Synthesis of Pyrimidine Derivatives of Amino Acids using Pig Liver Esterase and Pancreas Lipase

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The parent methyl ester of the N-1 substituted 5-chloropyrimidinone is hydrolysed by pig liver esterase to the corresponding carboxylic acid. The acid chloride was made with PCl_5 in toluene, and coupling with benzyl and methyl esters of selected L-amino acids to the corresponding amides was done in dichloromethane in the presence of triethylamine. The benzyl and methyl ester protecting groups were hydrolysed with pancreas lipase or esterase in a pH-stat to yield the corresponding carboxylic acids.

Certain 5-halo-2(1*H*)-pyrimidinones substituted at N-1 with hydrophobic moieties reversibly arrest eukaryotic cells in metaphase.[1] Acidic or basic functionalities in the N-1 substituent lead to loss of metaphase-arresting activity. Owing to cell kinetic requirements, the compound must have a fair solubility in water. In this paper we describe work aimed at the preparation of amino acid derivatives of N-1 substituted 5-halogeno-2(1*H*)-pyrimidinones in order to improve their water solubility and possibly the membrane-penetrating ability of the metaphase arrestors.

For our preparation of metaphase arresting pyrimidinones, we were interested in the synthesis of amino acid derivatives of N-1 substituted 5-chloro-2(1*H*)-pyrimidinone. The amino acid moiety was selected to achieve better cell membrane penetration to enhance the metaphase-arresting activity. Initially, our synthetic problems was selectively to hydrolyse the methyl ester **1** , in the presence of the acid-labile *O,N*-acetal function. Attempts to do this with base failed and resulted in cleavage of the acetal function and in adduct formation at the electrophilic 4- or 6-position of the halopyrimidinone. We have circumvented this problem by including enzymes in our synthetic scheme. The enzymes available to us were pig liver esterase (EC 3.1.1.1), wheat germ lipase, pancreas lipase (EC 3.1.1.3) commercially available from Sigma Chemical Company and acetyl esterase (EC 3.1.1.6), which was purified from orange peel. All the enzymes were tested for their ability to furnish our target molecules. Only pig liver esterase and pancreas lipase were useful with our substrates. By use of pig liver esterase, the hydrolysis of the methyl ester **1** proceeded smoothly when performed in a pH-stat at pH 8.4 at ambient temperature. This opened a pathway for new derivatives of **1** which seemed interesting to us. The acid **2** was activated by transformation to the acid chloride **3**, with PCl_5 in dichloromethane, and coupled with methyl and benzyl esters of the L-amino acids Phe, Glu and Asp to yield the corresponding amides **4**. Again the task was selectively to remove the blocking groups without cleaving the acetal function. Selective hydrolysis of the benzyl ester functionalities of **4** (Glu diOBz and Asp diOBz) failed with esterase, but was easily accomplished with pancreas lipase at pH 8.4 in a pH-stat at ambient temperature to give the slightly more water soluble target molecules **5**. The methyl esters were again cleaved by esterase.

Pig liver esterase[2] and porcine pancreas lipase[3] have a broad substrate specificity[4] and are cited in the literature for both synthesis and hydrolysis of esters. Pyrimidine derivatives of the kind reported in this paper are not previously cited in the literature as substrates for pig liver esterase and lipase, and are thus new to us.

