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Regioselective Lipase-Catalyzed Synthesis of L-Glutamic α-Monoamide Derivatives. Effect of the N-Blocking Group

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Abstract: Several Block-L-Glu(OEt)-OEt (Block = amides, carbamates and trityl group) have been subjected to an aminolysis reaction catalyzed by the lipase B of *Candida antarctica*. The reaction took place in a regioselective manner and α-monoamides were obtained in all cases (except the bulky trityl group that did not react). Besides the synthetic value of the method, the results may empirically point out to some features of the active site of the enzyme as the reaction rate was severely affected by volume and electronic characteristics of the blocking group. © 1997 Elsevier Science Ltd.

The lipase-catalyzed amidation of esters is not a common reaction although there are some significant precedents. In 1987, Klibanov[1] and Wong[2] recorded the synthesis of dipeptide derivatives catalyzed by porcine pancreatic and *Candida rugosa* (*C. cylindracea*) lipases. Gotor's group developed a general survey of the reaction including a variety of nucleophiles such as chiral amines,[3] amino groups of nucleosides,[4] hydrazines[5] and, nearly simultaneously with Sheldon,[6] ammonia.[7] The acyl donor moiety has also been investigated: besides the original Klibanov and Wong's resolution of α-aminoacids, amidation of esters of linear aliphatic,[8] multibond,[3] α-[9] or β-substituted[10] chain acids catalyzed by lipases have been reported.

Our group is also involved in the study of this reaction, in particular when the substrates are esters of dicarboxylic aminoacid derivatives. We have previously reported[11] that the amidation of Cbz-Glu(OEt)-OEt catalyzed by the lipase B[12] from *Candida antarctica* (referred to as CAL)[13] is not only effective but also enantioselective, and consequently regioselective also, with respect to the acyl donor (α-amidation in L-(*S*)- and γ-amidation in D-(*R*)-derivatives). Another important aspect in the aminoacid chemistry concerns the N-protective group; it would be highly interesting to investigate if the above mentioned reaction can be extended to the usual blocking groups and how its steric and electronic features affect the regioselectivity and rate of the reaction. On the other hand, a scandinavian multigroup team has recently published[14] the three-dimensional structure of *Candida antarctica* lipase B co-crystallized, first with the detergent Tween 80, and second with a covalently bound inhibitor. They conclude that the alcohol side of the active site pocket is a small and well-defined tunnel, only suitable for sterically restricted nucleophiles while the acyl side is more spacious and the stereoselectivity with respect to this part of the molecule is lower than for the alcohol moiety.

In this paper we report the results of the CAL-catalyzed amidation of several diethyl L-glutamate derivatives that only differ in the N-blocking group, using pentylamine (*n*-$PnNH_2$) and anhydrous diisopropylether as unique nucleophile and medium respectively, and identical experimental conditions. The aim of this work was to investigate the synthetic limits of the reaction and how the rate and, eventually, regioselectivity were affected by different groups that could add new data to the empirical understanding of the structure of the CAL active site.

RESULTS AND DISCUSSION

SUBSTRATES AND INTERNAL STANDARDS

Initially, the following model diethyl L-glutamate derivatives were synthesized: N-acetyl (Ac) **1a**, N-benzyloxycarbonyl (Cbz) **1b**, N-*t*-butyloxycarbonyl (Boc) **1c**, N-diphenylacetyl (Ph_2Ac) **1d** and N-triphenylmethyl (trityl, Tr) **1e** and, at a second stage of the work, N-phenylacetyl (PhAc) **1f** and N-trimethylacetyl (pivaloyl, Piv) **1g**. They were readily synthesized by treatment of commercial diethyl L-glutamate with the corresponding halide (anhydride in the case **c**) and triethylamine in an organic solvent. We were not specially concerned with the yields of the reactions but with obtaining pure compounds. All the five blocking groups are commonly used in aminoacid chemistry and are inert under the mild experimental conditions of the enzymatic reactions. They were initially selected because of their lack of reactivity as CAL substrates and increasing size from the small acetyl to the bulky trityl although are chemically not equivalent because **1a,d** are amides, **1b,c** carbamates and **1e** is an amine.

