* lipase (3000 U/g) were generous gifts from Biocatalysts. Chirazyme L-1 carrier-fixed lyophilized (235000 U/g, *Burkholderia cepacia*), Chirazyme L-5 carrier-fixed lyophilized (*Candida antarctica* B, 13000 U/g) Chirazyme L-9 c.f. C2 lyophilized, (*Rhizomucor miehei*, 80000 U/g), Chirazyme L-9 c.f. lyophilized (*Rhizomucor miehei*, 66000 U/g) were obtained from Boehringer Mannheim, Lipase AK (*Pseudomonas fluorescens*, 20000 U/g), Lipase G (*Penicilium cyclopium*, 50000 U/g), Lipase N (*Rhizopus sp.*, 80000 U/g) Lipase AP6 (*Aspergillus sp.*, 60000 U/g), Lipase PS-800 (*Pseudomonas cepacia*, 840000 U/g) Lipase R-10 (*Humicola lanuginosa*, 4000 U/g), Lipase AH (*Pseudomonas cepacia*, 12000 U/g), and Lipoproteine Lipase Amano 100s (*Burkholderia sp.*, 30000 U/g) were generous gifts from Amano Enzymes, Inc.

General Methods. *General Method A: Synthesis of Ethyl 3-((2-Aminoethyl)amino)butanoate (**16**). This Method Is Representative for Compounds **16**–**23**.* Ethylenediamine (0.6 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol) or ethyl crotonate (1.15 g, 10

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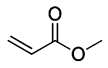
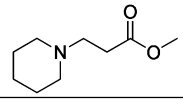
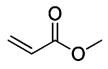
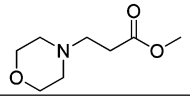
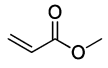
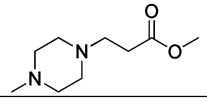
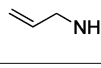
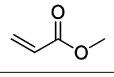
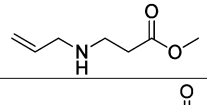
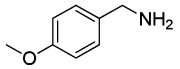
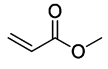
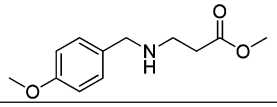
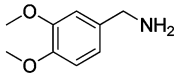
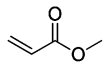
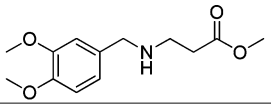
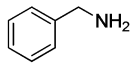
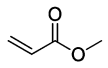
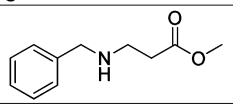
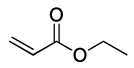
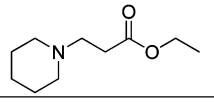
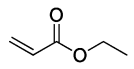
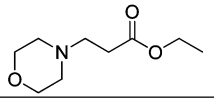
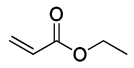
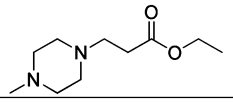
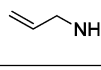
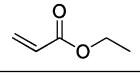
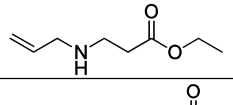
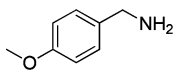
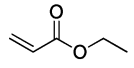
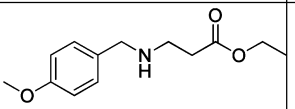
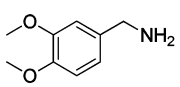
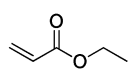
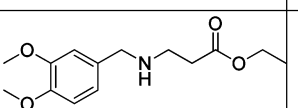
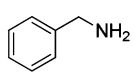
Table 6. Yields for PSL-Catalyzed Aza-Michael Additions of Various Amines to Methyl and Ethyl Acrylate

Entry	Amine	Acrylate	Product structure	Product number	Yield (mol%)[a,b]
1				**24**	99
2				**25**	95
3				**26**	95
4				**27**	n.d.
5				**28**	74
6				**29**	71
7				**3**	81
8				**30**	95
9				**31**	95
10				**32**	95
11				**33**	n.d.
12				**34**	65
13				**35**	89
14				**36**	75
15				**37**	70

[a]Based on GC−MS analysis. [b]Reaction conditions: 0.1 mmol of amine, 0.1 mmol of MMA, 5 mg of enzyme, and 0.3 mL of hexane were added to a sealed vessel and stirred at 60 °C for 4 h.

mmol) were dissolved in 6 mL of hexane, and 50 mg enzyme was added. The reaction mixture was stirred at 60 °C, 250 rpm in a sealed vial for 4 days. Enzymes were filtered off and washed with EtOAc (3 × 5 mL). Evaporation of solvents gave a yellow to light-brown oil. The crude product was purified by bulb-to-bulb distillation.

*General Method B: Synthesis of Methyl 3-(Benzylamino)-Propanoate (**3**). This Method Is Representative for Compounds **24**–**45** and **3**.* Benzylamine (1.07 g, 10 mmol) and methyl acrylate (0.86 g, 10 mmol) were dissolved in 6 mL of hexane, and 50 mg enzyme was added. The reaction mixture was stirred at 60 °C, 250 rpm in a sealed vial for 4 h. Enzymes were filtered off and washed with

