Synthetic Communications

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

Regioselective Enzymatic Synthesis of Glycerol Derivatives

G. Aranda & L. Blanco

To cite this article: G. Aranda & L. Blanco (1992) Regioselective Enzymatic Synthesis of Glycerol Derivatives, Synthetic Communications, 22:1, 135-144, DOI: 10.1080/00397919208021084

To link to this article: http://dx.doi.org/10.1080/00397919208021084

Published online: 23 Sep 2006.

Submit your article to this journal

Article views: 10

View related articles

Citing articles: 5 View citing articles

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=lsyc20

Download by: [University of Cambridge] **Date:** 13 June 2016, At: 01:06

SYNTHETIC COMMUNICATIONS, 22(1), 135-144 (1992)

REGIOSELECTIVE ENZYMATIC SYNTHESIS OF GLYCEROL DERIVATIVES

G. Aranda[a] and L. Blanco[b]

[a] Laboratoire de Synthèse Organique (URA 1308 associée au C.N.R.S.) Ecole Polytechnique - 91128 PALAISEAU Cedex - FRANCE

[b] Laboratoire des Carbocycles (URA D 0478 associée au C.N.R.S.) I.C.M.O., Bâtiment 420 - Université de Paris-Sud 91405 ORSAY Cedex - FRANCE

Downloaded by [University of Cambridge] at 01:06 13 June 2016

ABSTRACT : *The regioselective synthesis of 3-acyl-1-(O)-tetradecyl glycerol derivatives was realized by enzymatic transesterification from 1-(O)-tetradecyl glycerol and various acylating reagents. Numerous details on the experimental procedure and isolated 1,3-derivatives are given.*

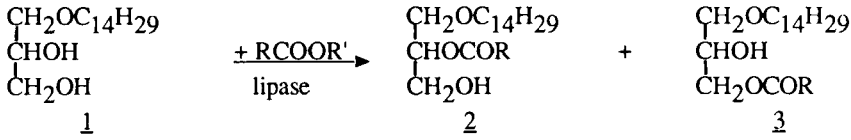
We have recently presented a valuable general (1) solution to the synthesis of photolabile and active biologically ligands. The possibility we have adopted requieres glycerol as multifunctional and biocompatible transport vector. The notion of modulable transporter has been borrowed from nature. We need for particular synthesis 1,3-disubstituted glycerol derivatives. To our knowledge, the number of reactions giving directly and regioselectively these compounds is very small (1) (2) (3) and usually, over all procedures of esterification gave mixtures of 1,2-, 1,3-di- and trisubstituted compounds from 1-substituted glycerol (4). 1,3-Disubstituted glycerol derivatives are usually obtained by a meticulous procedure, involving among others steps, solide state isomerization of 1,2-disubstituted derivatives (5) (6). Furthermore, chromatography enhances dramatically the complexity of the starting mixture (7) (8), although addition of boric acid to silicagel prevents partially acyl migration (9) (10).

In order to realize a regioselective monoacylation of 1-(O)-substituted glycerol compounds, we have envisionned to use enzymatic reactions. Numerous reports are devoted to the regioselectivity in the enzyme-catalyzed hydrolysis of triacyl glycerol derivatives. Many hydrolases work on the 1 and 3 positions (11) and some ones are able to cleave selectively the 2-acyl substituent (12) (13).

Copyright © 1992 by Marcel Dekker, Inc.

Results and discussion

After the understanding that hydrolases can be used in organic media (14) it was shown on glycol derivatives the facile acylation of a primary alcohol function in the presence of a secondary one (15). In the field of glycerol derivatives, was also reported the synthesis of chiral 2-(O)-alkylglycerol monoesters by transesterification of 2-(O)-alkylglycerol with a lipase in organic medium (16) or by enzyme-catalyzed hydrolysis of the corresponding diester (17). We now wish to describe the efficient lipase-catalyzed synthesis of 3-acyl-1-(O)-alkylglycerol compounds 3 following the traditional transesterification process applied to 1-tetradecyl glycerol 1 and various acylating agents.