N-Methylacetanilide and N-methylbutyranilide synthesized by us were used as internal standards. They were selected because tertiary amides are not expected to react in CAL-catalyzed processes and are and quickly synthesized from N-methylaniline, an acyl chloride and pyridine. Thus, lipophilic inert standard can be easily obtained in an overnight reaction by just changing the acyl chloride: acetyl, butyryl, etc.

MOLECULAR SURFACES AND VOLUMES

These were determined following the Conolly program[15] that uses the Van der Waals radii of its atoms. The term molecular surface was introduced by Richards[16] and defined as consisting of two parts: the contact and the re-entrant surface. The contact one is that part of the Van der Waals surface of each atom accessible to a probe sphere of a given radius (the solvent-accessible surface). The re-entrant surface is the inward-facing part of the probe sphere when it is simultaneously in contact with more than one atom.

The surfaces and volumes of the blocking groups were studied as the full molecules Block-H (aldehydes for amides, formates for carbamates and triphenylmethane for trityl) and assuming that the hydrogen atom adds a negligible amount to the real data (Table 1). After the results obtained with **1a-e**, we calculated also the areas and volumes of phenylacetyl (PhAc, **f**) and trimethylacetyl (pivaloyl, Piv, **g**). The values obtained must be taken as an approach to the steric hindrance but not necessarily as a direct relation with it . For example, a linear long chain will display bigger volume but, in many cases, less steric hindrance than the smaller *t*-butyl.

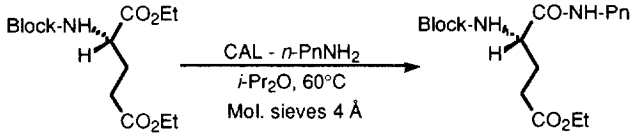
TABLE 1. Molecular Areas and Volumes

Group	**a**:Ac-H	**b**:Cbz-H	**c**:Boc-H	**d**:Ph_2Ac-H	**e**:Tr-H	**f**:PhAc-H	**g**:Piv-H
Area ($Å^2$):	62.31	143.12	116.89	197.17	254.51	132.90	111.38
Volume ($Å^3$):	38.58	109.82	92.79	170.39	237.33	103.19	88.71

CAL-CATALYZED AMIDATIONS OF 1a-e

The kinetic study was initially carried out by performing the reactions of the substrates **1a-e** (Scheme 1) at an analytical scale. Aliquots were periodically withdrawn and analyzed by HPLC to plot and compare the progress curves of the reactions that are directly related to the reactivity of the substrates. The following step

was to scale-up the reactions and isolate and identify the products obtained in order to find out any possible variation in the expected α-regioselectivity of the process on changing the N-blocking group. The structural studies were mainly accomplished by ^{1}H- and ^{13}C-NMR techniques. All the reactions, including those without enzyme, were carried out at the same experimental conditions (see *Experimental Part*).



1a-e **2a-d**

SCHEME 1

In the absence of enzyme, the mixtures remained unchanged for at least 24 hours, which means that any detected transformation had an enzymatic origin. Substrate **1e** did not react but the four derivatives **1a-d** reacted in a quick and clean way, yielding in all cases the α-monoamide **2a-d** (see below, *Structural Elucidation Part*) although there are significant kinetic differences among them (Figure 1).

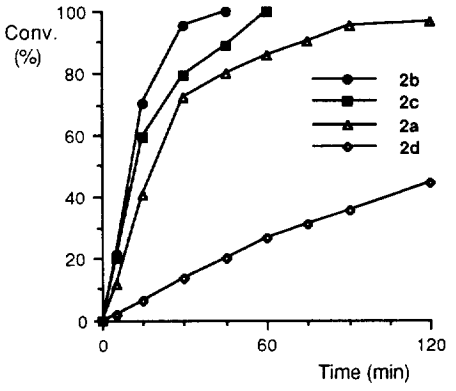


FIGURE 1. Conversion (%) of **1a-d**. Analytical scale, HPLC data. **1e** did not react.

Figure 1 shows the progress curves of the reactions, products formed (%) *vs.* time, when performed at an analytical scale. The remarkable point is that the reactivity of the substrates does not correspond with the volume of the blocking groups, at least not in all cases: bulky **1d** reacts slowly and the even bigger trityl derivative **1e** remains unchanged for at least 5 days, but the rate of conversion of **1a**, bearing the small acetyl group appears lower than that of **1b** and **1c**.

