EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201402749

Lipase-Catalyzed Synthesis of Substituted Phenylacetamides: Hammett Analysis and Computational Study of the Enzymatic Aminolysis

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Keywords: Enzyme catalysis / Molecular modeling / Reaction mechanisms / Amides / Esters

A series of hydroxy-, methoxy-, and nitrophenylacetamides was synthesized by enzyme catalysis. The 28 new products were obtained through a lipase-catalyzed two-step reaction in very good to excellent yield. In the case of nitro derivatives, a one-pot, two-step methodology allowed the desired products to be obtained in high yields. The influence of various reaction parameters in the lipase-catalyzed reactions, such as enzyme source, nucleophile (alcohol or amine)/substrate ratio, enzyme/substrate ratio, solvent and temperature were studied. It was observed that nitro-substituted phenylacetates were more reactive in the aminolysis reaction than phenylacetates substituted with a hydroxyl group. To study this substituent effect, a Hammett analysis and the determination of the ρ parameter were carried out. Moreover, a computational study was applied to the most representative systems, performing an exploration of the potential energy surface for the catalyzed and noncatalyzed aminolysis reaction for nitro- and hydroxyphenylacetates. Both analysis showed that the presence of a strongly electron-attracting group favors the activity of the enzyme, in complete agreement with the experimental results of the enzymatic catalysis.

1. Introduction

The exploration of new molecules that can have potency in multiple biological targets remains an intriguing scientific endeavor. In a continuation of our ongoing program aimed at the development of bioactive compounds using enzymes as catalysts, we became interested in the preparation of derivatives of phenylacetamides. In recent years, much attention has focused on the synthesis of this type of compounds, and a significant number of them have been tested for a range of biological activities. For example, one of the first antimalarial hits identified was the polyamine diamide orthidine F[1] and, very recently, it was reported that diphenylacetamides are potent and selective antimalarial agents.[2]

Substituted phenylacetamides showed a diverse range of biological activities, such as antimicrobial activity,[3,4] selective α-1a adrenergic receptor antagonists used for the clinical management of benign prostatic hyperplasia,[5] functional antagonists at the human CCR5 receptor,[6,7] non-SCFA allosteric agonists of FFA2,[8] anticonvulsant activity,[9] and analgesic effects.[10]

The use of enzymes and whole cells of microorganisms in the synthesis of pharmaceutical derivatives is a continuously increasing field.[11–13] It is recognized that enzymes are capable of accepting a wide array of substrates, and catalyze reactions in a chemo- and regioselective way. As a result, biocatalysts allow different chemical transformations to be carried out without the need for tedious protection and deprotection steps, especially in compounds with several functional groups. Over the last years, biocatalysis using lipases in non-aqueous media has been widely used for several synthetic reactions such as esterification, transesterification, aminolysis, and polymerization.[14–16] Enzymes are also well-known for their highly enantioselective behavior, and this property has formed the basis for their widespread use in the synthesis of enantiomerically pure compounds.[17,18]

Several studies carried out in our laboratory on the esterification and transesterification of multiple substrates have shown that lipases are useful in the synthesis of biologically active compounds.[19] Recently, in the field of pharmaceuticals, we reported the synthesis of a series of 2- and 3-hydroxypyridine derivatives with application as potential antiparasitic agents.[20] Moreover, lipases showed high chemo- and regioselectivity in aminolysis reactions,[21] particularly in the preparation of an intermediate in the synthesis of alfuzosin by a one-pot, two-step aminolysis of esters,[22] and the application of this methodology to the synthesis of the bactericide lapyrium chloride.[23] Encouraged by these results, in the present work we report an enzymatic

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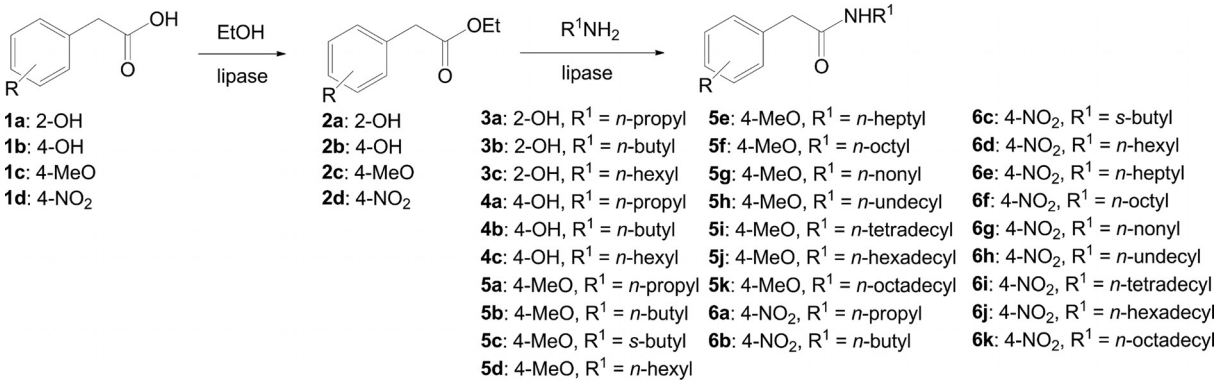
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201402749.



Scheme 1. Synthesis of substituted phenylacetamides.

strategy for the synthesis of a series of new substituted phenylacetamides, as summarized in Scheme 1.

In addition, with the aim of finding an explanation for the effect of substituents in the aromatic ring on the enzymatic aminolysis, a Hammett correlation study was carried out and the ρ parameter was determined. Finally, a computer simulation study was applied involving a combination of molecular mechanics and quantum calculations.

2. Results and Discussion

2.1. Enzymatic Synthesis

Phenylacetamides are usually synthesized by conventional chemical methods involving reagents that are not friendly to the environment such as acid chlorides and pyridine. Biocatalysis allows the use of esters or the direct use of carboxylic acid to obtain amides, which is advantageous from economical and environmental viewpoints. Regarding the biocatalytic approach, to date, little has been reported on the enzymatic synthesis of phenylacetamides. By using penicillin G acylase immobilized on glyoxyl agarose, it was possible to perform the direct condensation between (±)-2-hydroxy-2-phenylethylamine and different acyl donors in the presence of high concentrations of organic cosolvent.[24] In the present work, we applied a lipase-catalysis method, previously reported in our laboratory,[22,23] which afforded 28 substituted phenylacetamides with various alkyl chain length and substituent groups on the aromatic ring.

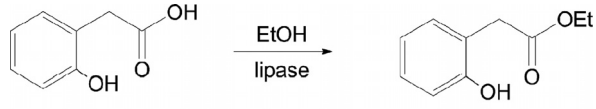
The enzymatic approach involved two steps: (i) the reaction of substituted phenylacetic acids with ethanol to obtain the corresponding ethyl phenylacetates, and (ii) the aminolysis of esters with a variety of amines to yield substituted phenylacetamides (Scheme 1). Moreover, a one-pot, two-step procedure to obtain phenylacetamides was performed. With the aim of achieving the optimal conditions, we studied the behavior of various lipases and reaction parameters such as solvent, temperature, enzyme/substrate ratio (E/S) and nucleophile (ethanol)/substrate ratio (A/S). In every case, (2-hydroxyphenyl)acetic acid (**1a**) was used as substrate.

2.1.1. Esterification of Phenylacetic Acids

2.1.1.1. Enzyme Screening and Solvent Effect

Three commercial lipases were evaluated in the esterification reaction of **1a** with ethanol: *Candida rugosa* lipase (CRL), *Candida antarctica* lipase B (CAL B) and Lipozyme, a lipase from the fungus *Rhizomucor miehei* (LIP). The solvents tested were acetonitrile, hexane, diisopropyl ether, and toluene, and the reaction was also tested without co-solvent using ethanol as both nucleophile and solvent. Reactions were carried out at 35 °C using an E/S ratio of 10, an A/S ratio of l0, and the time necessary to achieve 100% conversion (Table 1). In the absence of biocatalyst, no product was

Table 1. Optimization of reaction parameters for lipase-catalyzed preparation of ethyl 2-hydroxyphenylacetate (**2a**).[a]



1a **2a**

Entry	Enzyme	Solvent	E/S[b]	*t* [h][c]
Lipase				
1	CAL B	ethanol	10	24
2	LIP	ethanol	10	> 96
3	CRL	ethanol	10	n.r.
Solvent				
4	CAL B	AcCN	10	n.r.
5	CAL B	hexane	10	4
6	CAL B	DIPE	10	10
7	CAL B	toluene	10	16
8	LIP	hexane	10	> 96[d]
9	LIP	DIPE	10	> 96
10	LIP	toluene	10	> 96
11	CRL	hexane	10	n.r.
E/S				
8	**CAL B**	**ethanol**	**5**	**24**
9	CAL B	ethanol	2	72
10	CAL B	ethanol	1	96

[a] Reactions were performed at 35 °C and 200 rpm. [b] E/S: enzyme amount in mg/substrate amount in mg. [c] Time required to achieve 100% conversion; n.r.: no reaction. [d] 46% conversion.

obtained, and it was observed that enzyme activity was variable, with CAL B giving the most satisfactory results in hexane; with this enzyme, 100% conversion into product **2a** was achieved at 4 h of reaction (Table 1, entry 5). This lipase was also active in toluene and DIPE, but to a lesser extent (Table 1, entries 6 and 7). LIP in hexane showed a lower performance than CAL B, showing 45% conversion after 96 h, whereas no enzyme activity was observed with CRL. Working without co-solvent, in ethanol, CAL B also afforded the desired product with 100% conversion at 24 h reaction (Table 1, entry 1). Although the reaction was faster using ethanol/hexane (4 h), because of the advantages of ethanol regarding economy and reduced toxicity, lipase-catalyzed esterification of substituted phenylacetic acids was carried out with ethanol as nucleophile and solvent.