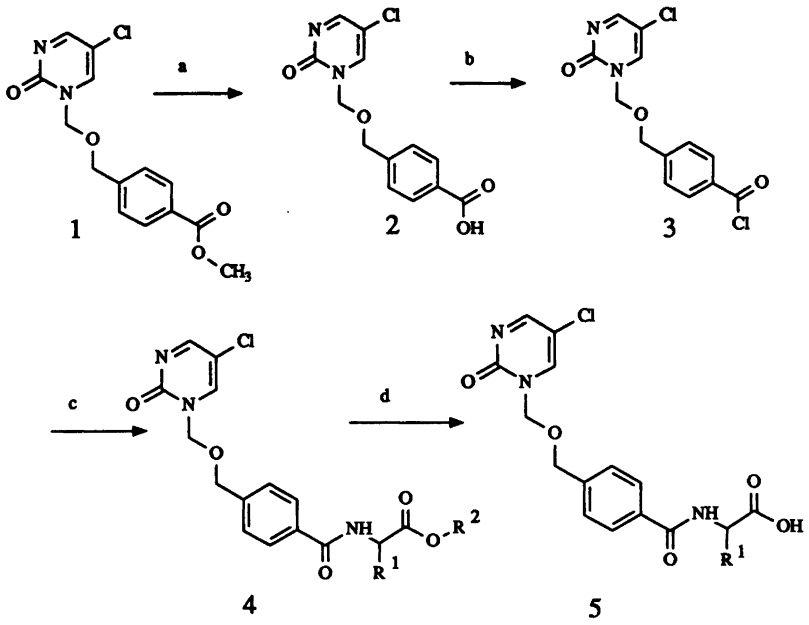
The enzymes have several advantages which can be exploited in synthetic chemistry. Besides being chiral catalysts, they catalyse reactions under mild conditions (pH 5–8) in aquous media containing up to 20% organic modifier. They show regio- and enantio-selectivity and are selective for functional groups, which permits selective deprotection of differently protected functional groups.

In this work ammonium carbonate was selected as the buffer medium, because it is volatile by lyophilization. This leads to a simple work-up of the reaction mixture.

No attempt was made to determine possible racemisation of the amino acid moiety in the final products **5**.

Experimental

NMR spectra were recorded in $CDCl_3$ at 300 MHz (^1H) and at 75 MHz (^{13}C) unless otherwise specified. EI Mass spectra were recorded at 70 eV, and FAB MS spectra were recorded on a Trio-2 (Quadropole), operated in FAB+ mode with thioglycerol and trifluoroacetic acid as the matrix, and



Scheme 1. Conditions: a, esterase, pH 8.4, 37 °C, 20 h; b, PCl_5, toluene, 20 °C, 24 h; c, $X^-H_3N^+CHR^1CO_2R^2$, CH_2Cl_2, 20 °C, 20 min; d, esterase/lipase, pH 8.4, 20 °C.

	X	R^1	R^2	Yield (%)	
				4	**5**
a	Cl	CH_2Ph	CH_3	93	79
b	Cl	$CH_2CO_2CH_3$	CH_3	92	84
c	Tos	$CH_2CO_2CH_2Ph$	CH_2Ph	94	89
d	Tos	CH_2Ph	CH_2Ph	95	90
e	Tos	$CH_2CH_2CO_2CH_2Ph$	CH_2Ph	93	88

8 kV Xe as the ionizing gas. The mass spectra are presented as *m/z* (% rel. int.).

Compounds available by literature methods. 4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzoic acid methyl ester (**1**).[5]

4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzoic acid (**2**). 4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzoic acid methyl ester (840 mg) was added in 100 mg portions over 60 min to a solution (700 ml) of 20 mM ammonium carbonate buffer pH 8.4 of the enzyme (5000 U porcine liver esterase E.C.1.1.1.3, 260 U/mg protein, E-3128 Sigma Comp.) at 37 °C with careful stirring overnight. The reaction mixture was lyophilized and the product extracted with methanol several times. The combined extracts were evaporated to give the title compound. Yield 641 mg (80 %). Anal. $C_{13}H_{11}ClN_2O_4$: C, H. ^{1}H NMR (DMSO-d_6): δ 4.85 (s, 2 H, CH_2O), 5.46 (s, 2 H, CH_2N), 7.53–8.04 (m, 4 H, ArH), 8.66 (d, 1 H, H-6 pyrimidinone), 8.76 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR (DMSO-d_6): δ 70.8 (CH_2O), 79.6 (CH_2N), 109.9 (C-5 pyrimidinone), 127.4, 129.4, 142.4 (Ar), 146.4 (C-6 pyrimidinone), 154.0 (C-2 pyrimidinone), 166.1 (C-4 pyrimidinone), 166.6 (CO_2H). MS (CI): 519 (19), 518 (39), 517 (100), 502 (5), 501 (8), 421 (16), 405 (16), 404 (37), 403 (100), 293 (7), 292 (12), 291 (58), 185 (5), 181 (7), 180 (5), 179 (52), 149 (10), 145 (6), 142 (14), 105 (6), 102 (16), 101 (9), 97 (10).

