



**HAL**  
open science

## The control of Novozym® 435 chemoselectivity and specificity by the solvents in acylation reactions of amino-alcohols

Florian Le Joubioux, Nicolas Bridiau, Yesmine Ben Henda, Oussama Achour, Marianne Graber, Thierry Maugard

### ► To cite this version:

Florian Le Joubioux, Nicolas Bridiau, Yesmine Ben Henda, Oussama Achour, Marianne Graber, et al.. The control of Novozym® 435 chemoselectivity and specificity by the solvents in acylation reactions of amino-alcohols. *Journal of Molecular Catalysis B: Enzymatic*, 2013, 95, pp.99-110. 10.1016/j.molcatb.2013.06.002 . hal-01070399

**HAL Id: hal-01070399**

**<https://hal.science/hal-01070399>**

Submitted on 1 Oct 2014

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **The control of Novozym<sup>®</sup> 435 chemoselectivity and specificity by the**  
2 **solvents in acylation reactions of amino-alcohols**

3 **Florian Le Joubioux, Nicolas Bridiau, Yesmine Ben Henda, Oussama Achour, Marianne**  
4 **Graber, Thierry Maugard\***

5

6 *UMR 7266 CNRS-ULR, LIENSs, Equipe Approches Moléculaires, Environnement-Santé,*  
7 *Université de La Rochelle, Avenue Michel Crépeau, 17042 La Rochelle, France.*

8

9

10 *\*Author for correspondence (Fax: +33 546458277; E-mail: tmaugard@univ-lr.fr)*

11

12 **Abstract**

13 The current work describes the differential behavior of Novozym<sup>®</sup> 435 (immobilized *Candida*  
14 *antarctica* lipase B) in *O*-acylation and *N*-acylation catalysis of bifunctional amino-alcohols  
15 acyl acceptors. We performed acylation experiments on three amino-alcohols (alaninol, 4-  
16 amino-1-pentanol and 6-amino-1-hexanol) using myristic acid as an acyl donor. Two organic  
17 solvents (*tert*-amyl alcohol and *n*-hexane) and one ionic liquid (1-butyl-3-methylimidazolium  
18 hexafluorophosphate: Bmim [PF<sub>6</sub>]) were used to determine the effect of the solvent. The  
19 influence of the amino-alcohol carbon chain length between the alcohol and amino groups on  
20 chemoselectivity  $C$  ( $k_{cat, app\ O\text{-acylation}}/k_{cat, app\ N\text{-acylation}}$ ) was highlighted. *N*-acylation is improved  
21 using alaninol, a short chain amino-alcohol (no mono-*O*-acylation in *tert*-amyl alcohol and  $C$   
22 = 0.12 in *n*-hexane) whereas *O*-acylation is improved using 4-amino-1-pentanol and 6-amino-  
23 1-hexanol which are amino-alcohols with longer chain ( $C = 10.5$  in *tert*-amyl alcohol and  $C =$   
24  $539$  in *n*-hexane). On the other hand, the production of the acylated amino-alcohols after 96  
25 hours of reaction was shown to be strongly affected by the solvent nature and the amino-  
26 alcohol structure: starting from alaninol as an acyl acceptor, the yield of amide synthesis  
27 reaches up to 98% in *tert*-amyl alcohol using 0.7 equivalents of myristic acid while the yield  
28 of amido-ester synthesis reaches up to 88% in Bmim [PF<sub>6</sub>] using 1.75 equivalents of myristic  
29 acid.

30 **Keywords:** *N*-acylation; *O*-acylation; Novozym<sup>®</sup> 435; Organic solvent; Ionic liquid;  
31 Chemoselectivity

32

## 33 **1. Introduction**

34 Acylated amino-alcohols such as ceramides, glucamides and amino-acid derivatives have  
35 found important applications, such as in pharmaceutical and cosmetic industries, as potential  
36 anti-viral or anti-tumor drugs [1-3], anti-oxidant stabilizers [4] and as active ingredients in  
37 hair and skin care products [5]. In addition, these compounds can also be used as surfactants  
38 in environmental, food and agricultural industries [6-8].

39 Many processes of chemical acylation of amino-alcohols have been developed so far but these  
40 methods have faced several limitations. One of the most serious restrictions during chemical  
41 processes is the necessity for fastidious steps of alcohol or amino group protection and  
42 deprotection, which are essential to control the chemoselectivity and stereoselectivity of  
43 acylation reactions [9-10].

44 Biocatalysis is considered as an interesting alternative for the preparation of synthetic  
45 compounds: it offers a clean way to perform chemical processes under mild reaction  
46 conditions, with a high degree of selectivity [11-12]. Lipases (E.C. 3.1.1.3) in particular  
47 provide several advantages when used in anhydrous organic media [13-15]. These include  
48 shifting of the thermodynamic equilibrium in favour of synthesis over hydrolysis reaction,  
49 increasing the solubility of non-polar substrates, eliminating side reactions, making enzyme  
50 recovery easier and increasing enzyme thermostability [16-18]. Lipases are excellent  
51 biocatalysts in *O*-acylation, transesterification and *N*-acylation reactions in the synthesis of  
52 various acylated derivatives used in pharmaceutical, cosmetic and food industries [19-22]. They  
53 have, therefore, been widely studied over the past two decades and a large amount of their key  
54 properties in biocatalysis have been highlighted [15, 23-25]. Recently, a new proton shuttle  
55 reaction mechanism was proposed to explain chemoselectivity for lipase-catalyzed *N*-  
56 acylation of amino-alcohols [26]. However, it is still necessary to optimize the output of

57 lipase-catalyzed reactions and to understand this type of selectivity in different reaction  
58 conditions.

59 The properties of lipases (activity, chemoselectivity, regioselectivity and stereoselectivity) can  
60 be modulated by many parameters such as genetic or chemical modification, enzyme  
61 immobilization [15, 27-29]. Solvent nature also particularly influences lipase-catalyzed  
62 acylation reactions. Recent studies have shown that the solvent affects the activity and  
63 selectivity of enzymes in a complex way, involving many interactions between the solvent  
64 and both the substrate and the enzyme [30-35]. Furthermore, the use of lipases in organic  
65 solvents makes many synthetic reactions possible that do not occur in the natural media of  
66 these enzymes. However, these solvents can display certain disadvantages, such as volatility  
67 and/or toxicity towards the environment [36], particularly when they are used on a large scale.  
68 An alternative to these organic solvents is the use of ionic liquids. Ionic liquids have recently  
69 emerged to replace organic solvents in biocatalytic transformations, especially in the case of  
70 polar substrates like amino-alcohols that are difficult to dissolve in organic solvents [37].  
71 Moreover, they show unique properties, including no vapor pressure and capacity to be  
72 recycled and to prevent the thermal deactivation of enzymes [30, 38]. These properties can be  
73 useful in lipase-catalyzed biotransformation. In many cases, these media have been shown to  
74 improve the efficiency of lipase-catalyzed acylation reactions [31, 39-41] and used to perform  
75 both ester and amide synthesis [38, 42-44].

76 Despite the attractive properties of organic solvents and ionic liquids, few studies have been  
77 devoted to the lipase-catalyzed acylation of bifunctional substrates, exhibiting both amino and  
78 alcohol groups, such as ethanolamine, diethanolamine, 2-amino-1-butanol, 6-amino-1-  
79 hexanol, serine and other amino-alcohols with variable carbon chain lengths [45-50]. In such  
80 reactions, the lipase catalyzes *O*-acylation or *N*-acylation with a chemoselectivity that is  
81 largely dependent on the amino-alcohol structure [26].

82 Among lipases used in organic synthesis, *Candida antarctica* lipase B is well known for its  
83 ability to convert alcohols and amines into esters and amides in various solvents [8, 51] and  
84 seems to be the ideal enzyme for the acylation of compounds such as amino-alcohols.  
85 Moreover, commercially available immobilized *Candida antarctica* lipase B, such as  
86 Novozym<sup>®</sup> 435, shows many advantages such as long-term stability at moderately high  
87 temperatures and a tolerance for polar and non-polar solvents [52]. In the present work, we  
88 investigated two organic solvents and one ionic liquid as reaction solvents in the acylation of  
89 three amino-alcohols, catalyzed by Novozym<sup>®</sup> 435, with myristic acid **1** as an acyl donor. In  
90 order to determine the optimum conditions for selective *N*-acylation and *O*-acylation of  
91 amino-alcohols and to maximize both the yield and the selectivity, the effects of amino-  
92 alcohol structure and solvent nature were compared.

## 93 **2. Materials and methods**

### 94 *2.1. Materials*

95 Novozym<sup>®</sup> 435 (*Candida antarctica* lipase B immobilized on acrylic resin), was kindly  
96 provided by Novozymes A/S, Bagsvaerd, Denmark. (±)-alaninol **2** (98%) and 6-amino-1-  
97 hexanol **6** (≥97%), as well as *tert*-amyl alcohol (99%) and 1-butyl-3-methylimidazolium  
98 hexafluorophosphate (Bmim [PF<sub>6</sub>]) (≥97%) were purchased from Sigma-Aldrich (St Louis,  
99 USA) while (±)-4-amino-1-pentanol **4** was from Santa Cruz Biotechnology (USA). Myristic  
100 acid **1** and acetic acid were from Fluka (St Quentin-Fallavier, Switzerland). All chemicals  
101 were dried over molecular sieves. Pure water was obtained via a Milli-Q system (Millipore,  
102 France). Acetonitrile, methanol, *n*-hexane, chloroform and 1-butanol were purchased from  
103 Carlo ERBA (Val-de-Reuil, France).

### 104 *2.2. Enzymatic acylation procedure*

105 *2.2.1. General procedure for lipase catalysed amino-alcohol acylation*

106 In all cases, reactions were carried out at 55°C in screw-caped tubes. 2 ml reaction mixtures  
107 containing various amounts of amino-alcohol (25-350 mM) and 175 mM of myristic acid **1** as  
108 an acyl donor were incubated for 10 minutes prior to addition of 5 g l<sup>-1</sup> of Novozym® 435.  
109 Reactions were conducted for 96 hours. Initial rate measurements were also performed  
110 according to a previously established procedure [53]. The initial rates were calculated from  
111 the linear relationship of the total concentration of products against reaction time (0-1 hour in  
112 *n*-hexane and 0-2 hours in *tert*-amyl alcohol or Bmim [PF<sub>6</sub>]).

113 *2.2.2. Procedure for amino-alcohol acylation in tert-amyl alcohol*

114 When using *tert*-amyl alcohol as a reaction solvent, 100 µl samples were taken at intervals  
115 and centrifuged at 18,000 g for a minute. The supernatants were then analyzed by LC-MS,  
116 leading to the determination and quantification of remaining substrates and synthesized  
117 products.

118 *2.2.3. Procedure for amino-alcohol acylation in n-hexane*

119 When using *n*-hexane as a reaction solvent, various samples containing the same  
120 concentration of reactants and enzyme were prepared and incubated under the same  
121 conditions. Reactions were then conducted in parallel and withdrawn at different times to  
122 determine the reaction progress. 6 ml of a methanol/chloroform (50/50, v/v) mixture were  
123 then added in each sample and the reaction medium was homogenized. 500 µl samples were  
124 taken and centrifuged at 18,000 g for a minute. The supernatants were then analyzed by LC-  
125 MS, leading to the determination and quantification of remaining substrates and synthesized  
126 products.