Regarding the other substrates, it is important to note that both (4-hydroxyphenyl)acetic acid (**1b**) and (4-methoxyphenyl)acetic acid (**1c**) showed a similar behavior to (2-hydroxyphenyl)acetic acid, whereas (4-nitrophenyl)acetic acid (**1d**) led to fast production of the ethyl ester, achieving 100% conversion at 12 h of reaction.

2.1.1.2. Effect of Enzyme/Substrate Ratio

The influence of the enzyme/substrate ratio in the enzymatic esterification was evaluated at 24 h, using ethanol as nucleophile and solvent at 35 °C and variable amounts of CAL B. From the results (Table 1, entries 8–10), it can be concluded that an E/S ratio of 5 was the most satisfactory.

2.1.1.3. Influence of Temperature

Temperatures of 25, 35, and 55 °C were tested, keeping the other reaction parameters at their optimal values (CAL B, ethanol, E/S ratio 5). Conversions of 75% at 25 °C, 98% at 35 °C and 100% at 55 °C were observed, showing the influence of temperature on the yield for the catalyzed reactions. We therefore decided to perform the reaction at 35 °C.

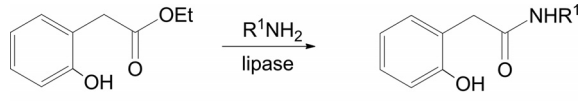
Considering the previously mentioned experiments, the following standard conditions for the enzymatic esterification of substituted phenylacetic acids were chosen: CAL B as biocatalyst, temperature: 35 °C, E/S ratio 5, and ethanol as nucleophile and solvent. All ethyl phenylacetate derivatives were obtained in quantitative yields, although the reaction time varied for each substrate. Whereas (2-hydroxyphenyl)acetic acid afforded the ester at 24 h, (4-hydroxyphenyl)acetic acid, (4-methoxyphenyl)acetic acid, and (4-nitrophenyl)acetic acid required 24, 16, and 12 h, respectively.

2.1.2. Aminolysis of Ethyl Phenylacetates

2.1.2.1. Enzyme Screening and Solvent Effect

Three commercial lipases were evaluated in the aminolysis of **2a**: CRL, CAL B, and LIP. The solvents tested were acetonitrile, hexane, diisopropyl ether, toluene, and acetone (Table 2). Reactions were carried out at 55 °C using E/S ratio 10 and a butylamine/substrate ratio (Nu/S) of 5. It was observed that CAL B was the only active enzyme: no product was detected with LIP and CRL. The most satisfactory results were obtained in DIPE, affording **3b** (100% conversion) at 5 h reaction (Table 2, entry 3). In the absence of biocatalyst, no product was obtained.

Table 2. Optimization of reaction parameters for lipase-catalyzed synthesis of *N*-*n*-butyl-(2-hydroxyphenyl)acetamide (**3b**).[a]



2a **3b**

Entry	Solvent	*T* [°C]	E/S[b]	Nu/S	*t* [h]	Conversion [%]
Solvent						
1	AcCN	55	10	5	24[c]	100
2	hexane	55	10	5	96[c]	100
3	**DIPE**	55	10	5	5[c]	100
4	toluene	55	10	5	96[c]	100
5	acetone	55	10	5	96[c]	100
E/S						
6	DIPE	55	5	5	5[c]	100
7	DIPE	55	2	5	6[c]	100
8	DIPE	55	**1**	5	6[c]	100
9	DIPE	55	0.5	5	48[c]	100
Nu/S						
10	DIPE	55	1	5	5	100
11	DIPE	55	1	2	5	100
12	DIPE	55	1	**1.2**	5	100
13	DIPE	55	1	1	5	89
14	DIPE	55	1	0.5	5	62
Temperature						
15	DIPE	25	1	1.2	5	51
16	DIPE	35	1	1.2	5	76
17	DIPE	**55**	1	1.2	5	100

[a] Reactions were performed at 200 rpm with CAL B. [b] E/S given as enzyme amount in mg/substrate amount in mg. [c] Time to achieve 100% conversion.

2.1.2.2. Effect of Enzyme/Substrate Ratio

The influence of E/S ratio on the enzymatic aminolysis was evaluated by using a Nu/S ratio of 5, DIPE as solvent at 55 °C and variable amounts of CAL B. From the obtained results, it was observed that an E/S ratio 5 was the best (Table 2, entry 6). Working at E/S ratio of 1, the reaction time was slightly longer but it was considered that it was preferable to use the lower amount of enzyme (Table 2, entry 8), therefore an E/S ratio of 1 was selected.

2.1.2.3. Effect of Nucleophile/Substrate Ratio

The influence of the Nu/S ratio on aminolysis yield was evaluated in DIPE using CAL B at 55 °C. It can be observed in Table 2 (entries 10–14) that a slight molar excess of butylamine (1.2) was sufficient to achieve the best results.

2.1.2.4. Influence of Temperature

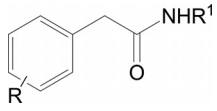
Investigating the influence of temperature on the enzymatic aminolysis, we performed the reaction at 25, 35, and

55 °C. The other reaction parameters were settled to their optimal values (CAL B, DIPE, E/S ratio 1, and Nu/S ratio 1.2). The results (Table 2, entries 15–17) show an increase in yield with increased temperature. We therefore selected 55 °C as the reaction temperature.

Taking into account these studies, we have chosen as standard conditions for the enzymatic aminolysis of substituted ethyl phenylacetates: CAL B as biocatalyst, DIPE as solvent, temperature: 55 °C, E/S ratio 1, and Nu/S ratio 1.2.

Having optimized the experimental conditions, we applied the enzymatic aminolysis to phenylacetates **2b**, **2c**, and **2d**. The results, expressed as yield of isolated product, for ethyl phenylacetates **2a**–**d** and for various amines, are summarized in Table 3. With the exception of **3c** (59%) and **3d** (52%), the products were obtained in yields ranging from approximately 70 to 99%.

Table 3. Substituted phenylacetamides **3**–**6**.[a]



Product	R	R^1	Yield [%]
3a	2-OH	*n*-propyl	80
3b	2-OH	*n*-butyl	72
3c	2-OH	*n*-hexyl	59
4a	4-OH	*n*-propyl	75
4b	4-OH	*n*-butyl	68
4c	4-OH	*n*-hexyl	52
5a	4-OCH_3	*n*-propyl	85
5b	4-OCH_3	*n*-butyl	80
5c	4-OCH_3	*s*-butyl	72
5d	4-OCH_3	*n*-hexyl	90
5e	4-OCH_3	*n*-heptyl	90
5f	4-OCH_3	*n*-octyl	96
5g	4-OCH_3	*n*-nonyl	94
5h	4-OCH_3	*n*-undecyl	98
5i	4-OCH_3	*n*-tetradecyl	95
5j	4-OCH_3	*n*-hexadecyl	90
5k	4-OCH_3	*n*-octadecyl	89
6a	4-NO_2	*n*-propyl	85
6b	4-NO_2	*n*-butyl	85
6c	4-NO_2	*s*-butyl	80
6d	4-NO_2	*n*-hexyl	95
6e	4-NO_2	*n*-heptyl	99
6f	4-NO_2	*n*-octyl	96
6g	4-NO_2	*n*-nonyl	98
6h	4-NO_2	*n*-undecyl	94
6i	4-NO_2	*n*-tetradecyl	92
6j	4-NO_2	*n*-hexadecyl	90
6k	4-NO_2	*n*-octadecyl	90

[a] Reactions were performed under standard conditions.

It can be seen that the best yields were obtained by aminolysis of ethyl 4-nitrophenylacetate **6a**–**k**, and the longest chain amides of ethyl 4-methoxyphenyacetate **5d**–**k**. Moreover, it was possible to prepare long chain amides until *n*-octadecyl by enzymatic aminolysis of these substrates. In contrast, the hydroxy-substituted esters gave the products in lower yield. A decrease in yield was observed with an increase in amine length chain, with **3c** and **4c** being the longest chain products obtained by aminolysis of 2- and 4-hydroxyphenylacetates.