The results are reported on the table ; yields and ratios were determined generally after, by separation column chromatography on silica gel impregnated with boric acid.With the low molecular weight acyl groups (entries 1,3-7), the corresponding methyl or ethyl esters were used as solvent and reagent in the transesterification. With the high molecular weight acyl groups, reactions were run in diisopropylether (entries 8 - 13) or in benzene (18) (entries 14 - 17) in the presence of an activated ester : in most cases esters of acetoxime (19) (20) were used because they are easily prepared with good yields from acetoxime by reaction with an acyl chloride in the presence of triethylamine or by direct coupling with the free acid in acetonitrile in the presence of N,N'-dicyclohexylcarbodiimide (DCC) (21).

With the straight chain acyl groups (entries 4, 9, 10, 11) a good regioselectivity was generally attained using Pig pancreatic lipase (PPL) : the ratios of the 3-acylated product 3 to the 2-acylated one 2 were ≥ 98/2 and practically no diacylated compounds were detected. The measure of the ratio between 1,2- and 1,3-derivatives was done from the ^{1}H NMR spectra. 1,2-Derivatives were characterized by a down-field signal of the secondary ester CHOCOR (m, 1H, 5.20) comparatively to the primary ester function CH_2OCOR (m, 2H, 4.16). In the ^{13}C NMR spectra the secondary ester was also shifted down-field (70 ppm) comparatively to the two glyceric methylene carbons (69 and 63 ppm) of the 1,3-derivative. The best result in the synthesis of the acetyl derivative was obtained using Lipozyme from NOVO (22) and ethyl acetate (entry 3),

Downloaded by [University of Cambridge] at 01:06 13 June 2016

Downloaded by [University of Cambridge] at 01:06 13 June 2016

TABLE

Preparation of 3-acyl-1-(O)-alkyl-glycerol by enzyme-catalyzed transesterification.

Entry	R	Acylating agent	Enzyme*	Conditions**		Monoester Yield (%)	3 / 2	Diol recovered (%)
1	CH_3	CH_3CO_2Et	PPL	1.75 d	A	50		46
2		$CH_3CO_2C(CH_3)=CH_2$	Lipozyme	4 d	B	31		66
3		CH_3CO_2Et	Lipozyme	3 d	A	94	98/2	
4	C_3H_7	$C_3H_7CO_2Et$	PPL	2 d	A	90	98/2	
5		"	LCC	4 d	A	47	83/17	52
6	$(CH_3)_2CH$	$(CH_3)_2CHCO_2Me$	PPL	1 d	A	no reaction		
7		"	LP	1.75 d	A	83	>98/2	
8	$(C_6H_5)_2CH$	$(C_6H_5)_2CHCO_2N=C(CH_3)_2$	PPL or LP	1 d	B	no reaction		
9	C_7H_{15}	$C_7H_{15}CO_2N=C(CH_3)_2$	PPL	1.1 d	B	89	>98/2	
10	$C_{15}H_{31}$	$C_{15}H_{31}CO_2N=C(CH_3)_2$	PPL	1 d	B	90	>98/2	
11	$Br(CH_2)_5$	$Br(CH_2)_5CO_2N=C(CH_3)_2$	PPL	0.8 d	B	83	>98/2	
12	$HO_2C(CH_2)_2$	$\overline{OC(CH_2)_2CO_2}$	PPL	1 d	B	no reaction		
13		"	LP	1.9 d	B	78	>98/2	
14	Z-(L)Phe	Z-(L)Phe-ON=$C(CH_3)_2$	PPL or LP	1 d	C	no reaction		
15		"	Lipozyme	0.6 d	C	48	95/5	34
16	CHO-(L)Phe	CHO-(L)Phe-ON=$C(CH_3)_2$	Lipozyme	1 d	C	no reaction		
17	CHO-(L,D)Val	CHO-(L,D)Val-ON=$C(CH_3)_2$	Lipozyme	1 d	C	no reaction		
18	C_3F_7	$C_3F_7CO_2CH_2$-CCl_3	LP	10 d	B	78	95/5	

* PPL : Pig pancreatic lipase (Sigma)
LCC : Lipase from Candida cylindracea (Sigma)
LP : Lipase from Pseudomonas cepacia (Amano)
Lipozyme : Lipase from Mucor miehei (Novo)

** A : Ester used as solvent
B : In diisopropyl ether
C : In benzene

comparatively with isopropenyl acetate (entry 2) or in the presence of PPL (entry 1) reaction rates were slower. With methyl isobutyrate (entry 6) and succinic anhydride (entry 12), no reaction was observed using PPL but the 3-acyl glycerol derivatives were obtained selectively with the lipase P from AMANO (LP) (23) (24) (entries 7, 13).

