# Chemoselective Hydrolysis of Esters by Fungal Lipase†

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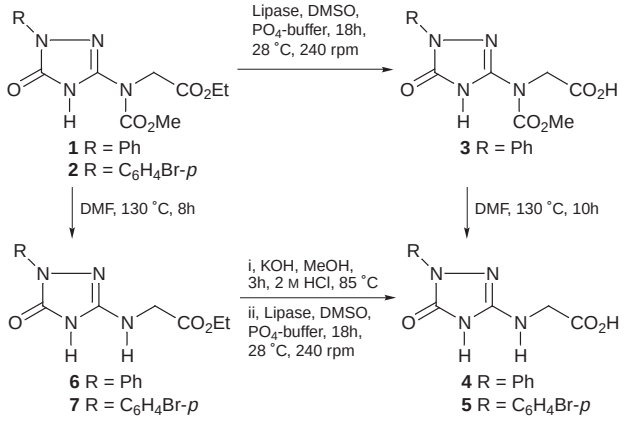
Lipase, isolated from *Aspergillus niger*, exhibits chemoselectivity towards hydrolysis of fatty acid esters and does not hydrolyse carbamates; this has been demonstrated with substrates such as 5 (*N*-methoxycarbonyl-*N*-ethoxycarbonylmethylamino)-2-aryl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones.

Enzymes as effective reagents in organic synthesis are well documented.[1] One well studied enzyme mediated reaction is the hydrolysis of esters by lipase.[2] These studies have revealed the usefulness of this class of enzyme and helped to market bacterial and fungal lipase. Despite this success, exploration of new lipases continues, to carry out chemo- and stereo-selective reactions.

Demonstration of chemoselective hydrolysis of an ester by a lipase demands critical choice of substrates. For example, a substrate possessing both carbamate and fatty acid ester groups is interesting because of the significant difference in the chemical reactivities of these groups.[3] It is well known that even controlled base or acid catalysed hydrolysis will invariably lead to the hydrolysis of carbamates with simultaneous decarboxylation.[4] If however, lipases hydrolyse only the fatty acid ester without any effect on the carbamate, their chemoselectivity and usefulness would be evident. No such study appears to have been made until now and the present work describes this possibility and presents our results.




**Scheme 1**

Reaction of **1**[5] with a newly isolated lipase in phosphate buffer (pH 7, 10 mM) gave compound **3** in which the *N*-methoxycarbonyl ($CO_2Me$) group remained unchanged (Scheme 1). The sensitivity of compounds **1** and **2** to very mild basic conditions is evident from the fact that they hydrolyse and decarboxylate even in refluxing DMF to yield products **6** and **7**, respectively. Compound **3** in refluxing DMF yields the *N*-substituted glycine **4**. Compounds **4** and **5** are also obtained by the alkaline hydrolysis of compounds **6** and **7**, respectively. The selective behaviour observed in the above experiments prompted us to hydrolyse compound **6** by lipase in a similar fashion, when reaction gave the desired free acid **4**. It is possible that the carbamate group is hydrogen bonded and may effect the hydrolysis *via* the lipase; however, our experiments with the NMe derivative (Me instead of $CO_2Me$ in **1**) gave the free acid while in another experiment, the derivative with Me instead of $CH_2CO_2Et$ in **1** did not show hydrolysis of the carbamate group. This confirms that the selectivity of the reagent depends on the nature of the ester which is to be hydrolysed. The present study also highlights the hydrolysing capability of lipase derived from the fungal isolate, as well as providing easy access to the synthesis of *N*-substituted glycine.

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## Experimental

Melting points were determined in a hot-stage apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer 881 spectrophotometer. $^1H$ NMR spectra were measured in Bruker 400 FT NMR and Bruker Avance RX 300 spectrometers. EI mass spectra were recorded on a JEOL-D-300 spectrometer. Elemental analysis was carried out on a Carlo-Erba EA1108 elemental analyser. Reactions were monitored by TLC on silica gel 60 (E. Merck) of 0.25 mm thickness. Column chromatography was carried out on Merck silica gel (70–230 mesh).

*Microorganism*.—The fungus used in the present investigation was isolated from contaminated mango pickle, employing agar plates containing triglycerides. Lipase-catalysed hydrolysis was assessed from the precipitates of the salts of produced fatty acids. The isolate was recognized as *Aspergillus niger*[7] (F24).