127 2.2.4. Procedure for amino-alcohol acylation in 1-butyl-3-methylimidazolium  
128 hexafluorophosphate

129 When using 1-butyl-3-methylimidazolium hexafluorophosphate (Bmim [PF<sub>6</sub>]) as a reaction  
130 solvent, various samples containing the same concentration of reactants and enzyme were  
131 prepared and incubated under the same conditions. Reactions were then conducted in parallel  
132 and withdrawn at different times to determine the reaction progress. 6 ml of 1-butanol, a  
133 solvent that is immiscible with Bmim [PF<sub>6</sub>], were added in order to extract substrates and  
134 products from Bmim [PF<sub>6</sub>]. 500 µl samples were taken from 1-butanol extracts and  
135 centrifuged at 18,000 g for a minute. The supernatants were then analyzed by LC-MS, leading  
136 to the determination and quantification of remaining substrates and synthesized products.

137 The partition coefficient between Bmim [PF<sub>6</sub>] and 1-butanol was determined for all  
138 compounds (myristic acid **1** and acylated products) using the following procedure: a solution  
139 of Bmim [PF<sub>6</sub>] with a known concentration of each compound was prepared and then  
140 extracted in 1-butanol. The partition coefficient was calculated as the ratio of the final  
141 quantity determined by HPLC to the known initial quantity. All samples were performed in  
142 duplicate and the averages of duplicate partition coefficients were mentioned in Table 1.  
143 Finally, it was used to correct the concentration values of all compounds in 1-butanol extracts.

144

145 **Table 1**

146 Partition coefficient of myristic acid **1** and acylated products in a Bmim [PF<sub>6</sub>]/1-butanol  
147 biphasic system.

Compound	Partition coefficient <sup>a</sup>
Myristic acid <b>1</b>	0.84
N-myristyl 2-amino-1-propanol <b>3a</b>	0.84
O-myristyl 2-amino-1-propanol <b>3b</b>	0.88
O,N-dimyristyl 2-amino-1-propanol <b>3c</b>	0.99
N-myristyl 4-amino-1-pentanol <b>5a</b>	0.82
O-myristyl 4-amino-1-pentanol <b>5b</b>	0.90
O,N-dimyristyl 4-amino-1-pentanol <b>5c</b>	0.99

N-myristyl aminohexanol <b>7a</b>	0.83
O-myristyl aminohexanol <b>7b</b>	0.87
O,N-dimyristyl aminohexanol <b>7c</b>	0.99

148 <sup>a</sup> The partition coefficient was calculated as the ratio of the final concentration determined by HPLC to the  
149 known initial concentration.

150

### 151 *2.3. Analytical methods*

#### 152 *2.3.1. HPLC-MS analysis*

153 Structural and quantitative analysis of reaction products were conducted using a LC/MS-ES  
154 (liquid chromatography–mass spectrometry) system from Agilent (1100 LC/MSD Trap mass  
155 spectrometer VL) with a C18 Prontosil 120-5-C18-AQ reversed-phase column (250×4 mm, 5  
156 μm; Bischoff Chromatography, Germany). The elution of reaction samples was carried out at  
157 room temperature and at a flow rate of 1 ml min<sup>-1</sup>, using a mobile phase consisting in a  
158 mixture of two solvents: acetonitrile/water/acetic acid (77/23/0.1, v/v/v) (A) and  
159 methanol/acetic acid (100/0.1, v/v) (B). The following variations of the mobile phase were  
160 used during the time interval of the analysis: 100% solvent A from 0 to 20 minutes; a linear  
161 gradient reaching 0% solvent A and 100% solvent B from 20 to 23 minutes; 100% solvent B  
162 from 23 to 80 minutes; a linear gradient reaching back 100% solvent A and 0% solvent B  
163 from 80 to 82 minutes; 100% solvent A from 82 minutes to the end of the run at 90 minutes.  
164 Products were detected and quantified by differential refractometry and UV detection at 210  
165 nm. An external calibration was performed with pure myristic acid. Then, calibrations for  
166 individual acylation products were obtained after mass balance in reaction conditions enabling  
167 to obtain only these products with myristic acid as an acyl donor. Low-resolution mass  
168 spectral analyses were obtained by electrospray in the positive detection mode. Nitrogen was  
169 used as the drying gas at 15 l min<sup>-1</sup> and 350 °C at a nebulizer pressure of 4 bars. The scan  
170 range was 50–1000 m/z using five averages and 13,000 m/z per second resolution. The  
171 capillary voltage was 4000 V. Processing was done offline using HP Chemstation software.

172

173 2.3.2. *Acylated-amino-alcohols purification and analysis for identification*

174 In order to perform the purification and characterization of acylated products, 20 ml reaction  
175 mixtures containing 50 mM of the amino-alcohol and 175 mM of myristic acid **1** in *tert*-amyl  
176 alcohol were incubated for 24 hours in presence of 15 g l<sup>-1</sup> of Novozym<sup>®</sup> 435. Purified  
177 products were then characterized by <sup>1</sup>H NMR (nuclear magnetic resonance spectroscopy) and  
178 IR (infrared spectroscopy) after purification via preparative HPLC using a ProntoPrep C18  
179 reversed-phase column (250×20 mm, 10 μm; Bischoff Chromatography, Germany) eluted via  
180 the mobile phase given in section 2.3.1, at room temperature and at a flow rate of 5 ml min<sup>-1</sup>.  
181 <sup>1</sup>H NMR were recorded on a JEOL-JNM LA400 spectrometer (400 MHz), with  
182 tetramethylsilane as an internal reference. Samples were studied as solutions in CDCl<sub>3</sub>.  
183 Infrared (IR) spectra were recorded from 400 to 4000 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> using a  
184 100 ATR spectrometer (Perkin-Elmer, United States).

185 ***N*-myristyl 2-amino-1-propanol 3a**: m/Z (LR-ESI<sup>+</sup>) C<sub>17</sub>H<sub>36</sub>NO<sub>2</sub> (M + H<sup>+</sup>), found: 286.4,  
186 calculated for: 286.48. IR ν<sub>max</sub> (cm<sup>-1</sup>): 3100-3500 (O-H, alcohol and N-H, amide), 2800-3000  
187 (CH of myristyl chain), 1638 (C=O, amide), 1543 (N-H, amide). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>,  
188 δ ppm): δ 0.88 (t, 3H, *J*= 6.06Hz, -CH<sub>2</sub>-CH<sub>3</sub>), 1.17 (d, 3H, *J*= 6.06Hz, -CH-CH<sub>3</sub>), 1.25 (m,  
189 20H, -CH<sub>2</sub>- of myristyl chain), 1.63 (m, 2H, -CH<sub>2</sub>-CH<sub>2</sub>-CO-NH- of myristyl chain), 2.19 (t,  
190 2H, *J*= 6.06Hz, -CH<sub>2</sub>-CH<sub>2</sub>-CO-NH- of myristyl chain), 3.04 (s, 1H, -OH), 3.27 (dd, 1H, *J*=  
191 5Hz, *J*= 10Hz, -CH-CH<sub>2</sub>-OH), 3.46 (dd, 1H, *J*= 3.7Hz, *J*= 11Hz, -CH-CH<sub>2</sub>-OH), 4.07 (m,  
192 1H, -CH-), 5.7 (s, 1H, -NH-).

193 ***O,N*-dimyristyl 2-amino-1-propanol 3c**: m/Z (LR-ESI<sup>+</sup>) C<sub>31</sub>H<sub>62</sub>NO<sub>3</sub>Na (M + Na<sup>+</sup>), found:  
194 518.6, calculated for: 518.85. IR ν<sub>max</sub> (cm<sup>-1</sup>): 3301 (N-H, amide), 2800-3000 (CH of myristyl  
195 chain), 1737 (C=O, ester), 1643 (C=O, amide), 1542 (N-H, amide). <sup>1</sup>H NMR (400 MHz,

196 CDCl<sub>3</sub>, δ ppm): δ 0.88 (t, 6H,  $J = 7.6\text{Hz}$ , 2x -CH<sub>2</sub>-CH<sub>3</sub>), 1.16 (d, 3H,  $J = 7.6\text{Hz}$ , -CH-CH<sub>3</sub>),  
197 1.25 (m, 40H, -CH<sub>2</sub>- of myristyl chain), 1.6 (m, 4H, 2x -CH<sub>2</sub>-CH<sub>2</sub>-CO- of myristyl chain),  
198 2.14 (t, 2H,  $J = 7.2\text{Hz}$ , -CH<sub>2</sub>-CH<sub>2</sub>-CO-O- of myristyl chain), 2.32 (t, 2H,  $J = 7.2\text{Hz}$ , -CH<sub>2</sub>-  
199 CH<sub>2</sub>-CO-NH- of myristyl chain), 4 (dd, 1H,  $J = 4.4\text{Hz}$ ,  $J = 10.7\text{Hz}$ , -CH-CH<sub>2</sub>-O-), 4.13 (dd,  
200 1H,  $J = 4.9\text{Hz}$ ,  $J = 10\text{Hz}$ , -CH-CH<sub>2</sub>-O-), 4.29 (m, 1H, -CH-), 5.54 (d, 1H,  $J = 7.3\text{Hz}$ , C-NH-  
201 CH<sub>2</sub>-).

202 ***N*-myristyl 4-amino-1-pentanol 5a**:  $m/Z$  (LR-ESI<sup>+</sup>) C<sub>19</sub>H<sub>40</sub>NO<sub>2</sub> (M + H<sup>+</sup>), found: 314.2  
203 calculated for: 314.53. IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3200-3500 (O-H, alcohol and N-H, amide), 2800-3000  
204 (CH of myristyl chain), 1639 (C=O, amide), 1545 (N-H, amide). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>,  
205 δ ppm): δ 0.88 (t, 3H,  $J = 6.58\text{Hz}$ , -CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (d, 3H,  $J = 6.23\text{Hz}$ , -CH-CH<sub>3</sub>), 1.25 (m,  
206 20H, -CH<sub>2</sub>- of myristyl chain), 1.53 (m, 4H, -CH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-OH), 1.63 (m, 2H, -CH<sub>2</sub>-  
207 CH<sub>2</sub>-CO-NH- of myristyl chain), 2.14 (t, 2H,  $J = 7.27\text{Hz}$ , -CH<sub>2</sub>-CH<sub>2</sub>-CO-NH- of myristyl  
208 chain), 2.94 (s, 1H, -OH), 3.67 (m, 2H, -CH<sub>2</sub>-CH<sub>2</sub>-OH), 4.06 (m, 1H, -CH-), 5.28 (s, 1H, -  
209 NH-).