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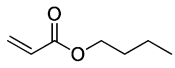
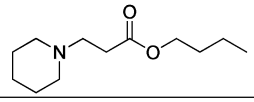
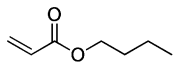
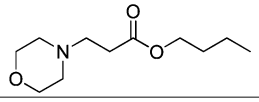
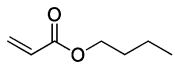
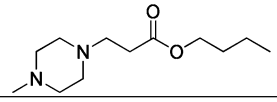
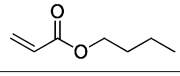
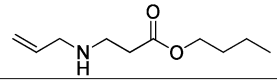
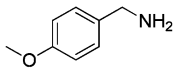
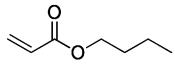
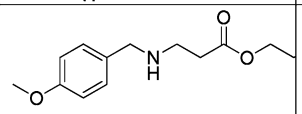
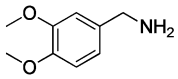
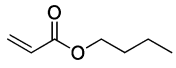
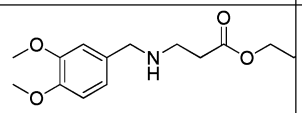
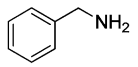
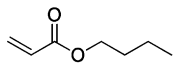
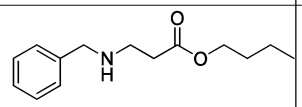
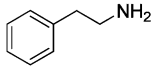
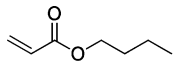
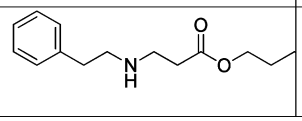
Table 7. Yields for PSL-Catalyzed Aza-Michael Additions of Various Amines to Butyl Acrylate

Entry	Amine	Acrylate	Product structure	Product number	Yield (mol%)[a,b]
1				**38**	94
2				**39**	90
3				**40**	>95
4				**41**	19
5				**42**	61
6				**43**	86
7				**44**	65
8				**45**	60

[a]Based on GC−MS analysis. [b]Reaction conditions: 0.1 mmol of amine, 0.1 mmol of MMA, 5 mg of enzyme, and 0.3 mL of hexane were added to a sealed vessel and stirred at 60 °C for 4 h.

EtOAc (3 × 5 mL). Evaporation of solvents gave a yellow, transparent oil. The crude product was purified over SiO_2 using DCM/MeOH 95:5 as mobile phase.

*General Method C: Synthesis of Methyl 3-(Benzylamino)-2-methylpropanoate (**9**). This Method Is Representative for Compounds **46**–**51** and **9**.* Benzylamine (1.07 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol) were dissolved in 6 mL of hexane, and 50 mg of enzyme was added. The reaction mixture was stirred at 60 °C, 250 rpm in a sealed vial for 4 days. Enzymes were filtered off and washed with EtOAc (3 × 5 mL). Evaporation of solvents gave a yellow to light-brown oil. The crude product was purified over SiO_2, using EtOAc/hexane 1:1 as mobile phase. Other compositions of mobile phases used are DCM/MeOH 95:5 and MTBE/MeOH 98:2, which yielded comparable results.

Compounds **25**,[20] **3**,[20] **36**,[22] **37**,[22] **44**,[22] and **45**[22] were described previously, and their NMR spectra are in accordance with literature.

*Ethyl 3-((2-Aminoethyl)amino)butanoate (**16**).* Prepared according to general method A from ethylenediamine (0.7 g, 10 mmol) and ethyl crotonate (1.15 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 79% (liquid, 1.39 g, 8.1 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.14 (q, 2H, J = 7.0 Hz), 3.09 (sm, 1H), 2.8−2.6 (m, 4H), 2.42 (dd, 1H, J = 7.5, J = 15.0 Hz), 2.35 (dd, 1H, J = 6.5, J = 15.0 Hz), 1.40 (bs, 3H, (NH)), 1.26 (t, J = 7.0 Hz, 3H) 1.11 (d, 2H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 171.9, 59.7, 49.6, 49.2, 41.6, 41.4, 20.1, 13.8 IR (neat) ν: 2928, 2974, 2853, 1727, 1447, 1295, 1179, 1028, 845, 801 cm^{-1}. HR-MS: mass calcd for $C_8H_{19}N_2O_2$ [M + H]$^+$ 175.1446, mass measured [M + H]$^+$ 175.1442.

*Methyl 3-((2-Aminoethyl)amino)-2-methylpropanoate (**17**).* Prepared according to general method A from ethylenediamine (0.7 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 69% (liquid, 1.11 g, 7.1 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.59 (s, 3H), 2.79 (m, 1H), 2.67 (t, 2H, J = 6.0 Hz), 2.5−2.4 (m, 4H), 1.28 (bs, NH), 1.07 (d, 2H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.0, 52.4, 52.0, 51.3, 41.3, 39.9, 15.0, IR (neat) ν: 3005, 2938, 2830, 1727, 1456, 1256, 1197, 1169, 832 cm^{-1}. HR-MS: mass calcd for $C_7H_{17}N_2O_2$ [M + H]$^+$ 161.1290, mass measured [M + H]$^+$ 161.1286.

*Ethyl 3-((3-Aminopropyl)amino)butanoate (**18**).* Prepared according to general method A from 1,3-propanediamine (0.75 g, 10 mmol) and ethyl crotonate (1.15 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 81% (liquid, 8.1 mmol, 1.53 g). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.14 (q, 2H, J = 7.0 Hz), 3.09 (m, 1H), 2.8−2.6 (m, 4H), 2.42 (dd, 1H, J = 6.8, J = 14.5 Hz), 2.34 (dd, 1H, J = 6.8, J = 14.5 Hz) 1,60 (sm, 2H), 1.26 (brt, 4H, J = 7.0 Hz) 1.12 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.2, 60.2, 50.2, 44.7, 41.4, 40.3, 33.8, 20.4, 14.1 IR (neat) ν: 3097, 2964, 2929, 2851, 1752, 1446, 1296, 1178, 845, 721 cm^{-1}. HR-MS: mass calcd for $C_9H_{21}N_2O_2$ [M + H]$^+$ 189.1603, mass measured [M + H]$^+$ 189.1599.

