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Study of the removal of allyl esters by *Candida antarctica* lipase B (CAL-B) and pig liver esterase (PLE)

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A number of allyl esters of various carboxylic acids and *N*-protected amino acids were synthesized and their hydrolysis by *Candida antarctica* lipase B and pig liver esterase was studied. In order to test the selectivity, the enzymatic hydrolysis of the corresponding methyl and ethyl esters was also examined. Both enzymes easily remove the allyl esters of monocarboxylates. The chemo- and regio-selectivity for the hydrolysis of glutamate diesters was studied, too, and it was found that the preference for the hydrolysis of a particular ester group depends not only on the ease of the hydrolysis observed for the esters of monocarboxylic acids, but also on the position (α- or γ-).

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1. Introduction

During recent years, enzymes have been extensively used in organic synthesis, not only in small-scale laboratory syntheses but also in the industrial production of fine chemicals. Biocatalysts offer great alternative to chemical methods. Moreover, they are environmentally beneficial and of increasing interest by chemical and pharmaceutical industries.

In particular, functional group deprotection is one of the procedures that usually demands strong alkaline, acidic or reductive conditions. Removal of esters by means of hydrolases takes place in almost neutral pH values, ambient temperature and aqueous solution. Due to high chemo-, regio-, and enantioselectivities of lipases and esterases, biocatalysis has found wide application mainly for the production of enantiopure organic molecules [1] but also in protecting group chemistry [2,3]. Methyl, heptyl, 2-*N*-(morpholino)ethyl, choline, (methoxyethoxy)ethyl, and methoxyethyl esters are some examples of enzymatically removable carboxyl protecting groups [4].

Recently, we demonstrated that an esterase from *Bacillus subtilis* (BS2, EC 3.1.1.1) and lipase A from *Candida antarctica* (CAL-A, EC 3.1.1.3) were able to cleave *tert*-butyl, methyl, benzyl, allyl, 2-chloroethyl, 2,2,2-trichloroethyl, phenacyl, and diphenylmethyl esters from a variety of substrates including simple carboxylic acids, *N*-protected amino acids and dipeptides, under mild and selective conditions, that avoid side-reactions [5–7]. To extend our studies, we investigated the applicability of various lipases and esterases for the removal of allyl esters from a variety of substrates. In particular, two enzymes, pig liver esterase (PLE, EC 3.1.1.1) and lipase B from *Candida antarctica* (CAL-B, EC 3.1.1.3), were found to be active toward allyl esters. Both enzymes are among the most efficient biocatalysts in organic synthesis. CAL-B is frequently used for the preparation of enantiopure alcohols [1]. PLE represents by far the most important enzyme and numerous examples in kinetic resolution and de-symmetrizations can be found [8,9]. Moreover, both enzymes are commercially available at low price. However, up to now none of them has been studied for the removal of the allyl ester group, which represents an important carboxyl protecting group, due to their ease of preparation and their stability under a variety of reaction conditions.

2. Results and discussion

The most common chemical cleavage method for allyl esters is catalytic hydrogenation using Pd^0 [10]. Catalytic hydrogen transfer [11], $Pd(OAc)_2$ [12], $PdCl_2(Ph_3P)_2$ [13], ruthenium [14] and rhodium [15] complexes have also been used in deallylation, as well as the

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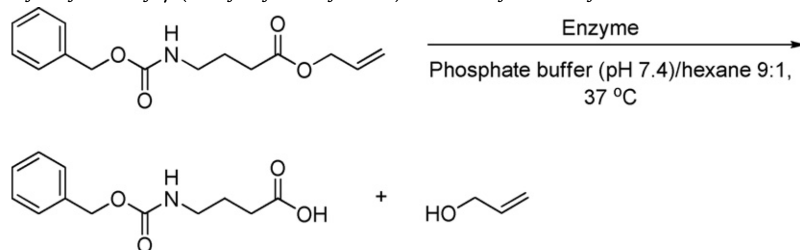
Table 1
Hydrolysis of allyl γ-(benzyloxycarbonylamino)butanoate by various hydrolases.