210 ***O*-myristyl 4-amino-1-pentanol 5b**:  $m/Z$  (LR-ESI<sup>+</sup>) C<sub>19</sub>H<sub>40</sub>NO<sub>2</sub> (M + H<sup>+</sup>), found: 314.2  
211 calculated for: 314.53. IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3291 (N-H, amine), 2800-3000 (CH of myristyl chain),  
212 1736 (C=O, ester), 1557 (N-H, amine). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): δ 0.88 (t, 3H,  $J =$   
213 6.99Hz, -CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (d, 3H,  $J = 8\text{Hz}$ , -CH-CH<sub>3</sub>), 1.25 (m, 20H, -CH<sub>2</sub>- of myristyl chain),  
214 1.52 (m, 4H, -CH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-O-), 1.62 (m, 2H, -CH<sub>2</sub>-CH<sub>2</sub>-CO-O- of myristyl chain),  
215 2.22 (t, 1H,  $J = 7.16\text{Hz}$ , -CH<sub>2</sub>-CH<sub>2</sub>-CO-O- of myristyl chain), 2.29 (t, 1H,  $J = 7.5\text{Hz}$ , -CH<sub>2</sub>-  
216 CH<sub>2</sub>-CO-O- of myristyl chain), 3.43 (m, 1H, -CH<sub>2</sub>-CH<sub>2</sub>-O-), 3.69 (m, 1H, -CH<sub>2</sub>-CH<sub>2</sub>-O-), 4.1  
217 (m, 1H, -CH-).

218 ***O,N*-dimyristyl 4-amino-1-pentanol 5c**:  $m/Z$  (LR-ESI<sup>+</sup>) C<sub>33</sub>H<sub>66</sub>NO<sub>3</sub>Na (M + Na<sup>+</sup>), found:  
219 546.2 calculated for: 546.9. IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3304 (N-H, amide), 2800-3000 (CH of myristyl

220 chain), 1732 (C=O, ester), 1640 (C=O, amide), 1546 (N-H, amide). <sup>1</sup>H NMR (400 MHz,  
221 CDCl<sub>3</sub>, δ ppm): δ 0.88 (t, 6H, *J*= 7.43Hz, 2x -CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (d, 3H, *J*= 6.83Hz, -CH-CH<sub>3</sub>),  
222 1.25 (m, 40H, -CH<sub>2</sub>- of myristyl chain), 1.53 (m, 4H, -CH-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-O-C), 1.6 (m, 4H,  
223 2x -CH<sub>2</sub>-CH<sub>2</sub>-CO- of myristyl chain), 2.14 (t, 4H, *J*= 7.08Hz, -CH<sub>2</sub>-CH<sub>2</sub>-CO- of myristyl  
224 chain), 3.68 (m, 2H, -CH<sub>2</sub>-CH<sub>2</sub>-O-C), 4.07 (m, 1H, -CH-), 5.27 (d, 1H, *J*= 6.86Hz, -NH-).

225 ***N*-myristyl aminohexanol 7a:** m/Z (LR-ESI<sup>+</sup>) C<sub>20</sub>H<sub>42</sub>NO<sub>2</sub> (M + H<sup>+</sup>), found: 329.5 calculated  
226 for: 328.56. IR ν<sub>max</sub> (cm<sup>-1</sup>): 3385 (O-H, alcohol), 3314 (N-H, amide), 2800-3000 (CH of  
227 myristyl chain), 1634 (C=O, amide), 1534 (N-H, amide). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm):  
228 δ 0.88 (t, 3H, *J*= 7.5Hz, -CH<sub>2</sub>-CH<sub>3</sub>), 1.25 (m, 20H, -CH<sub>2</sub>- of myristyl chain), 1.51 (m, 2H, -  
229 CH<sub>2</sub>-CH<sub>2</sub>-CO-O- of myristyl chain), 1.59 (m, 4H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-OH), 2.26 (t, 2H, *J*=  
230 7.65Hz, -CH<sub>2</sub>-CH<sub>2</sub>-CO-OH of myristyl chain), 2.72 (s, 1H, -OH), 3.25 (t, 2H, *J*= 7.07Hz, -  
231 CH<sub>2</sub>-CH<sub>2</sub>-OH), 3.63 (t, 2H, *J*= 7.29Hz, -CH<sub>2</sub>-CH<sub>2</sub>-NH-CO-CH<sub>2</sub>), 5.41 (s, 1H, -NH-).

232 ***O*-myristyl aminohexanol 7b:** m/Z (LR-ESI<sup>+</sup>) C<sub>20</sub>H<sub>42</sub>NO<sub>2</sub> (M + H<sup>+</sup>), found: 329.5 calculated  
233 for: 328.56. IR ν<sub>max</sub> (cm<sup>-1</sup>): 3400 (N-H, amine), 2800-3000 (CH of myristyl chain), 1736  
234 (C=O, ester), 1544 (N-H, amine). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): δ 0.88 (t, 3H, *J*=  
235 7.28Hz, -CH<sub>2</sub>-CH<sub>3</sub>), 1.25 (m, 20H, -CH<sub>2</sub>- of myristyl chain), 1.55 (m, 2H, -CH<sub>2</sub>-CH<sub>2</sub>-CO-O-  
236 of myristyl chain), 1.62 (m, 4H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-NH<sub>2</sub>), 2.28 (t, 2H, *J*= 7.65Hz, -CH<sub>2</sub>-  
237 CH<sub>2</sub>-CO-O- of myristyl chain), 2.81 (s, 2H, -NH<sub>2</sub>), 3.64 (t, 2H, *J*= 6.47Hz, -CH<sub>2</sub>-CH<sub>2</sub>-NH<sub>2</sub>),  
238 4.04 (t, 2H, *J*= 6.47Hz, -CH<sub>2</sub>-CH<sub>2</sub>-O-CO-CH<sub>2</sub>).

239 ***O,N*-dimyristyl aminohexanol 7c:** m/Z (LR-ESI<sup>+</sup>) C<sub>34</sub>H<sub>67</sub>NO<sub>3</sub>Na (M + Na<sup>+</sup>), found: 560.7,  
240 calculated for: 560.93. IR ν<sub>max</sub> (cm<sup>-1</sup>): 3298 (N-H, amide), 2800-3000 (CH of myristyl chain),  
241 1726 (C=O, ester), 1635 (C=O, amide), 1547 (N-H, amide). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, δ  
242 ppm): δ 0.88 (t, 6H, *J*= 6.48Hz, 2x -CH<sub>2</sub>-CH<sub>3</sub>), 1.25 (m, 40H, -CH<sub>2</sub>- of myristyl chain), 1.5  
243 (m, 4H, -CH<sub>2</sub>-CH<sub>2</sub>-CO- of myristyl chain), 1.6 (m, 4H, -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-O-C), 2.15 (t,

244 2H,  $J = 7.8\text{Hz}$ ,  $-\text{CH}_2-\underline{\text{CH}_2}-\text{CO}-\text{NH}-$ ), 2.29 (t, 2H,  $J = 7.8\text{Hz}$ ,  $-\text{CH}_2-\underline{\text{CH}_2}-\text{CO}-\text{NH}-$ ), 3.24 (q, 2H,  
245  $J = 6.5\text{Hz}$ ,  $-\text{CH}_2-\underline{\text{CH}_2}-\text{NH}-$ ), 4.06 (t, 2H,  $J = 5.9\text{Hz}$ ,  $-\text{CH}_2-\underline{\text{CH}_2}-\text{O}-\text{CO}-\text{CH}_2$ ), 5.4 (s, 1H,  $-\text{NH}-$ ).

#### 246 2.3.3. Analysis of the ionization state of substrates in *tert*-amyl alcohol

247 The ionization state of myristic acid **1** in *tert*-amyl alcohol was investigated using infrared  
248 spectroscopy analysis. Infrared (IR) spectra of samples containing 175 mM of myristic acid **1**  
249 and from 0 to 250 mM of alaninol **2** were recorded from 1500 to 1800  $\text{cm}^{-1}$  with a resolution  
250 of 4  $\text{cm}^{-1}$  using a 100 ATR spectrometer (Perkin-Elmer, United States). Before the  
251 interpretation of data, a treatment (base line correction, smoothing and normalization min-  
252 max) was applied to spectra.

#### 253 2.4. Titration of Novozym<sup>®</sup> 435 active sites

254 In order to determine the amount of immobilized *Candida antarctica* lipase B (Novozym<sup>®</sup>  
255 435) active sites, a suicide inhibitor (4-methylumbelliferyl hexylphosphonate) was used  
256 according to the method developed by Fujii *et al.* [54-55]. This inhibitor was added to 10 mg  
257 of immobilized lipase (immobilized on beads of acrylic support), to a final concentration of  
258 50  $\mu\text{M}$ . In this sample, acetonitrile was added to final volume of 1 mL. 100  $\mu\text{L}$  sample  
259 solution was adding to 900  $\mu\text{L}$  of buffer (100 mM Tris-HCl, 1 mM  $\text{CaCl}_2$ , pH 8.0), then  
260 fluorescence intensity was analyzed using a luminescence spectrometer (Luminescence  
261 Spectrometer Model LS-50B, PerkinElmer, MA, USA). The excitation wavelength was  
262  $\lambda = 360\text{ nm}$  and the emission wavelength was  $\lambda = 445\text{ nm}$ . The active site amount was establish  
263 from the linear relationship between fluorescence intensity and the concentration of the  
264 leaving group 4-methylumbelliferone. Finally, the relationship between fluorescence intensity  
265 and the amount of beads containing immobilized lipase was linear. The resulting active  
266 *Candida antarctica* lipase B load on beads was found to be 4.7% (%weight/weight) which  
267 equals 1.4  $\mu\text{mol}$  of lipase active site per gram of beads.

## 268 2.5. Determination of the kinetic parameters

269 The kinetic parameters from amino-alcohol acylation were determined using Lineweaver-  
270 Burk reciprocal plots of initial rates versus amino-alcohol concentrations. The apparent  
271 maximum rates ( $V_{max,app}$ ) of myristic acid conversion, *O*-acylation and *N*-acylation were  
272 obtained by using y-intercept which correspond to the  $1/V_{max,app}$  value. The apparent catalytic  
273 constants ( $k_{cat,app}$ ) were then calculated as the ratio of the  $V_{max,app}$  to the total amount of  
274 Novozym<sup>®</sup> 435 active sites in the reaction medium, which was previously determined by  
275 titration (1.4  $\mu\text{mol}$  of lipase active sites per gram of Novozym<sup>®</sup> 435: see section 2.4). In  
276 parallel, the apparent Michaelis constants ( $K_{m,app}$ ) were obtained by using x-intercept which  
277 correspond to the  $-1/K_{m,app}$  value.

