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Regioselective lipase-catalysed γ-monoamidation of D-glutamic acid diesters: effect of the *N*-protecting group

Santiago Conde,* Paloma López-Serrano† and Ana Martínez

Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

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Abstract

N-Blocked-D-glutamic acid diethylesters are γ-regioselectively amidated in a reaction catalysed by *Candida antarctica* lipase B, carried out in an anhydrous organic solvent. The ratio of γ:α-monoamides, as a measure of regioselectivity, depends on the *N*-protecting group present in the substrate. In the examples reported in this work, we have found that γ:α ranges from about 4 (*N*-group = Cbz) to 35 (*N*-group = isobutyryl). No diamides were detected.

1. Introduction

Amino acid derivatives generally are a cheap and easy source of chiral building blocks,[1] although this availability is not so clear in the case of differently functionalised (acid–ester, ester–amide, etc.) dicarboxylic amino acid derivatives. The reason for this is that the synthesis of these compounds requires selective protection/deprotection steps, generally leading to low yields. The study of the *Candida antarctica* lipase B (CAL from here on) catalysed amidation of diesters of dicarboxylic amino acids is one of our research lines, with the double aim of a convenient synthetic method to obtain these 'asymmetric' derivatives and an increase of the empirical understanding of the enzyme active site. We have followed the strategy of keeping constant all the experimental conditions while a single point of the substrate was changed in order to study its effect on the activity and selectivity of the reaction. The research was initially centred on the *N*-blocked-L-glutamic[2] acid derivatives as a representative model. We found that the protecting group (P.G.) affected the reaction rate but not the regioselectivity of the reaction[3] and α-monoamides were exclusively obtained in all cases. However, the regioisomer obtained depended on the stereochemistry of the amino acid moiety. Low γ-selectivity was found in a unique example when *N*-Cbz-D-Glu(OEt)OEt was amidated under the same experimental conditions: a 3.9:1 γ:α ratio of monoamides was obtained[4] at a lower reaction rate than its L-counterpart.

* Corresponding author. E-mail: sconde@iqm.csic.es

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The high reaction rate and α-regioselectivity exhibited by L-glutamic acid derivatives indicates that the substrate's preferred way of approaching the catalytic serine in the active site is the conformation **I** represented, in a very simplified way, in Fig. 1. This favoured conformation **I** is not possible for the corresponding D-glutamic acid derivatives: they must reach the catalytic serine in an enantiomerically opposite conformation **II**, among others, to yield α-monoamides. The γ-substitution would take place through a conformation type **III**, where the serine 'sees' the stereogenic carbon approaching in the geometrically convenient position of the groups but at a two methylene chain longer distance.

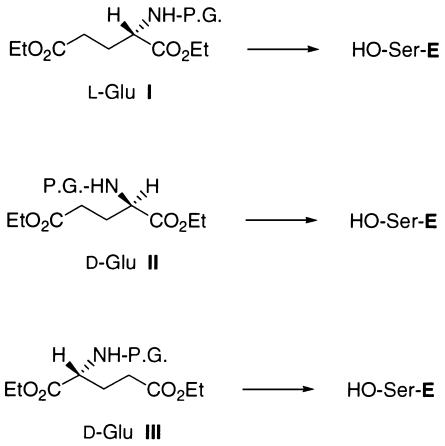


Figure 1. Representative L- and D-glutamic derivative configurations approaching the enzyme catalytic serine to afford α- (**I**, **II**) and γ-monoamides (**III**)

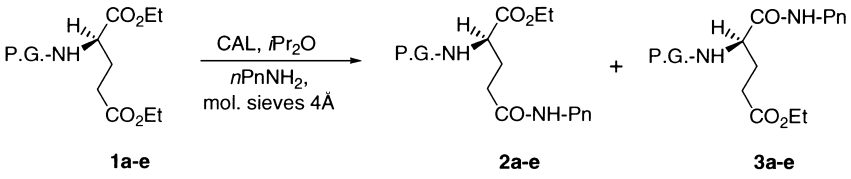
The acyl binding pocket of the CAL active site has been reported[5] to be wider than the nucleophile one, involving less restrictive sterical requirements for acceptance of a substrate. So, while the enantioselective amidation of esters is a useful method to separate racemic mixtures of chiral amines,[6] it is not as efficient for chiral esters. The enzyme can frequently accommodate both enantiomers of chiral acyl groups with little kinetic differences between them,[7] resulting in a low enantioselectivity, although some resolutions of racemic esters have also been reported.[8] It can be expected that the *N*-protecting group of the D-glutamic acid diester derivative plays an important role in the relative importance of conformations **II** (α-substitution) over **III** (γ-substitution), and hence lower or raise the regioselective γ:α ratio of the reaction. In this paper we report the results of our studies on the CAL-catalysed amidation of *N*-blocked-D-glutamic acid diethylesters and the dependence of the γ-regioselectivity on the *N*-protecting group. The double aim of this work is a deeper knowledge of the CAL active site interactions with this kind of substrates (dicarboxylic amino acids) and a synthetically useful γ-regioselective reaction of D-glutamic acid derivatives.