Entry	Enzyme[a]	Time (h)	Yield (%)[b]
1	BstE	>24	n.d.[c]
2	CRL	>24	n.d.[c]
3	RMIM	4	34
4	TLIM	4	15
5	Amano-PS	4	65
6	PFEI	4	81
7	SDE	3	81
8	PLE	2	87
9	CAL-B	1	90
10	BS2	1	97

[a] For abbreviations, see text.
[b] Yield of isolated product.
[c] Not detected.

DMSO-iodine reagent [16], H-β zeolite [17], sulphated SnO_2 [18], natural kaolinitic clay and EPZG [19], and montmorillonite K-10 [20]. Papain has also reported to be able to cleave allyl esters [21]. In the present work, initially we studied the enzymatic hydrolysis of allyl esters by various hydrolases.

Allyl γ-(benzyloxycarbonylamino)butanoate was prepared, serving as a model compound, and its hydrolysis by a number of hydrolases was studied in an initial screening (Table 1). Recombinant esterase from *Bacillus stearothermophilus* (BstE) produced in *E. coli*, *Candida rugosa* lipase (CRL), immobilized lipase from

Table 2
Hydrolysis of allyl and other esters by CAL-B and PLE.

Entry	Substrate	Product	CALB/substrate 1:4		CALB/substrate 1:30		PLE/substrate 1:4	
			Time (h)	Yield (%)[a]	Time (h)	Yield (%)[a]	Time (h)	Yield (%)[a]
1			1	88	1.5	58	1	90
2			1	92	1.5	75	1	92
3			1	89	1.5	77	1	90
4			1	18	1.5	9		n.i.[b]
5			24	76		n.i.[b]		n.i.[b]
6			24	58		n.i.[b]		n.i.[b]
7	BocHN	BocHN OH	1.5	88		n.i.[b]	>24	n.d.[c]
8	BocHN	BocHN OH	1.5	83		n.i.[b]	4	58
9	BocHN	BocHN OH	1.5	76		n.i.[b]		n.i.[b]

[a] Yield of isolated product.
[b] Not investigated.
[c] Not detected.

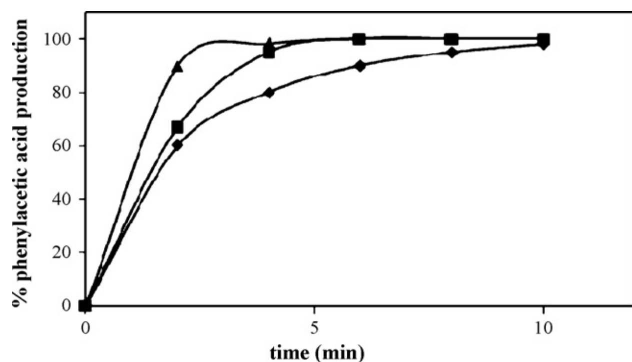


Fig. 1. Time course for the hydrolysis of various phenylacetates by CAL-B (enzyme/substrate 1:4, w/w). Conditions: phosphate buffer (pH 7.4)/hexane 9:1, 37 °C. Methyl phenylacetate (♦), allyl phenylacetate (■), ethyl phenylacetate (▲).

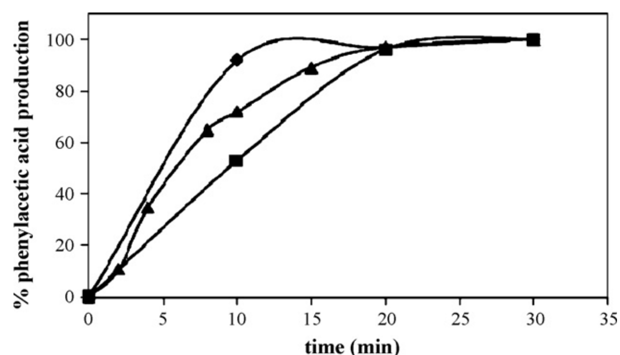


Fig. 2. Time course for the hydrolysis of various phenylacetates by PLE (enzyme/substrate 1:4, w/w). Conditions: phosphate buffer (pH 7.4)/hexane 9:1, 37 °C. Methyl phenylacetate (♦), allyl phenylacetate (■), ethyl phenylacetate (▲).

