Selective Synthesis of Unsaturated *N*-Acylethanolamines by Lipase-Catalyzed *N*-Acylation of Ethanolamine with Unsaturated Fatty Acids

Pierluigi Plastina*[,a], Jocelijn Meijerink[a], Jean-Paul Vincken[b], Harry Gruppen[b], Renger Witkamp[a] and Bartolo Gabriele*[,c]

[a]*Division of Human Nutrition, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands*

[b]*Laboratory of Food Chemistry, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands*

[c]*Dipartimento di Scienze Farmaceutiche, Università della Calabria, 87036, Arcavacata di Rende, Cosenza, Italy*

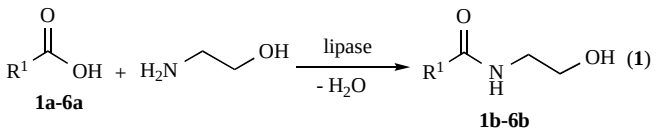
Received March 31, 2009: Revised April 20, 2009: Accepted April 20, 2009

Abstract: The selective synthesis of unsaturated *N*-acylethanolamines **1b-6b** by lipase-catalyzed direct condensation between unsaturated fatty acids **1a-6a** and ethanolamine is reported. Reactions were carried out in hexane at 40 °C, in the presence of *Candida antarctica* Lipase B as the catalyst, to give the corresponding amides **1b-6b** with yields ranging from 80 to 88%.

Keywords: Acylation, enzymatic catalysis, lipase, unsaturated *N*-acylethanolamines, unsaturated fatty acids.

Alkanolamides from fatty acids are an important class of compounds, with a wide range of applications. They are used as detergents, shampoos, cosmetics, lubricants, foam control agents, fungicides, corrosion inhibitors, and water repellents [1]. Moreover, some fatty acid ethanolamides (*N*-acylethanolamines, NAEs) are lipid mediators in animals and in plants [2]. For example, *N*-arachidonoylethanolamine (anandamide) is a well-known endogenous agonist for the cannabinoid receptors [2g,h]. These compounds are usually synthesized by the reaction of fatty acid chlorides or anhydrides with amines [3], or by the direct reaction between fatty acids and amines in the presence of a condensation agent, such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride or 1-cyano-1,2,4-triazole [4].

In this work, we report an alternative approach to the synthesis of unsaturated *N*-acylethanolamines (UNAEs) **1b-6b** based on enzyme-catalyzed *N*-acylation of ethanolamine with unsaturated fatty acids (UFAs) **1a-6a**, according to equation **1**.



1a-6a **1b-6b**

Enzymes have recently acquired a growing importance as catalysts for organic synthesis. In fact, the use of enzymes allows working under mild reaction conditions, leading to more selective and atom-economical processes. In particular, lipases (triacyl glycerol hydrolases, EC 3.1.1.3) are the most commonly used enzymes in organic synthesis due to their high stability in organic media and their ability to work with a great variety of substrates. Among the various lipases that have been reported to catalyze the reaction of amines with carboxylic acids [5], *Candida antarctica* lipase B proved to be the most effective catalyst for the synthesis of alkanolamides in organic solvents [6], in ionic liquids [7] or under solvent-free conditions [8]. It has also been reported that the chain length of the acyl donor affects the reaction rate [5c]. In particular, the C-14 alkyl chain resulted the optimal chain length for lipase QL, the reaction rate in the case of both shorter and longer chain fatty acids being lower. So far, lipase-catalyzed *N*-acylation of different amino alcohols has been carried out using short or medium chain fatty acids. Only little or no attention has been paid to the use of unsaturated fatty acids, especially the poly-unsaturated ones [5e, 6e].

Our method consists of the direct condensation reaction between ethanolamine and UFAs (UFA:ethanolamine molar ratio = 1:1), carried out at 40 °C in hexane as the solvent, in the presence of Novozym®435 (consisting of immobilized *Candida antarctica* Lipase B) as the catalyst. Typical results are shown in Table **1**. As can be seen, the methodology could be successfully applied to various UFAs **1a-6a**, with different unsaturation patterns and chain lengths. High yields of the corresponding UNAEs **1b-6b** were consistently obtained (80-88% entries 1-6). The reaction was completely selective towards the formation of UNAEs, as no formation of *O*-acylated products was observed [9]. Hexane was the solvent of choice for this reaction. In this solvent, all the fatty acids tested were easily soluble, and the system remained homogeneous even after the addition of ethanolamine. On the contrary, precipitation of the ammonium salt occurred when ethanolamine was added to a solution of the fatty acid in a more polar solvent, such as acetonitrile. Accordingly, less satisfactory results were obtained in this solvent (entry 7, to be compared with entry 6).

