

## Enzymatic Aminolysis of Non-activated Diesters with Diamines

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Selective aminolysis of diesters is catalysed by *Candida antarctica* lipase. Using this enzymatic reaction *N,N'*-polymethylenesuccinimides and *N,N'*-polymethyleneglutarimides can be obtained. When propane-1,2-diamine is used as the nucleophile, the enzyme catalyses the aminolysis of diesters with very good enantioselectivity.

Amide bond formation is of importance because of its involvement in the protection-deprotection of amino compounds, and also because of the ubiquity of the amide group in bioactive compounds.<sup>1</sup>

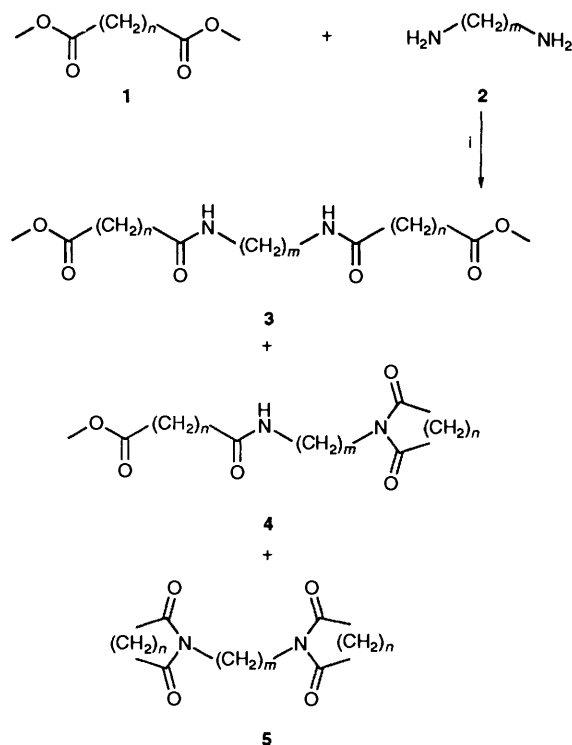
The employment of organic solvents in biotransformations has extended the application of enzymes to processes which are either difficult or impossible to carry out in water.<sup>2</sup> Lipases are the most widely used biocatalysts in non-aqueous media, and display considerable synthetic utility<sup>3</sup> as a result of their ability to accept a wide range of substrates.

Several years ago, we discovered that *Candida cylindracea* lipase (CCL) is an excellent catalyst for the aminolysis of activated esters such as 2-chloropropionates.<sup>4</sup> The enzymatic reaction of activated diesters with diamines has been investigated by Gutman *et al.*<sup>5</sup> who found that porcine pancreatic lipase (PPL) is the most efficient catalyst for the preparation of some macrobisactams. Recently, we have shown the potential of *C. antarctica* lipase (CAL) to catalyse the preparation of amides from non-activated esters<sup>6</sup> and from  $\beta$ -furyl and  $\beta$ -phenyl esters.<sup>7</sup> Now we report our results on the aminolysis of non-activated diesters using CAL as catalyst and diamines as nucleophiles. The enantioselectivity of this enzymatic aminolysis using racemic propane-1,2-diamine is also studied.

### Results and Discussion

First, we investigated the enzymatic aminolysis of non-activated diesters **1** with diamines **2**. Among the different lipases checked in this reaction, CCL and *Pseudomonas cepacia* lipase (PSL) showed a very low catalytic activity, while CAL (an acrylic resin-supported lipase) was very efficient in this process, amido esters **3** being obtained as the sole products in high yields at room temperature (Scheme 1, Table 1). The optimal yield for compounds **3** was obtained with the use of a 1:2 molar ratio of 2:1 and dioxane as solvent, no reaction taking place in the absence of the enzyme under similar reaction conditions. The yields in Table 1 were calculated after purification of the amido esters **3**. Conversion values were approximately 10% higher than yields but in no case could the reaction be completed by using longer reaction times. As is shown in Table 1, the size of the diamine had no influence on the catalytic activity of the lipase; similar results were obtained with ethylenediamine and butane-1,4-diamine. However, as the chain of the diester became longer, the reaction was slower and the yield for the amido ester **3** dropped.

We have tried the enzymatic aminolysis of **1** and **2** using other molar ratios in order to check if monoacylated products or macrobisactams are formed, but these compounds were never observed. Nevertheless, when the reaction was conducted within an excess of diamine and long reaction times (see Table 2), intramolecular heterocyclization products (**4** and **5**) were obtained, besides the amido ester **3**, in those cases in which five-



Scheme 1 Reagents and conditions: i, CAL, dioxane, 30 °C

Table 1 Reaction of diesters **1** with diamines **2**

Entry	<i>n</i>	<i>m</i>	t/h	Yield (%)
<b>3a</b>	2	2	30	66
<b>3b</b>	3	2	48	59
<b>3c</b>	4	2	48	56
<b>3d</b>	6	2	72	39
<b>3e</b>	2	4	30	70
<b>3f</b>	3	4	48	68
<b>3g</b>	4	4	48	50
<b>3h</b>	6	4	72	30
<b>3i</b>	2	6	30	67
<b>3j</b>	3	6	48	61
<b>3k</b>	4	6	48	46
<b>3l</b>	6	6	72	28

or six-membered rings could be formed (Scheme 1). Higher membered rings could not be obtained even if the reactions were carried out under conditions of high dilution.