In view to synthetize glycerol aminoester derivatives, the coupling of the diol 1 with the N-benzyloxycarbonylphenylalanine ester of acetoxime Z-(L)-Phe-ON=C(CH_3)$_2$ was attempted using α-chymotrypsin or subtilisin as reported previously (25,26). No glyceric ester was detected and only clean hydrolysis of the activated aminoester have occured. Using LPP or LP no reaction was noticed after one day (27). With lipase LN from AMANO (28) a slow reaction was observed but the best result was obtained with Lipozyme using only 1.4 equivalent of the activated aminoester (entry 15) : after 14 hours, the expected glyceric aminoester was isolated with 48 % yield after column chromatography on silica gel without boric acid. Thus, the detected 1,2-derivative (entry 15) can be correspond at least in part, to the chromatography. When silica gel impregnated with boric acid was used, degradation of the glyceric aminoester occurs widely.

In order to prepare an heptafluorobutyrate derivative of the diol 1 we have first attempted the transesterification with methyl heptafluorobutyrate used like solvent but no reaction has occured after one week in the presence of LPP, LP or Lypozyme. With the activated acetoxime heptafluorobutyrate ester the reaction in the presence of LPP gave after one week the 3-acylated and the 2-acylated isomers in the ratio 88/12 (≈ 90 % yield) and the diacylated compound (≈ 7 % yield). Control experiments without enzyme showed a similar reactivity but in the presence of dimethylaniline, the selectivity rose to 92/8 in favor of the 3-heptafluorobutyrate ester and no diester was detected. With the 3,3,3-trichloroethylheptafluorobutyrate a slow reaction was still observed without enzyme but the rate increase in the presence of LP and the selectivity was closed to 95/5 (entry 18) after ten days, although the second isomer was not shown after 4 days in the NMR spectra.

Finally, our results show clearly that the enzymatic transesterification is the method of choice to synthesize regioselectively 1,3-disubstituted glycerol derivatives. Although transesterifications were realized without significant enantiomeric excess, in our case, the enzymatic hydrolysis of 1-alkyl-2,3-diesters glycerol derivatives seems to be a promizing issue. In the context of another program, we will seek a simpler and more efficient procedure, starting with 1-alkyl-2,3-diesters glycerol derivatives.

Downloaded by [University of Cambridge] at 01:06 13 June 2016

EXPERIMENTAL

Melting points were determined on a Reichert microscope hot plate and are uncorrected. Infrared spectra were recorded in CCl_4 solution on a Perkin-Elmer 399 spectrometer. ^{1}H-NMR spectra were recorded at 200 and 400 MHz on Bruker AC.200 FT or AM.400 FT spectrometers. NMR samples were prepared in $CDCl_3$ or CD_3OD containing 1 % TMS as internal reference. ^{13}C-NMR spectra were recorded at 50.3 or 100.6 MHz respectively and assignments were made by polarization transfer using a DEPT 135 sequence. Full assignments of ^{1}H-spectra, and particularly assignments of individual chemical shifts of the methyl groups were obtained with the aid of 2D-^{1}H,^{13}C heteronuclear correlation analysis. Elemental analyses of crystalline samples were performed at the Central Microanalysis Laboratory, C.N.R.S., Gif-sur-Yvette, France. Optical rotations were measured on 1 dm-cells on a Perkin-Elmer 241 spectropolarimeter.

Downloaded by [University of Cambridge] at 01:06 13 June 2016

Synthesis of acylating reagents

Acylating reagents not described are commercially available. Esters realized with acetoxime are synthesized as follow starting with acid-chlorides or acids.