In conclusion, we have reported a simple and convenient approach for the selective synthesis of unsaturated *N*-

*Address correspondence to these authors at the Dipartimento di Scienze Farmaceutiche, Università della Calabria, 87036, Arcavacata di Rende, Cosenza, Italy; Fax: +39 984 49 20 44;
E-mail: p.plastina@unical.it; b.gabriele@unical.it

1570-1786/09 $55.00+.00

Table 1. Synthesis of Unsaturated *N*-Acylethanolamines (UNAEs) 1b-6b by Lipase-Catalyzed *N*-Acylation of Ethanolamine with Unsaturated Fatty Acids (UFAs) 1a-6a[a]

Entry	UFA[b]	t (h)	Conv (%)[c]	UNAE[d]	Yield (%)[e]
1	Oleic acid (**1a**)	6	90	**1b**	88
2	Linoleic acid (**2a**)	6	89	**2b**	85
3	CLA (**3a**)[f]	6	89	**3b**	80
4	Linolenic acid (**4a**)	6	90	**4b**	84
5	EPA (**5a**)[g]	15	91	**5b**	80
6	DHA (**6a**)[h]	15	91	**6b**	83
7[i]	DHA (**6a**)[h]	15	32	**6b**	20[c]

[a]Unless otherwise noted, all reactions were carried out in hexane (0.2 mmol UFA in 1 mL hexane) at 40 °C, in the presence of 50 mg of immobilized *Candida Antarctica* Lipase B. [b]UFA = Unsaturated Fatty Acid. [c]Determined by HPLC. [d]UNAE = Unsaturated *N*-acylethanolamine. [e]Unless otherwise noted, all yields are isolated. [f]According to the manufacturer, the conjugated linoleic acid (CLA) used was a complex mixture of octadecadienoic acids, namely: ~ 50% (9*E*,11*Z*) + (9*Z*,11*E*), ~ 40% (10*E*,12*Z*), ~ 10% (10*Z*,12*Z*). [g]EPA = *cis*-5,8,11,14,17-eicosapentaenoic acid. [h]DHA = *cis*-4,7,10,13,16,19-docosahexaenoic acid. [i]The reaction was carried out in acetonitrile.

acylethanolamines (UNAEs) **1b-6b**, by lipase-catalyzed *N*-acylation of ethanolamine with unsaturated fatty acids (UFAs) **1a-6a**. Formation of UNAEs occurs under mild conditions (40 °C) and with high yields (80-88%).

EXPERIMENTAL SECTION

General

Ethanolamine and UFAs **1a-6a** were purchased from Sigma-Aldrich. Novozym®435 was supplied by Novozymes A/S. HPLC grade acetonitrile (Sigma-Aldrich) was used for HPLC analysis and preparative-HPLC experiments. Water for HPLC was purified with a Milli-Q system (Millipore). Acetic acid and hexane were purchased from Merck.

Melting points were determined with a Reichert Thermovar melting point apparatus and are uncorrected. Elemental analyses were carried out with a Carlo Erba Elemental Analyzer Mod. 1106. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C on a Bruker AC 300 spectrometer in $CDCl_3$ solutions at 300 MHz and 75 MHz, respectively, with Me_4Si as internal standard. Chemical shifts (δ) and coupling constants (*J*) are given in ppm and in Hz, respectively. IR spectra were taken with a BIORAD FTS6000 spectrometer. Mass spectra were taken on a linear quadrupole ion trap mass spectrometer (LCQ Deca XP MAX, Thermo Fisher Scientific) equipped with an electrospray ionization source interface (positive ion mode). The operating parameters were as follows: the heated capillary temperature was set at 250 °C, the vaporizer temperature at 250 °C, the auxiliary gas at 2 arbitrary units, and the sheath gas at 40 psi. For MS/MS analysis, helium (He) was used in the collision cell; the collision energy was set at 25 eV.

All reaction mixtures were analyzed by HPLC using a Thermo Separation Spectra System P4000, with an XTerra RP18 column (3.5 μm, 4.6 x 150 mm, Waters) using: (A) MQ water + 0.1% acetic acid and (B) acetonitrile + 0.1% acetic acid as mobile phases. The optimized gradient elution program was as follows: 0-5 min: 65% B isocratic; 5-20 min: 65-80% B; 20-35 min: 80-88% B; 35-37 min: 88% B isocratic; 37-38 min: 65% B; The column was run at room temperature at a flow rate of 1.0 mL/min. UV detection was set at 205 nm. UNAEs were purified by preparative HPLC, using a Water Binary Gradient Module, equipped with a fraction collector, and using an XTerra Prep RP18OBD column (5 μm, 19 x 150 mm, Waters) with the gradient elution program described before.