Regarding the reactivity of the amines, it seems that the chain length did not influence significantly the reaction yields. The best performance was achieved with the normal chains of seven, nine, and eleven methylene groups, and their respective products **5h**, **6e**, and **6g**, were obtained in almost quantitative yield. A slight difference in product yield was observed in the case of *sec*-butylamine derivatives **5c** and **6c**, which could be attributed to some steric hindrance in the amine.

It was observed that the time required to achieve maximum conversion was variable and depended on the substituent in the aromatic ring of the ester. Performing the aminolysis of ethyl 2-hydroxy-, 4-hydroxy-, 4-methoxy-, and 4-nitrophenylacetate with *n*-butylamine, the reaction time for the first three substrates was 5 h; in the case of 4-ethyl nitrophenylacetate, the reaction required only 3 h. These reactions times were applied to the rest of the aminolysis substrates: 5 h to obtain **3a**–**c**, **4a**–**c**, **5a**–**k**, and 3 h for **6a**–**6k**.

2.1.3. One-Pot, Two-Step Procedure

In previous work, we developed an efficient one-pot, two-step procedure for the enzymatic preparation of *N*-substituted carboxamides from the corresponding carboxylic acids through the formation of carboxylic ethyl esters.[20,22,23] Considering that this procedure provides a simple and mild alternative method for the synthesis of substituted amides, we tried to apply it to obtain substituted phenylacetamides. To this end, we treated **1a**–**d** with ethanol in DIPE in the presence of CAL B. When the acid was converted into the corresponding ethyl ester, *n*-butylamine was added to the same reaction vessel to perform the aminolysis reaction. All the procedure was carried out in one pot, without isolation of the ethyl phenylacetate, which was obtained in quantitative yield through enzymatic catalysis.

It was observed that only in the case of the phenylacetamide derived from (4-nitrophenyl)acetic acid (**1d**) did this method give good results, affording **6b** in 85% yield after 16 h of reaction. The same procedure was applied to the synthesis of **6d** (90%), **6f** (89%) and **6i** (86%). The other three acids investigated (**1a**–**c**) were not good substrates to apply this procedure. Although esters were obtained in quantitative yield, aminolysis reaction gave the corresponding phenylacetamides in very low yield (less than 20%).

In summary, the enzymatic reactions offer a good alternative with which to prepare ester and amide derivatives from variously substituted phenylacetic acids. Although the synthesis of these compounds performed by chemical methods is not difficult, it has the disadvantage of using hazardous reagents such as acetic anhydride, pyridine, and thionyl chloride. The enzymatic approach shows interesting advantages. The reaction is simple, it is performed at low temperature, and the products are isolated by simple filtration and solvent evaporation. The lipase is biodegradable and, consequently, more friendly to the environment than chemical catalysts. In addition, because the enzyme is insoluble in the reaction medium, it is easily removed by filtration and can be reused. In the esterification reaction of phenylacetic acids and in the aminolysis reaction of ethyl phenylacetates,

CAL B retained 90 and 79% activity, respectively, after seven reaction cycles. The recycling of the lipase after the one-pot, two-step procedure did not show the same efficiency, keeping only 61% activity after the third cycle.

2.2. Hammett Analysis of Aminolysis of Ethyl Phenylacetates

As noted from the experimental results, the lipase activity in the aminolysis of ethyl phenylacetates is related to the substitution in the aromatic ring. Whereas a nitro substituent at the 4-position favors the reaction, the presence of a hydroxyl group at the same position gives the products in lower yield and requires longer reaction time. The effect of substituents on the ionization of benzoic acids and its application as a model system to estimate the electronic effects of substituents on similar reaction systems is well known.[25,26] Hence, we decided to study the effect of substituents on the lipase-catalyzed aminolysis of phenylacetates through a Hammett analysis.

First, the reaction rates between ethyl phenylacetate and ethyl 4-hydroxy-, 4-methoxy-, 4-amino-, and 4-nitrophenylacetates and *n*-butylamine were measured (data in Experimental Section) and the reaction parameter ρ was determined. Figure 1 shows a correlation between enzyme activity and electronic effect of the substituent in the aromatic ring (σ). The reaction rates appear to be slightly more sensitive to electron-withdrawing substituents (EWS) with $\rho = 1$, than to electron-donating substituents (EDS) with $\rho = 0.1$. The ρ for EWS is in agreement with ρ values obtained for similar chemical reactions and subtilisin-catalyzed cleavage of *p*-substituted phenyl acetates.[27] In the present work, the Hammett analysis allowed the stepwise mechanism to be applied to the case of substituted phenylacetates; this was previously reported for the lipase-catalyzed aminolysis of esters.[28] Furthermore, considering previous work in which the Hammett plot was used to analyze the lipase-catalyzed hydroysis of esters,[29] it could be suggested that the rate-determining step is the nucleophilic attack at the electrophilic center. The behavior of the EDS is described below.

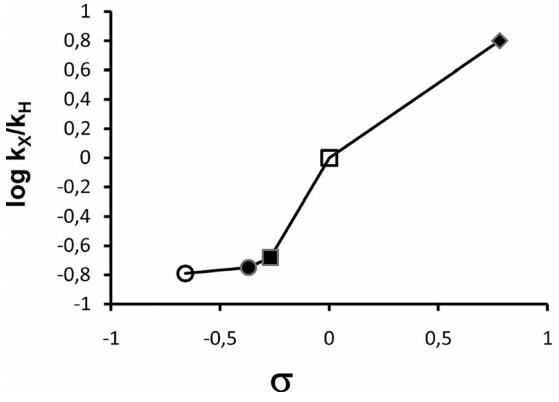


Figure 1. Hammett correlation for lipase-catalyzed aminolysis of substituted ethyl phenylacetates. *k* values in Experimental Section (♦ *p*-NO_2, □ H, ■ *p*-OCH_3, ● *p*-OH, ○ *p*-NH_2).

2.3. Molecular Modeling

To shed light to the molecular determinants of the enzymatic aminolysis reaction, a combination of docking calculations with molecular dynamic (MD) simulations was performed. We evaluated the ability of the catalytic pocket to accommodate ethyl 4-NO_2- (**2d**) and 4-OH- (**2b**) phenylacetates (as EWS and EDS model substrates, respectively). Additionally, a complete exploration of the potential energy surface (PES) for the catalyzed and non-catalyzed mechanisms was performed to explain the different yield obtained by using both substrates.

2.3.1. Docking Calculations and Molecular Dynamic Simulations of 4-Nitro- and 4-Hydroxyphenylacetates with CAL B

Inspection of the docking poses revealed that both ethyl phenylacetates **2d** and **2b** have two different main favorable conformations in the enzymatic gorge. As expected, the first corresponds to that in which the acetate side chain is accommodated near to the catalytic triad. On the other hand, there is a group of solutions that implies the interaction of the enzymatic amino acids with the 4-substituent (nitro and hydroxyl, respectively). For **2d**, these solutions have low population and they are energetically less favorable. However, for **2b**, the hydroxyl substituent can establish strong hydrogen bonds with polar amino acids in the enzyme. For this reason, these relevant conformations of **2b** were considered.

For each substrate, four main orientations, according to cluster population, binding energy, and proximity to the catalytic residues, were selected from the docking results (Figure 2). In the case of ethyl (4-nitrophenyl)acetate, the most favorable conformations show interactions between the amino acids in the catalytic pocket and the acetate side chain (Figure 2, a), whereas in the case of the 4-hydroxy substrate, some conformations show H-bonding with the phenolic hydroxyl group (grey and pink structures in Figure 2, b). All structures were considered for further analysis in molecular dynamic simulations (MD).

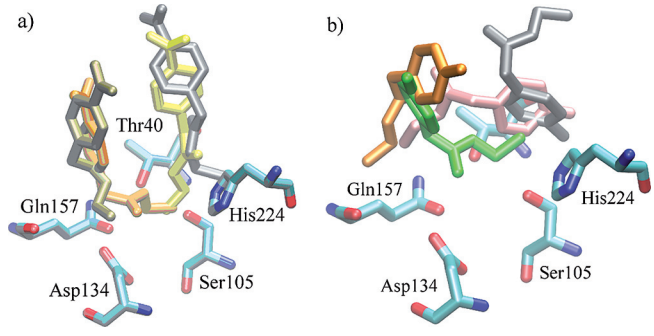


Figure 2. Most favorable poses from docking calculations for: (a) ethyl 4-nitrophenylacetate (**2d**), and (b) ethyl 4-hydroxyphenylacetate (**2b**). Thr40, Ser105, Asp134, Gln157, and His224 from CAL B are also represented.

The structural integrity of the selected poses for the ethyl phenylacetates **2d** and **2b** and CAL B was examined by means of 10 ns molecular dynamic simulations. Inspection

of the substrate–enzyme system during the simulation time reveals that two distinct initial conformations for **2d** adopt a very similar conformation in the early simulation, which remains steady during the simulation time (Figure 3). The profile for the positional root-mean square deviation (RMSD) of the ligand and enzyme is shown in the Supporting Information (Figure SI-1). This conformation, which is consistent with the accepted CAL B enzymatic mechanism,[28] allows ester **2d** to be attacked by Ser105 to form the acyl enzyme intermediate. The substrate is stabilized by H-bond interaction between the carbonyl oxygen of **2d** and the backbone N-H group and the OH group of the Thr40 (Figure 3).