Significantly, the two preferred substrates **1b** and **1c** were carbamates while the amide **1a** reacted slower in spite of its low steric hindrance.

These results suggested that the size of the blocking group is not the unique feature involved in the differences of reactivity among the substrates **1a-e** and, although carbamates and amides have relative affinities from a chemical point of view, the oxygen atom of the carbamate group plays an important role in the substrate-enzyme interactions and would produce a positive effect on the reaction rate.

EFFECT OF THE N-BLOCKING GROUP ON THE REACTION RATE

This hypothesis was confirmed by contrasting the carbamates **1b,c** in parallel reactions with their corresponding amides **1f** and **1g** respectively (Figure 2).

Obviously, amides have a smaller volume than their equivalent carbamates (Table 1) but, **1b** and **1c** reacted quicker than their counterparts **1f** and **1g** respectively. We deduced from these results that, as suggested above, there are interactions between the oxygen atom of the carbamates and the active site of the enzyme that

produce a positive effect on the reactivity. This effect agrees with Uppenberg's recent description[17] of the lipase B structure: the active site is located at the end of a narrow and hydrophobic tunnel but the region around the catalytic triad is remarkably polar and, in addition to the His of the triad, there are three polar residues (Thr, Asp and Gln) with their side chains within 5Å of the O_γ of the catalytic Ser. Thus, the oxygen atom of the carbamate group would be integrated in the hydrogen bond network formed by these polar residues and the energy needed to bind the substrate to the active site would be, consequently, lower than that of amides. The role of the oxygen atom could also be a mere link to elongate the chain with the subsequent decrease of their steric hindrance, but this idea was discarded because the derivative **1a** reacted in a slower manner than the carbamates **1b** and **1c**.

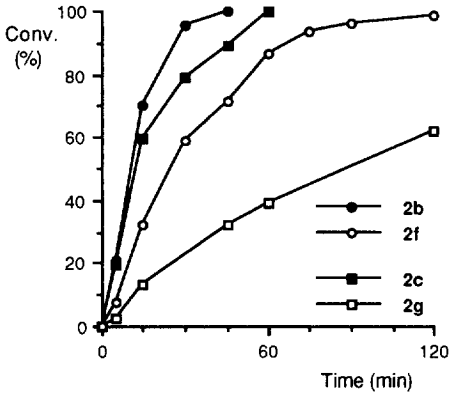


FIGURE 2. Comparison between the carbamates **1b,c** and their corresponding amides **1f,g**. Analytical scale. HPLC data.

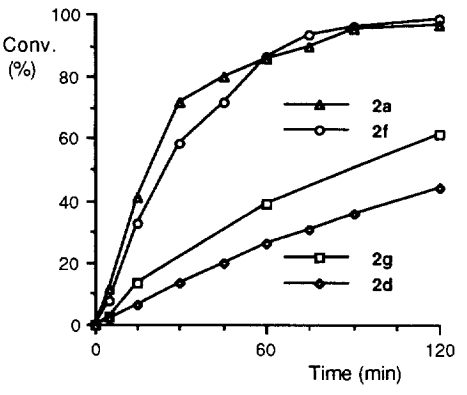


FIGURE 3. Reactivity of the N-amides **1 a, d, f, g**. Analytical scale. HPLC data.

These results also show that the presence of aryl rests in the N-blocking group exerts a minor but also positive effect on the reactivity. Although we have not found a comprehensible explanation, the effect is clear when comparing the progress curves of the reaction of the four amides **1a, d, f** and **g** (Figure 3).

Despite the great differences of molecular volume and steric hindrance between them, **1a** and **1f** displayed quite similar rates. At a lower level, **1g** and **1d** exhibited rates of the same order although diphenylacetyl group is more than twice bigger than pivaloyl one. Parallel to the carbamates and amides discussed above, this order of reactivities can only be explained if there are some helpful electronic interactions of the phenyl group with a precise point of the active site.