4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxy)-benzoyl chloride (**3**). To a solution of PCl_5 (400 mg) in anhydrous toluene, was added in one portion at 24 °C 4-(5-chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)-benzoic acid (500 mg) and the reaction was stirred for 24 h. Addition of petroleum ether (100 ml, b.p. 100–140 °C), followed by filtration gave the title compound. Yield: 520 mg (98 %). Anal. $C_{13}H_{10}Cl_2N_2O_3$: C, H. ^{1}H NMR: δ 4.81 (s, 2 H, CH_2O), 5.42 (s, 2 H, CH_2N), 7.75 (d, 4 H, ArH), 7.88 (d, 1 H, H-6 pyrimidinone), 8.15 (m, 2 H, ArH), 8.60 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR: δ 72.0 (CH_2O), 79.0 (CH_2N), 112.0 (C-5 pyrimidinone), 128.0, 131.0, 133.0 (Ar), 143.0 (C-4 pyrimidinone), 144.0 (CCl), 154.0 (C-2 pyrimidinone), 166.0 (C-4 pyrimidinone). IR (KBr): 1740 (COCl), 2500 cm^{-1} (hydrochloride at N1). MS (EI): 279 (5), 277 (16), 249 (11), 247 (31), 219 (18), 185 (28), 183 (79), 155 (20), 153 (62), 146 (32), 144 (100).

General procedure for the preparation of the carboxamides (**4**). To a solution of the amino acid methyl or benzyl ester

hydrochloride or tosylate (0.57 mmol) and triethylamine (116 mg, 1.14 mmol) in 20 ml dry dichloromethane, was added a solution of 4-(5-chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzoyl chloride (**3**, 0.57 mmol) and triethylamine (58 mg, 0.57 mmol) in 5 ml dichloromethane at room temperature. The reaction mixture was allowed to stir for 20 min and then washed with 10 mM brine (4×). The combined water phases were extracted once with dichloromethane. The combined organic phases were dried ($MgSO_4$) and evaporated at 20 °C to give an oily material which was purified by flash chromatography on silica gel 60 using acetonitrile for elution.

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)-benzamido]-3-phenylpropionic acid methyl ester (**4a**). Yield: 304 mg (93 %). ^{1}H NMR: δ 3.20–3.75 (m, 2 H, CH_2Ph), 3.80 (s, 3 H, CH_3O), 4.73 (s, 2 H, CH_2O), 5.00–5.45 (m, 1 H, CH), 5.43 (s, 2 H, CH_2N), 6.58 (d, 1 H, NH), 7.12 (d, 1 H, H-6 pyrimidinone), 7.31–7.75 (m, 9 H, Ar), 7.88 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR: δ 37.9 (CH_2Ph), 52.4 (CH_3O), 53.5 (CH), 72.0 (CH_2O), 79.0 (CH_2N), 110.9 (C-5 pyrimidinone), 127.2, 127.4, 127.8, 128.7, 129.4, 133.9, 135.8, 140.1 (Ar), 143.0 (C-6 pyrimidinone), 154.2 (C-2 pyrimidinone), 166.2 (CONH), 166.3 (C-4 pyrimidinone), 172.0 (CO_2). MS (EI): 457/455 (3/9, *M*), 295 (29), 162 (38), 147 (70), 144 (51), 133 (38), 119 (40), 118 (100).

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]succinic acid dimethyl ester (**4b**). Yield: 230 mg (92 %). ^{1}H NMR: δ 2.95–3.20 (m, 2 H, CH_2CO), 3.74 (s, 3 H, CH_3O side group amino acid), 3.80 (s, 3 H, CH_3O), 4.75 (s, 2 H, CH_2O), 5.13 (m, 1 H, CH), 5.47 (s, 2 H, CH_2N), 7.25 (d, 1 H, H-6 pyrimidinone), 7.40 (d, 2 H, Ar), 7.80 (d×2, 3 H, Ar and H-4 pyrimidinone). ^{13}C NMR: δ 35.9 (CH_2–C_α), 48.8 (CH), 51.9 (CH_3O side group amino acid), 52.8 (CH_3O), 71.9 (CH_2N), 78.9 (CH_2O), 112.0 (C-5 pyrimidinone), 127.4, 127.7, 133.5, 140.1 (Ar), 142.9 (C-6 pyrimidinone), 154.2 (C-2 pyrimidinone), 166.0 (C-4 pyrimidinone), 166.3 (CO, amide), 171.0 (CO side group amino acid), 171.5 (CO–C_α). MS (EI): 437 (0.2, *M*), 376 (3), 350 (2), 294 (30), 278 (31), 247 (30), 236 (12), 234 (16), 144 (100).