(a) General procedure with acid chlorides corresponding to octanoic, palmitic and 6-bromo-hexanoic acids.

$Br(CH_2)_5COCl$ (1.50 g) was dissolved in anhydrous methylene chloride (8 ml). Under nitrogen atmosphere, a solution of acetoxime (1.0 g) in CH_2Cl_2 (8 ml) and triethylamine (2 ml) was added with care under magnetic stirring at ice bath temperature. After one day at room temperature, CH_2Cl_2 (100 ml) was added. The organic solution was washed with cold 5 % HCl solution (10 ml), then with cold 5 % NaOH solution (10 ml), then with water (3 x 10 ml) and dried over $MgSO_4$. Solvent was removed under vacuum : a colorless oil was isolated (1.46 g).

I.R. (CCl_4) cm^{-1} : 2941, 1768, 1548, 1252, 1218, 1005 and 978.

^{1}H NMR ($CDCl_3$) : 1.55, 1.72 and 1.87 (3q, 6H, $CH_2CH_2CH_2$, J = 7.2 Hz) ; 2.01 and 2.05 (2s, 6H, $2CH_3$) ; 2.44 (t, 2H, J = 7.4 Hz, CH_2CO) ; 3,42 (t, 2H, $BrCH_2$, J = 6.6 Hz) . ^{13}C NMR ($CDCl_3$) : 16.6 and 21.6 (2 CH_3) ; 23.7 ; 27.3 and 32.0 (C_5, C_3 and C_4, 32.3 (CH_2CO) ; 33.15 ($BrCH_2$) ; 163.4 and 170.4 , CN and CO.
O-octanoyl acetoxime : b.p. = 94-95°C/0.10 Torr. O-palmitoyl acetoxime : m.p. = 32.5-33°C.

(b) General procedure with heptafluorobutyryl chloride and acetoxime or trichloroethanol. Acetoxime (1.0 g) was dissolved in anhydrous CH_2Cl_2 (15 ml) and $(C_2H_5)_3N$ (2 ml) under nitrogen atmosphere. At ice bath temperature, 2 ml of heptafluorobutyryl chloride (3.11 g) dissolved in CH_2Cl_2 (10 ml) were added with care. After one day at room temperature, anhydrous and cold ethylic ether (50 ml) was added. The solution was filtered on Celite. After standing in the refrigerator, the solution was filtered again, twice. Solvents were removed under ambiant pressure, then to reduced pressure (200-100 Torr). Acetoximic ester was then distilled. b.p = 62-64°C/16 Torr.

I.R. (neat) : 1800, 1646, 1437, 1357, 1220, 1077, 971, 871 and 755.

^1H NMR ($CDCl_3$) : 2.25 (s, 2 CH_3). ^{13}C NMR ($CDCl_3$) : 17.1 and 21.5 ($2CH_3$) ; 107.8 (tt, CF_3, J_{C-F} = 267 Hz and 33,2 Hz)) ; 108.0 (m, $CF_3\underline{C}F_2CF_2$) ; 117.45 (qt, CF_2CO, J_{C-F} = 286 Hz and 33,0 Hz) ; 155.6 (t, $CF_2\underline{C}O$, J_{C-F} = 29.0 Hz) and 168.6 (s, CN).

Trichloroethyl heptafluorobutyrate was characterized by b.p. = 96-100°C/80-85 Torr.

(c) General procedure with acids corresponding to diphenylacetic acid and N-protected amino-acids.

Commercially crystallized Z-(L)-phenylalanine (0.363 g) was dissolved in anhydrous acetonitrile (10 ml)) in presence of acetoxime (0.100 g). Under nitrogen atmosphere, at room temperature, dicyclohexyl carbodiimide (DCC) was added (0.30 g). One day later, the solution was filtered on celite and diluted with anhydrous ethylic ether. After standing in the refrigerator, the solution was filtered again, twice. Solvents were removed under vacuum and the acetoximic ester homogeneous on plate, (0.428 g), was recrystallized twice from the mixture ethylic ether and CH_2Cl_2. m.p. = 82.5-83°C. $[\alpha]_D^{20}$ = + 14.7° (c = 2.25, $CHCl_3$).