In addition, compounds **5** could also be obtained when the amido esters **3** were allowed to react in the presence of CAL and a small amount of diamine. In this reaction both the enzyme

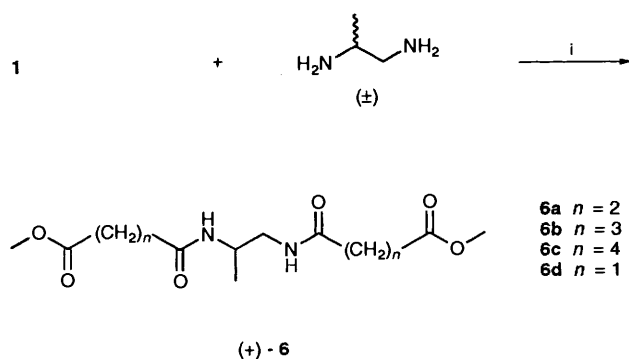
Table 2

Entry	<i>n</i>	<i>m</i>	<i>t/d</i>	Yield (%)
4a	2	2	6	27
5a	2	2	6	42
5b	3	2	6	31
5e	2	4	6	38

and the diamine are necessary since no cyclization of **3** took place in the absence of one of them. From this, we think that the diamine acts as a basic catalyst of the enzyme.

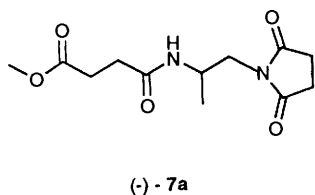
In connection with our interest in obtaining macrocyclic imides, we have attempted the heterocyclization of compounds **3** by conventional methods. Thus, the treatment of **3a** and **3b** with NaH in THF at reflux yielded *N,N'*-ethylenedisuccinimide **5a** and *N,N'*-ethylenediglutarimide **5b** respectively, but this method was found to be ineffective for the heterocyclization of longer amido esters.

Finally, the CAL-catalysed aminolysis of some of the diesters **1** with propane-1,2-diamine has been investigated (Scheme 2).



Scheme 2 Reagents and conditions: i, CAL, dioxane, 30 °C

Several solvents were tested in these reactions, but the best results were obtained with dioxane. As is shown in Table 3, the enzyme catalysed the formation of the amido ester **6** with very good enantioselectivity, except in the aminolysis of dimethyl adipate, for which the amido ester **6c** was obtained in moderate optical and chemical yields. The reactions were conducted at room temperature using equimolecular amounts of reagents and they were stopped when no further progress was observed by TLC. However, when the diester **1a** and the diamine were allowed to react over *ca.* 24 h, the heterocyclization of compound **6a** took place, **7a** being obtained in moderate yield



and very high enantiomeric excess (e.e.). Although two cyclization patterns could be envisaged for **6a**, only that involving the less hindered nitrogen atom actually took place leading to **7a**.

We have also checked the reaction of **1** and propane-1,2-diamine with PS lipase as catalyst. When hexane was used as solvent, the enzyme was completely inactive and no reaction took place. With diisopropyl ether or dioxane, PSL catalysed the formation of **6** but with very low enantioselectivity. Moreover, side products were observed as a result of polyamidation.

In conclusion, we have developed a method of obtaining amido esters and cyclic imides through enzymatic aminolysis of

Table 3 Amidation of the diesters **1** with propane-1,2-diamines

Entry	<i>n</i>	<i>t/h</i>	Yield (%)	E.e. (%)
<b>6a</b>	2	12	27	90
<b>6b</b>	3	22	21	> 95
<b>6c</b>	4	24	19	54
<b>6d</b>	1	9	39	> 95
<b>7a</b>	2	24	23	93

diesters with diamines. Similar compounds with very high enantiomeric excesses were obtained when propane-1,2-diamine was used as nucleophile.

## Experimental

*Candida cylindracea* (Type VII crude) lipase was purchased from Sigma Chemical Co. and *Pseudomonas cepacia* from Amano Pharmaceutical Co. *Candida antarctica* lipase, SP 435 L, was a gift from Novo Nordisk Co. All reagents were of commercial quality and purchased from Aldrich Chemie. Solvents were distilled over a suitable desiccant and stored under nitrogen. For column chromatography, Merck silica gel 60/230–400 mesh was used. Melting points were taken using a Gallenkamp apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter and are quoted in units of 10<sup>-1</sup> deg cm<sup>2</sup> g<sup>-1</sup>. IR spectra were recorded on a Perkin-Elmer 1720-X FTIR spectrophotometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained with TMS (tetramethylsilane) as internal standard using a Bruker AC-300 (<sup>1</sup>H 300 MHz and <sup>13</sup>C 75.5 MHz) spectrometer. *J* Values are given in Hz. Mass spectra were recorded on a Hewlett-Packard 5987 A spectrometer. Microanalyses were performed on a Perkin-Elmer 240B elemental analyser. Compound yields have been calculated on the basis of percentage conversion.