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]succinic acid dibenzyl ester (**4c**). Yield: 318 mg (94 %). ^{1}H NMR: δ 3.00–3.20 (m, 2 H, CH_2CO), 4.75 (s, 2 H, CH_2O), 5.06 (d, 2 H, CH_2Ph), 5.08 (m, 1 H, CH), 5.23 (s, 2 H, CH_2Ph), 5.40 (s, 2 H, CH_2N), 7.16 (d, 1 H, H-6 pyrimidinone), 7.26–7.75 (m, 14 H, Ar), 7.80 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR: δ 36.4 (CH_2CO), 49.1 (CH), 66.9 (CH_2Ph), 67.7 (CH_2Ph), 72.1 (CH_2O), 79.5 (CH_2N), 127.5, 127.8, 128.3, 128.4, 128.5, 128.6, 133.7, 135.1, 135.3, 140.2 (Ar), 142.7 (C-6 pyrimidinone), 166.4 (CONH), 170.5 (CO), 170,8 (CO). MS (EI): 446 (2), 392 (3), 144 (14), 135 (12), 118 (12), 108 (18), 107 (17), 91 (100).

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]-3-phenylpropionic acid benzyl ester (**4d**). Yield: 291 mg (95 %). ^{1}H NMR: δ 3.21–3.30 (m, 2 H, CH_2Ph), 4.72 (s, 2 H, CH_2O), 5.10 (m, 1 H, CH), 5.25 (m, 2 H, OCH_2Ph), 5.48 (s, 2 H, CH_2N), 7.00–7.75 (m, 14 H, Ar), 7.85 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR: δ 37.7 (CH_2Ph), 53.4 (CH), 67.3 (OCH_2Ph), 71.9 (CH_2O), 78.9 (CH_2N), 127.0, 127.2, 127.7, 128.4, 128.5, 129.3, 133.8, 134.9, 135.5, 139.9 (Ar), 142.8 (C-6 pyrimidinone), 166.0 (C-4 pyrimidinone), 166.1 (CONH), 171.3 (CO_2). MS (EI): 533/531 (2/4, *M*), 410 (2), 280 (5), 236 (6), 149 (9), 147 (29), 144 (34), 133 (20), 91 (100).

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]pentanedioic acid dibenzyl ester (**4e**). Yield: 324 mg (93 %). ^{1}H NMR: δ 2.10–2.58 (m, 4 H, CH_2 × 2), 4.73 (s, 2 H, CH_2O), 4.85 (m, 1 H, CH), 5.07 (s, 2 H, OCH_2Ph side group amino acid), 5.20 (s, 2 H, OCH_2Ph), 5.38 (s, 2 H, CH_2N), 7.09 (d, 1 H, H-6 pyrimidinone), 7.26–7.40 (m, 14 H, Ar), 7.79 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR: δ 27.0 (CH_2), 30.3 (CH_2CO side group amino acid), 52.3 (CH), 66.5 (CH_2O side group amino acid), 67.3 (CH_2O), 71.9 (CH_2O), 78.9 (CH_2N), 111.5 (C-5 pyrimidinone), 127.3, 127.7, 128.0, 128.2, 128.4, 128.5, 133.5, 135.0, 135.5, 140.0 (Ar), 142.9 (C-6 pyrimidinone), 154.4 (C-2 pyrimidinone), 166.0 (C-4 pyrimidinone), 166.5 (CONH), 171.6 (CO side group amino acid), 172.9 (CO). MS (EI): 336 (7), 247 (4), 220 (5), 147 (14), 146 (15), 144 (47), 135 (24), 133 (16), 91 (100).

