DOI: 10.1002/adsc.200900676

Stereoselective Chemoenzymatic Preparation of β-Amino Esters: Molecular Modelling Considerations in Lipase-Mediated Processes and Application to the Synthesis of (*S*)-Dapoxetine

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Received: September 29, 2009; Revised: December 22, 2009; Published online: February 9, 2010

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.200900676.

Abstract: A wide range of optically active 3-amino-3-arylpropanoic acid derivatives have been prepared by means of a stereoselective chemoenzymatic route. The key step is the kinetic resolution of the corresponding β-amino esters. Although the enzymatic acylations of the amino group with ethyl methoxyacetate showed synthetically useful enantioselectivities, the hydrolyses of the ester group catalyzed by lipase from *Pseudomonas cepacia* have been identified as the optimal processes concerning both activity and enantioselectivity. The enantiopreference of this lipase in these reactions has been explained, at the molecular level, by using a fragment-based approach in which the most favoured binding site for a phenyl ring and the most stable conformation of the 3-aminopropanoate core nicely match the (*S*)-configuration of the major products. The conversion and enantioselectivity values of the enzymatic reactions have been compared in order to understand the influence of the different substitution patterns present in the phenyl ring. This chemoenzymatic route has been successfully applied to the preparation of a valuable intermediate in the synthesis of (*S*)-dapoxetine, which has been chemically synthesised in excellent optical purity.

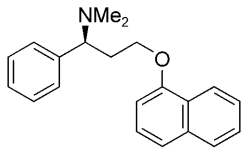
Keywords: amino acids; dapoxetine; enzyme catalysis; hydrolysis; lipases; molecular modelling

Introduction

Although enantiomerically pure β-amino acids are present in a high number of natural products they do not occur in nature as frequently as α-amino acids. They possess remarkable biological properties and are also considered as very important chiral building blocks for the synthesis of pharmaceuticals.[1] For example, the 3-amino-3-phenylpropionic acid core is present in anticancer agents such as Taxol,[2] and it also offers important synthetic possibilities as a valuable precursor of biologically active molecules such as Maraviroc (UK-427,857), a CCR-5 receptor antagonist.[3] Since their presence in nature is low, their stereoselective syntheses have recently attracted very much attention.[4]

Biocatalytic stereoselective methods are now recognised as an efficient tool for the preparation of pharmaceuticals at both industrial and laboratory scales.[5] Enzymes can exquisitely distinguish both enantiomers of a racemic mixture by reacting faster with one of them. This fact becomes of utmost importance in areas such as chemical biology and medicinal chemistry because enantiomers show different properties when they are exposed to the chiral environment of living organisms and, hence, highly different pharmacological profiles and physiological properties.[6]

Lipases have been extensively used in the kinetic resolution of carboxylic acids and their derivatives through mainly hydrolysis, alcoholysis, aminolysis, ammonolysis and transesterification processes.[7] Although in the synthesis of organic compounds organic solvents are usually preferred to water due to higher solubilities and easier isolation of the reactants and products,[8] hydrolytic processes in aqueous media have often afforded better results. Especially β-amino acids are easily accessible through enantioselective



(*S*)-dapoxetine (**1**)

Figure 1. Structure of (*S*)-dapoxetine.

lipase-catalysed hydrolyses of β-amino esters[9] or the ring cleavage of β-lactams.[10]

(*S*)-Dapoxetine {(*S*)-(+)-*N*,*N*-dimethyl-α-[2-(1-naphthalenyloxy)ethyl]benzenemethanamine, (**1**), Figure 1} is a potent serotonin re-uptake inhibitor used for the treatment of a variety of disorders as depression, bulimia or anxiety.[11] Besides, the use of its hydrochloride salt has been approved for the treatment of premature ejaculation, the most common male sexual dysfunction. For that reason original investigations have recently appeared in the literature describing the synthesis of this interesting drug.[12] These approaches involve asymmetric steps such as the Sharpless epoxidation and dihydroxylation, or lipase-catalysed aminolysis processes.