*Methyl 3-((3-Aminopropyl)amino)-2-methylpropanoate (**19**).* Prepared according to general method A from 1,3-propanediamine (0.75 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) or CvL was used as catalyst. Yield: 68% (liquid, 1.20 g, 7.0 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.69 (s, 3H), 2.90 (m, 1H), 2.74 (t, 2H, J = 7.0 Hz), 2.6−2.5 (m, 4H), 1,61 (sm, 2H), 1.21 (d, 2H, J = 6.8 Hz) 1.1 (bs, (NH)). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.1, 52.7, 51.4, 47.5, 40.3, 39.8, 33.4, 15.2 IR (neat) ν: 3099, 2934, 2844, 1726, 1458, 1258, 1197, 832, 757 cm^{-1}. HR-MS: mass calcd for $C_8H_{19}N_2O_2$ [M + H]$^+$ 175.1446, mass measured [M + H]$^+$ 175.1441.

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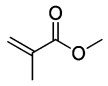
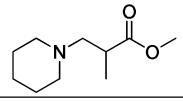
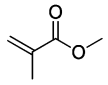
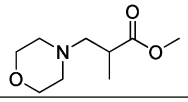
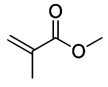
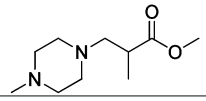
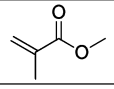
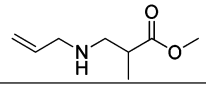
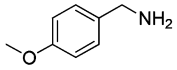
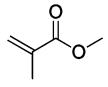
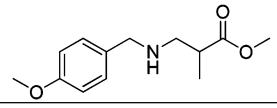
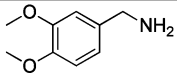
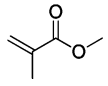
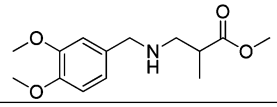
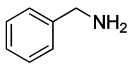
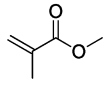
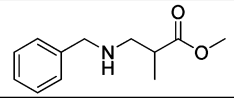
Table 8. Yields for PSL-Catalyzed Aza-Michael Additions of Various Amines to Methacrylates

Entry	Amine	Acrylate	Product structure	Product number	Yield (mol%)[a,b]
1				**46**	60
2				**47**	68
3				**48**	84
4				**49**	18
5				**50**	64
6				**51**	80
7				**9**	81

[a]Based on GC−MS analysis. [b]Reaction conditions: 0.1 mmol of amine, 0.1 mmol of MMA, 5 mg of enzyme, and 0.3 mL of hexane were added to a sealed vessel and stirred at 60 °C for 4 days.

*Ethyl 3-((4-Aminobutyl)amino)butanoate (**20**).* Prepared according to general method A from 1,4-butanediamine (0.88 g, 10 mmol) and ethyl crotonate (10 mmol, 1.15 g). PSL (50 mg) or CvL was used as catalyst. Yield: 75% (liquid, 7.5 mmol, 1.52 g). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.11 (q, 2H, J = 7.5 Hz), 3.08 (sm, 1H), 2.6−2.4 (m, 4H), 2.42 (dd, 1H, J = 7.5, J = 15.0 Hz), 2.34 (dd, 1H, J = 6.5, J = 15.0 Hz), 1.41 (m, 4H), 1.14 (t, 3H, J = 7.5 Hz), 1.14 (bs, 3H, (NH)), 1.09 (d, 3H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.3, 60.2, 50.6, 44.7, 41.5, 40.3, 33.6, 20.4, 14.1. IR (neat) ν: 2958, 2930, 2871, 1728, 1462, 1308, 1175, 1120, 1064, 738 cm^{-1}. HR-MS: mass calcd for $C_{10}H_{23}N_2O_2$ [M + H]$^+$ 203.1759, mass measured [M + H]$^+$ 203.1753

*Methyl 3-((4-Aminobutyl)amino)-2-methylpropanoate (**21**).* Prepared according to general method A from 1,4-butanediamine (0.9 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) or CvL was used as catalyst. Yield: 64% (liquid, 1.21 g, 6.5 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.59 (s, 3H), 2.82 (m, 1H), 2.67 (t, 2H, J = 7.0 Hz), 2.4−2.3 (m, 4H), 1,41 (m, 4H), 1.21 (bs, (NH)) 1.06 (d, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.3, 52.8, 52.0, 49.6, 42.1, 39.9, 31.5, 27.4, 15.3, IR (neat) ν: 2929, 2849, 1728, 1457, 1361, 1495, 1196, 1167, 1125, 833 cm^{-1} HR-MS: mass calcd for $C_9H_{21}N_2O_2$ [M + H]$^+$ 189.1603, mass measured [M + H]$^+$ 189.1600.

*Ethyl 3-((2-Hydroxyethyl)amino)butanoate (**22**).* Prepared according to general method A from ethanolamine (0.8 g, 10 mmol) and ethyl crotonate (1.15 g, 10 mmol). PSL (50 mg) or CvL was used as catalyst. Yield: 82% (liquid, 1.44 g, 8.1 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.14 (q, 2H, J = 7.0 Hz), 3.63 (m, 2H), 3.15 (m, 1H), 2.82 (m, 1H), 2.72 (m, 1H), 2.39 (m, 2H), 1.59 (m, 1H, (NH)), 1.28 (t, 3H, J = 7.0 Hz) 1.14 (d, 3H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.4, 60.9, 60.4, 49.9, 48.3, 41.5, 20.3, 14.1 IR (neat) ν: 3295, 2933, 2873, 1725, 1637, 1560, 1459, 1182, 1054, 1027 cm^{-1}. HR-MS: mass calcd for $C_8H_{18}NO_3$ [M + H]$^+$ 176.1286, mass measured [M + H]$^+$ 176.1282.