Acylation Procedure

The appropriate unsaturated fatty acid (0.2 mmol) and ethanolamine (12.2 mg, 0.2 mmol) were diluted with hexane (1.0 mL) in a stoppered glass bottle. The resulting mixture was placed in a thermoconstant orbital shaker and held at 40 °C for 5 minutes. Novozym®435 (50 mg) was then added, and the mixture shaken at 200 r.p.m. for the required time (see Table **1**). After cooling, the solvent was evaporated under reduced pressure, and the products purified by preparative HPLC as described above. The product yields, obtained in each experiment, are reported in Table **1**.

Characterization of Products

(Z)-Octadec-9-enoic acid (2-hydroxyethyl)amide (1b)

Colorless solid, mp 60-61 °C (lit. [4b]: 60-61 °C). IR (KBr) 3297 (s), 2919 (m), 2850 (w), 1643 (s), 1564 (m) cm^{-1}; ^{1}H NMR δ 5.95 (s, br, 1 H, NH), 5.37-5.32 (m, 2 H, HC=CH), 3.73 (t, *J* = 5.0, 2 H, C*H*$_2$OH), 3.46-3.39 (m, 2 H, C*H*$_2$NH), 2.36 (s, br, 1 H, OH), 2.21 (t, *J* = 7.7, 2 H, CH$_2$C=O), 2.06-1.96 (m, 4 H, C*H*$_2$CH=CHC*H*$_2$), 1.68-1.58 (m, 2 H, C*H*$_2$CH$_2$C=O), 1.38-1.20 [m, 20 H, CH$_3$(C*H*$_2$)$_6$ + =CHCH$_2$(C*H*$_2$)$_4$], 0.88 (t, *J* = 6.9, 3 H, CH$_3$); ^{13}C NMR δ 174.4, 130.1, 129.8, 62.7, 42.6, 36.8, 32.0, 29.84, 29.77, 29.6, 29.38, 29.33, 29.29, 29.19, 27.32, 27.25, 25.8, 22.7, 14.1; MS *m/z* 326.34 [(M+H)$^+$, 65], 308.30 (25), 265.10 (16), 223.06 (11), 198.07 (100), 155.16 (14). Anal. Calcd for $C_{20}H_{39}NO_2$: C, 73.79; H, 12.08; N, 4.30. Found C, 73.95; H, 12.05; N, 4.29.

(Z,Z)-Octadeca-9,12-dienoic acid (2-hydroxyethyl)amide (2b)

Colorless solid, mp 38-40 °C (lit. [3d]: 38-39.5 °C). IR (KBr) 3297 (s), 2917 (m), 2848 (w), 1643 (s), 1559 (m) cm^{-1}; ^{1}H NMR δ 6.27 (s, br, 1 H, NH), 5.44-5.27 (m, 4 H, 2 HC=CH), 3.70 (t, *J* = 5.0, 2 H, C*H*$_2$OH), 3.44-3.36 (m, 2 H,

CH_2NH), 2.77 (t, *J* = 5.7, 2 H, HC=CHCH_2CH=CH), 2.20 (t, *J* = 7.7, 2 H, CH_2C=O), 2.09-2.00 (m, 4 H, CH_2CH=CHCH_2CH=CHCH_2), 1.69-1.57 (m, 2 H, C$H_2$$CH_2$C=O), 1.42-1.22 [m, 14 H, CH_3(CH_2)$_3$ + =CHCH_2(CH_2)$_4$], 0.89 (t, *J* = 6.8, 3 H, CH_3). (Note: the OH signal was too broad to be detected); ^{13}C NMR δ 174.5, 130.3, 130.1, 128.2, 128.0, 62.4, 42.5, 36.7, 31.6, 29.7, 29.4, 29.3, 29.2, 27.3, 25.8, 25.7, 22.6, 14.0; MS *m/z* 324.34 [(M+H)$^+$, 71], 306.30 (22), 263.20 (16), 240.06 (11), 202.07 (100), 145.16 (14). Anal. Calcd for $C_{20}H_{37}NO_2$: C, 74.25; H, 11.53; N, 4.33. Found C, 74.42; H, 11.51; N, 4.30.