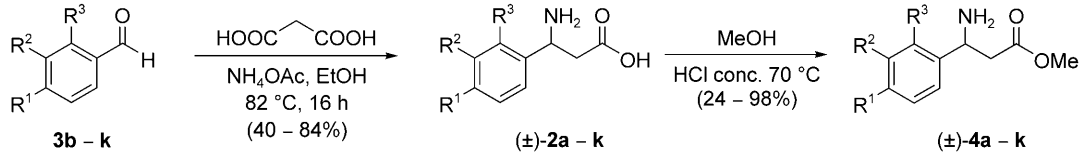
Herein we wish to report the study and set-up of a practical enzymatic method for the production of enantiomerically pure β-amino acid derivatives. The lipase enantiorecognition mechanism will be studied from a theoretical point of view in order to gain an insight into the experimental results obtained during these investigations. Subsequently, one of the so-obtained amino acids will be employed as starting material for the synthesis of (*S*)-dapoxetine [(*S*)-**1**] in high enantiomeric purity, which highlights the importance and potential of the methodology described here.

Results and Discussion

From a synthetic point of view, β-amino esters are very attractive compounds due to their intrinsic bifunctionality. Thus, concerning lipase catalysis, while the nucleophilic amino group is susceptible to undergo acylation, the electrophilic ester functionality allows the preparation of amides, carboxylic acids and related compounds by means of the typical addition-elimination mechanism. First we have synthesised a wide range of racemic β-amino acids [(±)-**2b–k**] starting from the corresponding commercially available aldehydes (**3b–k**). To do so a similar procedure to that already described in the literature and which makes use of malonic acid and ammonium acetate in refluxing EtOH was followed (Scheme 1).[9f,13] After 16 h, amino acids (±)-**2b–k**[14] were isolated with yields ranging from 40 to 84% (Supporting Information). We have attributed the dependence of the reaction efficiency on the substrate used to the final recrystallisation purification step.

Then, we carried out the chemical esterification of the so-obtained racemic carboxylic acids (±)-**2a–k** affording the corresponding 3-amino-3-arylpropionic methyl esters (±)-**4a–k** in low to excellent yields (24–98%, Supporting Information) depending on the type and, especially, the position of the substituent present in the phenyl ring, the *ortho*- or *meta*-substituted compounds being the ones obtained in lower yields.

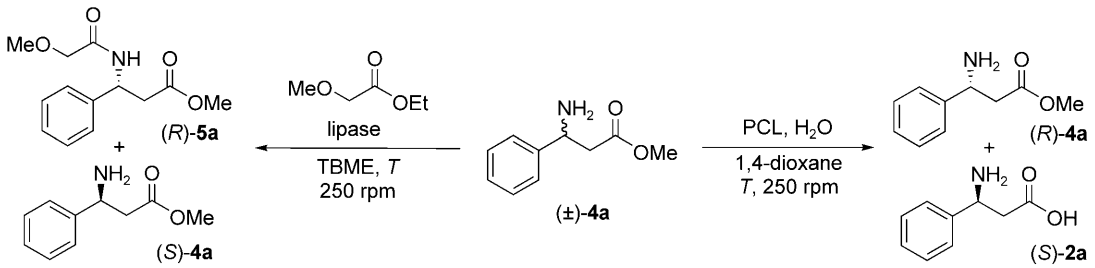
For the resolution of this set of β-amino esters we decided to use **4a** as the model substrate. The reason is two-fold: on the one hand it is the parent compound of the series, and, on the other hand, it is an adequate precursor for the synthesis of (*S*)-dapoxetine. For this reason, we initially focused our efforts on the optimisation of the reaction conditions for its successful kinetic resolution. The so-determined optimum conditions were later taken into account for the stereoselective preparation of the whole series of β-amino esters. Initially, we attempted the kinetic resolution of (±)-**4a** by acylation of the amino group using ethyl acetate as acyl donor, *tert*-butyl methyl ether as solvent, and lipase B from *Candida antarctica* (CAL-B) as catalyst (Scheme 2). However, after 48 h of reaction, no product was detected. This result is in accordance with the substrate requirements of this enzyme:[15] i) medium-sized substituents with more than two non-hydrogen atoms lead to a drop in the catalytic activity and selectivity, and ii) the presence of electronegative atoms in the medium-sized substituents is even more deleterious for enantioselectivity.