The corresponding e.e.s of compounds **6** and **7a** were calculated by <sup>1</sup>H NMR spectroscopy using the chiral shift reagent tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III).

*Reaction of the Diesters 1 with the Diamines 2 in Dioxane catalysed by CAL: General Procedure.*—CAL (0.3 g) was added to a solution of diester **1** (10 mmol) and diamine **2** (5 mmol) in dioxane (30 cm<sup>3</sup>). The suspension was stirred at 30 °C. When the reaction was complete, the enzyme was filtered off and washed with dichloromethane. The combined organic filtrate and workings were evaporated and flash chromatography on silica of the residue using acetonitrile–diethyl ether–propan-2-ol 10:10:0.2 yielded the corresponding compound **3**. For compounds **4** and **5** acetonitrile–diethyl ether–hexane 2:2:1 was used as eluent.

*Dimethyl 4,9-dioxo-5,8-diazadodecanedioate 3a.* Yield, 66%; m.p. 161–163 °C (Found: C, 50.1; H, 6.9; N, 9.8. C<sub>12</sub>H<sub>20</sub>N<sub>2</sub>O<sub>6</sub> requires C, 49.99; H, 6.99; N, 9.72%); *v*<sub>max</sub>(KBr)/cm<sup>-1</sup> 1659 (C=O) and 1728 (C=O); *δ*<sub>H</sub>(CDCl<sub>3</sub>) 2.43 (t, 4 H, CH<sub>2</sub>, *J* 6.7), 2.64 (t, 4 H, CH<sub>2</sub>, *J* 6.7), 3.31–3.36 (m, 4 H, CH<sub>2</sub>NH), 3.76 (s, 6 H, OCH<sub>3</sub>) and 6.63 (br s, 2 H, NH); *δ*<sub>C</sub>(CDCl<sub>3</sub>) 29.0 (CH<sub>2</sub>), 30.5 (CH<sub>2</sub>), 39.3 (CH<sub>2</sub>), 51.6 (CH<sub>3</sub>), 172.4 (C=O) and 173.4 (C=O); *m/z* 288 (M<sup>+</sup>, < 1%) and 115 (100).

*Dimethyl 5,10-dioxo-6,9-diazatetradecanedioate 3b.* Yield, 59%; m.p. 125–127 °C (Found: C, 53.25; H, 7.5; N, 8.9. C<sub>14</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub> requires C, 53.15; H, 7.65; N, 8.85%); *v*<sub>max</sub>(KBr)/cm<sup>-1</sup> 1642 (C=O) and 1738 (C=O); *δ*<sub>H</sub>(CDCl<sub>3</sub>) 1.98 (m, 4 H, CH<sub>2</sub>), 2.28 (t, 4 H, CH<sub>2</sub>, *J* 7.4), 2.41 (t, 4 H, CH<sub>2</sub>, *J* 7.2), 3.38–3.44 (m, 4 H, CH<sub>2</sub>NH), 3.68 (s, 6 H, OCH<sub>3</sub>) and 6.32 (br s, 2 H, NH); *δ*<sub>C</sub>(CDCl<sub>3</sub>) 20.6 (CH<sub>2</sub>), 32.9 (CH<sub>2</sub>), 35.1 (CH<sub>2</sub>), 39.7 (CH<sub>2</sub>), 51.5 (CH<sub>3</sub>) 173.2 (C=O) and 173.5 (C=O); *m/z* 316 (M<sup>+</sup>, < 1%) and 129 (100).

**Dimethyl 6,11-dioxo-7,10-diazahexadecanedioate 3c.** Yield, 56%; m.p. 134–136 °C (Found: C, 55.9; H, 8.1; N, 8.2).  $C_{16}H_{28}N_2O_6$  requires C, 55.80; H, 8.19; N, 8.13%;  $\nu_{\max}$  (KBr)/ $cm^{-1}$  1640 (C=O) and 1730 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.68 (m, 8 H, CH<sub>2</sub>), 2.23 (m, 4 H, CH<sub>2</sub>), 2.35 (m, 4 H, CH<sub>2</sub>), 3.37–3.43 (m, 4 H, CH<sub>2</sub>NH), 3.67 (s, 6 H, OCH<sub>3</sub>) and 6.56 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 24.2 (CH<sub>2</sub>), 25.0 (CH<sub>2</sub>), 33.5 (CH<sub>2</sub>), 36.0 (CH<sub>2</sub>), 40.0 (CH<sub>2</sub>), 51.6 (CH<sub>3</sub>), 173.8 (C=O) and 174.1 (C=O);  $m/z$  344 ( $M^+$ , <1%) and 55 (100).