2. Results and discussion

Reports on lipase-catalysed reactions of D-glutamic acid derivatives are very scarce[9] and, to our knowledge, there are none related to amidation reactions apart from our own precedent. The CAL-catalysed α-amidation of *N*-P.G.-L-Glu(OEt)OEt with *n*-pentylamine showed differences in the reaction rates depending on the protecting group: while the most reactive derivative was the aromatic

carbamate *N*-Cbz, sterically hindered *N*-amides exhibited lower reactivity and bulky *N*-trityl derivative did not react at all.[3] Based on these results, the following *N*-P.G.-D-Glu(OEt)OEt derivatives were synthesised: *N*-benzyloxycarbonyl (Cbz) **1a**, *N*-acetyl (Ac) **1b**, *N*-*t*butyloxycarbonyl (Boc) **1c**, *N*-phenylacetyl (PhAc) **1d** and *N*-*i*butyryl (*i*PrCO) **1e**. They were selected because their corresponding L-series derivatives exhibited a gradually increasing **e** to **a** reactivity (except *N*-Boc that is slightly more reactive than *N*-Ac derivative) which may be accepted as a better fit of their conformation **I** in the acyl binding pocket of the enzyme active site. *i*Butyryl was synthesised instead of pivaloyl (checked as a substrate in the L-series) because of the expected low reaction rate of the corresponding D-derivative. All of them were easily obtained using standard procedures.

The reactions were initially performed on an analytical scale in screw-cap 2 ml vials containing a suspension of CAL and molecular sieves 4 Å in an anhydrous diisopropylether solution of the D-Glu diethylester **1a–e**, *n*-pentylamine and *N*-methylacetanilide or *N*-methylbutyranilide as internal standard. All the reactions afforded a mixture of two products at variable ratios that, when isolated from preparative scale reactions, were identified as the corresponding γ-monoamide **2a–e** as the major product in all cases, and the α-monoamide **3a–e** (Fig. 2). Aliquots were periodically withdrawn from the analytical scale reactions and analysed by HPLC in order to compare the progress of the reactions.



Protecting group (P.G.): **a**: Cbz, **b**: Ac, **c**: Boc, **d**: PhAc, **e**: *i*PrCO

Figure 2. CAL-catalysed amidation of *N*-protected-D-glutamic acid diesters. Theoretically possible diamide was not detected in any reaction

Although γ-monoamide was always the main product, there were remarkable differences in the reactivity and, more important, in the regioselectivity of the reactions. The substrates **1a** and **1b**, bearing the well-reacting groups Cbz and Ac, reached a level of about 90% conversion in a 7 hour period while **1c–e** needed 18 hours to achieve a similar conversion (Table 1).

Table 1
Amidation[a] of diesters **1a–e**. Conversion[b] (%) at an analytical scale

Time (h)	**2a**	**3a**	**2b**	**3b**	**2c**	**3c**	**2d**	**3d**	**2e**	**3e**
1	33	8	47	8	16	1	18	2	23	2
3	56	13	64	12	32	2	42	5	32	2
5	68	16	71	12	43	3	53	5	43	2
7	70[c]	18[c]	81[c]	12[c]	50	3	59	5	51	3
18					86	4	79	5	85	3
24					92	5	87	5	90	3

[a]. No diamides were detected.
[b]. Internal standard, HPLC data.
[c]. The reaction hardly progressed after 7 hours.

