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Promiscuous Behavior of *Rhizomucor miehei* Lipase in the Synthesis of *N*-Substituted β-Amino Esters

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A mild and efficient procedure for the aza-Michael addition of amines to acrylates by using lipases as catalysts is reported. Various lipases, mono- and bifunctional amines, alkyl acrylates, and reaction parameters were studied. Under the optimal conditions, *Rhizomucor miehei* lipase showed high selectivity. It catalyzed the formation of the Michael monoadduct as the only product in high yield and purity. Moreover, when diamines were used as nucleophiles, the lipase catalyzed the addition of only one of the two amino groups, showing in this case high substrate specificity. This promiscuous and highly selective behavior displayed by *Rhizomucor miehei* lipase allowed us to obtain 22 *N*-substituted β-amino esters, 15 of them being new products.

Introduction

β-Amino esters are an important group of compounds, among which natural products with biological activities can be found.[1] These compounds are also useful as synthetic precursors for many bioactive heterocyclic[2–4] and β-peptoid[5] compounds. Moreover, β-amino esters are monomers in the synthesis of poly(β-aminoester)s, linear cationic polymers used as efficient gene delivery vectors.[6,7] These polymers contain both an amino group interacting with polyanionic DNA through electrostatic interaction and a degradable region, such as a hydrolyzable linkage.[8] These characteristics make them useful for targeted delivery of antitumor agents, such as cisplatin, minimizing its toxicity to healthy tissues and increasing its drug efficacy.[9]

The synthesis of β-amino esters can be carried out by following several strategies.[10,11] The most widely used methods are the Mannich reaction,[12] the *N*-alkylation of amines with β-haloesters.[4] and the Michael addition of amines to α,β-unsaturated esters.[3,13–15]

In particular, the aza-Michael addition is a powerful tool for carbon–nitrogen bond formation. The reaction between an amine and an α,β-unsaturated carbonyl compound can occur spontaneously in some cases in the absence of solvent[16] or if solvents with high dielectric constants such as methanol, ethanol, or tetrahydrofuran are employed.[3,14,15] An excess amount of the amine is often employed in these cases to maximize product yield. Various catalysts have also been used for Michael additions, including acids,[17] bases,[18] Lewis acids,[9,13] and ionic liquids.[19] Higher reaction yields can be obtained when catalysts are employed. Moreover, they shorten the reaction times, allow a broader range of solvents, and generally require milder reaction conditions and stoichiometric substrate loadings.

It is well known that enzymes are useful as biocatalysts in a variety of reactions. In particular, enzyme promiscuity has been thoroughly studied over the last years. For instance, many hydrolases are able to catalyze reactions that are completely different from those they originally evolved to perform: Michael addition, Markovnikov addition, and aldol condensation are a few examples.[20] Regarding the aza-Michael reaction, it is interesting to mention the aspartase-catalyzed addition of ammonia to fumaric acid was scaled-up for the commercial production of aspartic acid.[21] Using secondary amines and fluorine as double-bond activators, lipases have also been used as catalysts in Michael-type additions.[22] In the presence of lipases, Michael addition can compete with aminolysis of the ester to afford amides, and it is interesting to control the prevalence of one of these reactions over the other by applying several strategies. The optimization of the reaction conditions to minimize Michael-type side products allowed substituted acrylamides to be obtained in high yield.[23,24] On the other hand, a solvent engineering strategy was used to control the lipase selectivity in a Michael addition reaction,[25] and a polyamidoamine oligomer containing a completely regular structure was synthesized through both aminolysis and Michael addition reactions catalyzed by a lipase.[26]

Most of the previous reports on enzyme-catalyzed aza-Michael reactions show the results obtained on the conjugate addition of primary and secondary amines to various acceptors,[27–29] but the application of bifunctional amines such as alkanolamines or diamines as nucleophiles remains unexplored. Considering this fact, in this work we studied

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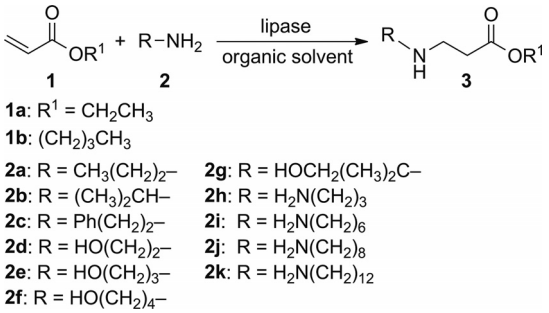
the chemoselective behavior of lipases in the synthesis of several *N*-substituted β-amino esters by using various mono- and bifunctional amines as nucleophiles in aza-Michael addition reactions (Scheme 1).



Scheme 1.

Results and Discussion

Reaction Conditions

In a previous report, by using ethyl acrylate and alkanolamines as reactants, we obtained *N*-hydroxyalkylacrylamides through an aminolysis reaction on the ester, catalyzed by *Candida antarctica* lipase (CAL B).[24] The enzymatic reaction of ethyl acrylate and the alkanolamines was carried out at 30 °C by using an alkanolamine/ester ratio equal to 1, 0.08 M ester concentration, a ratio enzyme/substrate (E/S) equal to 2, diisopropyl ether as solvent, and *p*-benzoquinone as radical inhibitor. Under these reaction conditions CAL B worked in a highly chemoselective way to afford the amide as the only product in high yield. It was possible to avoid the production of addition products and a polymeric material that were detected as secondary products working under less-controlled conditions.

With the aim to favor the synthesis of addition products, in the present work we studied the enzymatic aza-Michael reaction. In this case, we used three types of nucleophiles as Michael donors: alkylamines **2a**–**c**, alkanolamines **2d**–**g**, and diamines **2h**–**k**. To begin, it was necessary to determine the conditions under which lipase-catalyzed Michael addition prevailed over amide or polymer formation with the different nucleophiles. Therefore, the reactions of ethyl acrylate (**1a**) as the Michael acceptor and phenethylamine (**2c**), ethanolamine (**2d**), or 1,3-propanediamine (**2h**) as the nucleophile were chosen as model reactions. The influence of various reaction parameters such as lipase source, solvent, E/S ratio, substrate concentration, and temperature was studied.