## 278 2.6. Evaluation of the chemoselectivity

279 The chemoselectivity of Novozym<sup>®</sup> 435 during the acylation of amino-alcohols was studied  
280 by comparing the alcohol group *O*-acylation and the amino group *N*-acylation, and then  
281 calculated via the apparent catalytic efficiency ratio (Eq. (1)) [56], which was transformed  
282 into an apparent catalytic constant ratio (Eq. (2)) owing to the fact that there is a unique  $K_{m,app}$   
283 for each bifunctional amino-alcohol.

$$284 \quad C = (k_{cat, app \text{ } O\text{-acylation}} / K_{m,app}) / (k_{cat, app \text{ } N\text{-acylation}} / K_{m,app}) \quad (1)$$

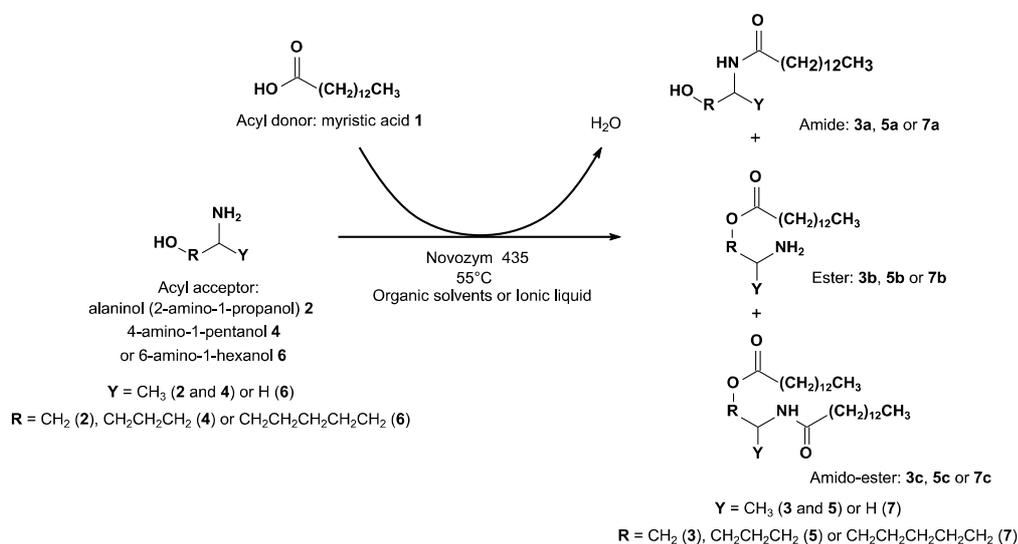
$$285 \quad C = k_{cat, app \text{ } O\text{-acylation}} / k_{cat, app \text{ } N\text{-acylation}} \quad (2)$$

286 **3. Results and discussion**

287 *3.1. Effect of the solvent on the apparent kinetic parameters and the*  
 288 *chemoselectivity of Novozym<sup>®</sup> 435 in the acylation of amino-alcohols*

289 Two organic solvents (*tert*-amyl alcohol and *n*-hexane) and one ionic liquid (1-Butyl-3-  
 290 methylimidazolium hexafluorophosphate; Bmim [PF<sub>6</sub>]) were used for the selective acylation  
 291 of alaninol (**2**), 4-amino-1-pentanol (**4**) and 6-amino-1-hexanol (**6**) using myristic acid **1** as an  
 292 acyl donor (Scheme 1). Bmim [PF<sub>6</sub>] was chosen as a model ionic liquid on the basis of several  
 293 earlier studies which revealed the efficiency of Bmim [PF<sub>6</sub>] for *O*-acylation,  
 294 transesterification and *N*-acylation reactions catalyzed by *Candida antarctica* lipase B [57,  
 295 58]. The two organic solvents were chosen from previous works found in the literature, which  
 296 have demonstrated the efficacy of these solvents during the acylation of amino-polyol  
 297 substrates catalyzed by lipases [8, 53, 59].

298



299

300 **Scheme 1.** Acylation of three amino-alcohols by Novozym<sup>®</sup> 435 (immobilized *Candida*  
 301 *antarctica* lipase B).

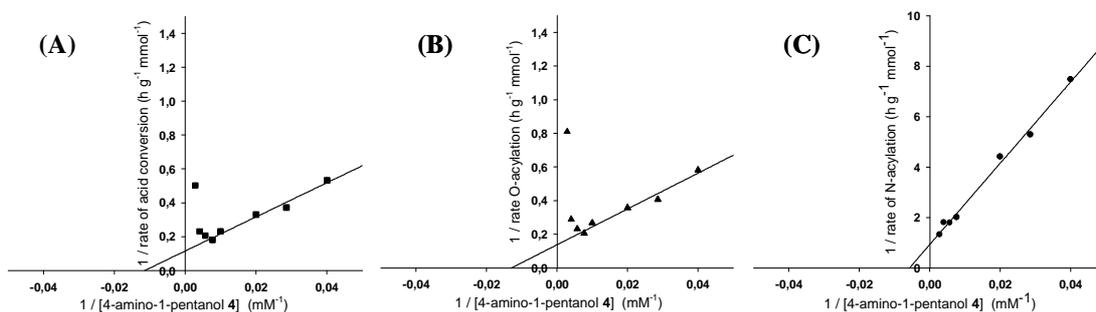
302

303 All experiments were performed in media containing various amounts of the amino-alcohol  
304 (25-350 mM) as an acyl acceptor and 175 mM of myristic acid **1** as an acyl donor. In the  
305 absence of enzyme, no product was detected within 2 days. LC-MS analysis demonstrated  
306 that the decrease in myristic acid **1** concentration is always concomitant with the synthesis of  
307 acylated products. The purification and the structural elucidation by MS, IR and NMR  
308 analyses identified the structure of the acylated products.

309 The kinetic parameters for the acylation of amino-alcohols **2**, **4** and **6** were determined using  
310 Lineweaver-Burk reciprocal plots.

311

312



313

314 **Fig. 1.** Reciprocal initial rates of myristic acid conversion (A), 4-amino-1-pentanol *O*-  
315 acylation (B) and 4-amino-1-pentanol *N*-acylation (C) versus reciprocal 4-amino-1-pentanol  
316 concentrations. Reactions were carried out at 55°C using a fixed concentration of myristic  
317 acid (175 mM) and 5 g l<sup>-1</sup> of Novozym<sup>®</sup> 435 in 2 ml of *tert*-amyl alcohol. The data represent  
318 the averages of triplicate runs whose standard deviations were always lower than 15%.

319

320

321 As shown on Fig. 1, which describes the acylation of 4-amino-1-pentanol **4** in *tert*-amyl  
322 alcohol, a decrease in the initial rates of myristic acid **1** conversion, *O*-acylation and *N*-  
323 acylation, occurred when amino-alcohol was in excess ( $\geq 175$  mM). This phenomenon was

324 also noticed on the acylation kinetic profile of alaninol **2** and 6-amino-1-hexanol **6**, in both  
325 organic solvents and ionic liquid and could be attributed to an inhibitor effect similar to  
326 excess substrate inhibition. However, it was not observed in a previous work in which we  
327 performed the *O*-acylation of 2-butanol starting from myristic acid as an acyl donor under  
328 similar experimental conditions [60]. Thus, the decrease in initial rates is most likely due to an  
329 interaction between myristic acid **1** and the amino group of the amino-alcohol. Indeed, the  
330 presence of an amino substrate and a fatty acid substrate in an organic solvent generally leads  
331 to the formation of an ion-pair complex between both substrates, depending on the acido-  
332 basic conditions of the medium [46, 53, 59]. This salt complex makes the ion forms of both  
333 substrates non reactive ( $\text{NH}_3^+$  amine form and  $\text{COO}^-$  fatty acid form) and therefore leads to  
334 the overestimation of the substrate concentrations that are actually available to the enzyme in  
335 the reaction medium. Maugard et al. [53] previously described this ion-pair complex as a  
336 limiting factor in lipase-catalyzed acylation, under conditions where it is less soluble than free  
337 substrates. To verify this hypothesis, the composition of the medium, in particular the  
338 carbonyl species was analyzed by IR spectroscopy at the start of the reaction using various  
339 concentrations of amino-alcohol **2** in *tert*-amyl alcohol as a reaction solvent. When myristic  
340 acid **1** alone was totally solubilized, only one carbonyl band was observed at  $1710\text{ cm}^{-1}$   
341 (Table 2, entry 1), corresponding to the acid form. When the concentration of amino-alcohol **2**  
342 was increased in the media, a decrease in the area of the carbonyl acid band ( $1710\text{ cm}^{-1}$ ) was  
343 concomitant with an increase in the area of the carboxylate band ( $1562\text{ cm}^{-1}$ ) (Table 2, entries  
344 2 to 5). This demonstrates the formation of an ion-pair complex between substrates **1** and **2**.

345

346 **Table 2**

347 Quantification of IR spectrum bands of mixtures containing 175 mM of myristic acid **1** and  
348 various concentrations of alaninol **2** in *tert*-amyl alcohol.

<u>Entry</u>	[Alaninol <b>2</b> ]	IR band
--------------	----------------------	---------

	(mM)	1710 cm <sup>-1</sup> (-COOH) (Area in %)	1562 cm <sup>-1</sup> (-COO <sup>-</sup> ) (Area in %)
1	0	100	0
2	50	64	36
3	100	26	74
4	175	7	93
5	250	5	95

349

350 Due to the inhibitor effect of the ion-pair complex at high substrate concentrations, the  
351 apparent kinetic parameters ( $k_{cat,app}$  and  $K_{m,app}$ ) were obtained from Lineweaver-Burk  
352 reciprocal plots corresponding to amino-alcohols concentrations lower than 175 mM. Indeed,  
353 no decrease in initial rates and de facto no inhibition due to the ion-pair complex formation  
354 was observed for these amino-alcohol concentrations. Thus, for amino-alcohol **4** acylation in  
355 *tert*-amyl alcohol (Fig. 1), the  $k_{cat,app}$  values were 86 min<sup>-1</sup> for *O*-acylation (Fig. 1B) and 13  
356 min<sup>-1</sup> for *N*-acylation (Fig. 1C) and 99 min<sup>-1</sup> for myristic acid **1** conversion (Fig. 1A) and the  
357  $K_{m,app}$  value of amino-alcohol **4** acylation was 75 mM (Fig. 1A). The apparent kinetic  
358 parameters, the chemoselectivity ratio (C), as well as the log P values of amino-alcohols, are  
359 summarized in Table 3. The log P value is defined as the logarithm of the partition coefficient  
360 of a given compound in a standard octanol/water biphasic system [61]. This parameter  
361 characterizes the hydrophobicity of a compound: the higher the log P value, the more  
362 hydrophobic the compound [62, 63].

363

364 **Table 3**

365 Apparent kinetic parameters and chemoselectivity ratios of the Novozym<sup>®</sup> 435 catalyzed  
366 acylation of amino-alcohols **2**, **4** and **6** carried out at 55°C in *n*-hexane, *tert*-amyl alcohol or  
367 Bmim [PF<sub>6</sub>] as a reaction solvent, using 175 mM of myristic acid **1** as an acyl donor.

Entry	Solvent	Amino-alcohol	$k_{cat,app}$ of myristic acid <b>1</b> conversion (min <sup>-1</sup> )	$k_{cat,app}$ of <i>O</i> - acylation (min <sup>-1</sup> )	$k_{cat,app}$ of <i>N</i> - acylation (min <sup>-1</sup> )	$K_{m,app}$ (mM)	C <sup>c</sup>
-------	---------	---------------	--	--	--	---------------------	----------------

1	<i>n</i> -hexane		357	14	114	18.7	0.12
2	<i>tert</i> -amyl alcohol	(±)-alaninol <b>2</b> (log P -0.96)	58	nd <sup>a</sup>	51	182	<0.001
3	Bmim [PF <sub>6</sub> ]		33	2	27	69.7	0.07
4	<i>n</i> -hexane		241	231	0 <sup>b</sup>	35.3	>1000
5	<i>tert</i> -amyl alcohol	(±)-4-amino-1-pentanol <b>4</b> (log P -0.43)	99	86	13	75	6.61
6	Bmim [PF <sub>6</sub> ]		38	27	0 <sup>b</sup>	72.2	>1000
7	<i>n</i> -hexane		551	539	1	53	539
8	<i>tert</i> -amyl alcohol	6-amino-1-hexanol <b>6</b> (log P -0.01)	136	126	12	63	10,5
9	Bmim [PF <sub>6</sub> ]		44	41	0 <sup>b</sup>	45	>1000

368  
369  
370

<sup>a</sup> Not detected.