**Dimethyl 8,13-dioxo-9,12-diazaicosanedioate 3d.** Yield, 39%; m.p. 129–131 °C (Found: C, 60.1; H, 9.15; N, 6.9).  $C_{20}H_{36}N_2O_6$  requires C, 59.98; H, 9.06; N, 6.99%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1640 (C=O) and 1740 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.35 (m, 8 H, CH<sub>2</sub>) 1.64 (m, 8 H, CH<sub>2</sub>), 2.19 (m, 4 H, CH<sub>2</sub>), 2.30 (m, 4 H, CH<sub>2</sub>), 3.36–3.44 (m, 4 H, CH<sub>2</sub>NH), 3.68 (s, 6 H, OCH<sub>3</sub>) and 6.51 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 24.7 (CH<sub>2</sub>), 25.4 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 33.9 (CH<sub>2</sub>), 36.4 (CH<sub>2</sub>), 40.1 (CH<sub>2</sub>), 51.5 (CH<sub>3</sub>), 174.2 (C=O) and 174.4 (C=O);  $m/z$  400 ( $M^+$ , <1%) and 171 (100).

**Dimethyl 4,11-dioxo-5,10-diazatetradecanedioate 3e.** Yield, 70%; 135–137 °C (Found: C, 53.2; H, 7.6; N, 8.7).  $C_{14}H_{24}N_2O_6$  requires C, 53.15; H, 7.65; N, 8.85%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1642 (C=O) and 1730 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.53 (m, 4 H, CH<sub>2</sub>), 2.47 (t, 4 H, CH<sub>2</sub>, *J* 6.7), 2.69 (t, 4 H, CH<sub>2</sub>, *J* 6.7), 3.27 (q, 4 H, CH<sub>2</sub>NH, *J* 6.0), 3.69 (s, 6 H, OCH<sub>3</sub>) and 6.09 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 26.6 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>), 30.8 (CH<sub>2</sub>), 39.0 (CH<sub>2</sub>), 51.8 (CH<sub>3</sub>), 171.6 (C=O) and 173.6 (C=O);  $m/z$  316 ( $M^+$ , <1%) and 116 (100).

**Dimethyl 5,12-dioxo-6,11-diazahexadecanedioate 3f.** Yield, 68%; m.p. 119–121 °C (Found: C, 55.9; H, 8.15; N, 8.3).  $C_{16}H_{28}N_2O_6$  requires C, 55.80; H, 8.19; N, 8.13%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1642 (C=O) and 1738 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.53 (m, 4 H, CH<sub>2</sub>), 1.95 (m, 4 H, CH<sub>2</sub>), 2.25 (t, 4 H, CH<sub>2</sub>, *J* 7.3), 2.38 (t, 4 H, CH<sub>2</sub>, *J* 7.2), 3.26 (q, 4 H, CH<sub>2</sub>NH, *J* 6.0), 3.67 (s, 6 H, OCH<sub>3</sub>) and 6.25 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 20.9 (CH<sub>2</sub>), 26.8 (CH<sub>2</sub>), 33.1 (CH<sub>2</sub>), 35.3 (CH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 51.6 (CH<sub>3</sub>), 172.4 (C=O) and 173.6 (C=O);  $m/z$  344 ( $M^+$ , <1%) and 129 (100).

**Dimethyl 6,13-dioxo-7,12-diazaoctadecanedioate 3g.** Yield, 50%; m.p. 129–130 °C (Found: C, 58.1; H, 8.6; N, 7.6).  $C_{18}H_{32}N_2O_6$  requires C, 58.05; H, 8.66; N, 7.52%;  $\nu_{\max}$  (KBr)/ $cm^{-1}$  1640 (C=O) and 1730 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.52 (m, 4 H, CH<sub>2</sub>), 1.64 (m, 8 H, CH<sub>2</sub>), 2.20 (m, 4 H, CH<sub>2</sub>), 2.34 (m, 4 H, CH<sub>2</sub>), 3.25 (q, 4 H, CH<sub>2</sub>NH, *J* 6.2), 3.64 (s, 6 H, OCH<sub>3</sub>) and 6.10 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 24.2 (CH<sub>2</sub>), 24.9 (CH<sub>2</sub>), 26.7 (CH<sub>2</sub>), 33.5 (CH<sub>2</sub>), 36.1 (CH<sub>2</sub>), 38.8 (CH<sub>2</sub>), 51.4 (CH<sub>3</sub>), 172.7 (C=O) and 173.9 (C=O);  $m/z$  372 ( $M^+$ , 8%) and 70 (100).