Rhizomucor miehei (RMIM, Novozymes), *Thermomyces lanuginosa* lipase (TLIM), Amano lipase PS from *Pseudomonas cepacia* (Amano-PS), recombinant esterase from *Pseudomonas fluorescens* (PFEI) produced in *E. coli*, *Streptomyces diastatochromogenes* esterase produced in *E. coli* (SDE), pig liver esterase (PLE, Roche) and lipase B from *Candida antarctica* (CAL-B, Novozymes) were tested toward the model substrate. The reaction took place in a mixture of buffer/hexane (9:1) containing a small amount of methanol. BS2 was also employed and the results are presented in Table 1. Apart from BS2 (entry 10, Table 1), PLE (entry 8, Table 1) and CAL-B (entry 9, Table 1), showed the best activity, within a maximum reaction time of 2 h. Based on the results of this initial screening, CAL-B and PLE were chosen for further studies.

The next step was the preparation of various allyl esters of carboxylic acids and *N*-protected amino acids. For comparison reasons, the hydrolysis of the corresponding methyl and ethyl esters was also studied. The results of their hydrolysis by CAL-B and PLE, using a 1:4 enzyme/substrate ratio, are summarized in Table 2. Both enzymes removed allyl, methyl, and ethyl esters from phenylacetic acid in high yields (entries 1–3, Table 2). CAL-B seemed to be inefficient to cleave the *tert*-butyl group from phenylacetic acid within the same time (entry 4, Table 2). Cinnamic and octanoic acid were isolated in moderate yields from the corresponding allyl esters in the presence of CAL-B, demanding longer reaction time (entries 5 and 6, Table 2). Concerning the allyl and methyl ester of Boc-β-alanine and allyl ester of Boc-γ-aminobutanoic acid, CAL-B was effective, hydrolyzing all three substrates in high yields within 1.5 h (entries 7–9, Table 2). On the contrary, PLE needed longer reaction time in the case of Boc-β-alanine methyl ester, showing no activity at all in the case of Boc-β-alanine allyl ester (entries 7 and 8, Table 2).

The hydrolysis of allyl, methyl and ethyl phenylacetate was also monitored by HPLC and the results are presented in Figs. 1 and 2. As shown, ethyl and allyl esters were quickly hydrolyzed by CAL-B in less than 5 min, while the methyl ester was hydrolyzed quantitatively within 10 min (Fig. 1). Monitoring employing PLE showed a slightly longer reaction time and no differentiation between the three esters (Fig. 2). Similar curves were obtained when lower CAL-B/substrate ratios (1:10 or 1:20) were used (data not shown).

Since the hydrolysis of the above mentioned esters was very quick and non-selective under the conditions used, the removal of these groups using an even lower ratio of CAL-B/substrate (1:30) was examined and the results are presented in Table 2. It is clear that longer reaction times were needed and the methyl ester was slower hydrolyzed by CAL-B, than the allyl and ethyl esters (entries 1–3, Table 2). As expected, *tert*-butyl phenylacetate was a very poor substrate for the lipase (entry 4, Table 2), as it is known that CAL-B is not active towards esters of tertiary alcohols [5]. HPLC monitoring, as depicted in Fig. 3, is consistent with the results taken from Table 2. Hydrolysis of phenylacetic acid allyl ester by CAL-B went to completion within 20 min, while that of ethyl ester needed 1 h (Fig. 3). Removal of methyl ester was 50% within 90 min. When PLE was used in a ratio enzyme/substrate 30:1, the hydrolysis of methyl ester was quantitative in 20 min, while in the same time, ethyl ester was very slowly hydrolyzed (not more than 5%) (Fig. 4).