<sup>b</sup> The acylated product was detected at a concentration too low to be quantified.

<sup>c</sup> The chemoselectivity ratio calculated via the apparent catalytic constant ratio (Eq. (2)).

371

372 Table 3 provides a number of key information. In order to facilitate the comparison of the  
373 parameters described in this table, the effect of solvent nature on the  $K_{m,app}$ ,  $k_{cat,app}$  and  
374 chemoselectivity is discussed separately.

375 • *Effect on the  $K_{m,app}$*

376 The  $K_{m,app}$  of amino-alcohol **2** in *tert*-amyl alcohol (182 mM) was 2- and 2.5-fold higher than  
377 the  $K_{m,app}$  of long chain amino-alcohols **4** (75 mM) and **6** (63 mM), respectively (Table 3,  
378 entries 2, 5 and 8). This indicates a lower affinity of Novozym<sup>®</sup> 435 for short chain amino-  
379 alcohol **2**. The same phenomenon is observed using Bmim [PF<sub>6</sub>] (Table 3, entries 3, 6 and 9).  
380 In contrast, the  $K_{m,app}$  of short chain amino-alcohol **2** in *n*-hexane (18.7 mM) was 1.5- and 2-  
381 fold lower than the  $K_{m,app}$  of long chain amino-alcohols **4** (35.3 mM) and **6** (53 mM),  
382 respectively (Table 3, entries 1, 4 and 7), which indicates a switch in the affinity of  
383 Novozym<sup>®</sup> 435 in favour of the short chain amino-alcohol **2**.

384 To date, a clear consensus has not yet emerged on the parameters to quantitatively describe  
385 solvents and their influence on enzymatic reactions. However, it is well known that solvent

386 hydrophobicity is a key factor, whose influence can be evaluated using the log P value [62,  
387 64-65]. The solvent effect can be analysed using the probable relationship between enzyme  
388 activity and substrate solvation. In a hydrophilic solvent such as *tert*-amyl alcohol (log P 0.89)  
389 or Bmim [PF<sub>6</sub>] (log P -2.36), the solvation of the highly hydrophilic amino-alcohol **2** (log P -  
390 0.96) is favoured to the detriment of its availability for the enzyme. Thus, the affinity of the  
391 lipase for amino-alcohol **2** is lower than its affinity for other amino-alcohols in hydrophilic  
392 solvents, resulting in a higher  $K_{m,app}$  value (Table 3). In contrast, in a hydrophobic solvent  
393 such as *n*-hexane (log P 3.90), the solvation of an amino-alcohol is favoured when its  
394 hydrophilicity decreases, the solvation of the more hydrophilic amino-alcohol **2** is therefore  
395 not favoured and this benefits its availability for the enzyme. Thus, the affinity of the lipase  
396 for amino-alcohol **2** is higher than its affinity for other amino-alcohols in hydrophobic  
397 solvents, resulting in a lower  $K_{m,app}$  value (Table 3). In addition, Novozym<sup>®</sup> 435 consisting in  
398 *Candida antarctica* lipase B immobilized on a positively charged acrylic polar resin, solvation  
399 of the biocatalyst would not be favoured in a hydrophobic solvent: as a consequence, this  
400 promote biocatalyst availability for polar substrates such as amino-alcohols in *n*-hexane,  
401 resulting in a lower  $K_{m,app}$  value. These results are in accordance with several studies that have  
402 demonstrated that the use of various supports (polar or apolar) allows to modulate activity and  
403 selectivity of a particular immobilized lipase used in various solvents [17].

404 • *Effect on the  $k_{cat,app}$  of myristic acid conversion*

405 In this work, the  $k_{cat,app}$  of myristic acid conversion is a representative parameter of the  
406 biocatalyst activity for the amino-alcohol acylation. Indeed, the  $k_{cat,app}$  of myristic acid  
407 conversion corresponds to the sum of  $k_{cat,app}$  of mono-*N*-acylation, mono-*O*-acylation and  
408 amido-ester synthesis.

409 *n*-Hexane was the most effective reaction solvent, in terms of  $k_{cat,app}$  of myristic acid **1**  
410 conversion, to enhance the activity of Novozym<sup>®</sup> 435, regardless of the three amino-alcohols

411 tested. Catalytic activity was lowest using Bmim [PF<sub>6</sub>]. The  $k_{\text{cat,app}}$  of myristic acid **1**  
412 conversion from the acylation of amino-alcohol **2** in *n*-hexane (357 min<sup>-1</sup>) was 6- and 11-fold  
413 higher than the  $k_{\text{cat,app}}$  of myristic acid **1** conversion in *tert*-amyl alcohol (58 min<sup>-1</sup>) and in  
414 Bmim [PF<sub>6</sub>] (33 min<sup>-1</sup>), respectively (Table 3, entries 1 to 3). These results are not surprising  
415 given that *tert*-amyl alcohol and Bmim [PF<sub>6</sub>] are the most polar of the three solvents used.  
416 Indeed, the polarity of solvents is well known to dramatically influence the catalytic activity  
417 of enzymes [64-69]. Extremely or intermediary hydrophilic polar solvents, such as *tert*-amyl  
418 alcohol, interact with the absolute amount of water, indispensable in the acquisition and the  
419 maintenance of enzyme conformation. These solvents strip off water molecules from the  
420 enzyme, resulting in either a change in the conformation and flexibility of the enzyme or a  
421 negative effect on the transition state stability and, thus, on catalytic activity. Apart from their  
422 potential interactions with water molecules, hydrophilic polar solvents also interact with the  
423 secondary structure of the enzyme via multiple hydrogen bonds and via other strong  
424 interactions. This can lead to an alteration in the protein conformation and thus a negative  
425 effect on catalytic activity.

426 However, the low  $k_{\text{cat,app}}$  of myristic acid **1** conversion obtained in Bmim [PF<sub>6</sub>] (Table 3,  
427 entries 3, 6 and 9) can also be explained by limiting factors such as diffusion and mass-  
428 transfer limitations associated with the high viscosity of this ionic liquid [70, 71]. Moreover,  
429 anions such as [PF<sub>6</sub>] can strongly interact with the positively charged sites in the *Candida*  
430 *antarctica* lipase B structure, potentially causing conformational changes [72]. In addition, it  
431 must be taken into consideration that ionic liquids form ion-pairs that can interact with both  
432 the carboxyl group of fatty acid and amino groups of amino-alcohols. On a one hand, these  
433 interactions could modulate the ionization state of the substrates and the enzyme and thus  
434 affect catalytic activity. On the other hand, they may stabilize substrate ionic form and  
435 promote as a result the formation of an ion-pair complex between both substrates. This would

436 cause higher limitation of substrate availability in Bmim [PF<sub>6</sub>] than in *n*-hexane and *tert*-amyl  
437 alcohol.

438 • *Effect on the chemoselectivity*

439 The chemoselectivity of the Novozym<sup>®</sup> 435-catalyzed acylation of the three amino-alcohols  
440 was evaluated using the chemoselectivity ratio (C) (Eq. (2)). The acylation of amino-alcohol **2**  
441 in *n*-hexane and Bmim [PF<sub>6</sub>] gave similar C values, close to 0.1 (Table 3, entries 1 and 3), as  
442 no ester **3b** was detected in *tert*-amyl alcohol (Table 3, entry 2). In contrast, C values of 539  
443 and 10.5 were obtained for the acylation of amino-alcohol **6** in *n*-hexane and *tert*-amyl  
444 alcohol, respectively (Table 3, entries 7 and 8), when only trace amounts of amide **7a** were  
445 detected in Bmim [PF<sub>6</sub>] (Table 3, entry 9) (the experimental detection limit by LC-MS  
446 analysis was 2 μM). Similarly, C value of 6.61 was observed for the acylation of amino-  
447 alcohol **4** in *tert*-amyl alcohol (Table 3, entry 5) while amide **5a** was detected as trace  
448 amounts not only in Bmim [PF<sub>6</sub>] but also in *n*-hexane (Table 3, entries 4 and 6). According to  
449 these data, Novozym<sup>®</sup> 435 shows higher selectivity towards *N*-acylation of amino-alcohol **2**,  
450 which has two carbons between the alcohol and amino groups. Moreover, we can also  
451 conclude that Novozym<sup>®</sup> 435 is more chemoselective for *O*-acylation of substrates **4** and **6**,  
452 which are long chain amino-alcohols exhibiting four and six carbons between their alcohol  
453 and amino groups, respectively.

454 In addition, the fact that the higher  $k_{cat, app}$  of *O*-acylation was obtained with amino-alcohol **6**  
455 (Table 3, entries 7 to 9) in comparison with amino-alcohol **4** (Table 3, entries 4 to 6) can be  
456 explained not only by the increase in the carbon chain length but also by the fact that amino-  
457 alcohol **6** is a primary alcohol while amino-alcohol **4** is a secondary one. Indeed, it has been  
458 widely demonstrated in the literature that lipase-catalyzed acylation of primary alcohols  
459 generally occurs with a higher catalytic activity than the acylation of secondary alcohols [73-  
460 75].

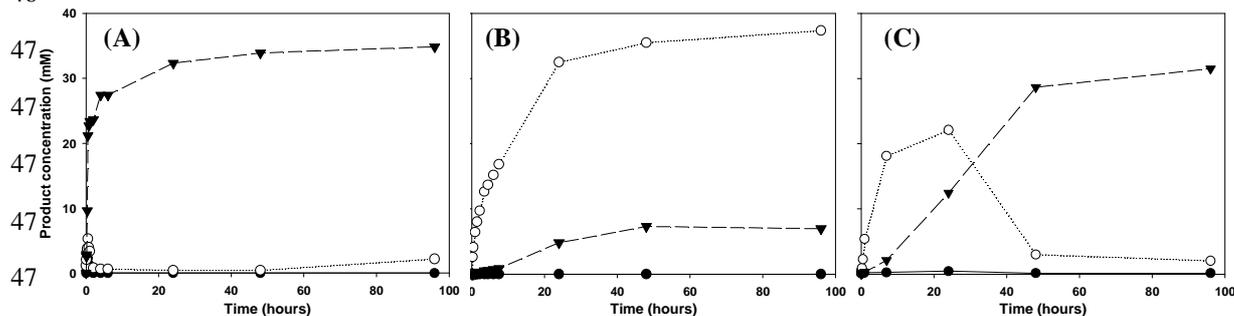
461 3.2. Effect of the solvent and of the amino-alcohol concentration on the  
462 production of acylated amino-alcohols catalyzed by Novozym<sup>®</sup> 435

463 • Time-course of alaninol acylation in *tert*-amyl alcohol, *n*-hexane and Bmim [PF<sub>6</sub>]

464 Prior to study the Novozym<sup>®</sup> 435-catalyzed acylation of amino-alcohols **2**, **4** and **6** in terms of  
465 production at a fixed time, the reaction was carried out in *n*-hexane, *tert*-amyl-alcohol and  
466 Bmim [PF<sub>6</sub>], using 35 mM amino-alcohol and 175 mM myristic acid **1**, and monitored over  
467 time.