On the contrary, the slowest substrates **1c**–**e** exhibited a higher regioselectivity than **1a**,**b**. Fig. 3 shows the ratio of γ- to α-monoamide obtained from analytical-scale reactions as a measure of the regioselectivity of the process. Diamide was not detected over a 24 hour period. Reactions **a** and **b** showed moderate γ:α ratios, 3.9 and 6.6, respectively, but **c**, **d** and **e** were highly regioselective and provided γ:α values of about 20 (**c**), 18 (**d**) and 35 (**e**). In an attempt to increase the regioselectivity, the reactions **a** and **b** were also carried out at 45 instead of 60°C, but only modest increments of γ:α ratio, up to 4.5 and 8.3, were obtained while conversion fell from 88 and 94 to 67 and 65%, respectively, in the same 7 hour reaction time.

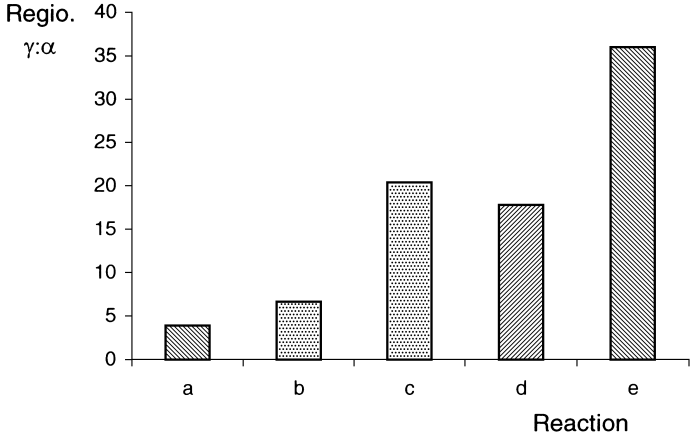


Figure 3. γ:α-Monoamide ratios as a measure of regioselectivity. Analytical scale reactions with internal standard, HPLC data

These data may be explained by the kinetic differences for approaching the catalytic serine of conformations **II** and **III** (Fig. 1) and confirm that the α- or γ-regioselectivity of the reaction basically depends on the enantiomer used. According to the high reactivity and regioselectivity exhibited by the L-Glu derivatives tested,[3] we can accept that CAL prefers L- over D-counterparts and hence, conformation **I** (α-amidation of L-Glu derivatives) fulfils the electronic and sterical requirements of the active site better than any other conformation. Considering the D-series, the spatial distribution of the groups with respect to the catalytic serine equivalent to **I** is found in **III**. It probably is the CAL's preferred conformation as the γ-regioselectivity of D-substrates suggests, although is not as favoured as **I** in the L-series (D- are less reactive than their analogous L-derivatives), probably due to the two methylene group separation of the stereogenic centre from the γ-ester. The CAL's modest enantioselectivity reported for the acyl moiety of the substrate[5] indicates that conformation **II** may also, to some extent, be accepted by the acyl binding site although it is opposite to the preferred **I**. The grade of acceptance, and hence the kinetic differences between **II** and **III** and the γ:α ratio of monoamides, will depend on the D-derivative protecting group.

In addition to the catalytic triad, there are three polar amino acids (Thr40, Asp134 and Gln157) that have their polar side chain atoms within 5 Å of the catalytic serine (Ser105) hydroxyl group,[10] forming a hydrogen bond network. We reported[3] that carbamates might interact with this electronic network and display higher reaction rates than the corresponding amides. This may also be true in the D-series: the whole -O-CO-NH- group also lies within 5 Å of the catalytic

ester bond in **II** and, although it is not located in the best position, has the possibility of interacting with the electronic network of the active site, affording the low regioselectivity and comparative high reactivity of this derivative. In the case of the other carbamate **1c**, the high sterical hindrance introduced by this derivative makes the proposed carbamate effect less important and increases the kinetic difference of **III** over **II**. Bulky derivatives **1d** and **1e** probably face even more problems than **1c** to fit in conformation **II**, increasing the regioselectivity while, due to its small size, **1b** (Ac) presents less impediment to bind **II** to the acyl site, giving rise to a lower regioselectivity and a comparatively higher reactivity. The amidation of the three **1c–e** derivatives provides a synthetically useful γ-regioselective process.