**Dimethyl 8,15-dioxo-9,14-diazadocosanedioate 3h.** Yield, 30%; m.p. 140–142 °C (Found: C, 61.8; H, 9.3; N, 6.7).  $C_{22}H_{40}N_2O_6$  requires C, 61.66; H, 9.41; N, 6.54%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1640 (C=O) and 1736 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.30–1.80 (m, 20 H, CH<sub>2</sub>), 2.17 (t, 4 H, CH<sub>2</sub>, *J* 7.4), 2.30 (t, 4 H, CH<sub>2</sub>, *J* 7.5), 3.15–3.36 (m, 4 H, CH<sub>2</sub>NH), 3.66 (s, 6 H, OCH<sub>3</sub>) and 5.84 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 24.7 (CH<sub>2</sub>), 25.5 (CH<sub>2</sub>), 26.9 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 33.9 (CH<sub>2</sub>), 36.6 (CH<sub>2</sub>), 39.0 (CH<sub>2</sub>), 51.5 (CH<sub>3</sub>), 173.2 (C=O) and 174.2 (C=O);  $m/z$  428 ( $M^+$ , <1%) and 143 (100).

**Dimethyl 4,13-dioxo-5,12-diazahexadecanedioate 3i.** Yield, 67%; m.p. 121–123 °C (Found: C, 55.7; H, 8.3; N, 8.2).  $C_{16}H_{28}N_2O_6$  requires C, 55.80; H, 8.19; N, 8.13%;  $\nu_{\max}$  (KBr)/ $cm^{-1}$  1640 (C=O) and 1731 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.26–1.40 (m, 4 H, CH<sub>2</sub>), 1.45–1.52 (m, 4 H, CH<sub>2</sub>), 2.47 (t, 4 H, CH<sub>2</sub>, *J* 6.6), 2.67 (t, 4 H, CH<sub>2</sub>, *J* 6.6), 3.23 (m, 4 H, CH<sub>2</sub>NH), 3.67 (s, 6 H, OCH<sub>3</sub>) and 5.98 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 25.8 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 30.9 (CH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 51.8 (OCH<sub>3</sub>), 171.3 (C=O) and 173.5 (C=O);  $m/z$  344 ( $M^+$ , <1%) and 115 (100).

**Dimethyl 5,14-dioxo-6,13-diazaoctadecanedioate 3j.** Yield, 61%; m.p. 117–119 °C (Found: C, 58.2; H, 8.5; N, 7.4).  $C_{18}H_{32}N_2O_6$  requires C, 58.05; H, 8.66; N, 7.52%;  $\nu_{\max}$  (KBr)/ $cm^{-1}$  1632 (C=O) and 1728 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.20–1.41

(m, 4 H, CH<sub>2</sub>), 1.50 (m, 4 H, CH<sub>2</sub>), 1.96 (m, 4 H, CH<sub>2</sub>), 2.24 (t, 4 H, CH<sub>2</sub>, *J* 7.3), 2.39 (t, 4 H, CH<sub>2</sub>, *J* 7.0), 3.23 (q, 4 H, CH<sub>2</sub>NH, *J* 6.5), 3.68 (s, 6 H, OCH<sub>3</sub>) and 5.90 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 21.0 (CH<sub>2</sub>), 25.9 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 33.0 (CH<sub>2</sub>), 35.4 (CH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 51.6 (CH<sub>3</sub>), 172.4 (C=O) and 173.7 (C=O);  $m/z$  372 ( $M^+$ , <1%) and 129 (100).

**Dimethyl 6,15-dioxo-7,14-diazaicosanedioate 3k.** Yield, 46%; m.p. 126–128 °C (Found: C, 60.85; H, 9.2; N, 7.1).  $C_{20}H_{36}N_2O_6$  requires C, 59.98; H, 9.06; N, 6.99%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1632 (C=O) and 1736 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.32 (m, 4 H, CH<sub>2</sub>), 1.48 (m, 4 H, CH<sub>2</sub>), 1.64 (m, 8 H, CH<sub>2</sub>), 2.18 (m, 4 H, CH<sub>2</sub>), 2.33 (m, 4 H, CH<sub>2</sub>), 3.23 (q, 4 H, CH<sub>2</sub>NH, *J* 6.5), 3.67 (s, 6 H, OCH<sub>3</sub>) and 5.84 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 24.4 (CH<sub>2</sub>), 25.1 (CH<sub>2</sub>), 25.9 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 33.7 (CH<sub>2</sub>), 36.3 (CH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 51.5 (CH<sub>3</sub>), 172.6 (C=O) and 174.0 (C=O);  $m/z$  400 ( $M^+$ , <1%) and 98 (100).

**Dimethyl 8,17-dioxo-9,16-diazatetracosanedioate 3l.** Yield, 28%; m.p. 122–124 °C (Found: C, 63.25; H, 9.6; N, 6.2).  $C_{24}H_{44}N_2O_6$  requires C, 63.13; H, 9.71; N, 6.14%;  $\nu_{\max}$  (KBr)/ $cm^{-1}$  1648 (C=O) and 1732 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.33 (m, 8 H, CH<sub>2</sub>), 1.49 (m, 4 H, CH<sub>2</sub>), 1.63 (m, 12 H, CH<sub>3</sub>), 2.16 (t, 4 H, CH<sub>2</sub>, *J* 7.5), 2.30 (t, 4 H, CH<sub>2</sub>, *J* 7.5), 3.23 (q, 4 H, CH<sub>2</sub>NH, *J* 6.9), 3.66 (s, 6 H, OCH<sub>3</sub>) and 5.61 (br s, 2 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 24.8 (CH<sub>2</sub>), 25.6 (CH<sub>2</sub>), 25.9 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 34.0 (CH<sub>2</sub>), 36.7 (CH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 51.5 (CH<sub>3</sub>), 173.0 (C=O) and 174.2 (C=O);  $m/z$  456 ( $M^+$ , <1%) and 98 (100).