Four commercially available lipases from various sources were screened for their catalytic activity towards the aza-Michael addition reaction: CAL B, *Rhizomucor miehei* lipase (LIP), *Pseudomonas cepacia* lipase (PSL), and *Candida rugosa* lipase (CRL). We observed that the most important differences in their performance were due to the chemoselectivity achieved in the reaction. In accordance with previous reports, CAL B-catalyzed aminolysis reactions were much faster than Michael additions.[24] PSL also catalyzed both reactions, and we obtained a mixture of esters and amides when this catalyst was used. On the other hand, CRL showed a poor performance. LIP was found to be the most efficient lipase to catalyze, in a chemoselective way, the addition reaction with alkylamine **2c**, alkanolamine **2d**, and diamine **2h**. Only the amino group of alkanolamine **2d** was reactive in the formation of the product, and the hydroxy function remained unaltered, as we observed in previous work with this class of compounds.[24,30]

Moreover, by using 1,3-diaminopropane (**2h**), LIP showed a high substrate specificity because the reaction product, ethyl *N*-(3-aminopropyl)-β-alaninate (**3h**), with a free NH_2 group in the molecule was unable to react with another ethyl acrylate molecule when this catalyst was employed. *N*-Substituted β-amino esters containing a free hydroxy or amino group are very attractive as monomers in the synthesis of polyesters with polar pendant groups.

Having established the optimal catalyst, screening of the solvent was performed to determine the optimal solvent for this biocatalytic aza-Michael reaction. With the belief that this reaction was only slightly affected by non-enzymatic acid/base catalysis and by considering previous reports,[25,28,29] three low-polarity solvents, hexane, toluene, and diisopropyl ether (DIPE), were tested.[31]

The performance of the solvent was not the same for every case and depended on the nature of the nucleophile. The experiments (Table 1, Entries 1–9) clearly identified hexane as the most efficient solvent for the reaction with ethyl acrylate and alkylamine **2c** (Table 1, Entry 1) and diamine **2h** (Table 1, Entry 7), whereas DIPE seemed to be the best choice when using alkanolamine **2d** as the nucleophile (Table 1, Entry 6).

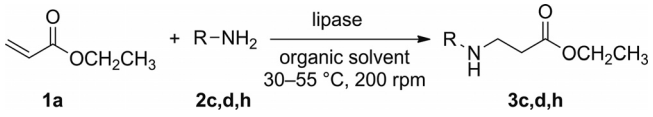
Interestingly, when the lipase-catalyzed Michael addition of ethanolamine (**2d**) to ethyl acrylate was attempted with the use of hexane as the solvent, we obtained double adduct **3d*** instead of **3d** (Table 1, Entry 4). This difference could be attributed to the scarce solubility of ethanolamine in hexane. A neat phase separation was observed even at a very low concentrations (0.012 M). This phenomenon should create a large excess of ethyl acrylate in the hexane phase and thus drive the reaction to the formation of double Michael adducts.

Regarding the optimum temperature (Table 1, Entries 10 and 11), we also performed the addition reaction with **2c** and **2d** as Michael donors at 55 °C. The results showed that an increase in temperature had no significant effect on the conversion to the desired products. Therefore, 30 °C was chosen as the reaction temperature in every experiment.

To determine the best enzyme/substrate (E/S) ratio, reactions with various biocatalyst concentrations were carried out (Table 1, Entries 12–16). An increase in conversion with an increase in E/S from 0.1 to 5 was observed, whereas E/S = 1 was enough to catalyze the reaction under the present parameters (Table 1, Entry 14).

Then, we decided to study the effect of substrate concentration in the spontaneous and enzymatic aza-Michael reaction. A high substrate concentration can have some conse-

Table 1. Optimization of reaction parameters for the lipase-catalyzed aza-Michael addition of ethyl acrylate (**1a**) to amines **2c**,**d**,**h**.[a]



1a **2c,d,h** **3c,d,h**

Entry	R	Solvent	*T* (°C)	E/S	Product (% conv.)
Solvent					
1	$(CH_2)_2Ph$	hexane	30	1	**3c** (92)
2	$(CH_2)_2Ph$	toluene	30	1	**3c** (75)
3	$(CH_2)_2Ph$	DIPE	30	1	**3c** (66)
4	$(CH_2)_2OH$	hexane	30	1	**3d*** (80)[b]
5	$(CH_2)_2OH$	toluene	30	1	**3d** (71)
6	$(CH_2)_2OH$	DIPE	30	1	**3d** (83)
7	$(CH_2)_3NH_2$	hexane	30	1	**3h** (100)
8	$(CH_2)_3NH_2$	toluene	30	1	**3h** (86)
9	$(CH_2)_3NH_2$	DIPE	30	1	**3h** (65)
Temperature					
10	$(CH_2)_2Ph$	hexane	55	1	**3c** (91)
11	$(CH_2)_2OH$	DIPE	55	1	**3d** (86)
E/S					
12	$(CH_2)_2OH$	DIPE	30	0.1	**3d** (12)
13	$(CH_2)_2OH$	DIPE	30	0.5	**3d** (40)
14	$(CH_2)_2OH$	DIPE	30	1	**3d** (84)
15	$(CH_2)_2OH$	DIPE	30	2.5	**3d** (91)
16	$(CH_2)_2OH$	DIPE	30	5	**3d** (90)

[a] Reaction conditions: LIP; substrate concentration = 0.12 M; ethyl acrylate/amine = 1. [b] **3d***: double Michael adduct, $HOCH_2CH_2N(CH_2CH_2COOEt)_2$.

quences: a spontaneous Michael reaction is more likely to occur due to an increase in the dielectric constant of the reaction medium, the lipase-catalyzed reaction rate may be limited due to diffusion processes to the heterogeneous catalyst, and loss of selectivity. We prepared solutions of different concentrations (0.012–1.2 M) of ethyl acrylate and ethanolamine (1:1) in DIPE. The solutions were incubated at 30 °C and the reaction was allowed to proceed in both the presence and absence of LIP. The conversion to product **3d** was monitored by GC, and the results are shown in Figure 1.