STRUCTURAL ELUCIDATION

We have shown in our previous work[11] that, in the CAL-catalyzed monoamidations of **1b**, the regioisomer obtained could be easily determined from the chemical shift differences between the monoamide and the starting diester. The highest differences were found in the nearest chain position (α or γ) to the amide group. Now, the structures of the new monoamides **2a, c, d, f** and **g** have been established by comparison of their ^1H and ^{13}C NMR spectra with those of their starting diethylesters **1a, c, d, f** and **g** respectively. In all

cases (Tables 2 and 3), higher chemical shift differences in the α-CH ($\Delta\delta \approx 0.2$ ppm in ^{1}H-NMR and $\Delta\delta \approx -0.7$ ppm in ^{13}C-NMR) than in the γ-CH_2 ($\Delta\delta \approx -0.01$ ppm and $\Delta\delta \approx -0.2$ ppm, respectively) were observed, pointing out to a regioselective α-amidation.

In addition, the monoamidation site was unequivocally confirmed by a HMBC (Heteronuclear Multiple Bond Correlation) experiment of **2d**. Firstly, we assigned the resonance at 170.64 ppm to the α-carbonyl group, from its correlation with the α-proton (4.47 ppm). The proposed structure for **2d** was confirmed as *n*-pentyl α-methylene protons (3.01 ppm) correlated exclusively with the α-CO (170.64 ppm), while γ-CO (173.36 ppm) correlated with the methylenic protons of the ethyl group (4.14 ppm).

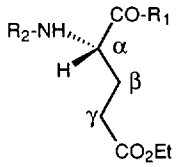


TABLE 2.–Selected ^{1}H[a] and ^{13}C[b] NMR data of Block-L-Glu derivatives **1** and **2**

Compd.	α-CH	β-CH_2	γ-CH_2	R_1(X^c-CH_2)	α-CH	β-CH_2	γ-CH_2	α–CO	γ-CO	R_2-CO	R_1(X^c-CH_2)
1a	4.56	2.17	2.35	4.16	51.71	27.39	30.31	171.96	172.82	169.93	61.55
2a	4.39	2.04	2.36	3.16	52.42	27.99	30.52	171.10	173.42	170.33	39.54
1b[d]	4.39	2.00	2.40	4.20	53.31	27.58	30.10	171.45	172.50	155.88	61.49
2b[d]	4.19	2.11	2.42	3.21	54.18	28.17	30.41	170.85	173.46	155.34	39.59
1c	4.27	2.13	2.36	4.16	53.01	27.79	30.32	172.16	172.68	155.25	61.41
2c	4.05	2.07	2.34	3.16	53.88	27.98	30.55	171.32	173.33	155.64	39.46
1d	4.61	2.13	2.28	4.14	51.95	27.07	30.16	171.50	172.58	171.80	61.50
2d	4.47	2.00	2.25	3.01	52.59	27.67	30.43	170.64	173.36	172.26	39.47
1e	3.36	2.03	2.36	4.12	55.28	30.17	30.54	173.05	174.48	—	60.53
1f	4.51	1.97	2.25	4.08	51.76	27.24	30.18	171.60	172.64	170.81	61.52
2f	4.41	1.96	2.31	3.17	52.47	27.80	30.49	171.22	173.50	170.74	39.53
1g	4.51	2.00	2.35	4.16	51.95	27.22	30.45	172.05	172.90	178.41	61.39
2g	4.38	2.06	2.38	3.15	52.34	27.72	30.49	171.20	173.57	178.82	39.46

[a]. 300 MHz, $CDCl_3$, δ ppm; [b]. 50 MHz, $CDCl_3$, δ ppm; [c]. X=O (compounds **1**) and NH (compounds **2**); [d]. Taken from Ref. 11

CONCLUSIONS

The regioselectivity of the CAL-catalyzed amidations of L-glutamic diesters is not affected by the N-blocking group as α-monoamides are obtained in all cases. However, there is a broad range of reaction rates depending on the size and electronic features of the group. CAL-active site may accept derivatives bearing an N-group as big as Ph_2Ac but not the biggest Tr. The molecular volume of the groups is not the only point to take into account as carbamates react faster than their corresponding amides and phenyl-supporting groups also react faster than expected. It suggests the existence of some electronic interactions that produce a positive effect on the reaction rate.