**Methyl 3-[N-(succinimidoethyl)carbamoyl]propanoate 4a.** Yield, 27%; m.p. 76–78 °C (Found: C, 51.6; H, 6.2; N, 11.0).  $C_{11}H_{16}N_2O_5$  requires C, 51.56; H, 6.29; N, 10.93%;  $\nu_{\max}$  (KBr)/ $cm^{-1}$  1694 (C=O) and 1736 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 2.41 (t, 2 H, CH<sub>2</sub>, *J* 6.6), 2.62 (t, 2 H, CH<sub>2</sub>, *J* 6.6), 2.72 (s, 4 H, CH<sub>2</sub>), 3.47 (q, 2 H, CH<sub>2</sub>NH, *J* 5.6), 3.64–3.70 (m, 2 H, CH<sub>2</sub>N), 3.67 (s, 3 H, OCH<sub>3</sub>) and 6.47 (br s, 1 H, NH);  $\delta_C$ (CDCl<sub>3</sub>) 28.0 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 30.4 (CH<sub>2</sub>), 37.9 (CH<sub>2</sub>), 38.2 (CH<sub>2</sub>), 51.6 (CH<sub>3</sub>), 171.9 (C=O), 173.3 (C=O) and 177.7 (C=O);  $m/z$  256 ( $M^+$ , 7%) and 115 (100).

**N,N'-Ethylenedisuccinimide 5a.** Yield, 42%; m.p. 257–259 °C (Found: C, 53.7; H, 5.35; N, 12.4).  $C_{10}H_{12}N_2O_4$  requires C, 53.57; H, 5.40; N, 12.49%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1695 (C=O);  $\delta_H$  (CDCl<sub>3</sub>) 2.59 (s, 8 H, CH<sub>2</sub>) and 3.66 (s, 4 H, CH<sub>2</sub>N);  $\delta_C$ (CDCl<sub>3</sub>) 28.1 (CH<sub>2</sub>), 37.0 (CH<sub>2</sub>) and 177.9 (C=O);  $m/z$  224 ( $M^+$ , 20%) and 125 (100).

**N,N'-Ethylenediglutaramide 5b.** Yield, 31%; m.p. 126–128 °C (Found: C, 57.2; H, 6.3; N, 11.2).  $C_{12}H_{16}N_2O_4$  requires C, 57.13; H, 6.39; N, 11.10%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1674 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.92 (m, 4 H, CH<sub>2</sub>), 2.65 (t, 8 H, CH<sub>2</sub>, *J* 6.6) and 4.06 (s, 4 H, CH<sub>2</sub>N);  $\delta_C$ (CDCl<sub>3</sub>) 16.7 (CH<sub>2</sub>), 33.1 (CH<sub>2</sub>), 37.9 (CH<sub>2</sub>) and 172.9 (C=O);  $m/z$  252 ( $M^+$ , 18%) and 139 (100).

**N,N'-Tetramethylenedisuccinimide 5c.** Yield, 38%; m.p. 163–165 °C (Found: C, 57.2; H, 6.45; N, 11.3).  $C_{12}H_{16}N_2O_4$  requires C, 57.13; H, 6.39; N, 11.10%;  $\nu_{\max}$ (KBr)/ $cm^{-1}$  1694 (C=O);  $\delta_H$ (CDCl<sub>3</sub>) 1.58 (m, 4 H, CH<sub>2</sub>), 2.71 (s, 8 H, CH<sub>2</sub>) and 3.51 (m, 4 H, CH<sub>2</sub>N);  $\delta_C$ (CDCl<sub>3</sub>) 24.8 (CH<sub>2</sub>), 28.0 (CH<sub>2</sub>), 38.0 (CH<sub>2</sub>) and 177.2 (C=O);  $m/z$  252 ( $M^+$ , <1%) and 140 (100).

**Reaction of the Diesters 1 with Propane-1,2-diamine in Dioxane catalysed by CAL: General Procedure.**—To a solution of diester 1 (5 mmol) and propane-1,2-diamine (0.37 g, 5 mmol) in dioxane (20 cm<sup>3</sup>), CAL (0.3 g) was added. The mixture was stirred at 30 °C. When no further progress of the reaction was observed by TLC, the enzyme was filtered off and washed with dichloromethane. The combined organic filtrate and workings were evaporated and flash chromatography on silica of the residue using acetonitrile–diethyl ether–hexane 2:2:1 as eluent yielded the corresponding compound 6. Compound 7a was also isolated by flash chromatography using the same eluent.