At the highest concentration (1.2 M), a spontaneous reaction was very fast and no difference was observed between the presence and absence of the enzyme in terms of a decrease in the ethyl acrylate and ethanolamine concentrations, but in both cases the reaction was not selective and a complex mixture of products including some polymeric material was obtained. At a very low concentration of the substrate (0.012 M), the enzymatic reaction was selective and only Michael adduct **3d** was obtained, but the conversion was low (30%, Figure 1). In the absence of enzyme, the amount of **3d** in the reaction was insignificant.

Through the results obtained working at a concentration of 0.12 M, we observed that the enzymatic reaction turned remarkably faster than the spontaneous one as the substrate concentration was increased. In this case, the conversion to **3d** reached 100% in 12 h, whereas in the absence of LIP, under the same reaction conditions, the conversion was

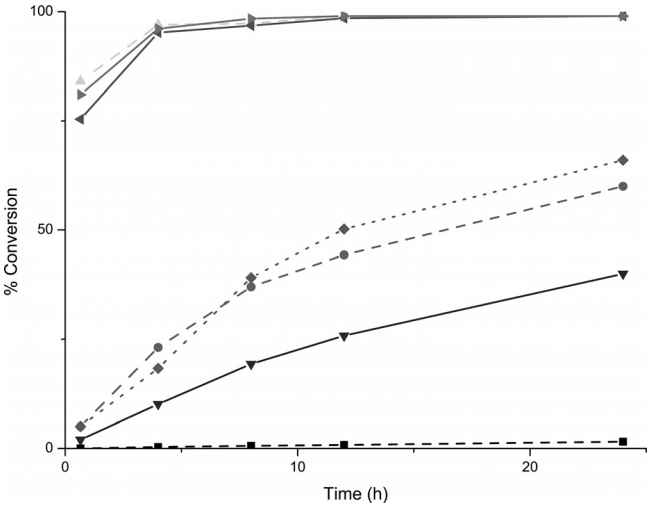


Figure 1. Conversion of **3d** with enzyme (solid lines) and without enzyme (dashed lines). Inactivated lipase was also used (dotted line). Reaction conditions: Enzyme: LIP, solvent = DIPE, E/S = 1, *T* = 30 °C. With enzyme: *c* = 0.12 M (◀), *c* = 0.012 M (▼). Without enzyme: *c* = 0.12 M (●), *c* = 0.012 M (■). Inactivated enzyme: *c* = 0.12 M (♦).

only 36%. From these experiments we concluded that the most adequate concentration for the enzyme-catalyzed reaction is 0.12 M.

Finally, with the aim of discarding the occurrence of unspecific catalysis by the protein or the enzyme support, we performed the addition of ethanolamine to ethyl acrylate by using a thermally inactivated LIP at a substrate concentration of 0.12 M. The results are also depicted in Figure 1 (dotted line). Comparison of these results with those obtained in the absence of the enzyme (Figure 1, dashed line) allowed us to conclude that neither denatured protein nor the support served as a catalyst for this reaction. Therefore, the active site of LIP is involved in the biocatalytic reaction.

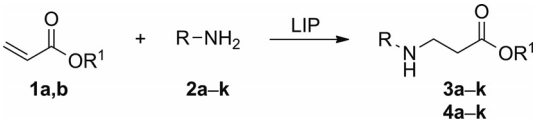
Preparation of *N*-Substituted β-Amino Esters

Once the experimental conditions were optimized, we decided to apply the enzymatic aza-Michael addition to other donors and acceptors. The results, expressed as yield of isolated product, for ethyl and butyl acrylate as Michael acceptors and various donors belonging to the above-mentioned three types, alkylamines, alkanolamines, and diamines, are summarized in Table 2.

A remarkable difference in product yield was observed for different alkylamines, and phenethylamine (**2c**) was found to be the best substrate for the reaction. Thus, product **3c** was obtained in higher yield (85%) than **3a** and **3b** by using *n*-propylamine and isopropylamine, respectively (Table 2, Entries 1–3).

It can be seen that the best yields were obtained with alkanolamines and diamines as donors. These nucleophiles have another polar group besides the nucleophilic amino group. With the exception of **3g** with 64% yield, which could be attributed to some steric hindrance in alkanol-

Table 2. *Rhizomucor miehei* lipase catalyzed aza-Michael addition reaction of amines **2a**–**k** to alkyl acrylates **1a** and **1b**.[a]



1a,b **2a–k** **3a–k** **4a–k**

Entry	Amine	Alkyl acrylate	Solvent	Product (% yield)
1	**2a**	**1a**	hexane	**3a** (55)
2	**2b**	**1a**	hexane	**3b** (45)
3	**2c**	**1a**	hexane	**3c** (85)
4	**2d**	**1a**	DIPE	**3d** (76)
5	**2d**	**1a**	hexane	**3d*** (76)[b]
6	**2e**	**1a**	DIPE	**3e** (71)
7	**2f**	**1a**	DIPE	**3f** (83)
8	**2g**	**1a**	DIPE	**3g** (64)
9	**2h**	**1a**	hexane	**3h** (100)
10	**2i**	**1a**	hexane	**3i** (89)
11	**2j**	**1a**	hexane	**3j** (91)
12	**2k**	**1a**	hexane	**3k** (99)[c]
13	**2a**	**1b**	hexane	**4a** (66)
14	**2b**	**1b**	hexane	**4b** (60)
15	**2c**	**1b**	hexane	**4c** (87)
16	**2d**	**1b**	DIPE	**4d** (75)
17	**2d**	**1b**	hexane	**4d*** (81)[d]
18	**2e**	**1b**	DIPE	**4e** (92)
19	**2f**	**1b**	DIPE	**4f** (80)
20	**2g**	**1b**	DIPE	**4g** (86)
21	**2h**	**1b**	hexane	**4h** (100)
22	**2i**	**1b**	hexane	**4i** (87)
23	**2j**	**1b**	hexane	**4j** (94)
24	**2k**	**1b**	hexane	**4k** (93)[c]

[a] Reaction conditions: Enzyme = LIP, substrate concentration = 0.12 M, E/S = 1, T = 30 °C. reaction time = 16 h, 200 rpm. [b] **3d***: double Michael adduct, $HOCH_2CH_2N(CH_2CH_2COOEt)_2$. [c] T = 55 °C. [d] **4d***: double Michael adduct, $HOCH_2CH_2N(CH_2CH_2COO\textit{n}Bu)_2$.

amine **2g**, the rest of the products were obtained in yields ranging from 71 to 100% (Table 2, Entries 4–12).