Conjugated N-(2-Hydroxyethyl)linoleamide (3b) (mixture of isomers)

Colorless solid, mp 49-52 °C. IR (KBr) 3298 (s), 2917 (m), 2848 (w), 1647 (s), 1551 (m) cm^{-1}; ^{1}H NMR δ 6.37-6.24 (m, 1 H, =CH), 6.04-5.89 (m, 2 H, =CH + NH), 5.72-5.58 (m, 1 H, =CH), 5.36-5.24 (m, 1 H, =CH), 3.74 (t, *J* = 5.0, 2 H, CH_2OH), 3.47-3.39 (m, 2 H, CH_2NH), 2.77 (s, br, 1 H, OH), 2.26-2.05 (m, 6 H, CH_2C=O + CH_2CH=CHCH=CHCH_2), 1.72-1.58 (m, 2 H, C$H_2$$CH_2$C=O), 1.50-1.30 (m, 16 H), 0.94-0.86 (m, 3 H, CH_3); MS *m/z* 324.36 [(M+H)$^+$, 88], 307.22 (44), 263.28 (9), 245.12 (52), 202.11 (100), 144.99 (27). Anal. Calcd for $C_{20}H_{37}NO_2$: C, 74.25; H, 11.53; N, 4.33. Found C, 74.31; H, 11.51; N, 4.32.

(Z,Z,Z)-Octadeca-9,12,15-trienoic acid (2-hydroxyethyl) amide (4b)

Colorless oil. IR (film) 3296 (s), 2918 (m), 2849 (s), 1643 (s), 1556 (m) cm^{-1}; ^{1}H NMR δ 6.12 (s, br, 1 H, NH), 5.45-5.27 (m, 6 H, 3 HC=CH), 3.71 (t, *J* = 4.9, 2 H, CH_2OH), 3.46-3.38 (m, 2 H, CH_2NH), 2.87-2.75 (m, 4 H, HC=CHCH_2CH=CHCH_2CH=CH), 2.20 (t, *J* = 7.6, 2 H, CH_2C=O), 2.14-2.01 (m, 4 H, CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2), 1.70-1.58 (m, 2 H, C$H_2$$CH_2$C=O), 1.40-1.25 [m, 8 H, =CHCH_2(CH_2)$_4$], 0.98 (t, *J* = 7.5, 3 H, CH_3). (Note: the OH signal was too broad to be detected); ^{13}C NMR δ 174.5, 132.1, 130.3, 128.41, 128.36, 127.8, 127.2, 62.5, 42.5, 36.7, 29.7, 29.3, 29.2, 27.3, 25.8, 25.7, 20.6, 14.3; MS *m/z* 322.30 [(M+H)$^+$, 100], 303.43 (65), 265.96 (42), 250.95 (35), 210.07 (15), 148.08 (11). Anal. Calcd for $C_{20}H_{35}O_2$: C, 74.72; H, 10.97; N, 4.36. Found C, 74.85; H, 10.94; N, 4.35.

(Z,Z,Z,Z,Z)-Eicosa-5,8,11,14,17-pentaenoic acid (2-hydroxyethyl)amide (5b)

Colorless oil. IR (film) 3297 (s), 2918 (m), 2849 (w), 1643 (s), 1556 (m) cm^{-1}; ^{1}H NMR δ 6.04 (s, br, 1 H, NH), 5.45-5.28 (m, 10 H, 5 HC=CH), 3.71 (t, *J* = 4.9, 2 H, CH_2OH), 3.45-3.37 (m, 2 H, CH_2NH), 2.88-2.77 (m, 8 H, 4 CH=CHCH_2CH=CH), 2.22 (t, *J* = 7.6, 2 H, CH_2C=O), 2.16-2.02 (m, 2 H, CH=CHC$H_2$$CH_2$$CH_2$C=O), 1.78-1.66 (m, 2 H, CH=CHC$H_2$$CH_3$), 1.34-1.23 (m, 2 H, C$H_2$$CH_2$C=O), 0.98 (t, *J* = 7.5, 3 H, CH_3). (Note: the OH signal was too broad to be detected); ^{13}C NMR δ 174.2, 132.1, 129.1, 128.9, 128.7, 128.4, 128.3, 128.2, 128.0, 127.1, 62.5, 42.5, 36.0, 31.6, 26.7, 25.7, 25.6, 25.5, 20.6, 14.3; MS *m/z* 346.13 [(M+H)$^+$, 100], 328.13 (84), 318.18 (30), 309.14 (27), 287.74 (28), 240.98 (14), 225.84 (9), 194.07 (9). Anal. Calcd for $C_{22}H_{35}O_2$: C, 76.47; H, 10.21; N, 4.05. Found C, 76.58; H, 10.17; N, 4.03.