3b – k (±)-**2a – k** (±)-**4a – k**

2, **4a** $R^1 = R^2 = R^3 = H$ | **2 – 4b** $R^1 = Me$, $R^2 = R^3 = H$ | **2 – 4c** $R^1 = OMe$, $R^2 = R^3 = H$ | **2 – 4d** $R^1 = OPh$, $R^2 = R^3 = H$ | **2 – 4e** $R^1 = CF_3$, $R^2 = R^3 = H$ | **2 – 4f** $R^1 = Ph$, $R^2 = R^3 = H$ | **2 – 4g** $R^1 = Br$, $R^2 = R^3 = H$ | **2 – 4h** $R^1 = Cl$, $R^2 = R^3 = H$ | **2 – 4i** $R^1 = F$, $R^2 = R^3 = H$ | **2 – 4j** $R^1 = H$, $R^2 = F$, $R^3 = H$ | **2 – 4k** $R^1 = R^2 = H$, $R^3 = F$

Scheme 1. Synthesis of racemic 3-amino-3-arylpropionic acid methyl esters (±)-**4a–k**.



Scheme 2. Enzymatic kinetic resolution of (±)-**4a** by lipase-mediated acylation using ethyl methoxycetate or through a hydrolytic procedure.

On the other hand, it is known that the employment of alkyl methoxyacetates as acylating agents leads to an impressive enhancement of the catalytic activity of lipases in aminolysis reactions.[12c,16] Therefore we decided to test ethyl methoxyacetate as acylating agent, and, to our surprise, optically active amide **5a** was obtained, after 27 h of reaction, with high *ee* and moderate conversion (Table 1, entry 1). This result demonstrates the high potential of this type of acyl donor in biocatalysis. Thus, for the case of CAL-B it considerably increases the range of racemic secondary alcohols and related nucleophiles that can be accessed, in high *ee*. In fact, amine **4a** is far beyond the limits of the substrate space determined for CAL-B by using conventional acyl donors.[15] Nevertheless, it can be resolved with an enantiomeric ratio value which can be regarded as acceptable for synthetic purposes.

In order to further explore this transformation we decided to use other lipases. Namely, we employed lipase from *Pseudomonas cepacia* (PCL) and lipase A from *Candida antarctica* (CAL-A). PCL is known to have a less restrictive pocket for the medium-sized substituent of racemic secondary alcohols with regard to both size and electronegativity,[18] and CAL-A has shown unique features among lipases, like the ability to accept tertiary and sterically hindered alcohols, and its N-chemoselectivity in the acylation of amino esters.[19] The experimental results showed that *Pseudomonas cepacia* type I immobilised on a ceramic support (PCL-C I, entry 2) led to the corresponding amide with higher enantiomeric ratio and conversion values than CAL-B. However, it is worthy of note that CAL-A did not show any activity in the acylation of (±)-**4a** (entry 3). Next, an increase of the temperature (45 °C) was attempted in order to obtain higher conversion values for those cases in which activity was observed. However, only a slightly higher conversion value for CAL-B was detected (entry 4), while the performance of PCL-C I remained constant (entry 5). Further increases of the temperature led to the inactivation of these two biocatalysts. Finally, the employment of ethyl methoxyacetate not only as acylating agent but also as solvent was also attempted. However, it slowed down the reaction considerably.

Table 1. Enzymatic kinetic resolution of (±)-**4a** through lipase-mediated acylation after 27 h.

Entry	Lipase	T [°C]	ee_S [%][a]	ee_P [%][a]	c [%][b]	E[c]
1	CAL-B	30	17	96	15	53
2	PCL-C I	30	32	98	25	90
3	CAL-A	30	–	–	–	–
4	CAL-B	45	37	97	28	92
5	PCL-C I	45	32	97	25	90

[a] Measured by HPLC, see further details in the Supporting Information.
[b] Conversion value: $c = ee_S/(ee_S + ee_P)$.
[c] $E = \ln[(1-c)\times(1-ee_P)]/\ln[(1-c)\times(1+ee_P)]$.[17]

Taking into account the bifunctionality of amino esters and that the enantioselectivities obtained so far *via* *N*-acylation of **4a** are moderate, we decided to turn our attention to the enzymatic hydrolysis of the ester group. In fact, PCL has already allowed the successful preparation of β-amino acids in optically pure form.[9a,f,i] Unfortunately, the structural diversity of the acyl binding sites of lipases[20] is probably responsible for the fact that rules explaining the chiral preference of lipases towards racemic acyl donors have only been formulated for a few cases[21] and cannot be generalised. Lipase from *Candida rugosa* (CRL) is, by far, the most studied enzyme in this kind of transformation and an empirical rule for the resolution of α-substituted carboxylic acid derivatives has been formulated.[21b] However, it is only valid for hydrolysis reactions and with purified CRL.