Regarding the reactivity of the diamines, only **2k**, containing the longest alkyl chain, required a higher reaction temperature to reach the maximum product yield (Table 2, Entries 12 and 24). From the results it seems that the chain length between both amino groups did not influence mainly the reaction yields. The best performance was achieved with the shorter chain of the three methylene groups, as with both acceptors **1a** and **1b**, their respective products **3h** and **4h** were obtained in quantitative yield.

Concerning the structure of the Michael acceptor on the reaction performance, two tendencies were observed. First, increasing the *O*-alkyl chain length (Table 2, Entries 13–24) did not affect the reaction and the same type of product (i.e., **4a**–**k**) in similar yields to those obtained for **3a**–**k** were obtained. Second, if methyl substituents were attached to the double bond no reaction products were obtained. Neither methyl methacrylate (with a methyl substituent at C3) nor methyl crotonate (with a methyl substituent at C4) gave any product with ethanolamine (**2d**) or 1,3-diaminopropane (**2h**), which are nucleophiles that served as the best substrates for the reaction with acrylates **1a** and **1b**. According to the classification of lipases on the basis of their binding sites to ester substrates, LIP belongs to the group of lipases that have a large alcohol binding cleft but a narrow acyl binding cleft.[32] As it has been proposed, the lipase-catalyzed aza-Michael reaction would start with the accommodation of the Michael acceptor.[28] This could explain why ethyl and *n*-butyl acrylate, with the same small acyl group are good substrates and the bulky alkyl crotonate or methacrylate are not. Regarding the alcohol moiety, the structural geometry of LIP allowed both alkyl groups to be accepted.

LIP is applied in a variety of chemical processes due to its high activity and stability.[33] Under the above-mentioned conditions, its substrate specificity was also remarkable. Therefore, a single Michael addition product could be obtained in very good yield in every case without any secondary product and none of these products were able to react as Michael donors. In the case of diamines as donors, this effect highlights even more the substrate specificity, as the NH_2 group in reaction products **3h**–**k** remained unreactive.

Conclusions

In this work we describe for the first time the synthesis of *N*-substituted-β-amino esters by application of lipases in the aza-Michael addition of mono- and bifunctional amines to α,β-unsaturated esters. Twenty-two *N*-substituted-β-amino esters and two *N*,*N*-disubstituted double Michael adducts were obtained. Fifteen of them, including the double Michael adducts, are new products.

The influence of the enzyme source, the substrate structure, and various reaction parameters on the results was analyzed. After an enzyme screening it was concluded that LIP was the best biocatalyst in terms of yield and selectivity. All reactions, except that with 1,12-diaminododecane (55 °C), were performed at room temperature. A strong influence of the solvent on the nature of the donors was observed. Hexane was the solvent of choice for amines and diamines and DIPE for alkanolamines. With alkanolamines in hexane, the enzyme was not selective and double Michael adducts were obtained. The substrate concentration played an important role in enhancing the enzyme catalysis over the spontaneous reaction. Biocatalyst efficiency was limited by high substrate concentration in which a mixture of products was obtained.

By comparing the performances of mono- and bifunctional amines as Michael donors, the highest yields were achieved when the nucleophile had a polar group besides the nucleophilic amine. Regarding the structure of the esters, the chain length of the *O*-alkyl substituent did not influence the reaction performance. However, the presence of methyl groups on the double bond did not allow the reaction to proceed.

In summary, due to their high selectivity and promiscuous behavior, the *Rhizomucor miehei* lipase proved to be an efficient biocatalyst to synthesize a variety of *N*-substituted-

β-amino esters with potential application as starting materials in the synthesis of biomedical polymers.

Experimental Section

Enzymes and Materials: Hexane, ethyl acetate, and toluene were purchased from Merck Argentina and diisopropyl ether (DIPE) from J. T. Baker. Chemical reagents were purchased from Sigma–Aldrich de Argentina. Ethyl acrylate, *n*-butyl acrylate, and methyl methacrylate were a generous gift from Clariant Argentina. *Candida rugosa* lipase (CRL, type VII, 706 U/mg solid) was purchased from Sigma–Aldrich de Argentina, *Pseudomonas* lipase: Lipase PS Amano (PSL, 33,200 U/g) was purchased from Amano Enzyme USA Co., and *Candida antarctica* B lipase (CAL B, Novozym 435, 7400 PLU/g) and *Rhizomucor miehei* lipase (Lipozyme RM IM, 7800 U/g) were a generous gift from Novozymes Spain. Enzymes were used "straight from the bottle". Inactivated enzyme was prepared by heating the biocatalyst at 105 °C in an oven for 2 h.

Analytical Methods: Reactions were carried out in a Sontec incubator shaker (Scientifica Argentina) at 200 rpm at the indicated temperature. All reactions were monitored by TLC with Merck TLC sheets (Silica gel 60 F254, aluminum support). Flash column chromatography was carried out by using silica gel 60 (0,040–0.063 mm) purchased from Merck. The percentage of conversion was determined by GC analysis with a Finnigan Focus GC, Thermo Electron Co. instrument, the capillary column being HP-ULTRA-1, 25 m × 0.2 mm, film thickness 0.11 μm, (5 min at 50 °C, 10 °C/min, 280 °C, inlet 180 °C, detector 300 °C). t_R: ethyl acrylate: 5.18 min, ethyl *N*-(2-hydroxyethyl)-β-alaninate (**3d**): 15.92 min, ethanolamine: 24.21 min. BHT (in DIPE as stabilizer) was used as internal standard (t_R: 19.59 min). ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AC 200 NMR instrument by using $CDCl_3$ as solvent. Chemical shifts were reported in δ units relative to TMS set at 0 ppm. High-resolution mass spectra were recorded with a Bruker microTOF-Q II mass spectrometer (ionization mode: ESI). FTIR measurements were performed with a Shimadzu FTIR-8300 spectrophotometer in film with KBr windows. Melting points were determined with a Fisher-Jones melting point apparatus.