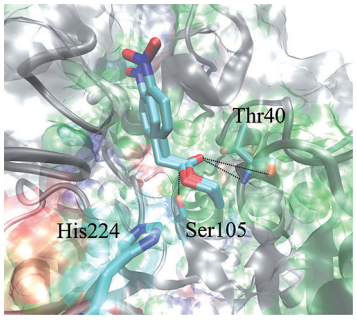
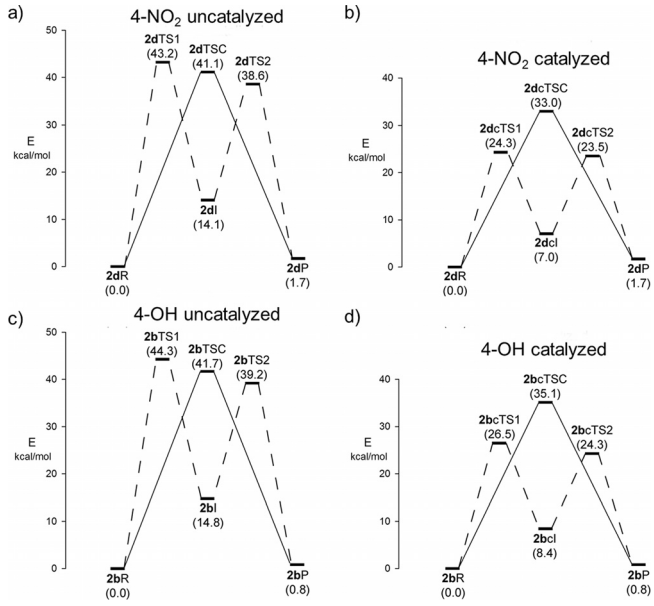


Figure 3. Most representative conformation adopted during the simulation time for two docking solutions of **2d**. Thr40, Ser105, and His224 are also shown.

In the case of **2b**, structures with the acetate side chain inside the catalytic gorge escape from the protein and change considerably during the MD simulation time. Furthermore, those initial structures that showed H-bond interactions between the 4-hydroxy group and the amino acids of the catalytic cavity, remain stable during the simulation time. This conformation is not suitable for the enzymatic reaction, because it does not allow the formation of the acyl enzyme intermediate. Therefore, substrates having substituents capable of forming H-bond with the amino acids in the enzyme have great difficulty adopting a reactive conformation.

2.3.3. Potential Energy Surface Analysis

In an attempt to clarify the determinants that govern reactivity, a study of the PES for the ethyl 4-nitro- and 4-hydroxyphenylacetates was also performed. Some studies of the enzymatic mechanism have been reported that reveal the importance of H-bonding and proton transfer assisting the aminolysis reaction.[28] Galabov and co-workers studied the ammonolysis reaction of methyl benzoate, which represents a more simplified model of the enzymatic aminolysis reaction.[30] The reported enzyme-catalyzed mechanism[28] supports the stabilization of the substrate in the enzymatic site by Thr40 in a similar way that ammonia does in the catalyzed mechanism.[30] Therefore, by using these studies as background, a complete PES analysis of the reactions of ethyl (4-nitro- and 4-hydroxyphenyl)acetates with ammonia was explored (Scheme 2).



Scheme 2. Potential energy surface (PES) with ZPE correction at 328 K for the aminolysis reaction of ethyl 4-nitrophenylacetate (**2d**) and ethyl 4-hydroxyphenylacetate (**2b**) with ammonia, Panels a/b and c/d, respectively. Both uncatalyzed and catalyzed reactions were considered for both stepwise and concerted mechanisms. Relative energy values are given in kcal/mol.

Quantum calculations show that, for the catalyzed mechanism, the ethyl (4-nitrophenyl)acetate is 2 kcal/mol more favorable than the 4-hydroxy substrate. In addition, this result was observed for both the concerted and the stepwise mechanisms. Description of the most favorable transition structures (TS1) is shown in Figure 4 and all transition structures obtained are described in the Supporting Information (Figure SI-2). To extend the study, a simplified PES analysis for the catalyzed reaction was performed by considering concerted and stepwise mechanisms for all

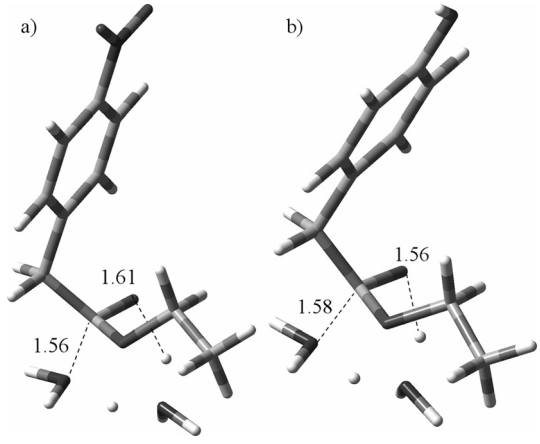


Figure 4. Description of the transition structures for the catalyzed aminolysis step mechanism for (a) ethyl 4-nitrophenylacetate (**2d**), and (b) ethyl 4-hydroxyphenylacetate (**2b**) optimized at the B3LYP/6-31+G(d,p) level of theory. Distances are in angstroms. Imaginary frequencies associated with the transition states of the first step in the stepwise mechanism are –963 and –1029 cm^{-1}, for **2d** and **2b**, respectively.

substrates involved in the Hammett analysis. Energetic values are summarized in Table 4. The results show a lower activation energy for the ethyl 4-nitrophenylacetate than for the rest of the substrates.

Table 4. Relative energy for biocatalyzed aminolysis of ethyl 4-substituted phenylacetates.[a]

Stationary points	4-NO_2	4-H	4-OCH_3	4-OH	4-NH_2
R	0.0	0.0	0.0	0.0	0,0
cTSC	33.0	35.0	35.2	35.1	35.6
P	1.7	0.8	3.3	0.8	0.5
R	0.0	0.0	0.0	0.0	0.0
cTS1	24.4	26.4	26.6	26.5	27.0
cI	7.2	8.5	8.5	8.4	8.7
cTS2	23.5	24.3	24.4	24.3	24.3
P	1.7	0.8	3.3	0.8	0.5

[a] Optimizations were performed at the B3LYP/6-31+G(d,p) level of theory. Data include ZPE correction at 328 K. Concerted (cTSC) and stepwise mechanisms (cTS1, cI, and cTS2) were considered. Energy values are given in kcal/mol.

Considering MD simulations and PES explorations, two factors seem to be responsible for the different reactivity observed. First, the energetic barrier associated with the aminolysis reaction for the 4-nitro substrate is between 2 and 2.6 kcal/mol more favored than that for the rest of substrates (Table 4). On the other hand, MD simulations showed that the capability of phenyl substituents to form hydrogen bonds with amino acids of the enzyme hinders the accommodation and stabilization of the substrate in the enzymatic site. In the case of ethyl 4-nitrophenylacetate and the unsubstituted ethyl phenylacetate, no hydrogen bonds are formed and, consequently, the energetic barriers determine the reaction rates.

3. Conclusions

We have described for the first time the synthesis of substituted phenylacetamides by application of lipases in esterification and aminolysis reactions; 28 phenylacetamides and four phenylacetates were obtained. All phenylacetamides are new products.

The influence of the enzyme source, substrate substitution, and various reaction parameters on the results was analyzed. After an enzyme screening, it was concluded that CAL B was the best biocatalyst in terms of yield and reaction time. All reactions were performed at moderate temperature: 35 °C for esterification and 55 °C for aminolysis. Regarding the influence of the solvent, ethanol was used as alcohol and solvent in the esterification and DIPE was the solvent of choice in the aminolysis.

By comparison of the performance of hydroxy- and nitro substituents in phenylacetates as substrates in the aminolysis, the highest yields were achieved when the phenylacetate had a nitro group as substituent. Moreover, nitrophenylacetamides could also be obtained in high yield by following a one-pot, two-step procedure, which is more convenient than the two-stage process because of its straightforward handling.

This difference in reactivity between hydroxyl and nitro substituents in phenylacetates prompted us to carry out a Hammett analysis. The Hammett plot showed that reaction rates are more sensitive to electron-withdrawing substituents than to electron-donating substituents. Nearly no difference was observed with electron-donating substituents.

Computer simulation results led to the conclusion that the observed difference in reactivity of 4-substituted ethyl phenylacetates is determined by two facts: the activation energy in the biocatalyzed aminolysis reaction and the possibility of the ring substituents to form H-bonds with the amino acids of the enzyme. The latter seems to be the determinant for achieving a good interaction between the side chain of the substrate and the catalytic triad to reach a favorable conformation for enzymatic reaction. The CAL B enzymatic pocket is very deep in comparison with other lipases and for this reason hydrogen bonding between enzyme and substrate is highly relevant in the aminolysis reaction. This fact could explain the observed deviations in the Hammet plot for the electron-donating substituents studied.