2.1. Structural elucidation

We have shown in previous work that, in the CAL-catalysed monoamidations of L-glutamic derivatives,[4] 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 newly formed amide group. This chemical shift displacement rule was validated in some other dicarboxylic amino acid diesters as aminoadipic or aminopimelic acids.[11] Now, the structures of the γ-monoamides **2a–e** have been established by comparison of their ^{1}H and ^{13}C NMR spectra with those of their starting diethylesters **1a–e** (Table 2). In all cases, higher chemical shift differences in the γ-CH_2 ($\Delta\delta\approx0.15$ ppm in ^{1}H NMR and $\Delta\delta\approx-2$ ppm in ^{13}C NMR) than in the α-CH ($\Delta\delta\approx0.05$ ppm and $\Delta\delta\approx-0.3$ ppm, respectively) were observed, pointing out to a γ-monoamidation.

Table 2
Chemical shift differences of **2a–e** and **3e**

Comp.	^{1}H-NMR[a] (Δδ, ppm)[b]		^{13}C-NMR[c] (Δδ, ppm)[b]	
	α–CH	γ-CH_2	α–CH	γ-CH_2
2a	0.07	0.17	−0.33	−1.45
2b	0.09	0.13	−0.38	−2.33
2c	0.05	0.15	−0.13	−2.31
2d	0.09	0.12	−0.33	−2.33
2e	0.19	0.14	−0.56	−2.03
3e	0.11	−0.05	−0.93	−0.44

[a] 300 MHz, $CDCl_3$
[b] $\Delta\delta = \delta_{diester} - \delta_{monoamide}$
[c] 50 MHz, $CDCl_3$

The chemical structure of the α-monoamides **3a–e** obtained were assigned following this same rule and by comparison of their NMR spectra and HPLC retention time with their L-series enantiomers previously reported.[3] Chemical shift differences of α-monoamide **3e**, gathered in Table 2, clearly show that the monoamidation took place in the α position.

3. Conclusions

The α- or γ-regioselectivity of the CAL-catalysed amidation of *N*-protected glutamic acid diesters basically depends on the enantiomer used: α-monoamides are exclusively obtained in the L-series and, at a lower reaction rate, γ-monoamides are the major products in the D-series. The γ:α ratio of D-monoamides is related to the *N*-protecting group and it reaches synthetically useful values when suitable groups are employed. Diamides were not found in any of the cases.

4. Experimental

The structural assignment of all compounds was made according to their ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$ solution using a Varian-Gemini 200 or Varian XL-300 spectrometer. Elemental analyses were performed on a Perkin–Elmer 240C instrument. Melting points were determined with a Reichert–Jung apparatus equipped with a microscope and are uncorrected. Optical rotations were determined on a Perkin–Elmer 241C polarimeter. Analytical HPLC was performed on a Beckman Chromatograph (flow rate 1 ml/min) using a UV detector at $\lambda = 200$ nm; the column employed was a Deltapak C_{18} 5μ (3.9×15 mm) with acetonitrile:H_2O (containing trifluoroacetic acid 0.05% v/v) as eluent. Chromatographic separations were carried out in columns using the flash chromatographic technique on silica gel 230–240 mesh (Merck). Diisopropyl ether was refluxed on sodium wire, distilled and stored on molecular sieves 4 Å. *n*Pentylamine was distilled and stored on KOH pellets. Novozym 435, a Novo Nordisk commercial immobilised preparation of CAL was used as received.

4.1. N-*P.G.*-D-*Glu(OEt)OEt* ***1a–e***

The substrates were synthesised from commercial D-glutamic acid, following two general methods (for **1a–c** and **1d–e**, respectively) previously reported by us.[11]

4.1.1. N-*Cbz*-D-*Glu(OEt)OEt* ***1a***

Purified by silica gel column chromatography (hexane:AcOEt, 4:1). A syrup that changed into colorless needles when treated with cold hexane (70%), mp 48°C; $[\alpha]_D = -6.1$ (*c* 1, $CHCl_3$). ^{1}H NMR: 7.36 (m, 5H, Ar Cbz), 5.42 (d, 1H, α-NH), 5.09 (s, 2H, CH_2 Cbz), 4.39 (m, 1H, CH-α), 4.15 (q, 2H, CH_2 Et), 4.10 (q, 2H, CH_2 Et), 2.38 (m, 2H, CH_2-γ), 2.21 (m, 1H, CH_2-β_1), 2.00 (m, 1H, CH_2-β_2), 1.26 (t, 3H, CH_3 Et), 1.22 (t, 3H, CH_3 Et). ^{13}C NMR: 172.5, 171.5 (CO-α, CO-γ), 155.9 (CO-Cbz), 136.2–127.1 (Ar, Cbz), 67.0 (CH_2 Cbz), 60.5, 61.5 (CH_2 Et), 53.3 (C-α), 30.1 (C-γ), 27.6 (C-β), 14.0, 13.9 (CH_3 Et).