Preparation of *N*-Substituted-β-amino Esters: The typical procedure consisted in the preparation of a solution containing the α,β-unsaturated ester (0.9 mmol) and the corresponding amine (0.9 mmol) in the indicated solvent (7.5 mL). Lipozyme (100 mg) (or equivalent amount of lipases from various sources) was added to the solution, and the resulting suspension was shaken at 30 °C and 200 rpm. The course of the reaction was followed by TLC and GC. Once the reaction reached its maximum conversion, the enzyme was filtered and washed with the solvent (3×). The filtered solvents were evaporated under reduced pressure, and the residue was purified by flash chromatography and characterized by ^{1}H NMR, ^{13}C NMR, and FTIR spectroscopy and HRMS.

Ethyl *N*-Propyl-β-alaninate (3a): Colorless liquid. Yield: 87.5 mg (55%). ^{1}H NMR (200.1 MHz): δ = 0.81 (t, J = 7.3 Hz, 3 H), 1.15 (t, J = 6.9 Hz, 3 H), 1.42 (m, J = 7.3 Hz, 2 H), 2.46 (t, J = 6.6 Hz, 2 H), 2.51 (t, J = 7.3 Hz, 2 H), 2.81 (t, J = 6.6 Hz, 2 H), 3.49 (br. s), 4.03 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 11.4, 14.0, 22.4, 34.0, 44.5, 51.1, 60.2, 172.3 ppm. FTIR (film): $\tilde{\nu}$ = 3403, 1732, 1647 cm^{-1}. HRMS: calcd. for $C_8H_{18}NO_2$ [M + H] 160.13321; found 160.13379.

Ethyl *N*-Isopropyl-β-alaninate (3b): Colorless liquid. Yield: 71.7 mg (45%). ^{1}H NMR (200.1 MHz): δ = 1.01 (d, J = 6.2 Hz, 6 H), 1.16 (t, J = 6.9 Hz, 3 H), 2.48 (t, J = 6.6 Hz, 2 H), 2.76 (m, J = 6.2 Hz, 1 H), 2.82 (t, J = 6.6 Hz, 2 H), 3.19 (br. s), 4.04 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.0, 22.2, 34.2, 42.0, 48.5, 60.3, 172.3 ppm. FTIR (film): $\tilde{\nu}$ = 3397, 1735, 1632 cm^{-1}. HRMS: calcd. for $C_8H_{18}NO_2$ [M + H] 160.13321; found 160.13368.

Ethyl *N*-(2-Phenylethyl)-β-alaninate (3c): Colorless liquid. Yield: 188.8 mg (85%). Physical properties as described previously.[33] ^{1}H NMR (200.1 MHz): δ = 1.23 (t, J = 6.9 Hz, 3 H), 2.51 (t, J = 6.6 Hz, 2 H), 2.72 (t, J = 5.1 Hz, 2 H), 2.82 (t, J = 6.6 Hz, 2 H), 2.91 (t, J = 5.1 Hz, 2 H), 4.11 (q, J = 6.9 Hz, 2 H), 7.22 (dd, J = 6.9, 2.9 Hz,1 H), 7.27 (ddd, J = 6.9, 2.2, 2.9 Hz, 2 H), 7.30 (ddd, J = 6.9, 2.2, 2.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.1, 34.3, 35.9, 44.7, 50.7, 60.4, 126.1, 126.3, 128.4, 128.6, 172.5 ppm. FTIR (film): $\tilde{\nu}$ = 3313, 1733, 1650, 843 cm^{-1}. HRMS: calcd. for $C_{13}H_{20}NO_2$ [M + H] 222.14886; found 222.14874.

Ethyl *N*-(2-Hydroxyethyl)-β-alaninate (3d): Colorless liquid. Yield: 110.3 mg (76%). Physical properties and ^{1}H NMR and FTIR spectroscopic data as described previously.[14] ^{13}C NMR (50.2 MHz): δ = 14.1, 34.6, 44.4, 50.8, 60.5, 60.6, 172.6 ppm. HRMS: calcd. for $C_7H_{16}NO_3$ [M + H] 162.11247; found 162.11188.

Ethyl *N*-(3-Ethoxy-3-oxopropyl)-*N*-(2-hydroxyethyl)-β-alaninate (3d*): Colorless liquid. Yield: 89.7 mg (76%). ^{1}H NMR (200.1 MHz): δ = 1.27 (t, J = 6.9 Hz, 6 H), 2.47 (t, J = 6.6 Hz, 4 H), 2.61 (t, J = 6.6 Hz, 2 H), 2.82 (t, J = 6.6 Hz, 4 H), 3.60 (t, J = 6.6 Hz, 2 H), 4.15 (q, J = 6.9 Hz, 4 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.1, 32.6, 49.1, 55.9, 58.9, 60.5, 172.5 ppm. FTIR (film): $\tilde{\nu}$ = 3467, 1732 cm^{-1}. HRMS: calcd. for $C_{12}H_{24}NO_5$ [M + H] 262.16545; found 262.16517.

Ethyl *N*-(3-Hydroxypropyl)-β-alaninate (3e): Colorless liquid. Yield: 112.0 mg (71%). ^{1}H NMR (200.1 MHz): δ = 1.25 (t, J = 6.9 Hz, 3 H), 1.68 (m, J = 6.6 Hz, 2 H), 2.48 (t, J = 6.6 Hz, 2 H), 2.77 (t, J = 6.6 Hz, 2 H), 2.87 (t, J = 6.6 Hz, 2 H), 3.78 (t, J = 6.6 Hz, 2 H), 4.13 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.2, 30.6, 34.4, 44.9, 49.6, 60.5, 64.2, 172.6 ppm. FTIR (film): $\tilde{\nu}$ = 3409, 1724, 1643 cm^{-1}. HRMS: calcd. for $C_8H_{18}NO_3$ [M + H] 176.12818; found 176.12833.