Therefore, in order to gain an insight into the reasons of this high enantioselectivity at the molecular level, we have first carried out a visual inspection of the active site of PCL (Figure 2) and analysed the key interactions that the acyl chain of **4a** could establish when bound there. This inspection reveals that this lipase has an acyl binding site which offers an optimum environment to host aromatic rings. It is lined by the side chains of the hydrophobic amino acids Phe119, Leu167, Val266 and Val267, which can stabilise the binding of an aryl ring *via* van der Waals in-

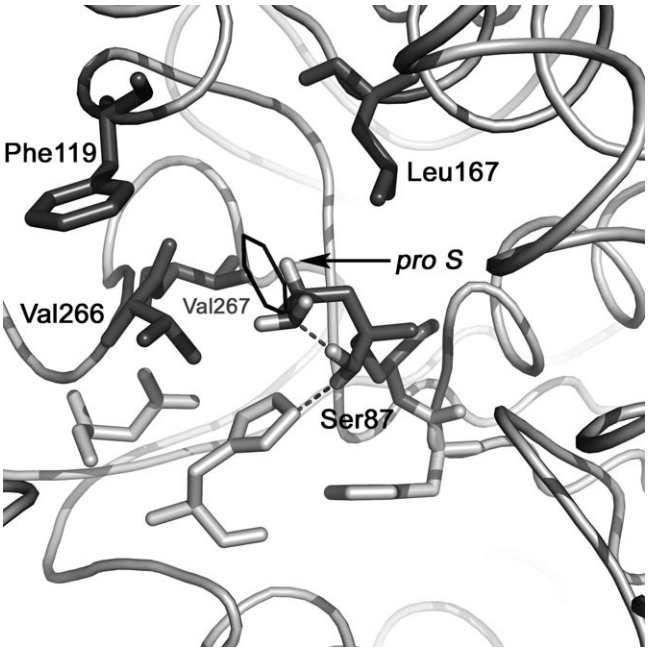


Figure 2. Binding mode proposal for the deacylation tetrahedral intermediate of the PCL-catalysed hydrolysis of 3-amino-propanoate (dark grey) superposed on the most favourable binding site for a benzene fragment (black). The amino acids of the acyl binding site and the catalytic serine (dark grey) are also shown.

teractions. To corroborate this hypothesis we have carried out a multi-fragment search simulation using a benzene molecule (see Experimental Section for details). The results did confirm, as it can be seen in Figure 2, that the most favoured location for a phenyl ring in the binding site of PCL is the aforementioned site. Moreover, the distance between the nucleophilic oxygen of the catalytic serine and the closest carbon atoms of the phenyl ring is between 3.5 and 3.75 Å. This suggests that such a position could be reached by the phenyl ring of a substrate like **4a**, in which it is attached to the reactive carbonyl carbon by a linker made of two carbon atoms.

Due to the structure of **4a** it is likely that intramolecular hydrogen bonds are established between the β-amino group and any of the heteroatoms bonded to the carbonylic carbon of the tetrahedral intermediates present during the enzymatic hydrolysis mechanism:[22] the oxyanion, the oxygen of the nucleophile or the leaving group, or the Oγ of the catalytic serine. Indeed, this type of intramolecular hydrogen bond has been already described to play a key role in both activity and selectivity of lipases towards related substrates.[16e,21g,23] For the case of the hydrolysis of **4a**, they would lead to intramolecular six-membered rings which would considerably reduce the degrees of freedom available to the binding modes of the substrate in the active site of PCL. If we additionally take into account that the substituent attached to the chiral centre is a bulky one (aryl) and the elongated shape of the acyl binding site, an inversion of configuration at the chiral centre should probably mean either a dramatic rearrangement of the binding mode thus leading to conformations higher in energy, or keeping the binding mode at the expense of the catalytic hydrogen bonding network. Accordingly, this binding scenario should be reflected into high enantiomeric ratios. We have modelled all possible deacylation tetrahedral intermediates corresponding to the hydrolysis of β-aminopropanoate which fulfilled the following requirements: i) the catalytic hydrogen bonding network is maintained and ii) an intramolecular hydrogen bond between the 3-amino group and any other oxygen atom of the tetrahedral intermediate is present. Subsequently they were analysed in terms of internal and interaction energy. In Figure 2 the intermediate with the lowest energy is shown. As it can be seen, the superposition of this binding mode with the optimal binding orientation of a benzene fragment shows that the latter could be linked to the *pro S* 3-position of the former with only a slight accommodation of the optimal binding conformations. However, for the *pro R* orientation this is not possible and it either requires a change in the plane of binding of the benzene fragment, or a rearrangement of the conformation of the 3-aminopropanoate chain.