(Z,Z,Z,Z,Z,Z)-Docosa-4,7,10,13,16,19-hexaenoic acid (2-hydroxyethyl)amide (6b)

Colorless oil. IR (film) 3289 (s), 2919 (m), 2847 (w), 1647 (s), 1551 (m) cm^{-1}; ^{1}H NMR δ 6.27 (s, br, 1 H, NH), 5.48-5.28 (m, 12 H, 6 HC=CH), 3.70 (t, *J* = 5.0, 2 H, CH_2OH), 3.44-3.36 (m, 2 H, CH_2NH), 2.91-2.77 (m, 10 H, 5 CH=CHCH_2CH=CH), 2.40 (t, *J* = 6.6, 2 H, CH_2C=O), 2.30-2.23 (m, 2 H, C$H_2$$CH_2$C=O), 2.14-2.01 (m, 2 H, CH=CHC$H_2$$CH_3$), 0.97 (t, *J* = 7.6, 3 H, CH_3). (Note: the OH signal was too broad to be detected); ^{13}C NMR δ 173.7, 132.1, 129.6, 128.7, 128.5, 128.4, 128.2, 128.1, 128.0, 127.2, 62.3, 42.5, 36.4, 29.8, 25.8, 25.72, 25.66, 23.5, 20.6, 14.3; MS *m/z* 372.20 [(M+H)$^+$, 100], 354.00 (49), 344.14 (47), 331.78 (11), 261.27 (18), 168.02 (14). Anal. Calcd for $C_{24}H_{37}O_2$: C, 77.58; H, 10.04; N, 3.77. Found C, 77.65; H, 10.01; N, 3.75.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. Henk Schols for his generous gift of Novozym®435, Ing. Mark Sanders for his professional technical support, and Fondazione Carical for a post-doctoral grant for P.P.

REFERENCES AND NOTES

[1] (a) Johansson I. *Amides, Fatty Acid. Kirk-Othmer Encyclopedia of Chemical Technology*. John Wiley & Sons: New York, **2003**; (b) Reck, R.A. In *Fatty Acids in Industry*; Johnson, R.W.; Fritz, E., Eds.; Dekker: New York, **1989**; pp. 177-199.

[2] (a) Kilaru, A.; Blancaflor, E.B.; Venables, B.J.; Tripathy, S.; Mysore, K.S.; Chapman, K.D. *Chem. Biodivers.*, **2007**, *4*, 1933-1955; (b) Okamoto, Y.; Wang, J.; Morishita, J.; Ueda N. *Chem. Biodivers.*, **2007**, *4*, 1842-1857; (c) Re, G.; Barbero, R.; Miolo, A.; Di Marzo V. *Vet. J.*, **2007**, *173*, 21-30; (d) Thabuis, C.; Tissot-Favre, D.; Bezelgues, J.-B.; Martin, J.-C.; Cruz-Hernandez, C.; Dionisi, F.; Destailla F. *Lipids*, **2008**, *43*, 887-894; (e) Terrazzino, S.; Berto, F.; Dalle Carbonare, M.; Fabris, M.; Guiotto, A.; Bernardini, D.; Leon, A. *FASEB J.*, **2004**, *18*, 1580-1582; (f) Dalle Carbonare, M.; Del Giudice, E.; Stecca, A.; Colavito, D.; Fabris, M.; D'Arrigo, A.; Bernardini, D.; Dam, M.; Leon, A. *J. Neuroendocrinol.*, **2008**, *20*, 26-34; (g) Devane, W.A.; Hanus, L.; Breuer, A.; Pertwee, R.G.; Stevenson, L.A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science*, **1992**, *258*, 1946-1949; (h) Matias, I.; Di Marzo V. *Trends Endocrinol. Metab.*, **2007**, *18*, 27-37.