EXPERIMENTAL SECTION

As a criterion of purity, elemental analyses of both substrates and products (except **1** and **2b**[11]) were carried out; they were performed on a Perkin Elmer 240C equipment. ^1H and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution, with a Varian XL-300, Bruker WP-200 or Varian Unity-500 spectrometers. Optical rotations were determined on a Perkin-Elmer 241-C polarimeter. Analytical HPLC was performed on a Beckman Chromatograph using a DeltaPak C_{18} column (3.9 x 150 mm, 5 μm), using different ratios of acetonitrile-H_2O with 0.5 mL/L of trifluoroacetic acid as eluent (flow rate 1 mL/min) and an UV detector. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of silicagel 60 F_{254} (Merck). Chromatographic separations were performed on columns using the flash chromatographic technique on silicagel 230-400 mesh (Merck). Diisopropylether was refluxed on sodium wire, distilled and stored on molecular sieves 4Å . Internal standards for HPLC and substrates **1a-g** were prepared according to a well-known general procedure.[18] The rest of the chemicals were obtained from normal commercial sources and used without any further purification.

Internal standards: N-methylacetanilide and N-methylbutyranilide. A solution of N-methylaniline (50 mmol) and pyridine (52.5 mmol) in toluene (120 mL) stirred and cooled in an ice-bath, was treated with the corresponding acyl chloride (52.5 mmol), sealed and stirred overnight. The resulting mixture was poured onto ice-diluted HCl and the organic layer was separated, washed twice with water, dried and evaporated. The residue was crystallised from hexane (N-methylacetanilide, mp 92 °C, 71%) or obtained as a yellowish oil (N-methylbutyranilide, 83%) pure enough for our purposes.

General procedure for the synthesis of substrates 1a-g. The corresponding acyl chloride or Boc_2O (5 mmol) was added to a solution of L-Glu(OEt)-OEt hydrochloride (0.96 g, 4 mmol) in pyridine (10 mL) or CH_2Cl_2 (20 mL) containing Et_3N (10 mmol). The reaction mixture was stirred at room temperature for 48 hours and then processed following the above-described standard procedure. The products were purified by silicagel column chromatography using hexane:AcOEt (3, 4 or 6:1) as eluent. They were eluted as many times as needed, to obtain HPLC-pure substrates. ^1H and ^{13}C NMR for these substrates and the final products **2a-g** (not **2e**) are given in Table 2 .

Ac-L-Glu(OEt)OEt **1a**. White solid (52%): mp 44-45°C. $[\alpha]_D$= -13.8° (Lit.[19] $[\alpha]_D$= -10°) (c1, acetone). Anal. calcd. for $C_{11}H_{19}NO_5$(%): C, 53.88; H, 7.75; N, 5.71. Found(%): C, 54.10; H, 7.56; N, 5.65.

Cbz-L-Glu(OEt)OEt **1b** Yellowish oil that becomes a white solid after washing with cold hexane (65% yield): mp 48°C; $[\alpha]_D$= -22.2° (c1, MeOH).

Boc-L-Glu(OEt)OEt **1c**. White solid (34%): mp 46-47°C. $[\alpha]_D$= -16.4° (c1, acetone). Anal. calcd. for $C_{14}H_{25}NO_6$(%): C, 55.44; H, 8.25; N, 4.62. Found(%): C, 55.60; H, 8.20; N, 4.60.

Ph_2Ac-L-Glu(OEt)OEt **1d**. White solid (52%): mp 78-79°C. $[\alpha]_D$= -17.9° (c1, acetone). Anal. calcd. for $C_{23}H_{27}NO_5$(%): C, 69.52; H, 6.80; N, 3.53. Found(%): C, 69.54; H, 6.79; N, 3.45.

Tr-L-Glu(OEt)OEt **1e**. White solid (18%): mp 52°C. $[\alpha]_D$= +43.4° (c1, acetone). Anal. calcd. for $C_{28}H_{31}NO_4$(%): C, 75.48; H, 7.01; N, 3.14. Found(%): C, 75.26; H, 7.08; N, 3.09.

PhAc-L-Glu(OEt)OEt **1f**. White solid (16%): mp 38°C. $[\alpha]_D$= +12.3° (c1, CH_2Cl_2). Anal. calcd. for $C_{17}H_{23}NO_5$(%): C, 63.53; H, 7.21; N, 4.36. Found(%): C, 63.25; H, 7.55; N, 3.99.