Subsequently, we carried out the hydrolysis of the β-amino esters (±)-**4a–k**. Instead of using an aqueous system we decided to employ organic solvents as reaction medium because of the low solubility in water of some of them. Additionally, the amino acids obtained in the enzymatic processes would precipitate in the organic solvent leading to an easy separation of the final products. Initially, amino ester (±)-**4a** and 1,4-dioxane were selected as substrate and solvent, respectively, and 5 equivalents of water were used for the screening of enzymatic activity (Scheme 2).

The results obtained (Table 2) show that while in the absence of enzyme no reaction was detected (entry 1), in the presence of different preparations of PCL, immobilised on ceramics or diatomite (PCL-C I, PCL-C II and PCL-D; entries 2, 3 and 4, respectively) optically active amino acid **2a** was obtained. From all the preparations tested, PCL-C I (entry 2) and PCL-D (entry 4) turned out to be the most active, the latter also being the most enantioselective. These results highlight the importance of the support used to immobilise a given enzyme.

Therefore, PCL-D was selected as the catalyst of choice to continue with the optimisation of the hydrolytic kinetic resolution of the β-amino ester (±)-**4a**. Our goal then was the optimisation of the enzymatic activity. To do so we first explored the effect of the solvent. Thus, tetrahydrofuran (THF, entry 5) and acetonitrile (MeCN, entry 6) were also tested, but 1,4-

Table 2. Enzymatic kinetic resolution of the β-amino ester (±)-**4a** using H_2O as nucleophile in an organic solvent.[a]

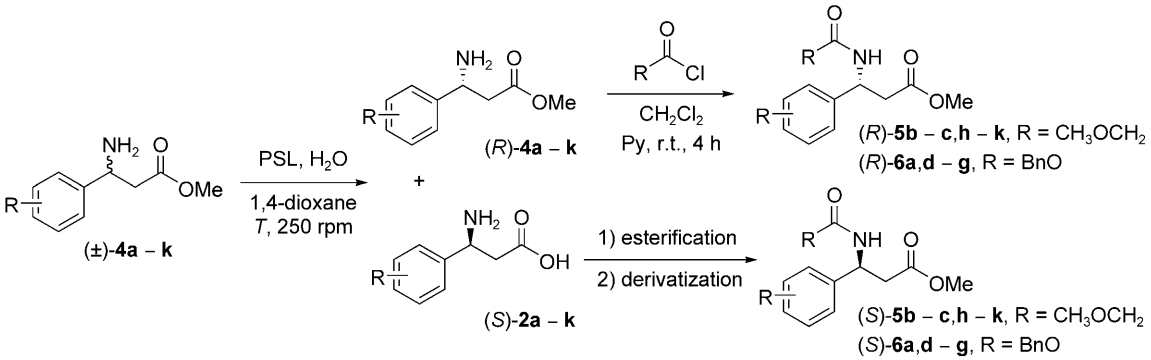
Entry	Lipase	Solvent	Ratio (**4a**: lipase)[b]	T [°C]	H_2O [equiv.]	t [h]	ee_S [%][c]	ee_P [%][c]	c [%][d]	E[e]
1	–	1,4-dioxane	1:1	30	5	24	0	–	0	–
2	PCL-C I	1,4-dioxane	1:1	30	5	24	58	98	37	198
3	PCL-C II	1,4-dioxane	1:1	30	5	24	31	97	24	92
4	PCL-D	1,4-dioxane	1:1	30	5	102.5	72	99	42	>200
5	PCL-D	THF	1:1	30	5	102.5	51	99	34	>200
6	PCL-D	MeCN	1:1	30	5	102.5	59	99	37	>200
7	PCL-D	1,4-dioxane	1:1	45	5	102.5	79	>99	44	>200
8	PCL-D	1,4-dioxane	1:1	60	5	102.5	69	>99	41	>200
9	PCL-D	1,4-dioxane	1:1	30	10	102.5	70	>99	41	>200
10	PCL-D	1,4-dioxane	1:2	30	5	102.5	89	98	48	>200
11	PCL-D	1,4-dioxane	1:2	45	5	102.5	96	98	49	>200
12	PCL-C Amano	1,4-dioxane	1:2	45	5	89	>99	97	51	>200

[a] For experimental details, see Experimental Section.
[b] Ratio **4a**: enzyme in weight.
[c] Measured by HPLC, see further details in the Supporting Information.
[d] Conversion value: $c = ee_S/(ee_S + ee_P)$.
[e] $E = \ln[(1-c)\times(1-ee_P)]/\ln[(1-c)\times(1+ee_P)]$.

dioxane was the one which afforded the highest degrees of conversion. From the analysis of the time courses of the reactions (see Figure S1 in the Supporting Information) it can be corroborated that the activity indeed increases as follows: 1,4-dioxane > MeCN > THF. Moreover, the enantioselectivity is very high in all cases, and the product is accordingly recovered in enantiomerically pure form.