4.1.2. N-*Ac*-D-*Glu(OEt)OEt* ***1b***

A colorless solid (87%), mp 39–40°C; $[\alpha]_D = -27.4$ (*c* 1, $CHCl_3$). ^{1}H NMR: 6.32 (d, 1H, α-NH), 4.54 (m, 1H, CH-α), 4.14 (q, 2H, CH_2 Et), 4.07 (q, 2H, CH_2 Et), 2.34 (m, 2H, CH_2-γ), 2.15 (m, 1H, CH_2-β_1), 1.96 (m, 1H, CH_2-β_2), 1.96 (s, 3H, CH_3 Ac), 1.23 (t, 3H, CH_3 Et), 1.18 (t, 3H, CH_3 Et). ^{13}C NMR: 172.8, 171.9 (CO-α, CO-γ), 170.0 (CO-Ac), 61.5, 60.6 (CH_2 Et), 51.7 (C-α), 30.3 (C-γ), 27.3 (C-β), 22.9 (CH_3 Ac), 14.0, 14.0 (CH_3 Et). Calcd for $C_{11}H_{19}NO_5$ (%): C, 53.88; H, 7.75; N, 5.71. Found (%): C, 53.71; H, 7.92; N, 5.55.

4.1.3. N-*Boc-D-Glu(OEt)OEt* ***1c***

Purified by silica gel column chromatography (hexane:AcOEt, 9:1). A colorless solid (54%), mp 45°C; $[\alpha]_D = -10.6$ (*c* 1, $CHCl_3$). ^1H NMR: 5.12 (d, 1H, α-NH), 4.21 (m, 1H, CH-α), 4.12 (q, 2H, CH_2 Et), 4.06 (q, 2H, CH_2 Et), 2.33 (m, 2H, CH_2-γ), 2.11 (m, 1H, CH_2-β_1), 1.88 (m, 1H, CH_2-β_2), 1.36 (s, 9H, CH_3 Boc), 1.20 (t, 3H, CH_3 Et), 1.18 (t, 3H, CH_3 Et). ^{13}C NMR: 172.6, 172.1 (CO-α, CO-γ), 155.2 (CO-Boc), 79.7 (C Boc), 61.3, 60.5 (CH_2 Et), 52.9 (C-α), 30.2 (C-γ), 28.2 (CH_3, Boc), 27.6 (C-β), 14.0, 14.0 (CH_3 Et). Calcd for $C_{14}H_{25}NO_6$ (%): C, 55.44; H, 8.25; N, 4.62. Found (%): C, 55.21; H, 8.40; N, 4.64.

4.1.4. N-*PhAc-D-Glu(OEt)OEt* ***1d***

Purified by silica gel column chromatography (hexane:AcOEt, 5:1). A colorless solid (34%), mp 40–41°C; $[\alpha]_D = -10.0$ (*c* 1, $CHCl_3$). ^1H NMR: 7.24 (m, 5H, Ar PhAc), 6.15 (d, 1H, α-NH), 4.51 (m, 1H, CH-α), 4.08 (q, 2H, CH_2 Et), 4.02 (q, 2H, CH_2 Et), 3.52 (s, 2H, CH_2 PhAc), 2.22 (m, 2H, CH_2-γ), 2.10 (m, 1H, CH_2-β_1), 1.86 (m, 1H, CH_2-β_2), 1.17 (t, 3H, CH_3 Et), 1.17 (t, 3H, CH_3 Et). ^{13}C NMR: 172.7, 171.6 (CO-α, CO-γ), 170.8 (CO-PhAc), 134.5, 129.3, 128.9, 127.3 (Ar, PhAc), 61.5, 60.6 (CH_2 Et), 51.7 (C-α), 43.5 (CH_2-Ph PhAc), 30.1 (C-γ), 27.2 (C-β), 14.1, 14.0 (CH_3 Et). Calcd for $C_{17}H_{23}NO_5$ (%): C, 63.53; H, 7.21; N, 4.36. Found (%): C, 63.80; H, 7.50; N, 4.44.