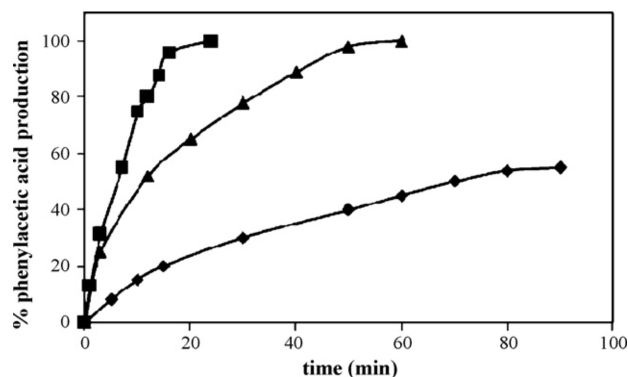


Fig. 3. Time course for the hydrolysis of various phenylacetates by CAL-B (enzyme/substrate 1:30, w/w). Conditions: phosphate buffer (pH 7.4)/hexane 9:1, 37 °C. Methyl phenylacetate (♦), allyl phenylacetate (■), ethyl phenylacetate (▲).

In order to further investigate the quick and possibly selective removal of the groups mentioned above, in case they are present in the same molecule, we synthesized a series of dicarboxylates, based on glutamic acid. The results obtained using CAL-B are summarized in Table 3, and those using PLE are shown in Table 4. In the case of α-ethyl, γ-allyl and α-allyl, γ-ethyl Z-L-glutamates (entries 1 and 2, Table 3), CAL-B shows a preference for ethyl esters, either in γ- or in

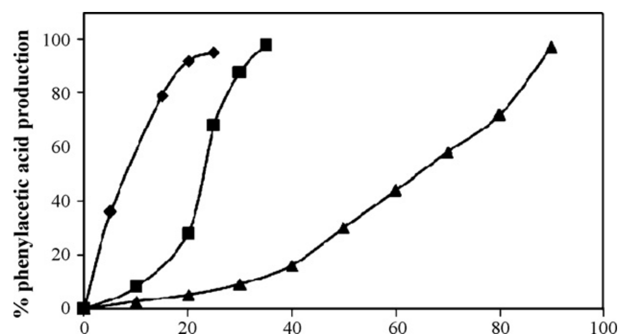


Fig. 4. Time course for the hydrolysis of various phenylacetates by PLE (enzyme/substrate 1:30, w/w). Conditions: phosphate buffer (pH 7.4)/hexane 9:1, 37 °C. Methyl phenylacetate (♦), allyl phenylacetate (■), ethyl phenylacetate (▲).

Table 3
Hydrolysis of various Z-L-glutamic acid diesters by CAL-B.

Entry	Substrate	Product	CAL-B/substrate 1:4		CAL-B/substrate 1:10		CAL-B/substrate 1:30	
			Time (h)	Yield (%)[a]	Time (h)	Yield (%)[a]	Time (h)	Yield (%)[a]
1			4.5	26	4.5	9	4.5	9
			4.5	50	4.5	34	4.5	24
2			4.5	22	4.5	12	4.5	16
			4.5	44	4.5	18	4.5	23
3			24	0		n.i.[b]		n.i.[b]
			24	17		n.i.[b]		n.i.[b]

[a] A mixture of the two products was isolated and the yield of each one was determined from the ^{1}H NMR spectrum of the mixture.
[b] Not investigated.

Table 4
Hydrolysis of various Z-L-glutamic acid diesters by PLE[a].

Entry	Substrate	Product	PLE	
			Time (h)	Yield (%)[b]
1			4.5	40
			4.5	18
2			4.5	47
			4.5	12
3			4.5	26
			4.5	14
4			4.5	30
			4.5	10

[a] Enzyme/substrate ratio 1:30 (w/w).
[b] A mixture of the two products was isolated and the yield of each one was determined from the ^{1}H NMR spectrum of the mixture.

α-position. The reaction rate is lower when the enzyme/substrate ratio is decreased, while the selectivity for ethyl toward allyl ester is not improving (entries 1 and 2, Table 3). The hydrolysis of α-allyl, γ-*tert*-butyl Z-L-glutamate (entry 3, Table 3) is slow and reaches a maximum of 17% yield within 24 h at a CAL-B/substrate ratio 1:4. Although the removal of allyl ester was selective, the yield of the isolated product was not improved even after 48 h (18%).