Piv-L-Glu(OEt)OEt **1g**. White solid (64%): mp 32°C. $[\alpha]_D$= +4.7° (c1, CH_2Cl_2). Anal. calcd. for $C_{14}H_{25}NO_5$(%): C, 58.52; H, 8.76; N, 4.87. Found(%): C, 58.72; H, 8.39; N, 4.87.

*CANDIDA ANTARCTICA CATALYZED AMIDATIONS OF **1a-g**. GENERAL PROCEDURE.*

All the enzymatic reactions were carried out in a diisopropylether solution containing the corresponding N-blocked-glutamic acid diethylester (20 mM), pentylamine (50 mM), 50 mg/mL of *Candida antarctica* lipase and 50 mg/mL of molecular sieves 4Å (powder). Reactions were carried out at 60°C and stirred in an orbital shaker at 250 rpm.

Analytical scale reactions were performed in 2 mL sealed vials containing also an appropriated concentration of N-methylacetanilide or N-methylbutyranilide as internal standards for HPLC analysis. Periodically, 20 μL aliquots were withdrawn, evaporated and diluted in acetonitrile, filtered off and analyzed by HPLC. Mobile phase composition and wavelength are specified in each case. Parallel blank reactions without enzyme were always performed but no changes were detected in any of them.

Preparative scale reactions were performed under the same above-specified experimental conditions except the absence of the internal standard. The enzyme and molecular sieves were filtered off and washed with acetonitrile. Combined organic extracts were evaporated to dryness and the resulting products were purified by recrystallization, extraction or column chromatography. Eluents, initial amounts, reaction times and purification methods are specified in each case.

Ac-L-Glu(OEt)-NH-n-Pn **2a.** Analytical reaction was performed with N-methylbutyranilide (3 mM) as internal standard. HPLC conditions: 30% AcN: 70% H_2O; λ: 200 nm. Preparative scale reaction was stopped after 2 hours, and the final product was purified by silicagel column (hexane:ethyl acetate 4:1 to 1:1). White solid (89%): mp 111-2°C. $[\alpha]_D$= –14.4° (c1, CH_2Cl_2). Anal. calcd. for $C_{14}H_{26}N_2O_4$(%): C, 58.74; H, 9.09; N, 9.79. Found(%): C, 58.52; H, 8.87; N, 9.49.

Cbz-L-Glu(OEt)-NH-n-Pn **2b.** Analytical scale: N-methylacetanilide (8 mM) was used as internal standard. HPLC: 40% AcN: 60% H_2O; λ: 215 nm. The preparative scale reaction was completed after 60 min., and final product was purified by silicagel column (hexane:ethyl acetate 4:1). White solid (94%): mp 98°C. $[\alpha]_D$= –13.4° (c1, MeOH).

Boc-L-Glu(OEt)-NH-n-Pn **2c.** Analytical scale: N-methylacetanilide (8 mM) was used as internal standard. HPLC: 40% AcN: 60% H_2O; λ: 240 nm (0-3 min), 200 nm (3-10 min.). Preparative scale reaction was completed after 90 min., and final product was purified by silicagel column (hexane:ethyl acetate 4:1). Colorless oil (95%) $[\alpha]_D$= –7.5° (c1, CH_2Cl_2). Anal. calcd. for $C_{17}H_{32}N_2O_5$(%): C, 59.28; H, 9.36; N, 8.13. Found(%): C, 58.49; H, 9.57; N, 8.25.

Ph_2Ac-L-Glu(OEt)-NH-n-Pn **2d.** Analytical scale: N-methylacetanilide (5 mM) was used as internal standard. HPLC: 50% AcN: 50% H_2O; λ: 254 nm. Preparative scale reaction was stopped after 5h. The final product was purified by silicagel column (hexane:ethyl acetate 4:1). White solid (82%): mp 101-2°C. $[\alpha]_D$= -14.4° (c1, acetone). Anal. calcd. for $C_{26}H_{34}N_2O_4$(%): C, 71.23; H, 7.73; N, 6.39. Found(%): C, 71.07; H, 7.93; N, 6.18.