4.1.5. N-i*PrCO-D-Glu(OEt)OEt* ***1e***

Purified by silica gel column chromatography (hexane:AcOEt, 5:1). A colorless solid (53%), mp 53–56°C; $[\alpha]_D = +26.0$ (*c* 1, $CHCl_3$). ^1H NMR: 6.31 (d, 1H, α-NH), 4.49 (m, 1H, CH-α), 4.10 (q, 2H, CH_2 Et), 4.04 (q, 2H, CH_2 Et), 2.35 (m, 1H, CH-CO), 2.30 (m, 2H, CH_2-γ), 2.13 (m, 1H, CH_2-β_1), 1.92 (m, 1H, CH_2-β_2), 1.19 (t, 3H, CH_3 Et), 1.18 (s, 9H, CH_3 *i*PrCO), 1.16 (t, 3H, CH_3 Et), 1.07 (d, 3H, CH_3-CH), 1.07 (d, 3H, CH_3-CH). ^{13}C NMR: 172.8, 171.9 (CO-α, CO-γ), 176.8 (CO-*i*PrCO), 61.4, 60.5 (CH_2 Et), 51.4 (C-α), 35.2 (C *i*PrCO), 30.2 (C-γ), 27.1 (C-β), 19.3, 19.2 (CH_3, *i*PrCO), 14.0, 13.9 (CH_3 Et). Calcd for $C_{13}H_{23}NO_5$ (%): C, 57.13; H, 8.48; N, 5.12. Found (%): C, 57.43; H, 8.70; N, 5.21.

4.2. Enzymatic reactions. General procedure

CAL (50 mg/ml) and molecular sieves 4 Å (50 mg/ml) were added to a diisopropyl ether solution containing the corresponding substrate **1a**–**e** (20 mM) and *n*pentylamine (50 mM) and the resulting mixture was incubated at 60°C in an orbital shaker at 250 r.p.m.

The reactions were initially performed at an analytical scale in 2 ml screw cap vials containing also 5 mM of *N*-methylacetanilide **1a**,**b** or *N*-methylbutyranilide **1c**–**e** as internal standards. Aliquots (20 μl) were periodically withdrawn, evaporated, dissolved in acetonitrile and centrifuged, and then analysed by HPLC (conditions specified for each case). Preparative reactions were carried out under the same experimental conditions on a 70–150 mg scale, without the internal standard. After conclusion, the enzyme and molecular sieves were filtered off and washed with acetonitrile, methanol and methylene chloride. The combined organic extracts were evaporated and the residue chromatographed (hexane/EtAcO) to yield the α- and γ-monoamides, always in this order of elution.

4.2.1. Amidation of N-*Cbz-D-Glu(OEt)OEt* ***1a***

HPLC: $\lambda = 200$ (0 to 6 min) and 215 nm (6 to 30 min), water:acetonitrile, 65:35. Preparative scale, 100 mg, the reaction was stopped after 12 hours and the final products were purified by silica gel column hexane:AcOEt, 3:1.

N-Cbz-D-Glu(NHPn)OEt **2a** (64%) and *N*-Cbz-D-Glu(OEt)NHPn **3a** (9%), in accordance to authentical samples previously synthesised by us.[4]

4.2.2. *Amidation of* N-*Ac-D-Glu(OEt)OEt* ***1b***

HPLC: $\lambda = 200$ nm, water:acetonitrile, 85:15. Preparative scale: 70 mg, 12 hours, silica gel column hexane:AcOEt, 1:1.

N-Ac-D-Glu(NHPn)OEt **2b** (53%): a colorless solid, m.p. 104°C; $[\alpha]_D = -9.2$ (*c* 1, $CHCl_3$). ^{1}H NMR: 6.84 (d, 1H, α-NH), 6.37 (s, 1H, NH-Pn), 4.45 (m, 1H, CH-α), 4.13 (q, 2H, CH_2 Et), 3.17 (m, 2H, CH_2-NH), 2.20 (m, 2H, CH_2-γ), 2.15 (m, 1H, CH_2-β_1), 1.98 (m, 1H, CH_2-β_2), 1.98 (s, 3H, CH_3 Ac), 1.22 (t, 3H, CH_3 Et), 0.83 (t, 3H, CH_3 Pn). ^{13}C NMR: 172.0, 172.0 (CO-α, CO-γ), 170.5 (CO-Ac), 61.5 (CH_2 Et), 52.1 (C-α), 39.6 (NH-CH_2), 32.6 (C-γ), 29.1, 29.0, 22.2 (CH_2-Pn), 28.4 (C-β), 23.0 (CH_3 Ac), 14.0 (CH_3 Et), 13.9 (CH_3 Pn). Calcd for $C_{14}H_{26}N_2O_4$ (%): C, 58.74; H, 9.09; N, 9.79. Found (%): C, 59.00; H, 9.12; N, 9.49.