I.R. (CCl_4) cm^{-1} : 3434, 3032, 1769, 1727, 1499, 1346, 1218, 1053 and 698.

^1H NMR ($CDCl_3$) : 1.77 (s, 3H, CH3) ; 2.00 (s, 3H, CH_3) ; 3.14 (d,2H, CH_2CH, J = 6.25 Hz) ; 4.75 (dt, 1H, CHN, J = 6.25 Hz and 8.75 Hz) ; 5.08 (s, 2H, CH_2OCO); 5.39 (d, 1H, NH, J = 8.75 Hz) ; 7.10-7.32 (m, 10H, 2 x C_6H_5). ^{13}C NMR ($CDCl_3$) : 16.85 and 21.9 (2 CH_3), 38.8 ($C_6H_5\underline{C}H_2$), 54.2 CH) ; 67.0 (OCH_2) ; 127.1-136.2 (2 x C_6H_5) ; 155.6 (NCO_2) ; 165.2 (CN) and 169.4 (OCO).

Enzymatic transesterification

1-Tetradecylglycerol was previously described and synthesized without detectable 1-(O)-alkyl migration (1).

Downloaded by [University of Cambridge] at 01:06 13 June 2016

General procedure for usual acylating reagents :

A 5 ml round bottomed flask was charged with 27 mg (0.093 mmol) of the diol 1, 90.5 mg (0.29 mmol) of O-palmitoyl acetoxime, 86 mg of 4 Å molecular sieves, 29.4 mg of Pig pancreatic lipase and 500 mg of diisopropyl ether (0.70 ml). The mixture was stirred during 24 hours at room temperature and the reaction was monitored by TLC. The enzyme was filtered, washed with ethylic ether and the organic solution was concentrated under reduced pressure (18 Torr). Column chromatography on silica gel impregnated with boric acid (9,10) (eluent : hexane/ethylic ether : 80/20) of the oily residue gave 44.1 mg (yield = 90 % with respect to diol 1) of the 2-hydroxy-3 (tetradecyloxy)propyl palmitate. m.p. = 58.5-59.5°C. $C_{33}H_{66}O_4$, calc. C % = 75.23, H % = 12.63 ; found : C % = 75.6, H % = 12.57.

I.R. (CCl_4) cm^{-1} : 3588, 2926, 2854, 1741, 1466 and 1177.

^1H NMR ($CDCl_3$) : 0.89 (t, 6H, J = 6.75 Hz, 2 CH_3) ; 1.27 (br.s, 23 CH_2) ; 1.58 (m, 2 CH_2) ; 2.35 (t, 2H, CH_2CO, J = 7.6 Hz) ; 3.49 (m, 4H, CH_2OCH_2) ; 4.0 (m, 1H, CHOH) ; 4.16 (m, 2H, CH_2OCO). ^{13}C NMR ($CDCl_3$) : 14.2 (CH_3) ; 22.8-34.3 (27 CH_2) ; 65.5 (CH_2OCO) ; 69.0 (CHOH) ; 71.5 (CH_2O) ; 71.9 [$CH_2O\underline{C}H_2(CH_2)_{12}CH_3$] ; 174.1 (CO).

General procedure for amino-esters :

A 5 ml round bottomed flask well capped was charged with 26.1 mg (0.09 mmol) of the diol 1, 46.2 mg (0.13 mmol) of the N-benzyloxycarbonyl-(L)-phenylalanine ester of acetoxime, 196 mg of Lipozyme (Novo) and 0.60 ml of benzene. The mixture was stirred at 35°C during 15 h. Lipozyme was removed by filtration, washed with ethylic ether and the organic solution was concentrated under reduced pressure. Silica gel column chromatography of the oily residue with hexane/ether (75/25 then 60/40), gave 24.7 mg (48 % yield with respect to diol 1) of N-benzoyloxycarbonyl-(L)-phenylalanine 2-hydroxy-3-tetradecyloxypropyl ester and 9 mg of starting racemic diol 1 (34 %). The glyceric aminoester derivative does not crystallize whatever solvents and conditions.