Once 1,4-dioxane was identified as the best solvent, we next investigated the role played by the temperature on the activity and selectivity of the enzyme. The results obtained show that at 45 °C (entry 7) the enantiomeric ratio increased with respect to the reaction carried out at 30 °C (entry 4). However, on further increasing the temperature (60 °C, entry 8) a slight deactivation of the enzyme was observed. On the other hand, an increase in the amount of water did not affect in any form the reactivity (compare entries 4 and 9), while the use of twice as much enzyme (compare entries 4 *vs.* 10, and 7 *vs.* 11; see Figure S2 in the Supporting Information) allowed conversions close to 50% and, hence, the recovery of the final products in almost enantiopure form (entry 11). After finding the best conditions for the hydrolysis of racemate **4a** using PCL supported on diatomite (PCL-D), a preparation from Amano Pharmaceuticals Co. (PCL-C Amano) was also tested showing an excellent enantiopreference and activity values in shorter reaction times (entry 12).

Next, the optimum conditions determined for **4a** were applied to the other β-amino esters of the series (Scheme 3) using both PCL-D and PCL-C Amano as biocatalysts. The data obtained are summarised in Table 3. Initially reactions were performed at 30 and 45 °C for compounds **4a**, **b** (R = 4-H and 4-Me) showing similar reactivities but slightly higher at 45 °C and using PCL-C Amano (entries 1–6). In this manner **4a** and **4b** were efficiently resolved although a shorter reaction time was required for **4b** (entry 6). The influence of electron-donor substituents in the aromatic ring such as *p*-methoxy (**4c**) or *p*-phenoxy (**4d**) led to



Scheme 3. Hydrolysis of (±)-**4a**–**k** by lipase-catalysed kinetic resolutions.

Table 3. Enzymatic kinetic resolution of β-amino esters **4a–k** using 1,4-dioxane as solvent and 5 equivalents of H_2O as nucleophile.[a]

Entry	Compound	Lipase[b]	R^1	R^2	R^3	T [°C]	t [h]	ee_S [%][c]	ee_P [%][c]	c [%][d]	E[e]
1	**4a**	PCL-D	H	H	H	30	102.5	89	98	48	>200
2	**4a**	PCL-D	H	H	H	45	102.5	96	98	49	>200
3	**4a**	PCL-C Amano	H	H	H	45	89	>99	97	51	>200
4	**4b**	PCL-D	Me	H	H	30	102.5	>99	98	50	>200
5	**4b**	PCL-D	Me	H	H	45	58	96	98	49	>200
6	**4b**	PCL-C Amano	Me	H	H	45	58	99	98	50	>200
7	**4c**	PCL-D	OMe	H	H	30	52.5	>99	98	50	>200
8	**4c**	PCL-D	OMe	H	H	45	51.5	>99	98	50	>200
9	**4c**	PCL-C Amano	OMe	H	H	45	120	88	90	49	58
10	**4d**	PCL-D	OPh	H	H	45	23	94	99	49	>200
11	**4d**	PCL-C Amano	OPh	H	H	45	23	98	97	50	>200
12	**4e**	PCL-D	CF_3	H	H	45	95	99	98	50	>200
13	**4e**	PCL-C Amano	CF_3	H	H	45	64.5	>99	98	50	>200
14	**4f**	PCL-D	Ph	H	H	45	102.5	77	99	44	>200
15	**4f**	PCL-C Amano	Ph	H	H	45	102.5	84	97	46	>200
16	**4g**	PCL-D	Br	H	H	45	32.5	>99	98	51	>200
17	**4g**	PCL-C Amano	Br	H	H	45	39	99	96	51	>200
18	**4h**	PCL-D	Cl	H	H	45	30	99	>99	50	>200
19	**4h**	PCL-C Amano	Cl	H	H	45	38.5	>99	96	51	>200
20	**4i**	PCL-D	F	H	H	45	71	99	97	51	>200
21	**4i**	PCL-C Amano	F	H	H	45	31	99	98	50	>200
22	**4j**	PCL-D	H	F	H	45	102	95	97	49	>200
23	**4j**	PCL-C Amano	H	F	H	45	74	99	98	50	>200
24	**4k**	PCL-D	H	H	F	45	102.5	74	>99	43	>200
25	**4k**	PCL-C Amano	H	H	F	45	102.5	97	>99	49	>200