468

469



475 **Fig. 2.** Enzymatic acylation of alaninol **2** (35 mM) by myristic acid **1** (175 mM) in *n*-hexane  
476 (A), *tert*-amyl alcohol (B) and Bmim [PF<sub>6</sub>] (C): ester (●), amide (○) and amido-ester (▼).  
477 Reactions were carried out at 55°C using 5 g l<sup>-1</sup> of Novozym<sup>®</sup> 435.

478

479 Fig. 2 shows the time-course of alaninol **2** acylation. During this reaction conducted in Bmim  
480 [PF<sub>6</sub>] (Fig. 2C), ester **3a** was detected only as trace amounts. On the other hand, amide **3a** was  
481 the main product synthesized in *tert*-amyl alcohol at a stationary state reached after 96 hours  
482 with a yield close to 100% (based on starting alaninol **2**) (Fig. 2B), whereas it was  
483 predominant only over the first 30 minutes and 24 hours of reactions effected in *n*-hexane and  
484 Bmim [PF<sub>6</sub>], respectively (Fig. 2 A and C). After these times, amide **3a** was then consumed to  
485 the benefit of amido-ester **3c** synthesis, which became the main product after 96 hours with

486 yields close to 99% and 90% in *n*-hexane and Bmim [PF<sub>6</sub>], respectively (based on starting  
487 alaninol **2**). Interestingly, this stationary phase was also reached after 96 hours for reactions  
488 involving amino-alcohols **4** and **6**, whatever the solvent (data not shown). On the other hand,  
489 the time-course of the increase in amido-ester concentration was clearly concomitant with the  
490 decrease in amide concentration.

491 These results suggest that the synthesis of the amido-ester proceeds in two steps which might  
492 follow two hypothetic sequences:

- 493       ▪ (1) either the *N*-acylation of alaninol **2** occurs first and is then followed by the  
494        *O*-acylation of the amide synthesized in the first step;
- 495       ▪ (2) or the *O*-acylation of alaninol **2** occurs first and is then followed by the *N*-  
496        acylation of the ester synthesized in the first step. This second hypothesis  
497        would, however, necessarily imply a reason to make the ester undetectable all  
498        along the reaction time-course. Two possibilities would explain this: either the  
499        rate of ester *N*-acylation is at least as fast as the rate of alaninol **2** *O*-acylation,  
500        or the ester is transacylated into the amide via a kinetically controlled process  
501        involving the formation of the acyl enzyme from the ester that would be  
502        released by the attack of the amine, as reported by Volker Kasche works to  
503        describe the mechanism of amidase-catalyzed amine acylation using esters as  
504        acyl donors [76].

505 Moreover, we focused in a previous work on the kinetic analysis of Novozym<sup>®</sup> 435-catalyzed  
506 acylation of a mono-alcohol and a mono-amine structurally related to alaninol **2** carried out in  
507 *tert*-amyl alcohol under similar experimental conditions. We demonstrated then that (R)-2-  
508 butanol *O*-acylation rate was 22-fold faster than (R)-*sec*-butylamine *N*-acylation rate [60]. On  
509 this basis, we would expect the ester intermediate to accumulate in the reaction medium

510 before being converted into the amido-ester, which is, however, not the case: this ester  
511 intermediate is detected at best as trace amounts whatever the reaction time or the solvent  
512 tested. In addition, we have also shown that the *N*-acylation rates obtained for the enzymatic  
513 acylation of bifunctional compounds such as 1-methoxy-2-propylamine and 1,2-  
514 diaminopropane, catalyzed by Novozym<sup>®</sup> 435 in *tert*-amyl alcohol at 55°C, were 8- and 38-  
515 fold higher, respectively, than the *N*-acylation rate of the related mono-amine (*sec*-  
516 butylamine) [77]. These bifunctional compounds are structurally related to alaninol **2** but  
517 exhibit no alcohol group in their structure and thus cannot possibly be *O*-acylated. For this  
518 reason, we formulated the following postulate for amino-alcohol acylation: the presence of an  
519 alcohol group in  $\beta$ -position of the amino group of the acyl acceptor would result in the  
520 enhancement in the rate of *N*-acylation. This may be due to the formation of an intramolecular  
521 interaction between the amino group and the alcohol group located in  $\beta$ -position, which is  
522 strengthened by the fact that this interaction could not occur for amino-alcohols **4** and **6**,  
523 considering the longer distance between both functional groups, giving as a result low rates of  
524 *N*-acylation. In order to confirm and develop this new postulate, molecular modelling studies  
525 were conducted to precisely understand the Novozym<sup>®</sup> 435-catalyzed *N*-acylation mechanism  
526 using a methoxy-amine, a di-amine, a short chain amino-alcohol or a long chain amino-  
527 alcohol as an acyl acceptor. These studies recently led to propose a new proton shuttle  
528 reaction mechanism to explain chemoselectivity for Novozym<sup>®</sup> 435-catalyzed *N*-acylation of  
529 alaninol **2** and 4-amino-1-pentanol **4** [26]. As a consequence, all these results seem to rule out  
530 the hypothetical sequence 2 and suggest that the amide is a better substrate than the amino-  
531 alcohol for enzymatic *O*-acylation, especially when using *n*-hexane: the highest amido-ester  
532 **3c** synthesis rate of 7 mmol.h<sup>-1</sup>.g<sup>-1</sup> was obtained in this solvent.

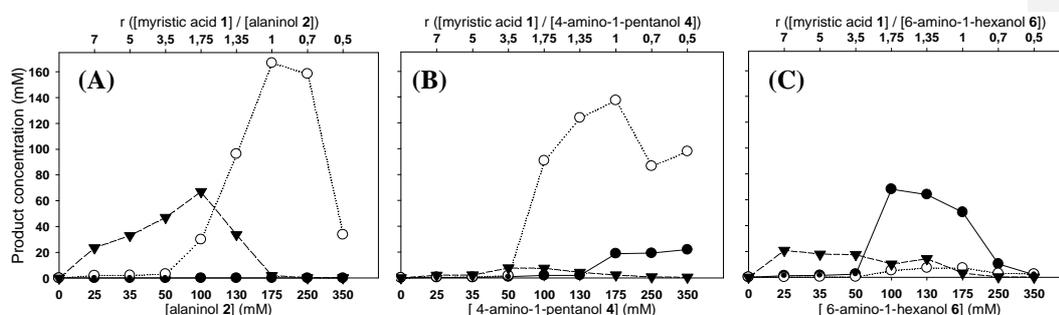
533 Finally, to identify optimal, highly selective conditions required to produce monoacylated and  
534 diacylated compounds, the influences of the amino-alcohol concentration and substrate molar

535 ratio  $r$  ([myristic acid **1**]/[amino-alcohol]) were investigated. All experiments were performed  
 536 using the same conditions as in section 3.1. Figs. 3, 4 and 5 show the product distribution in  
 537 *n*-hexane, *tert*-amyl alcohol and Bmim [PF<sub>6</sub>] solvents, respectively, after 96 hours of  
 538 Novozym<sup>®</sup> 435-catalyzed acylation of amino-alcohols **2**, **4** and **6**. This reaction time was  
 539 chosen to reach a stationary phase for the production of acylated amino-alcohols under all  
 540 experimental conditions such as previously described.

541 • *Production of acylated amino-alcohols in n-hexane*

542

543



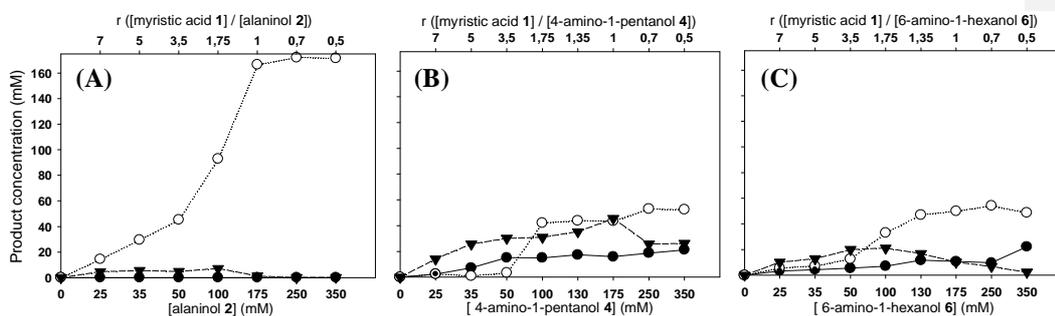
544 **Fig. 3.** Effect of amino-alcohol concentration and substrate molar ratio  $r$  on the enzymatic  
 545 production of acylated amino-alcohols in *n*-hexane, at 96 hours: ester (●), amide (○) and  
 546 amido-ester (▼). Reactions were carried out at 55°C using amino-alcohols **2** (A), **4** (B) or **6**  
 547 (C), with a fixed concentration of myristic acid **1** (175 mM) and 5 g l<sup>-1</sup> of Novozym<sup>®</sup> 435.

548

549 The data in Fig. 3 demonstrate that the use of *n*-hexane gives two major products, depending  
 550 on the  $r$ -value. At initial amino-alcohol concentrations inferior or equal to 100 mM ( $r \geq 1.75$ ),  
 551 the selective production of amido-esters **3c**, **5c** or **7c** is improved, up to 49 mM for amido-  
 552 ester **3c** production (56% yield based on starting myristic acid **1**). Amino-alcohol  
 553 concentrations superior to 100 mM ( $r < 1.75$ ) enhances the production of monoacylated  
 554 compounds. The synthesis of amides from amino-alcohols **2** and **4** reached 166 mM for amide  
 555 **3a** and 138 mM for amide **5a** (95% and 79% yields based on starting myristic acid **1**) under

556 stoichiometric conditions (Fig. 3A and 3B). Moreover, ester **3b** was not detected and ester **5b**  
557 was negligible. Hence, stoichiometric conditions are the optimal conditions for acylation of  
558 amino-alcohols **2** and **4**, and favour both the synthesis yield and the chemoselectivity of amide  
559 production. On the other hand, the production of ester **7b** from the longest amino-alcohol **6** (6  
560 carbons) (Fig. 3C) was improved and reached up to 68 mM (39% yield based on starting  
561 myristic acid **1**) using 100 mM starting amino-alcohol **6**. Again, this change in the  
562 chemoselectivity of Novozym<sup>®</sup> 435 at 96 hours, which is shown to be dependent on the  
563 carbon chain length between the alcohol and amino groups of the amino-alcohol, was in  
564 accordance with its chemoselectivity under initial velocity conditions, previously described in  
565 section 3.1. An interesting result of this study, which should be highlighted, is the decrease in  
566 the global conversion yield observed when a large excess (350 mM) of amino-alcohol ( $r <$   
567  $0.7$ ) is used. This is true for all amino-alcohols tested. The decrease could be explained by the  
568 formation of a non-reactive ion-pair complex between myristic acid **1** and the amino-alcohol  
569 as also described in section 3.1. Other studies have already demonstrated that the use of non-  
570 polar solvents such as *n*-hexane promotes and stabilizes the formation of an ion-pair complex  
571 because polar amino-alcohols are slightly solubilized while ion-pair complex forms of amino-  
572 alcohols are highly solubilized in these solvents [46]. This is due to the carboxylic acid  
573 function playing the role of phase transfer agent for the amino-alcohol, which improves its  
574 solubility. The formation of this complex would indeed drastically affect the availability of  
575 both substrates for the enzyme, especially in excess of the amino-alcohol.