*Methyl 3-(Piperidin-1-yl)propanoate (**24**).* Prepared according to general method B from piperidine (0.85 g, 10 mmol) and methyl acrylate (0.86 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 95% (liquid, 1.62 g, 9.5 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.60 (s, 3H), 2.62 (m, 2H), 2.44 (m, 2H), 2.33 (m, 4H), 1.50 (m, 4H), 1.40 (m, 2H). ^{13}C NMR ($CDCl_3$) δ (ppm): 173.2, 54.2, 51.5, 32.1, 26.1, 24.3. IR (neat) ν: 2934, 2853, 2776, 1738, 1437, 1198, 1169, 1113 cm^{-1}. HR-MS: mass calcd for $C_9H_{17}NO_2$ [M + H]$^+$ 172.1332, mass measured [M + H]$^+$ 172.1326.

*Methyl 3-Morpholinopropanoate (**25**).* Prepared according to general method B from morpholine (0.87 g, 10 mmol) and methyl acrylate (0.86 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 95% (liquid, 1.64 g, 9.5 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.63 (s, 3H), 3.62 (m, 4H), 2.62 (t, 2H, J = 6.5 Hz), 2.44 (t, 2H, J = 6.5 Hz), 2.38 (m, 4H). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.8, 67.2, 54.1, 52.9, 51.8, 31.9. IR (neat) ν: 3342, 2974, 2950, 2828, 1731, 1454, 1193, 1171, 734, 697 cm^{-1}. HR-MS: mass calcd for $C_8H_{16}NO_3$ [M + H]$^+$ 174.1125, mass measured [M + H]$^+$ 174.1119.

*Methyl 3-(4-Methylpiperazin-1-yl)propanoate (**26**).* Prepared according to general method B from 1-methylpiperazine (1.0 g, 10 mmol) and methacrylate (0.86 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 95% (liquid, 1.76 g, 9.5 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.61 (s, 3H), 2.65 (t, 2H, J = 7.4 Hz), 2.44 (t, 2H, J = 8.0 Hz), 2.37 (bs, 8H), 2.22 (s, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.5, 55.0, 53.5, 52.9, 51.6, 46.0, 32.1. IR (neat) ν: 2939, 2879, 2794, 2690, 1736, 1458, 1283, 1161, 1085 cm^{-1}. HR-MS: mass calcd for $C_9H_{18}N_2O_2$ [M + H]$^+$ 187.1441, mass measured [M + H]$^+$ 187.1434.

*Methyl 3-(Allylamino)propanoate (**27**).* Prepared according to general method B from allylamine (0.57 g, 10 mmol) and methacrylate (0.86 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: n.d. ^{1}H NMR ($CDCl_3$) δ (ppm): 5.88 (sm, 1H), 5.15 (m, 2H), 3.67 (s, 3H), 3.25 (dt, 2H, J = 6.0, J = 1.5 Hz), 2.87 (t, 2H, J = 6.5 Hz), 2.51 (t, 2H, J = 6.5 Hz), 1.65 (bs, (NH)). ^{13}C NMR ($CDCl_3$) δ (ppm): 173.2, 136.6, 116.0, 52.2, 51.6, 44.3, 34.5. IR (neat) ν: 3322, 3077, 2952, 1733, 1643, 1437, 1194, 995 cm^{-1}. HR-MS: mass calcd for $C_7H_{14}NO_2$ [M + H]$^+$ 144.1014, mass measured [M + H]$^+$ 144.1014.

*Methyl 3-((4-Methoxybenzyl)amino)propanoate (**28**).* Prepared according to general method B from 4-methoxybenzylamine (1.37 g, 10 mmol) and methacrylate (0.86 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 74% (liquid, 1.65 g, 7.4 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.19 (m, 2H), 6.78 (m, 2H), 3.72 (s, 3H), 3.66 (s, 2H), 3.60 (s, 3H), 2.81 (t, 2H, J = 6.5 Hz), 2.46 (t, 2H, J = 6.5 Hz), 1.52 (bs,

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(NH)). ^{13}C NMR ($CDCl_3$) δ (ppm): 173.2, 158.6, 132.3, 129.2, 113.8, 53.5, 53.1, 51.5, 44.4, 30.9. IR (neat) ν: 2997, 2952, 2835, 1731, 1611, 1511, 1457, 1437, 1243, 1169, 1032, 812 cm^{-1}. HR-MS: mass calcd for $C_{12}H_{18}NO_3$ $[M + H]^+$ 224.1268, mass measured $[M + H]^+$ 224.1274.

*Methyl 3-((3,4-Dimethoxybenzyl)amino)propanoate (**29**).* Prepared according to general method B from 3,4-dimethoxybenzylamine (1.67 g, 10 mmol) and methyl acrylate (0.86 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 71% (liquid, 1.88 g, 0.7 mmol) ^{1}H NMR ($CDCl_3$) δ (ppm): 6.84 (m, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.72 (s, 2H), 3.67 (s, 3H), 2.88 (t, 2H, J = 6.5 Hz), 2.52 (t, 2H, J = 6.4 Hz), 1.67 (bs, (NH)). ^{13}C NMR ($CDCl_3$) δ (ppm): 173.2, 149.0, 148.1 132.8, 120.1, 111.3, 111.1, 55.9, 55.8, 53.5, 51.5, 44.4, 34.5, IR (neat) ν: 3326.7, 3026, 2951, 2840, 1731, 1453, 1436, 1169, 734, 697 cm^{-1}. HR-MS: mass calcd for $C_{13}H_{20}NO_4$ $[M + H]^+$ 254.1392, mass measured $[M + H]^+$ 254.1357.