N-Ac-D-Glu(OEt)NHPn **3b** (less than 2%): identified by non-chiral HPLC and ^{1}H NMR by comparison with a sample of the L-enantiomer.[3]

4.2.3. *Amidation of* N-*Boc-D-Glu(OEt)OEt* ***1c***

HPLC: $\lambda = 240$ (0 to 10 min) and 200 nm (10 to 30 min), water:acetonitrile, 68:32. Preparative scale: 150 mg, 24 hours, silica gel column hexane:AcOEt, 2:1.

N-Boc-D-Glu(NHPn)OEt **2c** (86%): a colorless solid, m.p. 76–77°C; $[\alpha]_D = -5.4$ (*c* 1, $CHCl_3$). ^{1}H NMR: 6.34 (d, 1H, α-NH), 5.41 (s, 1H, NH-Pn), 4.16 (m, 1H, CH-α), 4.10 (q, 2H, CH_2 Et), 3.14 (m, 2H, CH_2-NH), 2.17 (m, 2H, CH_2-γ), 2.10 (m, 1H, CH_2-β_1), 1.85 (m, 1H, CH_2-β_2), 1.36 (s, 9H, CH_3 Boc), 1.19 (t, 3H, CH_3 Et), 0.80 (t, 3H, CH_3 Pn). ^{13}C NMR: 172.2, 171.8 (CO-α, CO-γ), 155.8 (CO-Boc), 79.8 (C Boc), 61.3 (CH_2 Et), 53.0 (C-α), 39.4 (NH-CH_2), 32.5 (C-γ), 29.0, 28.9, 22.2 (CH_2-Pn), 28.1 (C-β), 28.1 ((CH_3)$_3$ Boc), 14.0 (CH_3 Et), 13.8 (CH_3 Pn). Calcd for $C_{17}H_{32}N_2O_5$ (%): C, 59.27; H, 9.36; N, 8.13. Found (%): C, 59.60; H, 9.18; N, 8.12.

N-Boc-D-Glu(OEt)NHPn **3c** (less than 2%): identified by non-chiral HPLC and ^{1}H NMR by comparison with a sample of the L-enantiomer.[3]

4.2.4. *Amidation of* N-*PhAc-D-Glu(OEt)OEt* ***1d***

HPLC: $\lambda = 200$ nm, water:acetonitrile, 65:35. Preparative scale: 100 mg, 24 hours. The final products were purified by silica gel column hexane:AcOEt, 6:1, progressively changing to AcOEt:methanol, 30:1. Although α-monoamide **3d** was detected and evaluated at analytical scale (by comparison with its L-enantiomer in non chiral HPLC), only the γ-regioisomer could be isolated.

N-PhAc-D-Glu(NHPn)OEt **2d** (74%): a colorless solid, m.p. 108–109 °C; $[\alpha]_D = +1.2$ (*c* 4, $CHCl_3$). ^{1}H NMR: 7.23 (m, 5H, Ar PhAc), 6.72 (d, 1H, α-NH), 6.25 (s, 1H, NH-Pn), 4.42 (m, 1H, CH-α), 4.07 (q, 2H, CH_2 Et), 3.51 (s, 2H, CH_2 PhAc), 3.11 (m, 2H, CH_2-NH), 2.09 (m, 2H, CH_2-γ), 2.09 (m, 1H, CH_2-β_1), 1.82 (m, 1H, CH_2-β_2), 1.19 (t, 3H, CH_3 Et), 0.82 (t, 3H, CH_3 Pn). ^{13}C NMR: 171.8, 171.6 (CO-α, CO-γ), 171.3 (CO-PhAc), 134.5, 129.2, 128.8, 127.2 (Ar PhAc), 61.5 (CH_2 Et), 52.0 (C-α), 43.4 (CH_2 PhAc), 39.5 (NH-CH_2), 32.5 (C-γ), 29.1, 29.0, 22.2 (CH_2-Pn), 28.4 (C-β), 14.0 (CH_3 Et), 13.9 (CH_3 Pn). Calcd for $C_{20}H_{30}N_2O_4$ (%): C, 66.27; H, 8.34; N, 7.73. Found (%): C, 66.42; H, 8.60; N, 7.52.