Ethyl *N*-(4-Hydroxybutyl)-β-alaninate (3f): Colorless liquid. Yield: 141.4 mg (83%). ^{1}H NMR (200.1 MHz): δ = 1.21 (t, J = 6.9 Hz, 3 H), 1.60 (m, J = 6.6 Hz, 4 H), 2.51 (t, J = 6.6 Hz, 2 H), 2.63 (t, J = 6.6 Hz, 2 H), 2.85 (t, J = 6.6 Hz, 2 H), 3.52 (t, J = 6.6 Hz, 2 H), 4.10 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.1, 27.8, 31.9, 33.6, 44.3, 49.2, 60.5, 62.2, 172.8 ppm. FTIR (film): $\tilde{\nu}$ = 3417, 1716, 1638 cm^{-1}. HRMS: calcd. for $C_9H_{20}NO_3$ [M + H] 190.14377; found 190.14383.

Ethyl *N*-(2-Hydroxy-1,1-dimethylethyl)-β-alaninate (3g): Colorless liquid. Yield: 109.0 mg (64%). ^{1}H NMR (200.1 MHz): δ = 1.06 (s, 6 H), 1.26 (t, J = 6.9 Hz, 3 H), 2.17 (br. s), 2.47 (t, J = 6.6 Hz, 2 H), 2.77 (t, J = 6.6 Hz, 2 H), 3.30 (s, 2 H), 4.14 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.2, 27.1, 35.2, 37.1, 60.6, 67.7, 53.6, 171.0 ppm. FTIR (film): $\tilde{\nu}$ = 3403, 1732, 1652 cm^{-1}. HRMS: calcd. for $C_9H_{20}NO_3$ [M + H] 190.14377; found 190.14414.

Ethyl *N*-(3-Aminopropyl)-β-alaninate (3h): Colorless liquid. Yield: 156.8 mg (100%). ^{1}H NMR (200.1 MHz): δ = 1.15 (t, J = 6.9 Hz, 3 H), 1.53 (m, J = 7.0 Hz, 2 H), 2.39 (t, J = 6.6 Hz, 2 H), 2.57 (t, J = 7.0 Hz, 2 H), 2.65 (t, J = 7.0 Hz, 2 H), 2.77 (t, J = 6.6 Hz, 2 H), 4.02 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.0, 33.5, 34.5, 40.3, 44.9, 47.4, 60.1, 172.6 ppm. FTIR (film): $\tilde{\nu}$ = 3363, 1733, 1643, 1561 cm^{-1}. HRMS: calcd. for $C_8H_{19}N_2O_2$ [M + H] 175.14410; found 175.14397.

Ethyl *N*-(6-Aminohexyl)-β-alaninate (3i): Colorless liquid. Yield: 173.2 mg (89%). ^{1}H NMR (200.1 MHz): δ = 1.20–1.70 (m, 8 H),

1.25 (t, J = 6.9 Hz, 3 H), 2.50 (t, J = 6.6 Hz, 2 H), 2.59 (t, J = 6.9 Hz, 2 H), 2.67 (t, J = 6.9 Hz, 2 H), 2.86 (t, J = 6.6 Hz, 2 H), 4.13 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.1, 26.8, 27.2, 30.1, 33.6, 34.9, 41.9, 45.1, 49.5, 60.4, 172.7 ppm. FTIR (film): $\tilde{\nu}$ = 3360, 1735, 1653, 1560 cm^{-1}. HRMS: calcd. for $C_{11}H_{25}N_2O_2$ [M + H] 217.19105; found 218.19133.

Ethyl *N*-(8-Aminooctyl)-β-alaninate (3j): Colorless liquid. Yield: 200.1 mg (91%). ^{1}H NMR (200.1 MHz): δ = 1.26 (t, J = 6.9 Hz, 3 H), 1.30–1.43 (m, 12 H), 2.51 (t, J = 6.6 Hz, 2 H), 2.60 (t, J = 6.9 Hz, 2 H), 2.67 (t, J = 6.9 Hz, 2 H), 2.87 (t, J = 6.6 Hz, 2 H), 4.14 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.1, 26.8, 27.2, 29.3, 29.4, 30.0, 33.8, 34.7, 42.2, 45.0, 49.7, 60.3, 172.8 ppm. FTIR (film): $\tilde{\nu}$ = 3324, 1733, 1639, 1560 cm^{-1}. HRMS: calcd. for $C_{13}H_{29}N_2O_2$ [M + H] 245.22290; found 245.22235.

Ethyl *N*-(12-Aminododecyl)-β-alaninate (3k): White solid. Yield: 270.4 mg (99%). M.p. 93–95 °C. Physical properties as described previously.[35] ^{1}H NMR (200.1 MHz): δ = 1.21 (m, 19 H), 1.41 (m, J = 6.9 Hz, 4 H), 2.47 (t, J = 6.6 Hz, 2 H), 2.54 (t, J = 6.9 Hz, 2 H), 2.63 (t, J = 6.9 Hz, 2 H), 2.81 (t, J = 6.6 Hz, 2 H), 4.14 (q, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.1, 26.7, 27.2, 29.3, 29.4, 29.6, 32.8, 33.0, 34.2, 41.7, 44.7, 49.6, 60.4, 172.7 ppm. FTIR (film): $\tilde{\nu}$ = 3340, 1732, 1652, 1568 cm^{-1}. HRMS: calcd. for $C_{17}H_{37}N_2O_2$ [M + H] 301.28495; found 301.28507.