[3] (a) Astarita, G.; Di Giacomo, B.; Gaetani, S.; Oveisi, F.; Compton, T.R.; Rivara, S.; Tarzia, G.; Mor, M.; Piomelli, D. *J. Pharmacol. Exp. Ther.*, **2006**, *318*, 563-570; (b) Cravatt, B.F.; Lerner, R.A.; Boger, D.L. *J. Am. Chem. Soc.*, **1996**, *118*, 580-590; (c) Giuffrida, A.; Rodriguez de Fonseca, F.; Nava, F.; Loubet-Lescoulié, P.; Piomelli, D. *Eur. J. Pharmacol.*, **2000**, *408*, 161-168; (d) Lin, S.; Khanolkar, A.D.; Fan, P.; Goutopoulos, A.; Qin, C.; Papahadjis, D.; Makriyannis, A. *J. Med. Chem.*, **1998**, *41*, 5353-5361; (e) Arsenov, D.V.; Babitskaya, S.V.; Vashkevich, I.I.; Golubeva, M.B.; Kisel', M.A.; Kuz'mitskii, B.B.; Mashkovich, A.E.; Slabkevich, N.M.; Strel'chenok, O.A. *Pharm. Chem. J.*, **2002**, *36*, 474-477.

[4] (a) Williams, J.; Wood, J.A.; Pandarinathan, L.; Karanian, D.A.; Bahr, B.A.; Vouros, P.; Makriyannis, A. *Anal. Chem.*, **2007**, *79*, 5582-5593; (b) Park, Y.-S.; Jang, H.-J.; Lee, K.-H.; Hahn, T.-R.; Paik, Y.-S.; *J. Agric. Food Chem.*, **2006**, *54*, 1238-1242; (c) Klenova, N.A.; Belousova, Z.P. *Pharm. Chem. J.*, **2002**, *36*, 423-424.

[5] (a) Rantwijk, F.; Hacking, M.A.P.J.; Sheldon, R.A. *Monatsh. Chem.*, **2000**, *131*, 549-569; (b) Sharma, J.; Batovska, D.; Kuwamori, Y.; Asano, Y. *J. Biosci. Bioeng.*, **2005**, *100*, 662-666; (c) Furutani, T.; Furui, M.; Ooshima, H.; Kato, J. *Enzyme Microb. Technol.*, **1996**, *19*, 578-584; (d) Kanerva, L.T.; Kosonen, M.;

Vänttinen, E.; Huuhtanen, T.T.; Dahlqvist, M. *Acta Chem. Scand*., **1992**, *46*, 1101-1105; (e) Maugard, T.; Remaud-Simeon, M.; Petre, D.; Monsan, P. *Tetrahedron*, **1997**, *53*, 7587-7594.

[6] (a) Fernandez-Perez, M.; Otero, C. *Enzyme Microb. Technol*., **2001**, *28*, 527-536; (b) Fernandez-Perez, M.; Otero, C. *Enzyme Microb. Technol*., **2003**, *33*, 650-660; (c) Gotor-Fernandez, V.; Busto, E.; Gotor, V. *Adv. Synth. Catal*., **2006**, *348*, 797-812; (d) Levinson, W.E.; Kuo, T.M.; Kurtzman, C.P. *Enzyme Microb. Technol.*, **2005**, *37*, 126-130; (e) Liu, K.J.; Nag, A.; Shaw, J.F. *J. Agric. Food Chem*., **2001**, *49*, 5761-5764; (f) Levinson, W.E.; Kuo, T.M.; Weisleder, D. *J Am. Oil. Chem. Soc*., **2005**, *82*, 501-504; (g) Khare, S.K.; Kumar, A.; Kuo, T.M. *Bioresour. Technol.*, **2009**, *100*, 1482-1485.

[7] (a) Irimescu, R.; Kato, K. *J. Mol. Catal. B Enzym*., **2004**, *30*, 189-194; (b) Irimescu, R.; Kato, K. *Tetrahedron Lett*., **2004**, *45*, 523-525.

[8] (a) Prasad, A.K.; Husain, M.; Singh, B.K.; Gupta, R.K.; Manchanda, V.K.; Olsen, C.E.; Parmar, V.S. *Tetrahedron Lett.*, **2005**, *46*, 4511-4514; (b) Tufvesson, P.; Annerling, A.; Hatti-Kaul, R.; Adlercreutz, D. *Biotechnol. Bioeng*., **2007**, *97*, 447-453; (c) Couturier, L.; Taupin, D.; Yvergnaux, F. *J. Mol. Catal. B Enzym*., **2009**, *56*, 29-33.

[9] Previous studies have reported that in the enzyme-catalyzed acylation reaction of amino alcohols, with general formula $HO(CH_2)_nNH_2$, *O*-acylated products could be isolated only when n≥3, while *O*-acylation followed by spontaneous intramolecular *O*→*N* acyl migration was observed for n=2 (references [5c,d]).