4. Experimental Section

4.1. General: Chemicals were purchased from Sigma–Aldrich de Argentina and used without further purification. Lipase from *Candida rugosa* (CRL) (905 U/mg solid) was purchased from Sigma Chemical Co.; *Candida antarctica* lipase B (CAL B): Novozym 435 (7400 PLU/g) and Lipozyme RM 1M (LIP) (7800 U/g) were generous gifts of Novozymes Spain; all enzymes were used as received. Enzyme/substrate chemicals and solvents were purchased from Merck Argentina. E/S ratio are given as enzyme amount in mg/substrate amount in mg. Enzymatic reactions were carried out with an Innova 4000 digital incubator shaker, New Brunswick Scientific Co. at the corresponding temperature and 200 rpm. To monitor the progress of the reaction, aliquots were withdrawn and analyzed by TLC performed on commercial 0.2 mm aluminum-coated silica gel plates (F_{254}) and visualized by 254 nm UV or by immersion in an aqueous solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.04 M), $Ce(SO_4)_2$ (0.003 M) in concentrated H_2SO_4 (10%). Percent conversion was determined by monitoring the reactions with analytical reverse-phase HPLC employing a Phenomenex Phenogel column 5 μM 10E5A, 300 × 7.8 mm, eluting with MeOH/H_2O (80:20) at 1.00 mL/min. Melting points were measured with a Fisher Johns apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ as solvent with a Bruker AM-500 NMR instrument operating at 500.14 and 125.76 MHz for ^1H and ^{13}C, respectively. The ^1H NMR spectra are referenced with respect to the residual $CHCl_3$ proton of the solvent $CDCl_3$ at δ = 7.26 ppm. Coupling constants are reported in Hertz [Hz]. ^{13}C NMR spectra were fully decoupled and are referenced to the middle peak of the solvent $CDCl_3$ at δ = 77.0 ppm. Splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quadruplet; quint, quintet; dd, double doublet. IR spectra were recorded with a Nicolet Magna 550 spectrometer. High-resolution mass spectrometry was recorded with a Thermo Scientific EM/DSQ II – DIP. The results were within ±0.02% of the theoretical values.

4.2. Synthesis of Ethyl Phenylacetates. General Procedure: CAL B (2.2 g for **1a** and **1b**, 2.4 g for **1c**, and 2.7 g for **1d**) was added to a

solution of the corresponding substituted phenylacetic acid (3 mmol, **1a** and **1b**: 456 mg, **1c**: 498 mg, **1d**: 544 mg) in hexane (10 mL) and ethanol (0.7 mL) or ethanol (10 mL, without hexane). The mixture was shaken at 200 rpm and 35 °C. Once the reaction was finished, the enzyme was filtered off and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (silica gel; hexane/EtOAc, 9:1–3:2).

4.3. Synthesis of Phenylacetamides. General Procedure: To a solution of the corresponding phenylacetate (3 mmol, **2a** and **2b**: 540 mg, **2c**: 582 mg, **1d**: 627 mg) in DIPE (10 mL), the corresponding amine (3.6 mmol) and CAL B (540 mg, 582 mg, and 627 mg, respectively) were added. The mixture was shaken at 200 rpm and 55 °C. Once the reaction was finished, the enzyme was filtered off and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (silica gel; hexane/EtOAc, 9:1–3:2).

4.4. General One-Pot Procedure: CAL B (1.2 g) was added to a solution of the carboxylic acid (3 mmol) in ethanol (0.7 mL) and DIPE (10 mL). The suspension was shaken at 200 rpm at 35 °C and the progress of the reaction was monitored by TLC. When the acid was converted into the ethyl ester, the corresponding amine (3.6 mmol) was added and the temperature was increased to 55 °C. When the reaction finished, the enzyme was filtered off, the solvent was evaporated, and the crude residue was purified by column chromatography on silica gel (hexane/EtOAc, 9:1–3:2).

Ethyl 2-Hydroxyphenylacetate (2a): Yield 98%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.29 (t, J = 7.4 Hz, 3 H, CH_3CH$_2$O-), 3.67 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 4.20 (q, J = 7.3 Hz, 2 H, CH$_3$CH_2O-), 6.88 (dt, J = 1.1, 7.4 Hz, 1 H, 4-H), 6.95 (dd, J = 1.0, 7.6 Hz, 1 H, 6-H), 7.10 (dd, J = 1.2, 7.4 Hz, 1 H, 3-H), 7.20 (dt, J = 1.2, 7.5 Hz,1 H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (CH_3CH$_2$O-), 38.2 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 62.0 (CH$_3$CH_2O-), 117.8 (C-6), 120.6 (C-2), 120.9 (C-4), 129.2 (C-5), 131.0 (C-3), 155.3 (C-1), 174.1 (CH$_3$CH$_2$C OO-) ppm. HRMS: m/z calcd. for $C_{10}H_{13}O_3$ [M + H]$^+$ 181.0865; found 181.0859.

Ethyl 4-Hydroxyphenylacetate (2b): Yield 96%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.25 (t, J = 7.3 Hz, 3 H, CH_3CH$_2$O-), 3.54 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 4.15 (q, J = 7.3 Hz, 2 H, CH$_3$CH_2O-), 6.74 (d, J = 8.4 Hz, 2 H, 2-H, 6-H), 7.11 (d, J = 8.4 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (CH_3CH$_2$O-), 40.5 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 61.0 (CH$_3$CH_2O-), 115.5 (C-2, C-6), 125.9 (C-4), 130.4 (C-3, C-5), 154.9 (C-1), 172.6 (CH$_3$CH$_2$C OO-) ppm. HRMS: m/z calcd. for $C_{10}H_{13}O_3$ [M + H]$^+$ 181.0865; found 181.0872.

Ethyl 4-Methoxyphenylacetate (2c): Yield 98%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$O-), 3.56 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 3.80 (s, 1 H, CH_3O-), 4.15 (q, J = 7.1 Hz, 2 H, CH$_3$CH_2O-), 6.87 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.21 (d, J = 8.6 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.2 (CH_3CH$_2$O-), 40.5 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 55.2 (CH_3O-), 60.8 (CH$_3$CH_2O-), 113.9 (C-2, C-6), 126.2 (C-4), 130.2 (C-3, C-5), 158.6 (C-1), 172.0 (CH$_3$CH$_2$C OO-) ppm. HRMS: m/z calcd. for $C_{11}H_{14}NaO_3$ [M + Na]$^+$ 217.0841; found 217.0833.

Ethyl 4-Nitrophenylacetate (2d): Yield 99%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, J = 7.1 Hz, 3 H, CH_3CH$_2$O-), 3.72 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 4.17 (q, J = 7.1 Hz, 2 H, CH$_3$CH_2O-), 7.46 (dd, J = 1.8, 6.6 Hz, 2 H, 3-H, 5-H), 8.19 (dd, J = 2.0, 6.8 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (CH_3CH$_2$O-), 41.1 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 61.4 (CH$_3$CH_2O-), 123.7 (C-2, C-6), 130.3 (C-4), 130.2 (C-3, C-5), 158.6 (C-1), 172.0 (CH$_3$CH$_2$C OO-) ppm. HRMS: m/z calcd. for $C_{10}H_{11}NNaO_4$ [M + Na]$^+$ 232.0586; found 232.0582.

***N*-*n*-Propyl-(2-hydroxyphenyl)acetamide (3a):** Yield 80%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.90 (t, J = 7.4 Hz, 3 H, 3′-H), 1.52 (sext, J = 7.2 Hz, 2 H, 2′-H), 3.21 (q, J = 6.6 Hz, 2 H, 1′-H), 3.55 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 6.82 (dt, J = 1.2, 7.4 Hz, 1 H, 4-H), 6.96 (m, 2 H, 3-H, 6-H), 7.17 (dt, J = 1.2, 7.6 Hz, 1 H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.2 (C-3), 22.5 (C-2), 38.0 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 41.8 (C-1), 118.0 (C-6), 120.8 (C-4), 121.5 (C-2), 129.1 (C-5), 130.3 (C-3), 156.3 (C-1), 173.4 (C ONH-) ppm. HRMS: m/z Calcd. for $C_{11}H_{16}NO_2$ [M + H]$^+$ 194.1181; found 194.1145.

***N*-*n*-Butyl-(2-hydroxyphenyl)acetamide (3b):** Yield 72%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.91 (t, J = 7.3 Hz, 3 H, 4′-H), 1.33 (sext, J = 7.2 Hz, 2 H, 3′-H), 1.50 (q, J = 7.4 Hz, 2 H, 2′-H), 3.26 (dt, J = 6.2, 6.9 Hz, 2 H, 1′-H), 3.56 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 6.83 (dt, J = 1.1, 7.4 Hz, 1 H, 4-H), 6.98 (dd, J = 1.0, 8.0 Hz, 1 H, 6′-H), 7.01 (dd, J = 1.2, 7.5 Hz, 1 H, 3′-H), 7.18 (dt, J = 1.2, 7.6 Hz, 1 H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.6 (C-4), 19.9 (C-3), 31.2 (C-2), 39.9 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 41.2 (C-1), 118.1 (C-6), 120.2 (C-4), 121.5 (C-2); 129.1 (C-5), 130.3 (C-3); 156.3 (C-1), 173.3 (C ONH-) ppm. HRMS: m/z Calcd. for $C_{12}H_{18}NO_2$ [M + H]$^+$ 208.1338; found 208.1332.