*Methyl 3-(Benzylamino)propanoate (**3**).* Prepared according to general method B from benzylamine (1.07 g, 10 mmol) and methacrylate (0.86 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 78% (liquid, 1.51 g, 7.8 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.22 (m, 5H), 3.77 (s, 2H), 3.64 (s, 3H), 2.87 (t, 2H, J = 6.5 Hz), 2.50 (t, 2H, J = 6.5 Hz), 1.75 (bs, (NH)). ^{13}C NMR ($CDCl_3$) δ (ppm): 173.1, 140.2, 128.4, 128.0, 126.9, 53.7, 51.5, 44.5, 34.6. IR (neat) ν: 3326.7, 3026, 2951, 2840, 1731, 1453, 1436, 1169.5, 734, 697 cm^{-1}. HR-MS: mass calcd for $C_{11}H_{16}NO_2$ $[M + H]^+$ 194.1176, mass measured $[M + H]^+$ 194.1169.

*Ethyl 3-(Piperidin-1-yl)propanoate (**30**).* Prepared according to general method B from piperazine (0.86 g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 95% (liquid, 1.75 g, 9.5 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.07 (q, 2H, J = 7.5 Hz), 2.59 (t, 2H, J = 7.5 Hz), 2.44 (t, 2H, J = 7.6 Hz), 2.34 (m, 4H), 1.50 (m, 4H), 1.36 (m, 2H), 1.20 (t, 3H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) 172.8, 60.3, 54.3, 54.2, 32.3, 25.9, 24.3, 14.2. IR (neat) ν: 2980, 2934, 2853, 1734, 1171, 1153, 1075 cm^{-1}. HR-MS: mass calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$ 186.1489, mass measured $[M + H]^+$ 186.1482.

*Ethyl 3-Morpholinopropanoate (**31**).* Prepared according to general method B from morpholine (0.87 g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 95% (liquid, 9.5 mmol, 1.77 g). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.07 (q, 2H, J = 7.5 Hz), 3.62 (m, 4H), 2.62 (t, 2H, J = 7.5 Hz), 2.5−2.3 (m, 6H), 1.19 (t, 3H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 174.7, 69.2, 62.7, 56.3, 55.7, 34.5, 16.5. IR (neat) ν: 2958, 2854, 2810, 1731, 1255, 1185, 1115, 1010 cm^{-1}. HR-MS: mass calcd for $C_9H_{18}NO_3$ $[M + H]^+$ 188.1281, mass measured $[M + H]^+$ 188.1276.

*Ethyl 3-(4-Methylpiperazin-1-yl)propanoate (**32**).* Prepared according to general method B from 1-methylpiperazine (1.0 g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 95% (liquid, 1.90 g, 9.5 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.14 (q, 2H, J = 7.0 Hz), 2.70 (t, 2H, J = 7.0 Hz), 2.5−2.4 (m, 10H), 2.28 (s, 3H), 1.25 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.5, 60.3, 55.1, 53.5, 52.8, 46.0, 32.3, 14.2. IR (neat) ν: 2938, 2879, 2794, 1733, 1459, 1284, 1180, 1162, 1027 cm^{-1}. HR-MS: mass calcd for $C_{10}H_{21}N_2O_2$ $[M + H]^+$ 201.1598, mass measured $[M + H]^+$ 201.1593.

*Ethyl 3-(Allylamino)propanoate (**33**).* Prepared according to general method B from allylamine (0.57 g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: n.d. ^{1}H NMR ($CDCl_3$) δ (ppm): 5.90 (sm, 1H), 5.16 (m, 2H), 4.15 (q, 2H, J = 7.2 Hz), 3.27 (dt, J = 6.0, J = 1.5 Hz, 2H), 2.88 (t, 2H, J = 6.6 Hz), 2.50 (t, 2H, J = 6.6 Hz), 1.53 (bs, (NH)), 1.27 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.7, 136.7, 115.9, 60.4, 52.2, 44.4, 34.8, 14.2. IR (neat) ν: 3362, 3079, 2981, 2907, 2825, 1729, 1459, 1417, 1179 cm^{-1}. HR-MS: mass calcd for $C_8H_{16}NO_2$ $[M + H]^+$ 158.1176, mass measured $[M + H]^+$ 158.1170.

*Ethyl 3-((4-Methoxybenzyl)amino)propanoate (**34**).* Prepared according to general method B from 4-methoxybenzylamine (1.37 g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 71% (liquid, 1.68 g, 7.1 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.24 (m, 2H), 6.87 (m, 2H), 4.14 (q, 2H, J = 7.0 Hz), 3.80 (s, 3H), 3.74 (s, 2H), 2.89 (t, 2H, J = 6.4 Hz), 2.52 (t, 2H, J = 6.4 Hz), 1.60 (bs(NH)), 1.26 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.8, 158.6, 132.3, 129.2, 113.8, 60.4, 55.3, 53.2, 44.4, 34.8, 14.2. IR (neat) ν: 2980, 2906, 2835, 1727, 1611, 1511, 1243, 1172, 811 cm^{-1}. HR-MS: mass calcd for $C_{13}H_{20}NO_3$ $[M + H]^+$ 238.1424, mass measured $[M + H]^+$ 238.1430.

*Ethyl 3-((3,4-Dimethoxybenzyl)amino)propanoate (**35**).* Prepared according to general method B from 3,4-dimethoxybenzylamine (1.67 g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 89% (liquid, 2.37 g, 8.9 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.84 (m, 3H), 4.14 (q, 2H, J = 7.2 Hz), 3.89 (s, 3H), 3.88 (s, 3H), 3.73 (s, 2H), 2.88 (t, 2H, J = 6.4 Hz), 2.50 (t, 2H, J = 6.4 Hz), 1.66 (bs(NH)), 1.23 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.7, 148.9, 147.9, 132.8, 120.0, 111.2, 111.0, 60.3, 55.8, 53.3, 44.5, 34.7, 14.1. IR (neat) ν: 2937, 2906, 2835, 1727, 1514, 1260, 1233, 1155, 1026, 806 cm^{-1}. HR-MS: mass calcd for $C_{14}H_{22}NO_4$ $[M + H]^+$ 268.1530, mass measured $[M + H]^+$ 268.1535.