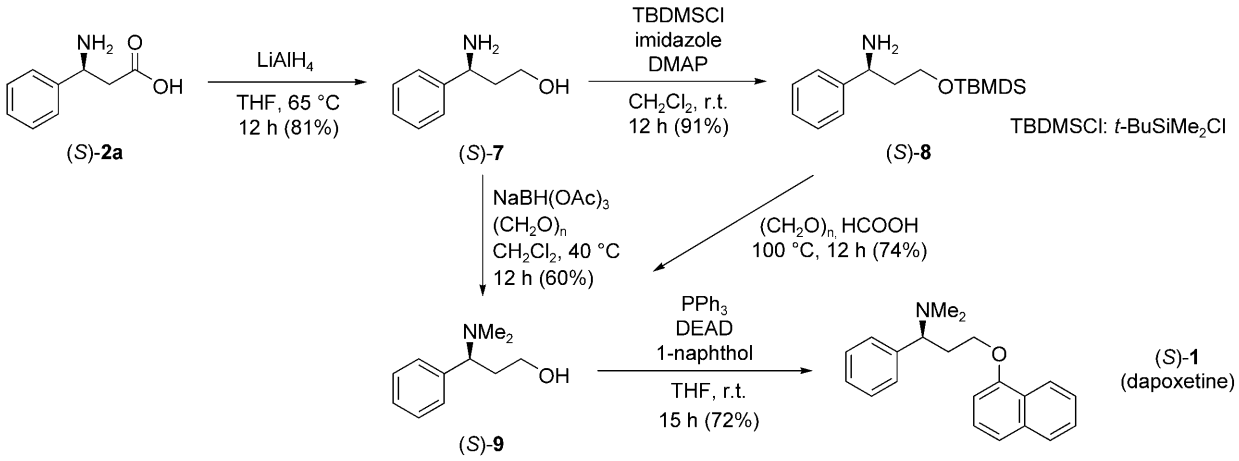
[a] For experimental details, see Experimental Section.
[b] Ratio 1:2 in weight of compound **4a–k**:enzyme.
[c] Measured by HPLC, see further details in the Supporting Information section.
[d] Conversion value: $c = ee_S/(ee_S + ee_P)$.
[e] $E = \ln[(1-c)\times(1-ee_P)]/\ln[(1-c)\times(1+ee_P)]$.

higher activities while maintaining an excellent selectivity with PCL-D (entries 7–11).

Amino esters with a trifluoromethyl (**4e**) and phenyl (**4f**) substituents in the *para* position of the aromatic ring were enantioselectively hydrolysed at reaction rates similar to that of the model substrate **4a** yielding the corresponding β-amino acids in almost enantiopure form (entries 12–15). The presence of a halogen atom in the *para* position was also studied by using β-amino esters with a bromine (**4g**), chlorine (**4h**) and fluorine (**4i**) substituent. Both the corresponding amino esters and amino acids were recovered in excellent enantiopurity after 32.5, 30 and 71 h, respectively, for PCL-D. Therefore, it seems that amino esters with bulkier and less electronegative substituents react faster than **4a** (entries 16–21). Finally, the influence of the substitution depending on the position of the phenyl ring to which it is bonded to was analysed by using a fluorine substituent (compounds **4i–k**). Again, for both PCL preparations an excellent enantiopreference was observed. Moreover, the reactions are faster the further the fluorine atom is from the alkyl rest (entries 22–25).