***N*-*n*-Hexyl-(2-hydroxyphenyl)acetamide (3c):** Yield 59%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, J = 7.1 Hz, 3 H, 6′-H), 1.20–1.26 (m, 6 H, 3′-H, 4′-H, 5′-H), 1.41 (q, J = 6.9 Hz, 2 H, 2′-H), 3.16 (q, J = 6.8 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 6.82 (dt, J = 1.0, 7.2 Hz, 1 H, 4-H), 6.99 (dd, J = 1.1, 7.9 Hz, 1 H, 6′-H), 7.00 (dd, J = 1.1, 7.4 Hz, 1 H, 3′-H), 7.17 (dt, J = 1.2, 7.5 Hz, 1 H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (C-6′), 22.5 (C-5′), 26.4 (C-4′), 29.4 (C-3′), 31.4 (C-2′), 39.7 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 43.0 (C-1′), 118.1 (C-6), 120.1 (C-4), 121.4 (C-2), 129.1 (C-5), 130.3 (C-3), 156.3 (C-1), 173.3 (C ONH-) ppm. HRMS: m/z Calcd. for $C_{14}H_{21}NNaO_2$ [M + H]$^+$ 258.1470; found 258.1459.

***N*-*n*-Propyl-(4-hydroxyphenyl)acetamide (4a):** Yield 75%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, J = 7.4 Hz, 3 H, 3′-H), 1.57 (m, 2 H, 2′-H), 3.19 (q, J = 6.7 Hz, 2 H, 1′-H), 3.46 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 6.83 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.03 (d, J = 8.6 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (C-3), 22.6 (C-2), 39.9 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 42.9 (C-1), 116.2 (C-2, C-6), 125.7 (C-4), 130.7 (C-3, C-5), 156.2 (C-1), 172.4 (C ONH-) ppm. HRMS: m/z Calcd. for $C_{11}H_{16}NO_2$ [M + H]$^+$ 194.1181; found 194.1162.

***N*-*n*-Butyl-(4-hydroxyphenyl)acetamide (4b):** Yield 68%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.94 (t, J = 7.4 Hz, 3 H, 4′-H), 1.28 (sext, J = 7.2 Hz, 2 H, 3′-H), 1.44 (q, J = 7.3 Hz, 2 H, 2′-H), 3.26 (q, J = 6.7 Hz, 2 H, 1′-H), 3.53 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 6.74 (d, J = 8.5 Hz, 2 H, 2-H, 6-H), 7.11 (d, J = 8.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.5 (C-4′), 19.2 (C-3′), 31.4 (C-2′), 39.4 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 42.7 (C-1′), 116.1 (C-2, C-6), 125.5 (C-4), 130.6 (C-3, C-5), 156.3 (C-1), 172.2 (C ONH-) ppm. HRMS: m/z Calcd. for $C_{12}H_{18}NO_2$ [M + H]$^+$ 208.1338; found 208.1328.

***N*-*n*-Hexyl-(4-hydroxyphenyl)acetamide (4c):** Yield 52%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.85 (t, J = 6.9 Hz, 3 H, 6′-H), 1.21–1.25 (m, 6 H, 3′-H, 4′-H, 5′-H), 1.40 (q, J = 7.0 Hz, 2 H, 2′-H), 3.19 (q, J = 6.8 Hz, 2 H, 1′-H), 3.48 (s, 2 H, Ar-CH_2-CO$_2$CH$_2$CH$_3$), 6.83 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.04 (d, J = 8.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.9 (C-6′), 22.5 (C-5′), 26.4 (C-4′), 29.3 (C-3′), 31.3 (C-2′), 39.8 (Ar-CH_2-CO$_2$CH$_2$CH$_3$), 42.8 (C-1′), 116.1 (C-2, C-6), 125.6 (C-4), 130.6 (C-3, C-5), 156.1 (C-1), 172.3 (C ONH-) ppm. HRMS: m/z Calcd. for $C_{14}H_{21}NNaO_2$ [M + Na]$^+$ 258.1470; found 258.1479.

***N*-*n*-Propyl-(4-methoxyphenyl)acetamide (5a):** Yield 85%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.84 (t, J = 7.4 Hz, 3 H, 3′-H), 1.44 (sext, J = 7.3 Hz, 2 H, 2′-H), 3.17 (q, J = 6.7 Hz, 2 H, 1′-H), 3.53 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.82 (s, 1 H, CH_3O-), 6.90 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.17 (d, J = 8.6 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.2 (C-3), 22.7 (C-2), 41.3 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1), 55.2 (*C*H_3O-), 114.4 (C-2, C-6), 126.9 (C-4), 130.6 (C-3, C-5), 158.9 (C-1), 171.4 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{12}H_{18}NO_2$ [M + H]$^+$ 208.1338; found 208.1326.

***N*-*n*-Butyl-(4-methoxyphenyl)acetamide (5b):** Yield 80%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, J = 7.3 Hz, 3 H, 4′-H), 1.25 (sext, J = 7.4 Hz, 2 H, 3′-H), 1.39 (q, J = 7.3 Hz, 2 H, 2′-H), 3.19 (q, J = 6.6 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 1 H, CH_3O-), 6.89 (d, J = 8.5 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.7 (C-4′), 20.0 (C-3′), 31.5 (C-2′), 39.4 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2, C-6), 126.9 (C-4), 130.6 (C-3, C-5), 158.8 (C-1), 171.4 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{13}H_{20}NO_2$ [M + H]$^+$ 222.1494; found 222.1452.

***N*-*s*-Butyl-(4-methoxyphenyl)acetamide (5c):** Yield 72%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.81 (t, J = 7.5 Hz, 3 H, 4′-H), 1.03 (d, J = 6.6 Hz, 3 H, 2′-H), 1.35 (m, 2 H, 3′-H), 3.50 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 1 H, CH_3O-), 3.89 (m, 2 H, 1′-H), 6.89 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.6 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.2 (C-4′), 20.3 (C-2′), 29.5 (C-3′), 43.1 (Ar-*C*H_2-$CO_2CH_2CH_3$), 46.6 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2, C-6), 127.0 (C-4), 130.5 (C-3, C-5), 158.8 (C-1), 171.4 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{13}H_{20}NO_2$ [M + H]$^+$ 222.1494; found 222.1481.

***N*-*n*-Hexyl-(4-methoxyphenyl)acetamide (5d):** Yield 90%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.85 (t, J = 7.0 Hz, 3 H, 6′-H), 1.21–1.27 (m, 6 H, 3′-H, 4′-H, 5′-H), 1.40 (q, J = 7.0 Hz, 2 H, 2′-H), 3.18 (q, J = 6.7 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (C-6′), 22.6 (C-5′), 26.4 (C-4′), 29.4 (C-3′), 31.4 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2, C-6), 127.0 (C-4), 130.6 (C-3, C-5), 158.9 (C-1), 171.3 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{15}H_{23}NNaO_2$ [M + Na]$^+$ 272.1627; found 272.1621.

***N*-*n*-Heptyl-(4-methoxyphenyl)acetamide (5e):** Yield 90%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, J = 7.1 Hz, 3 H, 7′-H), 1.21–1.27 (m, 8 H, 3′-H, 4′-H, 5′-H, 6′-H), 1.40 (q, J = 7.0 Hz, 2 H, 2′-H), 3.18 (q, J = 6.7 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (C-7′), 22.5 (C-6′), 26.7 (C-5′), 28.8 (C-4′), 29.4 (C-3′), 31.7 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2,C-6), 126.9 (C-4), 130.6 (C-3, C-5), 158.9 (C-1), 171.4 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{16}H_{26}NO_2$ [M + H]$^+$ 264.1964; found 264.1958.

***N*-*n*-Octyl-(4-methoxyphenyl)acetamide (5f):** Yield 96%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, J = 7.1 Hz, 3 H, 8′-H), 1.21–1.27 (m, 10 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H), 1.40 (q, J = 7.2 Hz, 2 H, 2′-H), 3.18 (q, J = 6.8 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-8′), 22.6 (C-7′), 26.8 (C-6′), 28.1 (C-4′, C-5′), 29.4 (C-3′), 31.7 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2,C-6), 127.0 (C-4), 130.6 (C-3, C-5), 158.8 (C-1), 171.3 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{17}H_{28}NO_2$ [M + H]$^+$ 278.2120; found 278.2112.