PLE at an enzyme/substrate ratio 1:30, shows a preference for allyl esters, either in α- or in γ-position (entries 1 and 2, Table 4). Interestingly, in the case of α-methyl, γ-ethyl and α-ethyl, γ-methyl Z-L-glutamates (entries 3 and 4, Table 4), there is a subtle, though noteworthy preference for the hydrolysis of methyl ester of both substrates. Such preference in the removal of methyl ester in the presence of ethyl ester can hardly be accomplished by chemical means.

3. Conclusions

Allyl, methyl and ethyl esters of monocarboxylic acids were easily hydrolyzed by CAL-B and PLE at a 1:4 enzyme/substrate ratio. The structure of the carboxylic acids played a crucial role in the hydrolysis rate. Specifically, when CAL-B was employed at a ratio 1:30 enzyme/substrate, the hydrolysis of allyl and ethyl esters was faster, in comparison to that of the methyl ester. Regarding PLE, the hydrolysis of allyl and methyl esters was significantly faster, compared to the ethyl ester, at the same enzyme/substrate ratio.

In the case of α-allyl, γ-ethyl or γ-allyl, α-ethyl L-glutamates, PLE showed a preference for the hydrolysis of allyl esters, though not quantitatively. On the contrary, when CAL-B was used, there was a preference for the cleavage of ethyl esters, either in α- or in γ-position. When α-ethyl, γ-methyl or α-methyl, γ-ethyl L-glutamates were used as substrates of PLE, the observed preference was in favor of methyl esters. Despite the limited yields, it must be noted that such differentiation between methyl and ethyl esters cannot be achieved by any chemical process. In the case of glutamate diesters, the preference for the hydrolysis of a particular ester group depends not only on the ease of the hydrolysis observed for the esters of monocarboxylic acids, but also on the position (α- or γ-).

4. Experimental

4.1. General

Melting points were determined on a Buchi 530 hot stage apparatus. Specific rotations were measured on a Perkin Elmer 343 polarimeter at 25 °C using a 10 cm cell. NMR spectra were recorded on a 200 MHz Varian spectrometer. TLC plates (silica gel 60 F254) and silica gel 60 (70–230 or 230–400 mesh) were used for column chromatography. Visualization of spots was effected with UV light and/or phosphomolybdic acid and/or ninhydrin, both in EtOH stain. Electron spray ionization (ESI) mass spectra were recorded on a Finnigan, Surveyor MSQ Plus spectrometer. All amino acid derivatives were purchased from Fluka Chemical Co., CAL-B, PLE, CRL, RMIM, TLIM and Amano-PS are commercially available (see Section 2). Details for recombinant enzymes are given in Section 2 and previous work [5–7].

4.2. General method for enzymatic hydrolysis

To a stirred solution of the substrate (0.15–0.40 mmol) in *n*-hexane (1 mL) and CH_3OH (100 μL) was added a solution of the enzyme (12–1.5 mg, as indicated in tables) in phosphate buffer (9 mL, 100 mM, pH 7.4). The reaction mixture was stirred at 37 °C. After acidification until pH 6 and extraction with EtOAc (3× 5 mL), the organic layers were combined and washed with 5% $NaHCO_3$ (3× 5 mL). The aqueous layer was acidified until pH 6 and extracted with EtOAc (3× 10 mL). The combined organic layers were dried over Na_2SO_4, and the organic solvent was removed under reduced pressure to give the product. All products of the enzymatic hydrolysis were identified by their analytical data in comparison with authentic samples.