*Ethyl 3-(Benzylamino)propanoate (**36**).* Prepared according to general method B from benzylamine (1.07g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 75% (liquid, 1.56 g, 7.5 mmol) ^{1}H NMR ($CDCl_3$) δ (ppm): 7.20 (m, 4H), 7.15 (m, 1H), 4.08 (q, 2H, J = 7.2 Hz), 3.73 (s, 2H), 2.83 (t, 2H, J = 6.4 Hz), 2.45 (t, 2H, J = 6.4 Hz), 1.58 (bs (NH)), 1.18 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.8, 140.2, 128.4, 128.1, 126.9, 60.4, 53.8, 44.5, 34.8, 14.2. IR (neat) ν: 3327, 3028, 2981, 2906, 2830, 1728, 1175, 1028, 734, 698 cm^{-1}. HR-MS: mass calcd for $C_{12}H_{18}NO_2$ $[M + H]^+$ 208.1332, mass measured $[M + H]^+$ 208.1326.

*Ethyl 3-(2-Phenylethylamino)propanoate (**37**).* Prepared according to general method B from 2-phenylethylamine (1.09 g, 10 mmol) and ethyl acrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 70% (liquid, 1.55 g, 7.1 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.21 (m, 5H), 4.08 (q, 2H, J = 6.5 Hz), 2.87 (m, 4H), 2.70 (t, 2H, J = 7.5 Hz), 2.44 (t, 2H, J = 7.5 Hz), 1.51 (bs, 1H, (NH)) 1.21 (t, 3H, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.4, 139.8, 128.5, 125.9, 60.1, 50.8, 44.1, 36.2, 34.6, 14.0. IR (neat) ν: 3027, 2974, 2950, 2829, 1731, 1495, 1255, 1194, 735, 698 cm^{-1}. HR-MS: mass calcd for $C_{14}H_{22}NO_2$ $[M + H]^+$ 222.1494, mass measured $[M + H]^+$ 222.1498.

*Butyl 3-(Piperidin-1-yl)propanoate (**38**).* Prepared according to general method B from piperidine (0.85 g, 10 mmol) and butyl acrylate (1.28 g, 10 mmol). PSL (50 mg) was used as a catalyst. Yield: 94% (liquid, 2.00 g, 9.4 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.01 (t, 2H, J = 6.6 Hz), 2.61 (t, 2H, J = 7.4 Hz), 2.42 (t, 2H, J = 7.4 Hz), 2.32 (m, 4H), 1.54 (m, 6H), 1.35 (m, 4H), 0.86 (t, 3H, J = 8.6 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.8, 64.2, 54.3, 54.2, 32.4, 30.7, 25.9, 24.3, 19.1, 13.7. IR (neat) ν: 2934, 2855, 2776, 1735, 1170, 1153, 1114 cm^{-1}. HR-MS: mass calcd for $C_{12}H_{24}NO_2$ $[M + H]^+$ 214.1788, mass measured $[M + H]^+$ 214.1794.

*Butyl 3-Morpholinopropanoate (**39**).* Prepared according to general method B from morpholine (0.87 g, 10 mmol) and butyl acrylate (1.28 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 90% (liquid, 1.93 g, 9.0 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.02 (t, 2H, J = 7.0 Hz), 3.62 (m, 4H), 2.61 (t, 2H, J = 7.5 Hz), 2.46 (t, 2H, J = 7.5 Hz) 2.44 (m, 4H), 1. 57 (m, 2H), 1.34 (m, 2H), 0.87 (t, 3H, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$) 172.4, 66.9, 64.3, 54.0, 53.4, 32.2, 30.7, 19.1, 13.7. IR (neat) ν: 2958, 2855, 2810, 1733, 1458, 1116, 1035, 868, 859 cm^{-1}. HR-MS: mass calcd for $C_{11}H_{22}NO_3$ $[M + H]^+$ 216.1594, mass measured $[M + H]^+$ 216.1588.

*Butyl 3-(4-Methylpiperazin-1-yl)propanoate (**40**).* Prepared according to general method B from 1-methylpiperazine (1.00 g, 10 mmol) and butyl acrylate (1.28 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 95% (liquid, 2.17 g, 9.5 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.09 (t, 2H, J = 6.6 Hz), 2.71 (t, 2H, J = 6.8 Hz), 2.51−2.36 (m, 10H), 2.28 (s, 3H), 1.64−1.57 (m, 2H), 1.43−1.33 (m, 2H), 0.94 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.6, 64.3, 55.1, 53.6, 52.9, 46.0, 32.4, 30.7, 19.1, 13.7. IR (neat) ν: 2936, 2794, 1734, 1458, 1178, 1013 cm^{-1}. HR-MS: mass calcd for $C_{12}H_{25}N_2O_2$ $[M + H]^+$ 229.1916, mass measured $[M + H]^+$ 229.1908.