***N*-*n*-Nonyl-(4-methoxyphenyl)acetamide (5g):** Yield 94%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, J = 7.0 Hz, 3 H, 9′-H), 1.22–1.25 (m, 12 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H), 1.39 (quint, J = 7.1 Hz, 2 H, 2′-H), 3.17 (q, J = 6.9 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.5 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-9′), 22.6 (C-8′), 26.8 (C-7′), 29.1 (C-5′, C-6′), 29.4 (C-4′), 29.7 (C-3′), 31.7 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2, C-6), 127.0 (C-4), 130.6 (C-3, C-5), 158.8 (C-1), 171.3 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{18}H_{29}NNaO_2$ [M + Na]$^+$ 314.2096; found 314.2104.

***N*-*n*-Undecyl-(4-methoxyphenyl)acetamide (5h):** Yield 98%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, J = 7.0 Hz, 3 H, 11′-H), 1.22–1.25 (m, 16 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H), 1.40 (quint, J = 7.0 Hz, 2 H, 2′-H), 3.18 (q, J = 6.7 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.6 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.6 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-11′), 22.7 (C-10′), 26.8 (C-9′), 29.2–29.6 (C-3′, C-4′, C-5′, C-6′, C-7′, C-8′), 31.9 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2, C-6), 126.9 (C-4), 130.6 (C-3, C-5); 158.7 (C-1), 171.2 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{20}H_{34}NO_2$ [M + H]$^+$ 320.2590; found 320.2586.

***N*-*n*-Tetradecyl-(4-methoxyphenyl)acetamide (5i):** Yield 95%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, J = 7.0 Hz, 3 H, 14′-H), 1.22–1.25 (m, 22 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H, 11′-H, 12′-H, 13′-H), 1.39 (m, 2 H, 2′-H), 3.18 (q, J = 6.1 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.3 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.3 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-14′), 22.7 (C-13′), 26.8 (C-12′), 29.2–29.7 (C-3′, C-4′, C-5′, C-6′, C-7′, C-8′, C-9′, C-10′, C-11′), 31.9 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2, C-6), 126.8 (C-4), 130.6 (C-3, C-5); 158.9 (C-1), 171.5 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{23}H_{40}NO_2$ [M + H]$^+$ 362.3059; found 362.3050.

***N*-*n*-Hexadecyl-(4-methoxyphenyl)acetamide (5j):** Yield 90%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, J = 7.1 Hz, 3 H, 16′-H), 1.23–1.26 (m, 26 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H, 11′-H, 12′-H, 13′-H, 14′-H, 15′-H), 1.39 (m, 2 H, 2′-H), 3.18 (q, J = 7.0 Hz, 2 H, 1′-H), 3.52 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.3 Hz, 2 H, 2-H, 6-H), 7.17 (d, J = 8.3 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-16′), 22.7 (C-15′), 26.8 (C-14′), 29.2 (C-13′), 29.4 (C-12′), 29.5 (C-9′, C-10′, C-11′), 29.6 (C-6′, C-7′, C-8′), 29.7 (C-3′, C-4′, C-5′), 31.9 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2,C-6), 126.9 (C-4), 130.6 (C-3, C-5), 158.9 (C-1), 171.3 (*C*ONH-) ppm. HRMS: *m*/*z* Calcd. for $C_{25}H_{44}NO_2$ [M + H]$^+$ 390.3372; found 390.3365.

***N*-*n*-Octadecyl-(4-methoxyphenyl)acetamide (5k):** Yield 89%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, J = 7.0 Hz, 3 H, 18′-H), 1.22–1.25 (m, 30 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H, 11′-H, 12′-H, 13′-H, 14′-H, 15′-H, 16′-H. 17′-H), 1.39 (q, 2 H, 2′-H), 3.18 (q, J = 6.9 Hz, 2 H, 1′-H), 3.51 (s, 2 H, Ar-CH_2-$CO_2CH_2CH_3$), 3.81 (s, 3 H, CH_3O-), 6.89 (d, J = 8.2 Hz, 2 H, 2-H, 6-H), 7.16 (d, J = 8.3 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-18′), 22.7 (C-17′), 26.8 (C-16′), 29.2 (C-15′), 29.4 (C-14′), 29.5 (C-11′, C-12′, C-13′), 29.7 (C-7′, C-8′, C-9′, C-10′), 29.7 (C-3′, C-4′, C-5′, C-6′), 31.9 (C-2′), 39.7 (Ar-*C*H_2-$CO_2CH_2CH_3$), 43.0 (C-1′), 55.3 (*C*H_3O-), 114.4 (C-2, C-6), 127.0 (C-4), 130.6 (C-3, C-5); 158.9 (C-1), 171.3 (*C*ONH-) ppm. HRMS:

m/*z* Calcd. for $C_{27}H_{47}NNaO_2$ [M + Na]$^+$ 440.3505; found 440.3515.

***N*-*n*-Propyl-(4-nitrophenyl)acetamide (6a):** Yield 85%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.84 (t, *J* = 7.2 Hz, 3 H, 3′-H), 1.44 (sext, *J* = 7.3 Hz, 2 H, 2′-H), 3.17 (q, *J* = 6.8 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.8 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.8 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.2 (C-3′), 22.7 (C-2′), 41.3 (C-3′), 43.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 123.7 (C-2, C-6), 130.3 (C-3, C-5), 141.2 (C-4), 148.9 (C-1), 170.1 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{11}H_{14}N_2NaO_3$ [M + Na]$^+$ 245.0902; found 245.0909.

***N*-*n*-Butyl-(4-nitrophenyl)acetamide (6b):** Yield 85% (one-pot: 81%); colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.84 (t, *J* = 7.3 Hz, 3 H, 4′-H), 1.30 (m, 2 H, 3′-H), 1.46 (m, 2 H, 2′-H), 3.25 (dt, *J* = 6.0, 7.2 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.7 Hz, 2 H, 3-H, 5-H), 8.21 (d, *J* = 8.7 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 13.7 (C-4′), 20.0 (C-3′), 31.5 (C-2′), 39.7 (C-1′), 43.4 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.7 (C-4), 148.8 (C-1), 170.9 (CH_3CH_2-*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{12}H_{17}N_2O_3$ [M + Na]$^+$ 237.1239; found 237.1231.

***N*-*s*-Butyl-(4-nitrophenyl)acetamide (6c):** Yield 80%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, *J* = 7.4 Hz, 3 H, 4′-H), 1.10 (d, *J* = 6.6 Hz, 3 H, 2′-H), 1.43 (m, 2 H, 3′-H), 3.62 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 3.91 (m, 2 H, 1′-H), 7.46 (d, *J* = 8.6 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.7 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.3 (C-4′), 20.3 (C-2′), 29.5 (C-3′), 43.5 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 47.1 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.6 (C-4), 148.6 (C-1), 171.2 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{12}H_{17}N_2O_3$ [M + Na]$^+$ 237.1239; found 237.1243.

***N*-Hexyl-(4-nitrophenyl)acetamide (6d):** Yield 95% (one-pot: 90%); colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, *J* = 7.1 Hz, 3 H, 6′-H), 1.25 (m, 6 H, 3′-H, 4′-H, 5′-H), 1.40 (q, *J* = 7.1 Hz, 2 H, 2′-H), 3.24 (q, *J* = 6.9 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.6 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.5 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (C-6′), 22.5 (C-5′), 26.5 (C-4′), 29.4 (C-3′), 31.4 (C-2′), 40.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 148.4 (C-1), 168.9 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{14}H_{20}N_2NaO_3$ [M + Na]$^+$ 287.1372; found 287.1378.

***N*-Heptyl-(4-nitrophenyl)acetamide (6e):** Yield 99%; colorless oil. ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7.0 Hz, 3 H, 7′-H), 1.25 (s, 8 H, 3′-H, 4′-H, 5′-H, 6′-H), 1.46 (quint, *J* = 7.1 Hz, 2 H, 2′-H), 3.24 (q, *J* = 7.0 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.6 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.6 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (C-7′), 22.5 (C-6′), 26.8 (C-5′), 28.8 (C-4′), 29.5 (C-3′), 31.7 (C-2′), 40.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 149.1 (C-1), 169.0 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{15}H_{22}N_2NaO_3$ [M + Na]$^+$ 301.1528; found 301.1535.

***N*-Octyl-(4-nitrophenyl)acetamide (6f):** Yield 96% (one-pot: 89%); white powder; m.p. 103–104 °C. ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, *J* = 7.1 Hz, 3 H, 8′-H), 1.25 (s, 10 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H), 1.46 (quint, *J* = 7.1 Hz, 2 H, 2′-H), 3.23 (q, *J* = 6.8 Hz, 2 H, 1′-H), 3.62 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.5 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.5 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.0 (C-8′), 22.6 (C-7′), 26.8 (C-6′), 29.1 (C-4′, C-5′), 29.5 (C-3′), 31.7 (C-2′), 39.9 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 147.2 (C-1), 168.9 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{16}H_{24}N_2NaO_3$ [M + Na]$^+$ 315.1685; found 315.1681.