General procedure for the preparation of carboxylic acids (**5**). All the hydrolyses were performed at 20 °C with careful stirring at constant pH 8.4 (pH-stat), with 0.1 M NH_3 as the titrant. The carboxamides **4a–e** (200–320 mg, 0.52–0.60 mmol) in 4 ml acetonitrile, were added to a solution of pancreas lipase (100 mg, E.C.3.1.1.3, L-3126 Sigma) in 160 ml 20 mM ammonium carbonate buffer pH 8.4 at room temperature. The hydrolysis began immediately and was allowed to run until the substrate was no longer present as evidenced by TLC. The reaction was stopped by being frozen in liquid nitrogen and lyophilized. The freeze-dried powder was purified on a column of C_8-silica (Lobar Fertig Seulen, Merck) using 40 % acetonitrile for elution.

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]-3-phenylpropionic acid (**5a**). **4a**: 275 mg (0.60 mmol), pancreas lipase, 100 mg. The majority of the substrate was converted after 10 min, but the hydrolysis was allowed to proceed for a further 10 min which was required for complete conversion. Yield: 210 mg (79 %). The product is unstable and had to be purified immediately. ^{1}H NMR (D_2O): δ 3.22 (m, 2 H, CH_2Ph), 4.65 (s, 2 H, CH_2O), 4.83 (m, 1 H, CH), 5.45 (s, 2 H, CH_2N), 7.24–7.63 (m, 9 H, Ar), 8.16 (d, 1 H, H-4 pyrimidinone). MS (FAB$^+$): 444/442 (33/100, *M*+H), 466/464 (2/5, *M*+Na), 412 (30), 300 (40), 282 (40), 253 (30).

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]succinic acid 4-methyl ester (**5b**). **4b**: 230 mg (0.52 mmol), pancreas lipase: 100 mg. Total conversion of substrate after 30 min. Yield: 179 mg (84%). ^{1}H NMR (D_2O–DMSO-d_6): δ 2.86–3.06 (m, 2 H, CH_2–C_α), 3.66 (s, 3 H, CH_3O side group amino acid), 4.77 (s, 2 H, CH_2O), 5.24 (m, 1 H, CH), 5.40 (s, 2 H, CH_2N), 7.38–7.75 (m, 4 H, Ar), 8.13 (d, 1 H, H-6 pyrimidinone), 8.45 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR: δ 37.6 (CH_2 side group amino acid), 52.0 (CH), 54.0 (CH_3O side group amino acid), 73.7 (CH_2O), 82.5 (CH_2N), 114.0 (C-5 pyrimidinone), 129.1, 129.4, 129.7, 134.4, 142.7 (Ar), 148.1 (C-6 pyrimidinone), 157.1 (C-2 pyrimidinone), 168.2 (CO amide), 170.4 (C-4 pyrimidinone), 174.7 (CO ester), 176.0 (CO acid). MS (FAB^+): 426/424 (32/100, *M*+H), 448/446 (10/30, *M*+Na), 394 (30), 282 (40), 264 (40), 126 (90).

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]succinic acid 4-benzyl ester (**5c**). **4c**: 318 mg (0.53 mmol), pig liver esterase: 1.0 ml. This hydrolysis was not effected by lipase. After 60 min the reaction was complete. Yield: 198 mg (89%). ^{1}H NMR (D_2O–DMSO-d_6): δ 2.92–3.10 (m, 2 H, CH_2CO), 4.85 (s, 2 H, CH_2O), 5.10 (s, 2 H, CH_2Ph), 5.34 (m, 1 H, CH), 5.48 (s, 2 H, CH_2N), 7.30–7.65 (m, 9 H, Ar), 8.15 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR: δ 38.7 (CH_2CO), 68.6 (CH_2Ph), 73.9 (CH_2O), 82.6 (CH_2N), 129.0, 129.7, 129.8, 130.1, 130.3, 137.1, 142.4 (Ar), 148.2 (C-6 pyrimidinone), 157.2 (C-2 pyrimidinone), 168.8 (CONH), 174.5 (CO_2). MS (FAB^+): 502/500 (30/100, *M*+H), 524/522 (3/10, *M*+Na), 472 (60), 470 (20), 299 (50), 250 (30), 225 (60).