*Culture Condition*.—For the production of extracellular lipase from strain F24,[7] a medium containing soybean meal 1% (w/v), $NaNO_3$ 1% (w/v), $Na_2CO_3$ 0.25% (w/v), $(NH_4)_2HPO_4$ 1% (w/v) and olive oil 0.25% (v/v) in distilled water was used. The pH was initially adjusted to 7.5. The cells were grown in 400 ml of the medium in a 1 l Erlenmeyer flask with reciprocal shaking (240 rpm) for 72 h at 30 °C. After the production period, the culture broth (4000 ml) was filtered through muslin cloth. The water soluble proteins contained in the cell free culture broth were precipitated using acetone. A two stage precipitation was carried out. Initially cold acetone was added in an ice-bath to give a concentration of 35% (v/v) and after 3 h the supernatant was collected by centrifugation at 10000 rpm at 4 °C for 30 min. To the resulting supernatant, cold acetone was added in an ice bath to give a final concentration of 60% (v/v). After 3 h the precipitate was collected by centrifugation at 10000 rpm at 4 °C for 30 min. The precipitate was then vacuum dried and dissolved in 10 mM sodium phosphate buffer (pH 7) and dialyzed overnight against the same buffer and vacuum dried. In order to achieve an economic use of the enzyme in organic biotransformation(s), the acetone precipitated powder of the crude enzyme preparation was used in the present studies. The protein content of the acetone powder (18.7 mg $g^{-1}$) was assayed by the method of Lowery *et al.*[9] with bovine serum albumin (Sigma) as a standard protein. The exolipase was assayed using *p*-nitrophenylpalmitate (PNP) as the substrate. One enzyme unit is defined as 1 nmole of *p*-nitrophenol enzymatically released from the substrate (ml$^{-1}$ min$^{-1}$) under the conditions described by Winkler and Stuckmann.[10]

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5-(N-*Methoxycarbonyl*-N-*carboxymethylamino*)-2-*aryl*-2,4-*dihydro*--3H-1,2,4-*triazol*-3-*one* (**3**).—*Procedure*: to a solution of compound **1** (0.005 mol) in DMSO (0.25 ml) and 10 mM phosphate buffer (pH 7) (25 ml) was added lipase (50 mg/5000 units towards *p*-nitrophenyl-palmitate) and the mixture incubated for 18 h at 28 °C with shaking at 240 rpm. The reaction mixture was extracted with EtOAc ($5 \times 20$ ml), the organic layer was dried ($Na_2SO_4$) and concentrated *in vacuo* to give an oily residue which was purified by column chromatography. Elution with $CHCl_3$–MeOH (95:5 v/v) yielded **3** (30.82%).

**3**: mp 168 °C; *m/z* 292 ($M^+$); IR (KBr) 1618 ($CO_2H$), 1664 (CO); $\delta_H$ ($CDCl_3$; 300 MHz) 3.78 (s, 3H, OMe), 4.14 (s, 2H, $CH_2$), 7.18 (t, 1H, *J* 7 Hz, ArH), 7.40 (t, 2H, *J* 7 Hz, ArH), 7.89 (d, 2H, *J* 7 Hz, ArH), 10.41 (brs, 1H, NH).

5-(N-*Ethoxycarbonylmethylamino*)-2-*aryl*-2,4-*dihydro*-3H-1,2,4-*triazol*-3-*ones* **6**,**7**.—Compounds **6** and **7** were prepared by the method reported earlier[5] in 64.28 and 56.75% yields, respectively.

5-(N-*Carboxymethylamino*)-2-*aryl*-2,4-*dihydro*-3H-1,2,4-*triazol*-3-*ones* **4**,**5**.—*Procedure A*: Compound **6** or **7** (0.005 mol) were refluxed in a solution of MeOH (10 ml) and KOH (0.001 mol) for 3 h at 85 °C, then the solvent was removed *in vacuo* and the residual solid acidified with 2 M HCl (2–3 ml) to yield **4** (47.86%) and **5** (43.58%).

*Procedure B*: compound **6** (0.00025 mol, 0.065 mg) was dissolved in DMSO (0.25 ml) and 10 mM phosphate buffer (pH 7) (25 ml) and to this lipase (50 mg/5000 units towards *p*-nitrophenylpalmitate) was added and the mixture incubated for 18 h at 28 °C at 240 rpm. After usual workup, an oily residue was obtained which was purified by column chromatography. Elution with $CHCl_3$–MeOH (92:8; v/v%) yielded **4** (0.038 g 65.56%).

**4**: mp 215 °C; *m/z* 234: IR (KBr) 1638 ($CO_2H$), 1660(CO), 3376(NH); $\delta_H$($CDCl_3$, 400 MHz) 3.95 (d, 2H, *J* 8 Hz, $CH_2$), 5.80 (brs, 1H, NH), 7.14 (t, 1H, *J* 7 Hz, ArH), 7.91 (t, 1H, *J* 7 Hz, ArH), 10.56 (brs, 1H, NH). Analysis. Calc. for $C_{10}H_{10}N_4O_3$: C, 51.28; H, 4.29; N, 23.92. Found: C, 50.77; H, 4.27; N, 23.17%.

**5**: mp 198 °C (decomp); *m/z* 313: IR (KBr) 1628 ($CO_2H$), 1684 (CO), 3324 (NH); $\delta_H$($CDCl_3$, 400 MHz) 4.84 (d, 2H, *J* 8 Hz, $CH_2$), 5.71 (brs, 1H, NH), 7.38 (d, 2H, *J* 7 Hz, ArH), 7.64 (d, 2H, *J* 8 Hz, ArH), 9.81 (brs, 1H, NH), Analysis. Calc. for $C_{10}H_9BrN_4O_3$ : C, 38.36; H, 2.89; N, 17.89. Found: C, 38.81; H, 3.01; N, 17.47%.

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