*Butyl 3-(Allylamino)propanoate (**41**).* Prepared according to general method B from allylamine (0.57 g, 10 mmol) and butyl acrylate (1.28 g, 10 mmol). PSL (50 mg) was used as a catalyst. Yield: 19% (liquid, 0.35 g, 1.9 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 5.83 (sm,

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1H), 5.07 (m, 2H), 4.02 (t, 2H, J = 6.6 Hz), 3.18 (dt, 2H, J = 6.0, J = 1.5 Hz), 2.81 (t, 2H, J = 6.6 Hz), 2.44 (t, 2H, J = 6.6 Hz), 1.57 (m, 2H), 1.46 (bs (NH)), 1.33 (m, 2H), 0.88 (t, 3H, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 170.4, 134.2, 113.5, 61.9, 49.8, 42.0, 32.4, 28.2, 16.7, 11.2. IR (neat) ν: 3327, 3078, 2960, 2934, 1730, 1461, 1177, 916 cm^{-1}. HR-MS: mass calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$ 186.1489, mass measured $[M + H]^+$ 186.1483.

*Butyl 3-((4-Methoxybenzyl)amino)propanoate (**42**).* Prepared according to general method B from 4-methoxybenzylamine (1.37 g, 10 mmol) and butyl acrylate (1.28 g, 10 mmol). PSL (50 mg) was used as a catalyst. Yield: 86% (liquid, 2.66 g, 8.6 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.24 (m, 2H), 6.87 (m, 2H), 4.08 (q, 2H, J = 7.5 Hz), 3.79 (s, 3H), 3.73 (s, 2H), 2.87 (t, 2H, J = 6.4 Hz), 2.53 (t, 2H, J = 6.4 Hz), 1.60 (bs, NH), 1.58 (m, 2H), 1.34, (m, 2H), 0.92 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.5, 158.3, 132.0, 129.4, 113.4, 60.5, 55.3, 53.2, 44.4, 34.8, 30.3, 18.8, 13.3. IR (neat) ν: 2957, 2934, 2834, 1728, 1510, 1462, 1243, 1169, 1033, 810 cm^{-1}. HR-MS: mass calcd for $C_{15}H_{24}NO_3$ $[M + H]^+$ 266.1756, mass measured $[M + H]^+$ 266.1748.

*Butyl 3-((3,4-Dimethoxybenzyl)amino)propanoate (**43**).* Prepared according to general method B from 3,4-dimethoxybenzylamine (1.67 g, 10 mmol) and butyl acrylate (1.28 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 86% (liquid, 2.53 g, 8.6 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.75 (m, 3H), 4.03 (t, 2H, J = 6.6 Hz), 3.86 (s, 3H), 3.80 (s, 3H), 3.67 (s, 2H), 2.83 (t, 2H, J = 6.6 Hz), 2.46 (t, 2H, J = 6.2 Hz), 1.52 (m, 3H), 1.32 (m, 2H), 0.88 (t, 3H, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 173.1, 148.2, 147.3, 133.0, 120.1 112.0, 111.1, 64.2, 55.9, 53.6, 44.6, 34.0, 30.8, 19.5, 13.8. IR (neat) ν: 2958, 2873, 2835, 1729, 1514, 1260, 1234, 1028 cm^{-1}. HR-MS: mass calcd for $C_{16}H_{26}NO_4$ $[M + H]^+$ 296.1843, mass measured $[M + H]^+$ 296.1849.

*Butyl 3-(Benzylamino)propanoate (**44**).* Prepared according to general method B from benzylamine (1.07 g, 10 mmol) and butyl acrylate (1.28 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 65% (liquid, 1.53 g, 0.66 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.28 (m, 5H), 4.09 (t, 2H, J = 6.8 Hz), 3.80 (s, 2H), 2.89 (t, 2H, J = 6.4 Hz), 2.52 (t, 2H, J = 6.4 Hz), 1.79 (bs, 1H), 1.60 (m, 2H), 1.36 (m,2H), 0.92 (t, 3H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.7, 140.1, 128.3, 128.0, 126.8, 64.2, 53.7, 44.5, 34.7, 30.5, 19.0, 13.6. IR (neat) ν: 3063, 3028, 2959, 2933, 2873, 1729, 1495, 1172, 734, 697 cm^{-1}. HR-MS: mass calcd for $C_{14}H_{22}NO_2$ $[M + H]^+$ 236.1632, mass measured $[M + H]^+$ 236.1638.

*Butyl 3-(2-Phenylethylamino)propanoate (**45**).* Prepared according to general method B from phenylethylamine (1.09 g, 10 mmol) and butyl acrylate (1.1 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 60% (liquid, 1.50 g, 6.1 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.21 (m, 5H), 4.07 (t, 2H, J = 7.5 Hz), 2.87 (m, 4H), 2.80 (t, 2H, J = 6.5 Hz) 2.50 (t, 2H, J = 6.5 Hz) 1.58 (m, 2H) 1.52 (brs, (NH)), 1.35 (m, 2H) 0.92 (t, 3H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 172.7, 140.0, 128.7, 128.4, 126.1, 64.3, 51.0, 45.0, 36.4, 34.0, 30.6, 19.1, 13.7. IR (neat) ν: 3027, 2957, 2932, 2872, 1729, 1454, 1250, 1166, 1065, 747, 698 cm^{-1}. HR-MS: mass calcd for $C_{15}H_{23}NO_2$ $[M + H]^+$ 250.1807, mass measured $[M + H]^+$ 250.1810.

*Methyl 2-Methyl-3-(piperidin-1-yl)propanoate (**46**).* Prepared according to general method B from piperidine (0.85 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 60% (liquid, 1.11 g, 6.0 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.69 (s, 3H), 2.65 (m, 1H), 2.53 (dd, 1H, J = 12.5, J = 8.5 Hz), 2.3−2.2 (m, 4H), 2.17 (dd, 1H, J = 12.5, J = 6.0 Hz), 1.45 (m, 4H), 1.32 (m, 2H), 1.06 (d, 3H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.8, 62.4, 54.7, 51.5, 32.9, 25.1, 24.4, 15.7. IR (neat) ν: 2934, 2854, 1739, 1457, 1435, 1352, 1169, 1157 cm^{-1}. HR-MS: mass calcd for $C_{10}H_{20}NO_2$ $[M + H]^+$ 186.1489, mass measured $[M + H]^+$ 186.1486.