N-PhAc-D-Glu(OEt)NHPn **3d** (less than 2%): identified by non-chiral HPLC by comparison with a sample of its L-enantiomer.[3]

4.2.5. Amidation of N-i*PrCO-D-Glu(OEt)OEt* ***1e***

HPLC: $\lambda = 200$ nm, water:acetonitrile, 80:20. Preparative scale: 150 mg, 17 hours, silica gel column hexane:AcOEt, 2:1, progressively changing to net EtOAc. Recovered starting material **1e** was initially isolated (13%), followed by the two monoamides **3e** and **2e**.

*N-i*PrCO-D-Glu(NHPn)OEt **2e** (72%): a colorless solid, m.p. 113–115°C; $[\alpha]_D = -2.3$ (*c* 2, $CHCl_3$). ^{1}H NMR: 6.78 (d, 1H, α-NH), 6.08 (s, 1H, NH-Pn), 4.30 (m, 1H, CH-α), 4.03 (q, 2H, CH_2 Et), 3.07 (m, 2H, CH_2-NH), 2.33 (m, 1H, CH *i*PrCO), 2.16 (m, 2H, CH_2-γ), 2.00 (m, 1H, CH_2-β_1), 1.87 (m, 1H, CH_2-β_2), 1.12 (t, 3H, CH_3 Et), 1.06 (d, 3H, CH_3 *i*PrCO), 1.04 (d, 3H, CH_3 *i*PrCO), 0.75 (t, 3H, CH_3 Pn). ^{13}C NMR: 177.4 (CO-*i*PrCO), 172.1, 171.8 (CO-α, CO-γ), 61.1 (CH_2 Et), 51.9 (C-α), 39.4 (NH-CH_2), 35.0 (CH *i*PrCO), 32.3 (C-γ), 28.9, 28.8, 22.1 (CH_2-Pn), 27.5 (C-β), 19.2 [$(CH_3)_2$ *i*PrCO]; 13.8 (CH_3 Et), 13.7 (CH_3 Pn). Calcd for $C_{16}H_{30}N_2O_4$ (%): C, 61.12; H, 9.62; N, 8.91. Found (%): C, 61.40; H, 9.92; N, 8.63.

*N-i*PrCO-D-Glu(OEt)NHPn **3e** (2–3%): a colorless solid, m.p. 117–119°C; $[\alpha]_D = +7.8$ (*c* 0.5, $CHCl_3$). ^{1}H NMR: 6.42 (d, 1H, α-NH), 6.42 (s, 1H, NH-Pn), 4.37 (m, 1H, CH-α), 4.12 (q, 2H, CH_2 Et), 3.21 (m, 2H, CH_2-NH), 2.52 (m, 1H, CH *i*PrCO), 2.35 (m, 2H, CH_2-γ), 2.08 (m, 1H, CH_2-β_1), 1.94 (m, 1H, CH_2-β_2), 1.24 (t, 3H, CH_3 Et), 1.14 (d, 3H, CH_3 *i*PrCO), 1.12 (d, 3H, CH_3 *i*PrCO), 0.87 (t, 3H, CH_3 Pn). ^{13}C NMR: 177.3 (CO-*i*PrCO), 173.7, 171.1 (CO-α, CO-γ), 60.8 (CH_2 Et), 52.3 (C-α), 39.5 (NH-CH_2), 35.5 (CH *i*PrCO), 30.7 (C-γ), 29.1, 29.0, 22.3 (CH_2-Pn), 27.8 (C-β), 19.5, 19.4 [$(CH_3)_2$ *i*PrCO]; 14.2 (CH_3 Et), 13.9 (CH_3 Pn). Calcd for $C_{16}H_{30}N_2O_4$ (%): C, 61.12; H, 9.62; N, 8.91. Found (%): C, 61.23; H, 9.50; N, 8.75.

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