***N*-Nonyl-(4-nitrophenyl)acetamide (6g):** Yield 98%; white powder; m.p. 123–124 °C. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, *J* = 7.0 Hz, 3 H, 9′-H), 1.23–1.25 (m, 12 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H), 1.46 (quint, *J* = 7.0 Hz, 2 H, 2′-H), 3.23 (q, *J* = 6.9 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.5 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.5 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-9′), 22.6 (C-8′), 26.8 (C-7′), 29.2 (C-5′, C-6′), 29.4 (C-4′), 29.5 (C-3′), 31.8 (C-2′), 40.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 148.5 (C-1), 168.9 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{17}H_{26}N_2NaO_3$ [M + Na]$^+$ 329.1841; found 329.1836.

***N*-Undecyl-(4-nitrophenyl)acetamide (6h):** Yield 94%; white powder; m.p. 107 °C. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, *J* = 7.0 Hz, 3 H, 11′-H), 1.24–1.29 (m, 16 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H), 1.46 (quint, *J* = 7.0 Hz, 2 H, 2′-H), 3.23 (q, *J* = 6.9 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.5 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.5 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-11′), 22.7 (C-10′), 26.8 (C-9′), 29.2 (C-8′), 29.3 (C-7′), 29.5 (C-4′, C-5′, C-6′), 29.6 (C-3′), 31.9 (C-2′), 40.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 147.4 (C-1), 168.9 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{19}H_{30}N_2NaO_3$ [M + Na]$^+$ 357.2154; found 357.2149.

***N*-Tetradecyl-(4-nitrophenyl)acetamide (6i):** Yield 92% (one-pot: 86%); white powder; m.p. 135 °C. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, *J* = 7.0 Hz, 3 H, 14′-H), 1.24–1.25 (m, 22 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H, 11′-H, 12′-H, 13′-H), 1.46 (quint, *J* = 7.0 Hz, 2 H, 2′-H), 3.23 (q, *J* = 6.9 Hz, 2 H, 1′-H), 3.62 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.5 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.5 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-14′), 22.7 (C-13′), 26.8 (C-12′), 29.2 (C-11′), 29.3 (C-10′), 29.5 (C-7′, C-8′, C-9′), 29.6 (C-5′, C-6′), 29.7 (C-3′, C-4′), 31.9 (C-2′), 40.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 148.2 (C-1), 169.0 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{22}H_{37}N_2O_3$ [M + H]$^+$ 377.2804; found 377.2799.

***N*-Hexadecyl-(4-nitrophenyl)acetamide (6j):** Yield 90%; white powder; m.p. 136 °C. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, *J* = 7.0 Hz, 3 H, 16′-H), 1.25–1.29 (m, 26 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H, 11′-H, 12′-H, 13′-H, 14′-H, 15′-H), 1.47 (quint, *J* = 7.0 Hz, 2 H, 2′-H), 3.23 (dt, *J* = 6.9, 7.5 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.6 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.5 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-16′), 22.7 (C-15′), 26.8 (C-14′), 29.2 (C-13′), 29.3 (C-11′, C-12′), 29.5 (C-8′, C-9′, C-10′), 29.6 (C-3′, C-4′, C-5′, C-6′, C-7′), 31.9 (C-2′), 40.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 148.0 (C-1), 168.9 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{24}H_{41}N_2O_3$ [M + H]$^+$ 405.3117; found 405.3111.

***N*-Octadecyl-(4-nitrophenyl)acetamide (6k):** Yield 90%; white powder; m.p. 129 °C. ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, *J* = 7.0 Hz, 3 H, 18′-H), 1.25–1.29 (m, 30 H, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H, 8′-H, 9′-H, 10′-H, 11′-H, 12′-H, 13′-H, 14′-H, 15′-H, 16′-H, H17′), 1.47 (quint, *J* = 7.0 Hz, 2 H, 2′-H), 3.23 (dt, *J* = 6.8, 7.4 Hz, 2 H, 1′-H), 3.63 (s, 2 H, Ar-C*H*$_2$-$CO_2CH_2CH_3$), 7.46 (d, *J* = 8.6 Hz, 2 H, 3-H, 5-H), 8.20 (d, *J* = 8.5 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (C-18′), 22.7 (C-17′), 26.8 (C-16′), 29.2 (C-14′, C-15′), 29.3 (C-11′, C-12′, C-13′), 29.5 (C-8′, C-9′, C-10′), 29.6 (C-3′, C-4′, C-5′, C-6′, C-7′), 31.9 (C-2′), 40.0 (Ar-*C*H$_2$-$CO_2CH_2CH_3$), 43.4 (C-1′), 124.0 (C-2, C-6), 130.2 (C-3, C-5), 142.5 (C-4), 148.0 (C-1), 168.9 (CH_3CH_2*C*OO-) ppm. HRMS: *m*/*z* Calcd. for $C_{26}H_{45}N_2O_3$ [M + H]$^+$ 433.3430; found 433.3439.

4.5. Hammett Analysis: Initial rates and rate constants for the reaction of substituted ethyl phenylacetates with an excess of *n*-butylamine were determined by monitoring the disappearance of ester and formation of the corresponding phenylacetamide by analytical reverse-phase HPLC using MeOH/H_2O (80:20) as mobile phase. Reactions were conducted at 55 °C with 7 mM ethyl phenylacetate and 70 mM amine in DIPE and E/S ratio of 1. To monitor the progress of the reaction, 20-μL aliquots of the above solution were removed at defined times during the reaction and were injected onto an analytical RP-C18 HPLC; the amount of ethyl phenylacetate derivative was determined by interpolation of the peak height and area values relative to standard curves. Pseudo-first-order rate constants were obtained from a plot of $\log(A/A_o)$ against time. Ethyl 4-aminophenylacetate, $k = 0.11\ s^{-1}$; ethyl 4-hydroxyphenylacetate, $k = 0.12\ s^{-1}$; ethyl 4-methoxyphenylacetate, $k = 0.14\ s^{-1}$; ethyl phenylacetate, $k = 0.67\ s^{-1}$; ethyl 4-nitrophenylacetate, $k = 4.22\ s^{-1}$.

4.6. Computational Details

4.6.1. Docking Calculations: The docking calculations were carried out with the Autodock 4.2 program.[31] The starting structure of CAL B was obtained from the chain A of the PDB entry 1LBS. The Autodock 4.2 method was applied considering as rotatables the torsion angles of the **2b** and **2d** ethyl phenylacetates side chain. A grid of $70 \times 70 \times 70$ points with a spacing of 0.25 Å centered in the enzymatic site of CAL B was calculated and used to obtain 250 runs of the genetic algorithm method. Finally, the best solutions of the most probable cluster, considering: (1) binding energy, (2) cluster population, and (3) H-bonding interaction with the catalytic triad, were selected to perform the MD simulations of the CAL B–substrate systems.

4.6.2. Molecular Dynamics Simulation: Initial structure of the enzyme was taken from the crystal structure of calB (PDB: 1LBS). The structure of reactants were optimized at the HF theory level with the 6-31G** basis set. Atomic partial charges were then computed at the same level to build the corresponding force field parameters of the reactants RESP (restraint electrostatic potential), by following the standard procedure of the Amber force field. All quantum calculations were performed in the Gaussian 03.33[32] suit of programs.

MD simulations were performed with the AMBER 12 software package[33] employing the GPU accelerated code.[34] The Amber99 force field parameters were used for all receptor residues.[35] The systems were immersed in an octahedral box of MOH methanol molecules using the Tleap module, giving final systems of around 25,400 atoms. The systems were initially optimized and then gradually heated to a final temperature of 300 K. Starting from these equilibrated structures, MD production runs of 10 ns were performed. All simulations were performed at 1 atm and 300 K, maintained with the Berendsen barostat and thermostat, respectively,[36] using periodic boundary conditions and the particle mesh Ewald method. The SHAKE algorithm was used to keep bonds involving H atoms at their equilibrium length, allowing the use of a 2 fs time step for the integration of Newton's equations.

4.6.3. Quantum Mechanics Calculations for the PES Exploration: All calculations were carried out with the Gaussian 03.33 suite of programs.[32] An extensive characterization of the PES was performed at the B3LYP/6-31+G(d,p)[37] level to ensure that all relevant stationary points were located and properly characterized. The optimizations were carried out by using the Berny analytical gradient optimization method.[38] All the optimized structure points were characterized by frequency calculations to verify that the transition structures have one and only one imaginary frequency, and stationary points (reactants, intermediates, and products) have no imaginary frequency. Zero-point correction for the vibrational energy at 328 K was included in all cases following the standard procedure in Gaussian 03.

Supporting Information (see footnote on the first page of this article): Spectroscopic data for compounds **2**–**6**, the root mean square deviations obtained in the MDs, and description of the transition structures obtained in the exploration of PES.

Acknowledgments

The authors thank the University of Buenos Aires: Science and Technology project (grant code X010) and the UBACYT project (grant number 20020100100304), the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET PIP) (grant number 112-200801-00801/09), the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT PICT) (grant number 2011-00595) and the Bunge y Born Foundation for partial financial support. A. B., P. A. M., and G. G. L. are Research Members of CONICET (Argentina). P. A. M. also thanks Prof. F. Javier Luque for his advice and computational resources.

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Received: June 12, 2014
Published Online: September 4, 2014