**Dimethyl 6-methyl-4,9-dioxo-5,8-diazadodecanedioate 6a.** Yield, 27%; m.p. 119–121 °C;  $[\alpha]_D^{25} + 29.1$  (c 0.76 in CHCl<sub>3</sub>), e.e. 90% (Found: C, 51.7; H, 7.5; N, 9.4).  $C_{13}H_{22}N_2O_6$  requires C,

51.65; H, 7.34; N, 9.27%);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  1643 (C=O) and 1738 (C=O);  $\delta_{\text{H}}(\text{CDCl}_3)$  1.18 (d, 3 H, CH<sub>3</sub>, *J* 6.6), 2.43–2.56 (m, 4 H, CH<sub>2</sub>), 2.65–2.76 (m, 4 H, CH<sub>2</sub>), 3.30–3.36 (m, 2 H, CH<sub>2</sub>NH), 3.68 (s, 3 H, OCH<sub>3</sub>), 3.69 (s, 3 H, OCH<sub>3</sub>), 4.09 (m, 1 H, CHNH), 6.23 (d, 1 H, NH, *J* 7.9) and 6.58 (br s, 1 H, NH);  $\delta_{\text{C}}(\text{CDCl}_3)$  18.1 (CH<sub>3</sub>), 29.1 (CH<sub>2</sub>), 30.5 (CH<sub>2</sub>), 30.8 (CH<sub>2</sub>), 44.8 (CH<sub>2</sub>), 45.9 (CH), 51.7 (CH<sub>3</sub>), 171.8 (C=O), 172.4 (C=O), 173.4 (C=O) and 173.6 (C=O); *m/z* 302 (M<sup>+</sup>, < 1%) and 115 (100).

**Dimethyl 7-methyl-5,10-dioxo-6,9-diazatetradecanedioate 6b.** Yield, 21%; m.p. 94–96 °C;  $[\alpha]_{\text{D}}^{22} + 27.7$  (*c* 0.55 in CHCl<sub>3</sub>), e.e. > 95% (Found: C, 54.5; H, 7.85; N, 8.6. C<sub>15</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub> requires C, 54.53; H, 7.93; N, 8.48%);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  1645 (C=O) and 1732 (C=O);  $\delta_{\text{H}}(\text{CDCl}_3)$  1.16 (d, 3 H, CH<sub>3</sub>, *J* 7.0), 1.94 (m, 4 H, CH<sub>2</sub>), 2.18–2.27 (m, 4 H, CH<sub>2</sub>), 2.36 (t, 4 H, CH<sub>2</sub>, *J* 7.2), 3.16–3.42 (m, 2 H, CH<sub>2</sub>NH), 3.63 (s, 6 H, OCH<sub>3</sub>), 3.92–4.17 (m, 1 H, CHNH), 6.17 (d, 1 H, NH, *J* 6.0) and 6.44 (br s, 1 H, NH);  $\delta_{\text{C}}(\text{CDCl}_3)$  18.0 (CH<sub>3</sub>), 20.6 (CH<sub>2</sub>), 32.9 (CH<sub>2</sub>), 35.0 (CH<sub>2</sub>), 35.2 (CH<sub>2</sub>), 45.0 (CH<sub>2</sub>), 45.9 (CH), 51.3 (CH<sub>3</sub>), 172.5 (C=O), 173.2 (C=O), 173.3 (C=O) and 173.4 (C=O); *m/z* 330 (M<sup>+</sup>, < 1%) and 129 (100).

**Dimethyl 8-methyl-6,11-dioxo-7,10-diazahexadecanedioate 6c.** Yield, 19%; m.p. 100–101 °C;  $[\alpha]_{\text{D}}^{22} + 21.4$  (*c* 0.42 in CHCl<sub>3</sub>), e.e. 54% (Found: C, 57.1; H, 8.5; N, 7.95. C<sub>17</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub> requires C, 56.97; H, 8.44; N, 7.82%);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  1641 (C=O) and 1734 (C=O);  $\delta_{\text{H}}(\text{CDCl}_3)$  1.18 (d, 3 H, CH<sub>3</sub>, *J* 6.8), 1.66 (m, 8 H, CH<sub>2</sub>), 2.21 (m, 4 H, CH<sub>2</sub>), 2.36 (t, 4 H, CH<sub>2</sub>, *J* 6.8), 3.17–3.45 (m, 2 H, CH<sub>2</sub>NH), 3.69 (s, 6 H, OCH<sub>3</sub>), 4.03–4.18 (m, 1 H, CHNH), 6.31 (d, 1 H, NH, *J* 7.0) and 6.56 (br s, 1 H, NH);  $\delta_{\text{C}}(\text{CDCl}_3)$  18.2 (CH<sub>3</sub>), 24.2 (CH<sub>2</sub>), 24.3 (CH<sub>2</sub>), 25.0 (CH<sub>2</sub>), 33.5 (CH<sub>2</sub>), 35.9 (CH<sub>2</sub>), 36.1 (CH<sub>2</sub>), 45.5 (CH<sub>2</sub>), 46.2 (CH), 51.5 (CH<sub>3</sub>), 173.1 (C=O), 173.7 (C=O) and 173.9 (C=O); *m/z* 358 (M<sup>+</sup>, < 1%) and 186 (100).