*Methyl 2-Methyl-3-morpholinopropanoate (**47**).* Prepared according to general method B from morpholine (0.87 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 68% (liquid, 1.27 g, 6.8 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.69 (s, 3H), 3.72 (m, 4H), 2.70 (m, 2H), 2.47 (m, 2H), 2.40 (m, 2H), 2.33 (m, 1H), 1.26 (d, 3H, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.4, 67.0, 62.0, 53.7, 51.5, 37.6, 15.4. IR (neat) ν: 2953, 2853, 2809, 1736, 1457, 1116, 1070, 985, 835 cm^{-1}. HR-MS: mass calcd for $C_9H_{18}NO_3$ $[M + H]^+$ 188.1281, mass measured $[M + H]^+$ 188.1275.

*Methyl 2-Methyl-3-(4-methylpiperazin-1-yl)propanoate (**48**).* Prepared according to general method B from 1-methylpiperazine (1.0 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 84% (liquid, 1.68 g, 8.4 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 3.61 (s, 3H), 2.62 (m, 2H), 2.4−2.2 (m, 9H), 2.19 (s, 3H), 1.14 (d, 3H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.5, 61.5, 55.2, 53.2, 51.5, 46.0, 37.8, 15.5. IR (neat) ν: 2938, 2878, 2794, 1737, 1458, 1245, 1168, 1013 cm^{-1}. HR-MS: mass calcd for $C_{10}N_{21}N_2O_2$ $[M + H]^+$ 201.1584, mass measured $[M + H]^+$ 201.1591.

*Methyl 3-(Allylamino)-2-methylpropanoate (**49**).* Prepared according to general method B from allylamine (0.57 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 18% (liquid, 0.28 g, 1.8 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 5.84 (sm, 1H), 5.05 (m, 2H), 3.63 (s, 3H), 3.19 (dt, 2H, J = 6.0, J = 1.5 Hz), 2.82 (m, 3H), 2.62 (m, 2H), 1.60 (bs (NH)), 1.18 (d, 3H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.3, 136.6, 116.0, 52.2, 52.0, 51.6, 40.0, 15.3. IR (neat) ν: 2945, 2827, 1725, 1520, 1477, 1237, 1190, 833 cm^{-1} HR-MS: mass calcd for $C_8H_{16}NO_2$ $[M + H]^+$ 158.1176, mass measured $[M + H]^+$ 158.1175.

*Methyl 3-((4-Methoxybenzyl)amino)-2-methylpropanoate (**50**).* Prepared according to general method B from 4-methoxybenzylamine (1.37 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 64% (liquid, 1.52 g, 6.4 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.17 (m, 2H), 6.78 (m, 2H), 3.72 (s, 3H), 3.65 (s, 2H), 3.61 (s, 3H), 2.81 (m, 1H), 2.61 (m, 2H), 1.46 (bs (NH)), 1.10 (d, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.3, 158.6, 132.5, 129.2, 113.8, 55.2, 53.1, 51.6, 40.0, 15.3. IR (neat) ν: 3340, 2950, 2835, 1730, 1511, 1459, 1243, 1171, 1065 cm^{-1}. HR-MS: mass calcd for $C_{13}H_{20}NO_3$ $[M + H]^+$ 238.1438, mass measured $[M + H]^+$ 238.1434.

*Methyl 3-((3,4-Dimethoxybenzyl)amino)-2-methylpropanoate (**51**).* Prepared according to general method B from 3,4-dimethoxybenzylamine (liquid, 1.67 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 80% (2.14 g, 8.0 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 6.77 (m, 3H), 3.88 (s, 3H), 3.79 (s, 3H), 3.66 (s, 2H), 3.61 (s, 3H), 2.82 (m, 1H), 2.62 (m, 2H), 1.51 (bs, (NH)), 1.17 (d, 2H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.3, 148.9, 148.0, 133.0, 120.1, 111.3, 111.0, 55.9, 55.8, 53.5, 52.1, 51.6, 40.0, 15.3. IR (neat) ν: 2950, 2834, 1730,1514, 1256, 1234, 1027 cm^{-1}. HR-MS: mass calcd for $C_{14}H_{22}NO_4$ $[M + H]^+$ 268.1543, mass measured $[M + H]^+$ 268.1540.

*Methyl 3-(Benzylamino)-2-methylpropanoate (**9**).* Prepared according to general method B from benzylamine (1.07 g, 10 mmol) and methyl methacrylate (1.0 g, 10 mmol). PSL (50 mg) was used as catalyst. Yield: 81% (liquid, 1.68 g, 8.1 mmol). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.31 (m, 5H), 3.72 (s, 2H), 3.61 (s, 3H), 2.79 (m, 1H), 2.67 (m, 2H), 1.56 (bs (NH)), 1.17 (d, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm): 176.3, 140.3, 128.35, 128.0, 126.9, 53.7, 52.1, 51.6, 40.1, 15.3. IR (neat) ν: 3027, 2974, 2950, 2829, 1731, 1495, 1255, 1194, 735, 698 cm^{-1}. HR-MS: mass calcd for $C_{12}H_{18}NO_2$ $[M + H]^+$ 208.1375, mass measured $[M + H]^+$ 208.1370.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Influence of enzyme loading on reaction mixture composition and enzymatic activity of enzyme from supplier vs inactivated enzyme; ^{1}H and ^{13}C NMR spectra for compounds **3**, **9**, and **16**−**51**. This material is available free of charge via the Internet, http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: maurice.franssen@wur.nl.

Notes

The authors declare no competing financial interest.

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