4.3. Synthesis of substrates

4.3.1. 3-tert-Butoxycarbonylamino-propanoic acid allyl ester (**7**, Table 2)

A mixture of $BocNH(CH_2)_2COOH$ (0.19 g, 1.0 mmol) and Cs_2CO_3 (0.16 g, 0.5 mmol) was dissolved in DMF (5 mL) and a few drops of water were added. The mixture was distilled under reduced pressure to dryness and the residue redistilled twice from DMF (10 mL), until all the water has been removed. The solid cesium salt was stirred with allyl bromide (0.1 mL, 1.15 mmol) in DMF (0.6 mL) overnight at room temperature. After removal of DMF, EtOAc (20 mL) was added, the organic layer was washed consecutively with a saturated solution of $NaHCO_3$, water and brine, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography using $CHCl_3$ as eluent, to give product as an oil (0.19 g, 81%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.91–5.71 (m, 1H, CH=), 5.40–5.16 (m, 3H, CH_2=, NH), 4.58–4.43 (m, 2H, =CHCH_2O), 3.39–3.23 (m, 2H, CH_2NH), 2.45 (t, 2H, *J* = 6.2, CH_2CO), 1.33 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.7, 155.5, 131.7, 118.1, 78.9, 65.0, 35.9, 34.3, 28.19; Anal. Calcd. for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11; Found: C, 57.46; H, 8.51; N, 6.02.

4.3.2. (S)-2-Benzyloxycarbonylamino-pentanedioic acid 5-allyl ester 1-ethyl ester (**1**, Table 3)

Compound **1** (Table 3) was prepared from Z-Glu(OH)-OEt [22] (0.31 g, 1 mmol) according to the procedure described in Section 4.3.1. The residue was purified by column chromatography using $CHCl_3$ as eluent, to give product as an oil (0.31 g, 89%); $[\alpha]_D^{25}$ + 89.6 (c 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.47–7.23 (m, 5H, Ph), 6.04–5.81 (m, 1H, CH=), 5.45 (d, 1H, *J* = 7.2, NH), 5.41–5.18 (m, 2H, CH_2=), 5.11 (s, 2H, CH_2Ph), 4.67–4.53 (m, 2H, =CHCH_2O), 4.46–4.33 (m, 1H, CH), 4.21 (q, 2H, *J* = 7.2, CH_3CH_2O), 2.57–2.35 (m, 2H, CH_2CO), 2.32–2.15 (m, 1H, C*H*HCH), 2.08–1.90 (m, 1H, CH*H*CH), 1.28 (t, 3H, *J* = 7.0, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.3, 171.8, 155.9, 136.1, 131.9, 128.5, 128.2, 128.1, 118.4, 67.0, 65.3, 61.7, 53.3, 30.1, 27.6, 14.1; Anal. Calcd. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01; Found: C, 61.55; H, 6.81; N, 3.91.

4.3.3. (S)-2-Benzyloxycarbonylamino-pentanedioic acid 1-allyl ester 5-ethyl ester (**2**, Table 3)

Compound **2** (Table 3) was prepared from Z-Glu(OEt)-OH (0.31 g, 1 mmol) according to the procedure described in Section 4.3.1. The residue was purified by column chromatography using $CHCl_3$ as eluent, to give product as a white solid (0.25 g, 73%); mp 41–43 °C; $[\alpha]_D^{25}$ + 35.4 (c 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.49–7.17 (m, 5H, Ph), 6.10–5.78 (m, 1H, CH=), 5.52–5.17 (m, 3H CH_2=, NH), 5.12 (s, 2H, CH_2Ph), 4.71–4.54 (m, 2H, =CHCH_2O), 4.49–4.31 (m, 1H, CH), 4.13 (q, 2H, *J* = 7.2, CH_3CH_2O), 2.56–2.29 (m, 2H, CH_2CO), 2.28–2.10 (m, 1H, C*H*HCH), 2.09–1.85 (m, 1H, CH*H*CH), 1.25 (t, 3H, *J* = 7.2, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.6, 171.5, 155.9, 136.1, 131.4, 128.5, 128.2, 128.1, 119.0, 67.0, 66.1, 60.7, 53.4, 30.2, 27.6, 14.1; Anal. Calcd. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01; Found: C, 61.51; H, 6.79; N, 3.91.