I.R. (CCl_4) cm^{-1} : 3614, 3436, 3032, 2927, 2854, 1727, 1555, 1500, 1197, 1119 and 1057.

^1H NMR ($CCDl_3$) : 0.88 (t, 3H, J = 6.75 Hz, CH_3) ; 1.25 (br.s, $(CH_2)_{12}$) ; 1.55 (m, 2H, CH_2) ; 3.12 (d, 2H, J = 6.2 Hz, $C_6H_5CH_2$) ; 3.40 (m, 4H, CH_2OCH_2) ; 3.92 (m, 1H, CHOH) ; 4.16 (m, 2H, CH_2OCO) ; 4.66 (dt, 1H, J = 6.75 Hz and 6.5 Hz, CHN) ; 5.09 (s, 2H,$C_6H_5CH_2O$) ; 5.27 (d, J = 6.5 Hz, 1H, NH) ; 7.10 to 7.33 (m, 10H, 2 C_6H_5) . ^{13}C NMR ($CDCl_3$) : 14.2 (CH_3) ; 22.8 to 32.0 $(CH)_{12}$; 38.3

Downloaded by [University of Cambridge] at 01:06 13 June 2016

($CH_2C_6H_5$) ; 55.05 (CH) ; 66.7 (CH_2OCO) ; 67.1 ($O\underline{C}H_2C_6H_5$) ; 68.5 (CHOH) ; 71.2 ($\underline{C}H_2OCH_2$) ; 71.9 ($CH_2O\underline{C}H_2(CH_2)_{12}CH_3$) ; 127.3, 128,2, 128;3, 128,6, 128,75, 129,4, 135.75 and 136.2 (2 C_6H_5) ; 155.8 (OCONH) and 171.7 (CH_2OCO) . $[\alpha]_D^{20}$ = + 16.4°(c = 2.52, $CHCl_3$).

When the transesterification was realized in presence of the Lipase N from *Rhizopus Niveus*, the same glycerol derivative was isolated after 8 days with 40 % yield and the recovered diol 1 was characterized by $[\alpha]_D^{20}$ = + 2.8° (c = 1.23, THF) and ee = 6.6 %.

Others particular glycerol derivatives isolated (entries 11 and 13) were previously described (1).

Acknowledgement

We thank warmly A. Tambuté for ee measurements, M. Bertranne for performing ^{13}C NMR spectroscopy, J. Seyden-Penne and J.Y. Lallemand for helpful discussions.

REFERENCES

1 . Aranda, G., and Lallemand, J.Y., Synthetic Communications,1990, 20, 85. Addition and corrections : p. 86, last line, $BrCH_2CH_2CH_2OH$; p. 91, 11th line, then crystallized derivative2c (0.835 g) ; p. 95, 6th line, 68.95 $[CH_2O(CH_2)_n]$.

2 . Cimetière, B., Jacob, L.and Julia, M., Tetrahedron Lett., 1986, 27, 6329.

3 . Guivisdalsky, P.N. and Bittman, R., ; Tetrahedron Lett., 1988, 29, 4393 ; 1989, 30, 2751.

4 . de Medeiros, E.F., Herbert, J.M. and Taylor,G., J. Chem. Soc., Chem. Comm.,1990, p. 1497 .

5 . Mank, A.P.J., Ward, J.P. and Van Dorp, D.A., Chem. and Phys. of Lipids,1976, 16, 107.

6 . Lok, C.M., Chem. and Phys. of Lipids, 1978, 22, 323 ; Lok, C.M., Mank, A.P.J. and Ward, J.P., Chem. and Phys. of Lipids, 1985, 86, 329.

7 . Burgos, C.E., Ayer D.E. and Johnson, R.A., J. Org. Chem., 1987, 52, 4973 .

8 . Bhatia, S.K. and Hajdu, J., Synthesis, 1989, p. 16.

9 . Thomas, A.E., Scharoun, J.E. and Ralston, H., J. Am. Oil Chem.Soc, 1965, 42, 789.

Downloaded by [University of Cambridge] at 01:06 13 June 2016

10 . Duralzki, A.A., P.J.R. Spooner, and Watts, A., Tetrahedron Lett., 1989, 30, 3585 .