Butyl *N*-Propyl-β-alaninate (4a): Colorless liquid. Yield: 11.2 mg (66%). ^{1}H NMR (200.1 MHz): δ = 0.93 (t, J = 7.3 Hz, 3 H), 0.94 (t, J = 6.9 Hz, 3 H), 1.38 (m, J = 6.9 Hz, 2 H), 1.57 (m, J = 7.3 Hz, 2 H), 1.58 (m, J = 6.9 Hz, 2 H), 2.58 (t, J = 6.6 Hz, 2 H), 2.62 (t, J = 7.3 Hz, 2 H), 2.92 (t, J = 6.6 Hz, 2 H), 4.09 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 11.4, 13.5, 18.9, 22.4, 30.4, 34.0, 44.6, 51.1, 64.2, 172.4 ppm. FTIR (film): $\tilde{\nu}$ = 3405, 1736, 1640 cm^{-1}. HRMS: calcd. for $C_{10}H_{22}NO_2$ [M + H] 188.16451; found 188.16408.

Butyl *N*-Isopropyl-β-alaninate (4b): Colorless liquid. Yield: 101.1 mg (60%). Physical properties and FTIR as described previously.[4] ^{1}H NMR (200.1 MHz): δ = 0.85 (t, J = 6.9 Hz, 3 H), 1.02 (d, J = 6.2 Hz, 6 H), 1.30 (m, J = 6.9 Hz, 2 H), 1.53 (m, J = 6.9 Hz, 2 H), 2.48 (t, J = 6.6 Hz, 2 H), 2.64 (t, J = 6.6 Hz, 2 H), 2.80 (m, J = 6.6 Hz, 1 H), 4.03 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.5, 18.1, 22.3, 30.5, 34.3, 42.2, 48.5, 64.2, 172.5 ppm. HRMS: calcd. for $C_{10}H_{22}NO_2$ [M + H] 188.16451; found 188.16496.

Butyl *N*-(2-Phenylethyl)-β-alaninate (4c): Colorless liquid. Yield: 195.8 mg (87%). ^{1}H NMR (200.1 MHz): δ = 0.90 (t, J = 6.9 Hz, 3 H), 1.31 (m, J = 6.9 Hz, 2 H), 1.52 (m, J = 6.9 Hz, 2 H), 2.51 (t, J = 6.6 Hz, 2 H), 2.69 (t, J = 5.1 Hz, 2 H), 2.82 (t, J = 6.6 Hz, 2 H), 2.92 (t, J = 5.1 Hz, 2 H), 4.03 (t, J = 6.9 Hz, 2 H), 7.22 (dd, J = 6.9, 2.9 Hz, 1 H), 7.27 (ddd, J = 6.9, 2.2, 2.9 Hz, 2 H), 7.32 (ddd, J = 6.9, 2.2, 2.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.8, 18.4, 30,9, 34.8, 36.2, 46.4, 49.1, 64.5, 126.1, 126.3, 128.5, 128.7, 172.3 ppm. FTIR (film): $\tilde{\nu}$ = 3315, 1733, 1650, 845 cm^{-1}. HRMS: calcd. for $C_{15}H_{24}NO_2$ [M + H] 250.18016; found 250.18044.

Butyl *N*-(2-Hidroxyethyl)-β-alaninate (4d): Colorless liquid. Yield: 127.7 mg (75%). Physical properties as described previously.[14] ^{1}H NMR (200.1 MHz): δ = 0.91 (t, J = 6.9 Hz, 3 H), 1.38 (m, J = 6.9 Hz, 2 H), 1.59 (m, J = 6.9 Hz, 2 H), 2.51 (t, J = 6.6 Hz, 2 H), 2.77 (t, J = 6.6 Hz, 2 H), 2.90 (t, J = 6.6 Hz, 2 H), 3.63 (t, J = 6.6 Hz, 2 H), 4.07 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.7, 19.1, 30.6, 34.6, 44.5, 50.8, 60.6, 64.5, 172.8 ppm. FTIR (film): $\tilde{\nu}$ = 3326 1732, 1638 cm^{-1}. HRMS: calcd. for $C_9H_{20}NO_3$ [M + H] 190.14377; found 190.14441.

***N*-(3-Ethoxy-3-oxopropyl)-*N*-(2-hydroxyethyl)-β-alaninate (4d*):** Colorless liquid. Yield: 115.7 mg (81%). ^{1}H NMR (200.1 MHz): δ = 0.93 (t, J = 6.9 Hz, 6 H), 1.36 (m, J = 6.9 Hz, 4 H), 1.61 (m, J = 6.9 Hz, 4 H), 2.47 (t, J = 6.6 Hz, 4 H), 2.61 (t, J = 6.6 Hz, 2 H), 2.82 (t, J = 6.6 Hz, 4 H), 3.60 (t, J = 6.6 Hz, 2 H), 4.07 (t, J = 6.9 Hz, 4 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.7, 19.1, 30.6, 32.5, 49.1, 56.0, 58.9, 64.5, 172.6 ppm. FTIR (film): $\tilde{\nu}$ = 3446, 1735 cm^{-1}. HRMS: calcd. for $C_{16}H_{32}NO_5$ [M + H] 318.22750; found 318.22839.

Butyl *N*-(3-Hydroxypropyl)-β-alaninate (4e): Colorless liquid. Yield: 168.3 mg (92%). ^{1}H NMR (200.1 MHz): δ = 0.87 (t, J = 6.9 Hz, 3 H), 1.31 (m, J = 6.9 Hz, 2 H), 1.48–1.70 (m, 4 H), 2.45 (t, J = 6.6 Hz, 2 H), 2.80 (t, J = 6.6 Hz, 2 H), 2.82 (t, J = 6.6 Hz, 2 H), 3.09 (br. s), 3.70 (t, J = 6.6 Hz, 2 H), 4.02 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.0, 19.0, 27.8, 30.7, 34.2, 44.8, 49.2, 60.5, 62.2, 172.5 ppm. FTIR (film): $\tilde{\nu}$ = 3314, 1732, 1646 cm^{-1}. HRMS: calcd. for $C_{10}H_{22}NO_3$ [M + H] 204.15942; found 204.15960.