*4.3.4. (S)-2-Benzyloxycarbonylamino-pentanedioic acid 1-allyl ester 5-tert-butyl ester (**3**, Table 3)*

To a stirred solution of Z-Glu(OBu^t)-OH (0.34 g, 1 mmol) and allyl alcohol (0.2 mL, 3 mmol) in CH_2Cl_2 (2 mL), 4-(dimethylamino)pyridine (0.01 g, 0.1 mmol) and subsequently *N,N′*-dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) were added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. After filtration, the solvent was evaporated under reduced pressure and EtOAc (20 mL) was added. The organic layer was washed consecutively with brine, 1N HCl, brine, 5% $NaHCO_3$, and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography using $CHCl_3$ as eluent, to give product as an oil (0.29 g, 76%); $[\alpha]_D^{25} + 0.74$ (c 1.1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.32–7.25 (m, 5H, Ph), 5.91–5.78 (m, 1H, CH=), 5.49 (d, 1H, *J* = 8.0, NH), 5.40–5.21 (m, 2H, CH_2=), 5.09 (s, 2H, CH_2Ph), 4.68–4.55 (m, 2H, =CHC*H*$_2$O), 4.45–4.31 (m, 1H, CH), 2.34–2.18 (m, 2H, CH_2CO), 2.16–2.06 (m, 1H, C*H*HCH), 2.01–1.67 (m, 1H, CH*H*CH), 1.41 [s, 9H, $C(CH_3)_3$]; ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.9, 171.6, 155.9, 136.1, 131.4, 128.4, 128.1, 128.0, 118.9, 80.7, 66.9, 66.0, 53.4, 31.3, 27.9, 27.5; Anal. Calcd. for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71; Found: C, 63.42; H, 7.34; N, 3.62.

*4.3.5. (S)-2-Benzyloxycarbonylamino-pentanedioic acid 5-ethyl ester 1-methyl ester (**3**, Table 4)*

Compound **3** (Table 4) was prepared from Z-Glu(OEt)-OH (0.31 g, 1 mmol) and MeOH (0.1 mL, 3 mmol) according to the procedure described in Section 4.3.4. The residue was purified by column chromatography using $CHCl_3$ as eluent, to afford product as an oil (0.23 g, 70%); $[\alpha]_D^{25} + 17.2$ (c 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.47–7.28 (m, 5H, Ph), 5.44 (d, 1H, *J* = 8, NH), 5.12 (s, 2H, CH_2Ph), 4.54–4.35 (m, 1H, CH), 4.12 (q, 2H, *J* = 7 Hz, CH_3C*H*$_2$O), 3.76 (s, 3H, CH_3O), 2.53–2.32 (m, 2H, CH_2CO), 2.30–2.14 (m, 1H, C*H*HCH), 2.09–1.90 (m, 1H, CH*H*CH), 1.25 (t, 3H, *J* = 7.2, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.0, 172.2, 155.9, 136.0, 128.4, 128.1, 128.0, 67.0, 60.8, 53.2, 52.4, 29.8, 27.5, 14.1; Anal. Calcd. for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.33; Found: C, 59.25; H, 6.73; N, 4.19.

*4.3.6. (S)-2-Benzyloxycarbonylamino-pentanedioic acid 1-ethyl ester 5-methyl ester (**4**, Table 4)*

Compound **4** (Table 4) was prepared from Z-Glu(OH)-OEt (0.31 g, 1 mmol) and MeOH (0.1 mL, 3 mmol) according to the procedure described in Section 4.3.4. The residue was purified by column chromatography using $CHCl_3$ as eluent, to afford product as an oil (0.15 g, 46%); $[\alpha]_D^{25} + 5.8$ (c 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.48–7.31 (m, 5H, Ph), 5.43 (d, 1H, *J* = 7.6, NH), 5.12 (s, 2H, CH_2Ph), 4.51–4.34 (m, 1H, CH), 4.13 (q, 2H, *J* = 7 Hz, CH_3C*H*$_2$O), 3.76 (s, 3H, CH_3O), 2.53–2.33 (m, 2H, CH_2CO), 2.30–2.14 (m, 1H, C*H*HCH), 2.05–1.90 (m, 1H, CH*H*CH), 1.25 (t, 3H, *J* = 7.2, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.3, 172.2, 155.9, 136.0, 128.4, 128.0, 127.9, 67.1, 60.6, 53.2, 52.3, 30.1, 27.3, 14.0; Anal. Calcd. for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.33; Found: C, 59.20; H, 6.69; N, 4.22.