- 576 • *Production of acylated amino-alcohols in tert-amyl alcohol*  
577  
578



579

580 **Fig. 4.** Effect of amino-alcohol concentration and substrate molar ratio  $r$  on the enzymatic  
 581 production of acylated amino-alcohols in *tert*-amyl alcohol, at 96 hours: ester (●), amide (○)  
 582 and amido-ester (▼). Reactions were carried out at 55°C using amino-alcohols **2** (A), **4** (B)  
 583 or **6** (C), with a fixed concentration of myristic acid **1** (175 mM) and 5 g l<sup>-1</sup> of Novozym® 435.

584

585

586 Similar experiments were performed using *tert*-amyl alcohol as a polar protic solvent (Fig. 4).

587 The acylation of amino-alcohol **2** in *tert*-amyl alcohol (Fig. 4A) leads predominantly to the

588 production of amide **3a** and was enhanced by an increase in amino-alcohol **2** concentration,

589 reaching a maximum value of 172 mM (98% yield based on starting myristic acid **1**) for

590 initial amino-alcohol **2** concentrations superior or equal to 250 mM. However, ester **3b** was

591 not detected and the production of amido-ester **3c** was negligible. Acylation of amino-

592 alcohols **4** and **6** (Fig. 4B and 4C) studies were also performed: esters **5b** and **7b** were

593 detected and quantified although their production remained low. On the other hand, the

594 production of amido-esters **5c** and **7c** were predominant and reached maxima of 30 mM and

595 20 mM (34% and 23% yields based on starting myristic acid **1**), respectively, for

596 concentrations in long chain amino-alcohol lower than 50 mM ( $r > 3.5$ ). The production of

597 amides **5a** and **7a** was improved for concentrations higher than 50 mM ( $r < 3.5$ ). Under the

598 best conditions used (250 mM starting amino-alcohol **4** or **6**), production close to 53 mM of

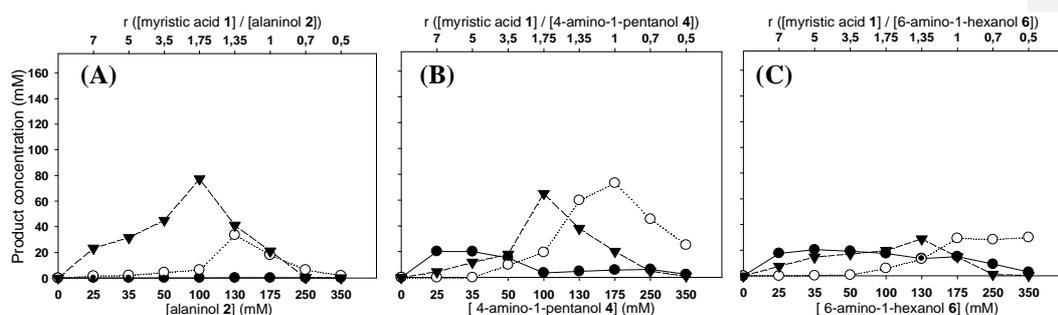
599 amides **5a** or **7a** (30% yield based on starting myristic acid **1**) was reached.

600 On the other hand, in contrast to the results obtained in *n*-hexane, no decrease of global yield  
 601 was observed in *tert*-amyl alcohol when a large excess (350 mM) of amino-alcohol ( $r < 0.7$ )  
 602 was used. This could be explained by the likely complete solubilization of substrates in this  
 603 solvent which would make the ion-pair complex formation less favoured and less stable than  
 604 in *n*-hexane. Both substrates would be therefore more available for the enzymatic reaction  
 605 than in *n*-hexane.

606 In summary, amide production at 96 hours is improved in *tert*-amyl alcohol for all amino-  
 607 alcohols tested, although acylation catalyzed by Novozym<sup>®</sup> 435 of amino-alcohols exhibits  
 608 higher  $k_{cat,app}$  of *O*-acylation than  $k_{cat,app}$  of *N*-acylation as described in section 3.1. These  
 609 results suggest that Novozym<sup>®</sup> 435 favours the synthesis of the amide as a thermodynamic  
 610 product whereas the ester, which is preferentially synthesized under initial velocity  
 611 conditions, can be considered as a kinetic product.

612 • *Production of acylated amino-alcohols in Bmim [PF<sub>6</sub>]*

613



614

615 **Fig. 5.** Effect of amino-alcohol concentration and substrate molar ratio  $r$  on the enzymatic  
 616 production of acylated amino-alcohols in Bmim [PF<sub>6</sub>], at 96 hours: ester (●), amide (○) and  
 617 amido-ester (▼). Reactions were carried out at 55°C using amino-alcohols 2 (A), 4 (B) or 6  
 618 (C), with a fixed concentration of myristic acid 1 (175 mM) and 5 g l<sup>-1</sup> of Novozym<sup>®</sup> 435.

619

620 Finally, Bmim [PF<sub>6</sub>] was tested as a solvent (Fig. 5). The use of 100 mM amino-alcohol (low  
621 excess of myristic acid **1**;  $r = 1.75$ ) in Bmim [PF<sub>6</sub>] gave the highest amido-ester production of  
622 all our experiments, reaching 77, 65 and 20 mM of amido-esters **3c**, **5c** and **7c** (88%, 74% and  
623 23% yields based on starting myristic acid **1**), respectively. However, when amino-alcohol  
624 concentration was higher than 130 mM ( $r < 1.35$ ) a sharp decrease in amido-ester production  
625 was observed. In contrast, the production of esters **5b** and **7b** (Fig. 5B and 5C) never  
626 exceeded 20 mM (11% yield based on starting myristic acid **1**) and the production of amides  
627 was enhanced when amino-alcohol concentration increased from 100 to 175 mM ( $1.75 > r >$   
628  $1$ ), reaching up to 74 mM of amide **5a** (42% yield based on starting myristic acid **1**) under  
629 stoichiometric conditions.

#### 630 **4. Conclusion**

631 In this work, we firstly evaluated the effect of reaction solvent on the activity and  
632 chemoselectivity of Novozym<sup>®</sup> 435 (immobilized *Candida antarctica* lipase B) for the  
633 acylation of three amino-alcohols (alaninol **2**, 4-amino-1-pentanol **4** and 6-amino-1-hexanol  
634 **6**) using myristic acid **1** as an acyl donor. Our results suggest that the nature of the solvent  
635 affects both the availability of substrates and the catalytic activity of the enzyme. In contrast,  
636 we also found that the chemoselectivity of Novozym<sup>®</sup> 435 under initial velocity conditions is  
637 mainly affected by amino-alcohol structure rather than by the reaction solvent. In particular,  
638 we have shown that the  $k_{cat,app}$  of *N*-acylation is improved when using the short chain amino-  
639 alcohol alaninol **2** whereas the  $k_{cat,app}$  of *O*-acylation is improved when using the longer chain  
640 amino-alcohols 4-amino-1-pentanol **4** and 6-amino-1-hexanol **6**. This demonstrates the strong  
641 influence of substrate structure on the chemoselectivity of Novozym<sup>®</sup> 435, and provides new  
642 insights into the selective synthesis of amides or esters produced from the acylation of  
643 bifunctional substrates.

644 Furthermore, we also investigated the impact of amino-alcohol structure, solvent nature and  
645 substrate molar ratio on enzymatic production obtained at a stationary state after 96 hours of  
646 reaction. From a general point of view, our work clearly demonstrates clearly that the  
647 production of acylated amino-alcohols catalyzed by Novozym<sup>®</sup> 435 after 96 hours is  
648 markedly influenced by three main parameters:

- 649 • Reaction solvent: *tert*-amyl alcohol improves the production of amides **3a**, **5a** and **7a**  
650 while Bmim [PF<sub>6</sub>] favours the formation of amido-esters **3a** and **5a**.
- 651 • [myristic acid **1**]/[amino-alcohol] molar ratio (**r**): under stoichiometric conditions and  
652 in excess of the amino-alcohol (**r** ≤ 1) in *tert*-amyl alcohol and *n*-hexane, the  
653 production of monoacylated amino-alcohols is improved in most cases. On the other  
654 hand, a large excess of myristic acid **1** (**r** > 1) enhances amido-ester production in *n*-  
655 hexane and Bmim [PF<sub>6</sub>].
- 656 • Amino-alcohol structure: chemoselective production of monoacylated amino-alcohols  
657 is markedly affected by the amino-alcohol structure. Using *n*-hexane and in excess of  
658 amino-alcohol, amide production from alaninol **2** and ester production from the longer  
659 chain amino-alcohol 6-amino-1-hexanol **6** were favoured.

660 Together with our previously published work [60, 77], these data give a clearer understanding  
661 of the parameters affecting the Novozym<sup>®</sup> 435-catalyzed acylation of amino-alcohols, which  
662 is important in order to maximize both the yields and the chemoselectivity of these reactions.  
663 In addition, *n*-hexane and *tert*-amyl alcohol used as solvents in this work can potentially be  
664 replaced by other organic solvents with similar properties but considered as more  
665 environmentally friendly, such as for example: cyclopentane and *tert*-butanol.

666 As prospect, it is also conceivable to test the influence of other parameters to modulate lipase  
667 properties, for example the use of various supports to immobilize *Candida antarctica* lipase

668 B. Indeed, the use of various immobilization protocols to produce a biocatalyst starting from a  
669 particular lipase has been shown to significantly affecting its catalytic activity and selectivity,  
670 depending on the solvent used [28-29]. On the other hand, it would also be interesting to test  
671 other lipases as biocatalysts, such as for instance *Rhizomucor miehei* lipase which has already  
672 been used to acylate amino-alcohol substrates [5].

673

674 **Acknowledgments**

675 This study was supported by the Centre National de la Recherche Scientifique and the French  
676 ANR (National Research Agency) through the EXPENANTIO project (CP2P program:  
677 Chimie et Procédés pour le Développement Durable).