Butyl *N*-(4-Hydroxybutyl)-β-alaninate (4f): Colorless liquid. Yield: 156.5 mg (80%). ^{1}H NMR (200.1 MHz): δ = 0.86 (t, J = 6.9 Hz, 3 H), 1.28 (m, J = 6.9 Hz, 2 H), 1.47–1.61 (m, 6 H), 2.47 (t, J = 6.6 Hz, 2 H), 2.60 (t, J = 6.6 Hz, 2 H), 2.81 (t, J = 6.6 Hz, 2 H), 3.50 (t, J = 6.6 Hz, 2 H), 3.65 (br. s), 4.02 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.5, 19.0, 28.0, 30.4, 32.0, 33.8, 44.4, 49.2, 62.2, 64.3, 172.5 ppm. FTIR (film): $\tilde{\nu}$ = 3312, 1733, 1646 cm^{-1}. HRMS: calcd. for $C_{11}H_{24}NO_3$ [M + H] 218.17507; found 218.17578.

Butyl *N*-(2-Hydroxy-1,1-dimethylethyl)-β-alaninate (4g): Colorless liquid. Yield: 169.0 mg (86%). ^{1}H NMR (200.1 MHz): δ = 0.92 (t, J = 6.9 Hz, 3 H), 1.06 (s, 6 H), 1.35 (m, J = 6.9 Hz, 2 H), 1.60 (m, J = 6.9 Hz, 2 H), 2.00 (br. s), 2.47 (t, J = 6.6 Hz, 2 H), 2.77 (t, J = 6.6 Hz, 2 H), 3.30 (s, 2 H), 4.09 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.6, 19.1, 24.0, 30.6, 34.9, 37.1, 51.7, 53.7, 64.5, 67.7, 173.4 ppm. FTIR (film): $\tilde{\nu}$ = 3363, 1735, 1653 cm^{-1}. HRMS: calcd. for $C_{11}H_{24}NO_3$ [M + H] 218.17507; found 218.17590.

Butyl *N*-(3-Aminopropyl)-β-alaninate (4h): Colorless liquid. Yield: 182.1 mg (100%). ^{1}H NMR (200.1 MHz): δ = 0.92 (t, J = 6.9 Hz, 3 H), 1.37 (m, J = 6.9 Hz, 2 H), 1.56 (m, J = 6.9 Hz, 4 H), 2.38 (t, J = 7.0 Hz, 2 H), 2.57 (t, J = 6.6 Hz, 2 H), 2.66 (t, J = 7.0 Hz, 2 H), 2.79 (t, J = 6.6 Hz, 2 H), 4.05 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 14.0, 19.1, 30.6, 33.5, 34.5, 40.3, 44.9, 47.4, 64.3, 172.3 ppm. FTIR (film): $\tilde{\nu}$ = 3381, 1735, 1653, 1558 cm^{-1}. HRMS: calcd. for $C_{10}H_{23}N_2O_2$ [M + H] 203.17540; found 203.17522.

Butyl *N*-(6-Aminohexyl)-β-alaninate (4i): Colorless liquid. Yield: 191.0 mg (87%). Physical properties as described previously.[15] ^{1}H NMR (200.1 MHz): δ = 0.93 (t, J = 6.9 Hz, 3 H), 1.20–1.65 (m, 12 H), 2.51 (t, J = 6.6 Hz, 2 H), 2.60 (t, J = 7.0 Hz, 2 H), 2.68 (t, J = 7.0 Hz, 2 H), 2.87 (t, J = 6.6 Hz, 2 H), 4.09 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.7, 19.1, 26.8, 27.2, 30.0, 30.6, 33.6, 34.7, 42.1, 45.1, 49.7, 64.3, 172.9 ppm. FTIR (film): $\tilde{\nu}$ = 3314, 1734, 1657, 1559 cm^{-1}. HRMS: calcd. for $C_{13}H_{29}N_2O_2$ [M + H] 245.22235; found 245.22221.

Butyl *N*-(8-Aminooctyl)-β-alaninate (4j): Colorless liquid. Yield: 228.1 mg (94%). ^{1}H NMR (200.1 MHz): δ = 0.91 (t, J = 6.9 Hz, 3 H), 1.30–1.41 (m, 14 H), 1.54 (m, J = 6.9 Hz, 2 H), 2.53 (t, J = 6.6 Hz, 2 H), 2.62 (t, J = 6.9 Hz, 2 H), 2.66 (t, J = 6.9 Hz, 2 H), 2.88 (t, J = 6.6 Hz, 2 H), 4.05 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.7, 19.2, 26.8, 27.2, 28.8, 29.2, 29.4, 30.1, 30.6, 33.9, 34.7, 42.3, 45.2, 49.9, 64.1, 172.7 ppm. FTIR (film): $\tilde{\nu}$ = 3333, 1733, 1630, 1560 cm^{-1}. HRMS: calcd. for $C_{15}H_{33}N_2O_2$ [M + H] 273.25365; found 273.25394.

Butyl *N*-(12-Aminododecyl)-β-alaninate (4k): White solid. Yield: 274.8 mg (93%). M.p. 97–98 °C. ^{1}H NMR (200.1 MHz): δ = 0.92 (t, J = 6.9 Hz, 3 H), 1.29 (m, 18 H), 1.41 (m, 6 H), 2.44 (t, J = 6.9 Hz, 2 H), 2.53 (t, J = 6.6 Hz, 2 H), 2.66 (t, J = 6.9 Hz, 2 H), 2.87 (t, J = 6.6 Hz, 2 H), 4.06 (t, J = 6.9 Hz, 2 H) ppm. ^{13}C NMR (50.2 MHz): δ = 13.7, 19.3, 27.2, 27.4, 28.7, 29.3, 29.5, 29.6, 29.8, 29.9, 30.4, 32.9, 34.4, 41.9, 44.8, 49.5, 64.4, 172.7 ppm. FTIR (film): $\tilde{\nu}$ = 3340, 1732, 1642, 1570 cm^{-1}. HRMS: calcd. for $C_{19}H_{41}N_2O_2$ [M + H] 329.31625; found 329.31579.

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