Compounds **1**–**3**, **5** and **6** (Table 2) are commercially available. The model substrate [7] (Table 1) and the substrates **4** [23], **8** [24] and **9** [7] (Table 2) were prepared according to known procedures of esterification and their spectroscopic data were in accordance with the given literature.

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References

[1] U.T. Bornscheuer, R.J. Kazlauskas, Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations, 2nd ed., Wiley-VCH, Weinheim, Germany, 2006.
[2] D. Kadereit, H. Waldmann, Chem. Rev. 101 (2001) 3367–3396.
[3] H. Waldmann, D. Sebastian, Chem. Rev. 94 (1994) 911–937.
[4] T.W. Greene, P.G.M. Wuts, Protective Groups in Organic Synthesis, 3rd ed., Wiley-Interscience, New York, 1999.
[5] M. Schmidt, E. Barbayianni, I. Fotakopoulou, M. Höhne, V. Constantinou-Kokotou, U.T. Bornscheuer, G. Kokotos, J. Org. Chem. 70 (2005) 3737–3740.
[6] E. Barbayianni, I. Fotakopoulou, M. Schmidt, V. Constantinou-Kokotou, U.T. Bornscheuer, G. Kokotos, J. Org. Chem. 70 (2005) 8730–8733.
[7] I. Fotakopoulou, E. Barbayianni, V. Constantinou-Kokotou, U.T. Bornscheuer, G. Kokotos, J. Org. Chem. 72 (2007) 782–786.
[8] J.B. Jones, Pure Appl. Chem. 62 (1990) 1445–1448.
[9] S.J. Phytian, Esterases, in: H.J. Rehm, G. Reed, A. Pühler, P.J.W. Stadler, D.R. Kelly (Eds.), Biotechnology Series, Vol. 8a, Wiley-VCH, Weinheim, 1998.
[10] H. Kunz, H. Waldmann, Angew. Chem. Int. Ed. Engl. 23 (1984) 71–72.
[11] P.J. Mandal, J.S. McMurray, J. Org. Chem. 72 (2007) 6599–6601.
[12] L.N. Jungheim, Tetrahedron Lett. 30 (1989) 1889–1892.
[13] H.X. Zang, F. Guibe, G. Balaviome, Tetrahedron Lett. 29 (1988) 623–626.
[14] S. Tanaka, H. Saburi, T. Murase, Y. Ishibashi, M. Kitamura, J. Organom. Chem. 692 (2007) 295–298.
[15] H. Kuntz, H. Waldmann, Helv. Chim. Acta 68 (1985) 618–622.
[16] K.N. Taksande, S.S. Sakate, P.D. Lokhande, Tetrahedron Lett. 47 (2005) 643–646.
[17] R.K. Pandey, V.S. Kadam, R.K. Upadhyay, M.K. Dongare, P. Kumar, Synth. Commun. 33 (2003) 3017–3024.
[18] S.P. Chavan, P.K. Zubaidha, S.W. Dantale, A. Keshavaraja, A.V. Ramaswamy, T. Ravindranathan, Tetrahedron Lett. 37 (1996) 237–240.
[19] A.S. Gajare, M.S. Shingare, V.R. Kulkarni, N.B. Barhate, R.D. Wakharkar, Synth. Commun. 28 (1998) 25–33.
[20] A.S. Gajare, N.S. Shaikh, B.K. Bonde, V.H. Deshpande, J. Chem. Soc. Perkin Trans. 1 (2000) 639–640.
[21] N. Xaus, P. Clapes, E. Bardaji, J.L. Torres, X. Jorba, J. Mata, G. Valencia, Tetrahedron 45 (1989) 7421–7426.
[22] W.J. Le Quesne, G.T. Young, J. Chem. Soc. (1950) 1954–1959.
[23] T. Hama, J.F. Hartwig, Org. Lett. 10 (2008) 1549–1552.
[24] J.A. McCubbin, M.L. Maddess, M. Lautens, Org. Lett. 8 (2006) 2993–2996.