11 . Haraldsson, G.G., . Höskuldsson, P.A., Sigurdsson, S.TH., Thorsteinsson, T. and Gudbjarsason, S., Tetrahedron Lett., 1989, 30, 1671

12 . Bhatia, S.K.and Hajdu, H., Tetrahedron Lett., 1987, 28, 1729 ; Bhatia, S.K. and Hajdu, H., Tetrahedron Lett., 1988, 29, 31 .

13 . Chakrabarti, P., Khorana, H.G., Biochemistry, 1975,14, 5021 ; Bunner, J. and . Richards, F.M., J. Biol. Chem., 1980, 255, 3319.

14 . Seo, C.W., Yamada, Y. and Okada, H., Biol. Chem., 1982, 46, 405 ; Cambou, B. and Klibanov, K., J. Am. Chem. Soc., 1984, 106, 2687 Langrand, G., Secchi, M., Buono, G., Barratti, J. and Triantaphyllides, C., Tetrahedron Lett., 1985, 26, 1857 .

15 . Cesti, P., Zaks, A. and Klibanov, A.M., Appl. Biochem. and Biotech., 1985, 11, 401 ;. Ramaswamy, S., Morgan, B. and Oehlshlager, A.C., Tetrahedron Lett., 1990, 31, 3405 ; Ottolina, Carrea, G. and Riva, S., J. Org. Chem., 1990, 55, 2366 .

16 . Wang, Y.F. and Wong, C.H., J. Org. Chem., 1988, 53, 3127 ; Wang, Y.F., Lalonde, J.L., Momongan, M., Bregbreiter, D.E. and Wong, C.H., J. Am. Chem. Soc., 1988, 110, 7200 ; Terao, Y., Murata, M., Achiwa, K., Nishio, T., Akamtsum, M. and. Kamimura,, M.,Tetrahedron Lett., 1988, 29, 5173 .

17 . Kerscher, V. and Kreiser, W., Tetrahedron Lett., 1987, 28, 531 ; Breitgoff, D.,Laumen, K. and Schneider, M.P., J. Chem. Soc., Chem. Comm., 1986, p. 1523.

18 . The solubility of the Z-L-phenylalanine ester of acetoxim was hardly in diisopropylether.

19 . Ghogare, A. and Sudesh Kumar, G., J. C. S. Chem. Comm., 1989, p. 1533.

20 . Enol esters were not employed because drastic conditions are used and low yields are often reported for their synthesis .See : Yang, Y.F., Lalonde, J.L., Momongan, M., Bregbreiter, D.E. and Wong, C.H., J. Am. Chem. Soc., 1988, 110, 7200 .

21 . Acetoximic esters can also be synthesized by enzymatic reaction ; see : Gotor, V. and Menendes, E., Synlett., 1990, p. 699 .

22 . Lypozyme : Lipase of Mucor Miehi immobilized on an anionic resin.

23 . LP : Lipase from Pseudomonas cepacia, see. ref. 6 in Rubio, E., . Fernandez-Mayorales, A. and Klibanov, A.M., J. Am. Chem. Soc., 1991, 113, 695 .

Downloaded by [University of Cambridge] at 01:06 13 June 2016

24 . Yamamoto, Y., Iwasa, M., . Sawada, S. and Oda, J.I., Agric. Biol. Chem., 1990, 54, 3269 ; Yamamoto, Y., Yamamoto, K., Nishioka, T. and Oda, J., Agric. Biol. Chem., 1988, 52, 3087 (1988) ; Yamamoto, K., Nishioka, T., Oda, J.I. and Yamamoto, Y., Tetrahedron Lett., 1988, 29, 1717.

25 . Pugniere, M., San Juan, C. and Previero, A., Tetrahedron Lett., 1990, 31, 4883 .

26 . Kuhl, P., Halling, P.J. and Jakubke, H.D., Tetrahedron Lett., 1990,31, 5213 .

27 . After one month the reaction with LPP had given a significant amount of the glyceric aminoester.

28 . LN : Lipase from Rhizopus niveus.

(Received in UK 10 July, 1991)

Downloaded by [University of Cambridge] at 01:06 13 June 2016