678

679 **References**

- 680 [1] M. Bakke, M. Takizawa, T. Sugai, H. Ohta, P. Herold, *J. Org. Chem.* 63 (1998) 6929-  
681 6938.
- 682 [2] H. Garg, N. Francella, K.A. Tony, L.A. Augustine, J.J. Jr Barchi, J. Fantini, A. Puri,  
683 D.R. Mootoo, R. Blumenthal, *Antiviral. Res.* 80 (2008) 54-61.
- 684 [3] M. Fillet, M. Bentires-Alj, V. Deregowski, R. Greimers, J. Gielen, J. Piette, V. Bours,  
685 M.P. Merville, *Biochem. Pharmacol.* 65 (2003) 1633-1642.
- 686 [4] J.F. Molina, *Household and personal care today* 2 (2008) 12-15.
- 687 [5] L. Lassalle, F. Yvergnaux, *FR Patent* 2,855,823 (2004).
- 688 [6] E.L. Soo, A.B. Salleh, M. Basri, R.N.Z.R.A. Rahman, K. Kamaruddin, *J. Biosci.*  
689 *Bioeng.* 95 (2003) 361-367.
- 690 [7] A. Singh, J.D. Van Hamme, O.P. Ward, *Biotech. Adv.* 25 (2007) 99-121.
- 691 [8] T. Maugard, M. Remaud-Simeon, P. Petre, P. Monsan, *Tetrahedron* 53 (1997) 5185-  
692 5194.
- 693 [9] K. Fukunaga, M. Yoshida, F. Nakajima, R. Uematsu, M. Hara, S. Inoue, H. Kondo, S.  
694 I. Nishimura, *Bioorg. Med. Chem. Lett.* 13 (2003) 813-815.
- 695 [10] J. Yin, H. Liu, C. Pidgeon, *Bioorg. Med. Chem. Lett.* 8 (1998) 179-182.
- 696 [11] J. Tao, J. H. Xu, *Curr. Opin. Chem. Biol.* 13 (2009) 43-50.
- 697 [12] B.M. Nestl, B.A. Nebel, B. Hauer, *Curr. Opin. Chem. Biol.* 15 (2011) 187-193.
- 698 [13] F. Björkling, S.E. Godtfredsen, O. Kirk, *Trends Biotechnol.* 9 (1991) 360-363.
- 699 [14] K.E. Jaeger, S. Ransac, B. Dijkstra, C. Colson, M. Van Heuvel, O. Misset, *Federation*  
700 *of European Microbiological Societies Microbiology Reviews* 15 (1994) 29-63.

- 701 [15] D. Sharma, B. Sharma, A.K. Shukla, *Biotechnol.* 10 (2011) 23-40.
- 702 [16] H. Ogino, H. Ishikawa, *J. Biosci. Bioeng.* 91 (2001) 109-116.
- 703 [17] E.P. Hudson, R.K. Eppler, D.S. Clark, *Curr. Opin. Biotechnol.* 16 (2005) 637-643.
- 704 [18] N. Doukyu, H. Ogino, *Biochem. Eng. J.* 48 (2010) 270-282.
- 705 [19] E.M. Anderson, K.M. Larsson, O. Kirk, *Biocatal. Biotransform.* 16 (1998) 181-204.
- 706 [20] R. Sharma, Y. Chisti, U. Chand Banerjee, *Biotechnol. Adv.* 19 (2001) 627-662.
- 707 [21] R.N. Patel, *J. Liposome Res.* 11 (2001) 355-393.
- 708 [22] R. Aravindan, P. Anbumathi, T. Viruthagiri, *Indian Journal of Biotechnology* 6 (2007)
- 709 141-158.
- 710 [23] L. Alberghina, M. Lotti, *Chem. Phys. Lipids* 93 (1998) 1-216.
- 711 [24] T. Ema, *Curr. Org. Chem.* 8 (2004) 1009-1025.
- 712 [25] L. Chebil, C. Humeau, A. Falcimaigne, J-M. Engasser, M. Ghoul, *Process Biochem.*
- 713 41 (2006) 2237-2251.
- 714 [26] P.O. Syren, F. Le Joubioux, Y. Ben Henda, T. Maugard, K. Hult, M. Graber,
- 715 *ChemCatChem* 5 (2013), doi: 10.1002/cctc.201200751.
- 716 [27] A.M. Gumel, M.S.M. Annuar, T. Heidelberg, Y. Chisti, *Process Biochem.* 46 (2011)
- 717 2079-2090.
- 718 [28] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente,
- 719 *Enzyme and Microbial Technol.* 40 (2007) 1451-1463.
- 720 [29] O. Barbosa, C. Ortiz, R. Torres, R. Fernandez-Lafuente, *J. Mol. Catal. B: Enzym.* 71
- 721 (2011), 124-132.
- 722 [30] F. Van Rantwijk, R.M. Lau, R.A. Sheldon, *Trends Biotechnol.* 21 (2003) 131-138.

- 723 [31] P. Lozano, T. De Diego, D. Carrie, M. Vaultier, J.L. Iborra, 23 (2001) 1529–1533.
- 724 [32] B. Fu, P.T. Vasudevan, *Energ. Fuel.* 24 (2010) 4646-4651.
- 725 [33] M. Graber, V. Leonard, Z. Marton, C. Cusatis, S. Lamare, *J. Mol. Catal. B: Enzym.*  
726 52-53 (2008) 121-127.
- 727 [34] V. Leonard, S. Lamare, M.D. Legoy, M. Graber, *J. Mol. Catal. B: Enzym.* 32 (2004)  
728 53-59.
- 729 [35] Z.Q. Duan, W. Du, D.H. Liu, *Bioresource Technol.* 102 (2011) 11048-11050.
- 730 [36] R. Irimescu, K. Kato, *J. Mol. Catal. B: Enzym.* 30 (2004) 189–94.
- 731 [37] R. Madeira Lau, F. Van Rantwijk, K.R. Seddon, R.A. Sheldon, *Org. Lett.* 2 (2000)  
732 4189–4191.
- 733 [38] F.J. Hernández-Fernández, A.P. De Los Ríos, L.J. Lozano-Blanco, C. Godínez, *J.*  
734 *Chem. Technol. Biot.* 85 (2010) 1423-1435.
- 735 [39] P. Lozano, T. De Diego, D. Carrie, M. Vaultier, J.L. Iborra, *J. Mol. Catal. B: Enzym.*  
736 21 (2003) 9–13.
- 737 [40] R.A. Sheldon, R. Madeira Lau, M.J. Sorgedraeger, F. Van Rantwijk, K.R. Seddon,  
738 *Green Chem.* 4 (2002) 147–151.
- 739 [41] C. Roosen, P. Müller, L. Greiner, *L. Appl. Microbiol. Biotechnol.* 81 (2008) 607-614.
- 740 [42] V. Gotor-Fernandez, V. Gotor, *Curr. Org. Chem.* 10 (2006) 1125–1143.
- 741 [43] N. Jain, A. Kumar, S. Chauhan, S.M.S. Chauhan, *Tetrahedron* 61 (2005) 1015–1060.
- 742 [44] C. Pilissão, P. De O. Carvalho, G. Da, M. Nascimento, *J. Brazil. Chem. Soc.* 21 (2010)  
743 973-977.
- 744 [45] M. Fernandez-Perez, C. Otero, *Enzyme Microb. Technol.* 33(2003) 650–660.

- 745 [46] M. Fernandez-Perez, C. Otero, *Enzyme Microb. Technol.* 28 (2001) 527–536.
- 746 [47] V. Gotor, R. Brieva, F. Rebolledo, *J. Chem. Soc., Chem. Commun.* (1988) 957-958.
- 747 [48] T. Furutani, M. Furui, H. Ooshima, J. Kato, *Enzyme Microb. Technol.* 19 (1996) 578–  
748 584.
- 749 [49] L.T. Kanerva, E. Vantinen, T.T. Huuhtanen, M. Dahlqvist, *Acta chem. Scand.* 46  
750 (1992) 1101–1105.
- 751 [50] O. Torre, V. Gotor-Fernandez, V. Gotor, *Tetrahedron Asymmetr.* 17 (2006) 860–866.
- 752 [51] S. Tawaki, A.M. Klibanov, *Biocatal. Biotransform.* 8 (1993) 3-19.
- 753 [52] J. A. Laszloa, M. Jacksona, R. M. Blancob, *J. Mol. Catal. B: Enzym.* 69 (2011) 60–65.
- 754 [53] T. Maugard, M. Remaud-Simeon, D. Petre, P. Monsan, *Tetrahedron* 53 (1997) 7587-  
755 7594.
- 756 [54] R. Fujiiia, Y. Utsunomiyaa, J. Hiratake, A. Sogabeb, K. Sakataa, *BBA-Mol. Cell. Biol.*  
757 L. 1631 (2003) 197–205.
- 758 [55] J. A. Laszloa, M. Jacksona, R. M. Blancob. *J. Mol. Catal. B-Enzym.* 69 (2011) 60–65.
- 759 [56] A. Fersht, *Enzyme Structure and Mechanism*, second ed., W. H. Freeman and  
760 Company, New York, (1985) 98-120.
- 761 [57] S.J. Nara, J.R. Harjani, M.M. Salunkhe, *Tetrahedron Lett.* 43 (2002) 2979.
- 762 [58] K.P. Dhake, Z.S. Qureshi, R.S. Singhal, B.M. Bhanage, *Tetrahedron Letters* 50 (2009)  
763 2811–2814.
- 764 [59] E. Husson, C. Humeau, F. Blanchard, X. Framboisier, I. Marc, I. Chevalot, *J. Mol.*  
765 *Catal. B: Enzym.* 55 (2008) 110-117.

- 766 [60] F. Le Joubioux, O. Achour, N. Bridiau, M. Graber, T. Maugard, J. Mol. Catal. B:  
767 Enzym. 70 (2011) 108-113.
- 768 [61] G. Carrea, G. Ottolina, S. Riva, Trends Biotechnol. 13 (1995) 63-70.
- 769 [62] S. Hazarika, P. Goswami, N.N. Dutta, A.K. Hazarika, Chem. Eng. J. 85 (2002) 61-68.
- 770 [63] W.S. He, J.J. Li, X.X. Pan, Y. Zhou, C.S. Jia, X.M. Zhang, Bioresource Technol.  
771 (2012) 114: 1-5.
- 772 [64] C.C. Akoh, L.N. Yee, J. Mol. Catal. B: Enzym. 4 (1998) 149-153.
- 773 [65] G. De Crescenzo, A. Ducret, M. Trani, R. Lortie, J. Mol. Catal. B: Enzym. 9 (2000)  
774 49-56.
- 775 [66] A. Zaks, A.M. Klivanov, J. Biol. Chem. (1988) 263: 3194.
- 776 [67] S. Hari Krishna, S. Divakar, S.G. Prapulla, N.G. Karanth, J. Biotechnol. 87 (2001)  
777 193-201.
- 778 [68] P.V. Iyer, L. Ananthanarayan, Process Biochem. 43 (2008) 1019-1032.
- 779 [69] M. Liaquat, J. Mol. Catal. B: Enzym. 68 (2011) 59-65.
- 780 [70] P. Hara, U. Hanefeld, L.T. Kanerva, Green Chem. 11 (2009) 250-256.
- 781 [71] Z. Yang, W. Pan, Enzyme Microb. Technol. 37 (2005) 19-28.
- 782 [72] J.L. Kaar, A.M. Jesionowski, J.A. Berberich, R. Moulton, A.J. Russell, J. Am. Chem.  
783 Soc. 125 (2003) 4125-4131.
- 784 [73] G. Ljunger, P. Adlercreutz, B. Mattiasson, Biotechnol. Lett. 16 (1994) 1167-1172.
- 785 [74] P. Ciuffreda, L. Alessandrini, G. Terraneo, E. Santaniello, Tetrahedron Asymmetr.  
786 14 (2003) 3197-3201.
- 787 [75] G.D. Yadav, P.S. Lathi, Enzyme Microb. Tech. 38 (2006) 814-820.
- 788 [76] V. Kasche, Enzyme Microb. Technol. 8 (1986), 4-16.

Mis en forme : Français (France)

789 [77] F. Le Joubioux, Y. Ben Henda, N. Bridiau, O. Achour, M. Graber, T. Maugard, J.  
790 Mol. Catal. B: Enzym. 85-86 (2013) 193-199.

791