**Dimethyl 5-methyl-3,8-dioxo-4,7-diazadecanedioate 6d.** Yield, 39%; m.p. 104–106 °C;  $[\alpha]_{\text{D}}^{22} + 21.7$  (*c* 0.36 in CHCl<sub>3</sub>), e.e. > 95% (Found: C, 48.1; H, 6.75; N, 10.3. C<sub>11</sub>H<sub>18</sub>N<sub>2</sub>O<sub>6</sub> requires C, 48.17; H, 6.62; N, 10.21%);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  1641 (C=O) and 1741 (C=O);  $\delta_{\text{H}}(\text{CDCl}_3)$  1.20 (d, 3 H, CH<sub>3</sub>, *J* 6.5), 3.31 (s, 2 H, CH<sub>2</sub>), 3.33 (s, 2 H, CH<sub>2</sub>), 3.35–3.40 (m, 2 H, CH<sub>2</sub>NH), 3.74 (s, 3 H, OCH<sub>3</sub>), 3.75 (s, 3 H, OCH<sub>3</sub>), 4.12 (m, 1 H, CHNH), 7.19 (d, 1 H, NH, *J* 7.0) and 7.46 (br s, 1 H, NH);  $\delta_{\text{C}}(\text{CDCl}_3)$  17.9 (CH<sub>3</sub>), 41.2 (CH<sub>2</sub>), 41.4 (CH<sub>2</sub>), 44.8 (CH<sub>2</sub>), 45.9 (CH), 52.3 (CH<sub>3</sub>), 165.4 (C=O), 166.0 (C=O), 169.3 (C=O) and 169.4 (C=O); *m/z* 274 (M<sup>+</sup>, < 1%) and 143 (100).

**Methyl 3-[N-(1-methyl-2-succinimidoethyl)carbamoyl]propionate 7a.** Yield, 23%; m.p. 76–78 °C;  $[\alpha]_{\text{D}}^{22} - 14.6$  (*c* 0.69 in CHCl<sub>3</sub>), e.e. 93% (Found: C, 53.4; H, 6.8; N, 10.2. C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub> requires C, 53.33; H, 6.71; N, 10.36%);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  1695 (C=O) and 1732 (C=O);  $\delta_{\text{H}}(\text{CDCl}_3)$  1.11 (d, 3 H, CH<sub>3</sub>, *J* 6.8), 2.30 (t, 2 H, CH<sub>2</sub>, *J* 6.6), 2.42–2.62 (m, 2 H, CH<sub>2</sub>), 2.65 (s, 4 H, CH<sub>2</sub>), 3.46 (d, 2 H, CH<sub>2</sub>N, *J* 7.0), 3.60 (s, 3 H, OCH<sub>3</sub>), 4.20–4.29 (m, 1 H, CHNH) and 5.82 (d, 1 H, NH, *J* 7.9);  $\delta_{\text{C}}(\text{CDCl}_3)$  18.3 (CH<sub>3</sub>), 28.0 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 30.6 (CH<sub>2</sub>), 43.7 (CH<sub>2</sub>), 44.2 (CH), 51.6 (CH<sub>3</sub>), 171.2 (C=O), 173.2 (C=O) and 177.9 (C=O); *m/z* 270 (M<sup>+</sup>, < 1%) and 115 (100).

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### References

- B. C. Challis and J. A. Challis, *Comprehensive Organic Chemistry*, Pergamon Press, 1979, vol. 2, p. 957; J. Zabicky, in *The Chemistry of Amides*, ed. S. Patai, 1970, p. 73.
- A. M. Klibanov, *CHEMTECH*, 1986, **16**, 354; A. Zaks and A. M. Klibanov, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 3192.
- K. Faber, *Biotransformations in Preparative Organic Chemistry*, Springer-Verlag, 1992; A. M. Klibanov, *Acc. Chem. Res.*, 1990, **23**, 114.
- V. Gotor, R. Brieva, C. González and F. Rebolledo, *Tetrahedron*, 1991, **47**, 9207.
- A. L. Gutman, E. Meyer, X. Yue and C. Abell, *Tetrahedron Lett.*, 1992, **33**, 3943.
- M. Quirós, V. M. Sánchez, R. Brieva, F. Rebolledo and V. Gotor, *Tetrahedron: Asymmetry*, 1993, **4**, 1105.
- V. Gotor, E. Menéndez, Z. Mouloungui and A. Gaset, *J. Chem. Soc., Perkin Trans. 1*, 1993, 2453.

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