Amidation of Tr-L-Glu(OEt)OEt **1e.** Analytical scale reaction was performed with N-methylacetanilide (20 mM) as internal standard. HPLC: 50% AcN: 50% H_2O (11 min.), 80% AcN: 20% H_2O (5 min.) ; λ: 254 nm but no changes were detected after 24 hours. A preparative scale reaction was carried out for 12 days but after this period of time initial substrate **1e** was recovered by silicagel column in 97% yield.

PhAc-L-Glu(OEt)-NH-n-Pn **2f.** Analytical scale: N-methylacetanilide (8 mM) as internal standard. HPLC: 40% AcN: 60% H_2O ; λ: 200 nm. Preparative scale: the reaction concluded at 150 min.. Then, the suspended

solid (enzyme, molecular sieves and precipitated **2f**) was filtered off and extracted with acetonitrile, yielding pure **2f** as a white solid (96%): mp 123-4°C. $[\alpha]_D$= –8.3° (c1, CH_2Cl_2). Anal. calcd. for $C_{20}H_{30}N_2O_4$(%): C, 66.30; H, 8.29; N, 7.73. Found(%): C, 65.95; H, 8.38; N, 7.56.

Piv-L-Glu(OEt)-NH-n-Pn **2g.** Analytical scale: N-methylacetanilide (9 mM) as internal standard. HPLC: 40% AcN: 60% H_2O ; λ: 200 nm. Preparative scale: the reaction time was 6h. and final product was purified by silicagel column (hexane:ethyl acetate 4:1). **2g** was isolated as a colorless syrup (83%): $[\alpha]_D$= –20.3° (c1, CH_2Cl_2). Anal. calcd. for $C_{17}H_{32}N_2O_4$(%): C, 62.17; H, 9.82; N, 8.53. Found(%): C, 61.77; H, 9.59; N, 8.46.

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REFERENCES

§. This Paper comprises a part of Miss López-Serrano PhD Thesis.
1. Margolin, A. M.; Klibanov, A. M. *J. Am. Chem. Soc.* **1987**, *109*, 3802-3804.
2. West, J. B.; Wong, C.-H. *Tetrahedron Lett.* **1987**, *28*, 1629-1632.
3. Puertas, S.; Brieva, R.; Rebolledo, F.; Gotor, V. *Tetrahedron* **1993**, *49*, 4007-4014.
4. Moris, F.; V Gotor, V. *J. Org. Chem.*. **1993**, *58*, 653-660.
5. Gotor, V.; Astorga, C.; Rebolledo, F. *Synlett.* **1990**, 387-388.
6. de Zoete, M. C.; Kock-van Dalen, A. C.; van Rantwijk, F.; R A Sheldon, R. A. *Biocatalysis* **1994**, *10*, 307-316.
7. García, M. J.; Rebolledo, F.; Gotor, V. *Tetrahedron Lett.* **1993**, *34*, 6141-6142.
8. Djeghaba, Z.; Deleuze, H.; de Jeso, J.; Messadi, D.; Maillard, B. *Tetrahedron Lett.* **1991**, *32*, 761-762.
9. Gotor, V.; Brieva, R.; Gonzalez, C.; F Rebolledo, F. *Tetrahedron* **1991**, *47*. 9207-9214.
10. Sánchez, V. M.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymm.* **1997**, *8*, 37-40.
11. Chamorro, C.; R González-Muñiz, R.; Conde, S. *Tetrahedron: Asymm.* **1995**, *6*, 2343-2352.
12. Theil, F.; Björkling, F. *Biotechnol Lett.* **1993**, *15*, 605-608.
13. We used Novozym 435, a Novo Nordisk's commercial immobilized preparation of that lipase
14 Patkar, S.; Uppenberg, J.; Öhrner, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Waagen, V.; Anthonsen, T.; Jones, T. A. *Biochemistry* **1995**, *34*, 16838-16851.
15. Conolly, M. L. *Science*, **1983**, *221*, 709-713.
16. Richards, F. M. *Ann. Rev. Biophys. Bioeng.*. **1977**, *6*, 151-176.
17. Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. *Structure* **1994**, *2*, 293-308.
18. Sandler, S. R.; Karo, W. "Organic Functional Groups Preparations". p 274-281. Academic Press **1968.**
19. Cherbuliez, E; Plattner, P. *Helv. Chim. Acta* **1929**, *12*, 317.

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