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]-3-phenylpropionic acid (**5d**). **4d**: 304 mg (0.57 mmol), pancreas lipase: 200 mg. 15 ml acetonitrile were added to enhance the solubility of the substrate. The reaction was stopped after 60 min. Yield: 228 mg (90%). Spectroscopic data identical with **5a**.

2-[4-(5-Chloro-2-oxo-1,2-dihydropyrimidin-1-ylmethoxymethyl)benzamido]pentanedioic acid dibenzyl ester (**5e**). **4e**: 321 mg (0.53 mmol), pig liver esterase: 2.2 ml. This hydrolysis was not effected by lipase. After 9 h, all substrate was consumed. Yield: 200 mg (88%). ^{1}H NMR (CD_3CN): δ 2.00–2.53 (m, 4 H, $CH_2 \times 2$), 4.50 (m, 1 H, CH), 4.73 (s, 2 H, CH_2O), 5.07 (s, 2 H, CH_2Ph side group amino acid), 5.36 (s, 2 H, CH_2N), 7.31–7.81 (m, 9 H, Ar), 8.11 (d, 1 H, H-6 pyrimidinone), 8.53 (d, 1 H, H-4 pyrimidinone). ^{13}C NMR (CD_3CN): δ 27.8 (CH_2), 31.4 (CH_2CO), 54.1 (CH), 67.2 (OCH_2Ph), 72.5 (CH_2O), 80.9 (CH_2N), 112.6 (C-5 pyrimidinone), 128.4, 128.6, 128.7, 128.8, 129.0, 129.1, 129.3, 129.5, 134.5, 137.2, 141.9 (Ar), 146.6 (C-6 pyrimidinone), 156.0 (C-2 pyrimidinone), 167.3 (C-4 pyrimidinone), 168.5 (CONH), 174.7 (CO). MS (FAB^+): 516/514 (20/60, *M*+H), 538/536 (5/15, *M*+Na), 484 (50), 372 (20), 264 (30), 225 (100).

References

1. (a) Undheim, K. In: van der Goot, H., Domany, G., Pallos, L. and Timmerman, H., Eds. *Trends in Medicinal Chemistry '88,* Elsevier, Amsterdam 1989, p. 781; (b) Gacek, M., Undheim, K., Oftebro, R. and Laland, S. G. *FEBS Lett. 98* (1979) 355.
2. (a) Whitesides, G. M. and Wong, C.-H. *Angew. Chem., Int. Ed. Engl. 24* (1985) 617; (b) Guanti, G., Banfi, L., Narisano, E., Riva, R. and Thea, S. *Tetrahedron Lett. 27* (38) (1986) 4639; (c) Fink, A. L. and Hay, G. M. *Can. J. Biochem. 47* (1969) 353; (d) Sariaslani, F. S. and Rosazza, J. P. N. *Enzym. Microbiol. Technol. 6* (1984) 242.
3. (a) Boyer, P. D., Ed., *The Enzymes,* Academic Press, New York 1972, 3rd ed, Vol. 7; (b) Willis, E. D. 'Lipases', *Adv. Lipid Res. 3* (1965) 197; (c) Langrand, G., Baratti, J., Buono, G. and Triantaphylides, C. *Tetrahedron Lett. 27 (1)* (1986) 29; (d) Langrand, G., Secchi, M., Buono, G., Baratti, J. and Triantaphylides, C. *Tetrahedron Lett. 26 (15)* (1985) 1857; (e) Aleksey, Z. and Klibanov, A. *Proc. Natl. Acad. Sci. USA 82* (1985) 3192.
4. (a) Morikawa, M., Inoue, M. and Tsuboi, M. *Chem. Pharm. Bull. (Tokyo) 24* (1976) 1661; (b) Barmann, T. E. *Enzyme Handbook, Vol. II,* Springer Verlag, Heidelberg 1985.
5. Undheim, K., Strande, P. and Benneche, T. Eur. Pat. Appl. 87326 (1983).

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