Both PCL preparations selectively hydrolysed the (*S*)-amino ester **4a** obtaining the amino acid (*S*)-**2a** with excellent enantiodiscrimination. The (*R*)-configuration for **4a** was assigned by comparison of the optical rotation value with the previously described one.[24] The (*S*)-enantiopreference of PCL has been also demonstrated by comparison of the optical rotation value for compounds 7, **8** and **1**, (Scheme 4) that were next synthesised, with the ones already described in the literature.[12c] PCL also catalysed the hydrolysis of the (*S*)-enantiomers of amino esters (±)-**4b–k** leading to amino acids (*S*)-**2b–k**, stereochemical trend also previously observed for the hydrolysis of structurally similar β-amino esters.[9a,f,i]

Thus we have successfully prepared a wide family of β-amino esters and β-amino acids, most of them in enantiopure form. From this moment we turned our attention to the preparation of (*S*)-dapoxetine using the precursor (*S*)-**2a** obtained by this enzymatic strategy in 98% *ee*. Previously we have reported the synthesis of this drug using as starting material the related 1,3-amino alcohol **7** obtained by a chemoenzymatic approach based on an enzymatic kinetic resolution of



Scheme 4. Chemical synthesis of (*S*)-dapoxetine from optically active β-amino acid (*S*)-**2a** obtained by an enzymatic hydrolysis process.

the O-protected amino alcohol using an acylation procedure mediated by *Candida antactica* lipase A (CAL-A).[12c] The free amino alcohol reacting with formaldehyde allows the dimethylation of the amino group at 100 °C but depending on the reaction conditions, an intramolecular condensation between the amino and the alcohol group can be observed leading to a decrease in the isolated yield of (*S*)-**9** and inconveniences in the isolation step of the final product.

In order to circumvent these problems we decided to investigate an alternative route for the preparation of compound (*S*)-**9** that is outlined in Scheme 4. In this manner, (*S*)-**2a** was chemically reduced using lithium aluminium hydride ($LiAlH_4$) in dry THF at 65 °C to the amino alcohol (*S*)-**7**. Subsequent O-selective protection using *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) and imidazole as base at room temperature provided (*S*)-**8** in 91% yield. Next dimethylation of the amino group and deprotection of the silyl ether in acidic conditions allowed the recovery of (*S*)-**9**. Dimethylation of compound (*S*)-**7** was also successfully achieved in only one synthetic step by using very mild reaction conditions such as sodium triacetoxyborohydride [$NaBH(OAc)_3$] in combination with formaldehyde at 40 °C. Finally, adequate functionalisation of the free hydroxy group using triphenylphosphine (PPh_3), diethyl azodicarboxylate (DEAD) and 1-naphthol under Mitsunobu conditions in dry THF as solvent led to (*S*)-dapoxetine as previously described.[12c] One clear advantage of this method is that (*S*)-dapoxetine can be obtained in higher enantiopurity than previously described, increasing the enantiomeric excess from 93 to 98% and allowing the recovery of some chiral intermediates through more simple isolation procedures.

Conclusions

In summary, we have chemically synthesised a family of racemic β-amino esters in good overall yields, which were further subjected to lipase-catalysed kinetic resolution. For the aminolysis reactions *via* the β-amino group, PCL and CAL-B afford synthetically useful activities and enantioselectivities if ethyl methoxyacetate is used as acylating agent. The result with CAL-B is especially interesting if we take into account that the enzyme successfully accepts a nucleophile with a medium-sized substituent made of five non-hydrogen atoms, thus widening the substrate space known for this lipase. However, the resolution of choice is the PCL-catalysed hydrolysis of the ester moiety. This way, the corresponding (*S*)-3-amino-3-arylpropanoic acids and methyl (*R*)-esters have been obtained in very high enantioselectivities, which has been rationalised in terms of a fragment-based approach. Thus, the optimum binding location and conformation of a benzene ring and the β-amino propanoate core, respectively, nicely match the fast-reacting (*S*)-configuration of methyl (±)-3-amino-3-phenylpropanoate.

The strategy reported here has been applied to the production of valuable optically active chiral precursors of (*S*)-dapoxetine, which has been chemoenzymatically prepared from racemic 3-amino-3-phenylpropionic acid by a straightforward manner in excellent optical purity under mild reaction conditions, in 19% isolated yield after 6 steps.

Experimental Section

Candida antarctica lipase type B (CAL-B, Novozyme 435, 7300 PLU/g) was a gift from Novo Nordisk Co. *Candida antarctica* lipase type A (CAL-A immobilized NZL-101, 2.6 U/

mg) was acquired from Codexis. *Pseudomonas cepacia* lipase in different immobilised forms was acquired from Sigma–Aldrich and Amano Pharmaceuticals Co. All other reagents were purchased from different commercial sources (Acros Organics, Fluka and Sigma–Aldrich) and used without further purification. Solvents were distilled over an adequate desiccant under nitrogen. Flash chromatographies were performed using silica gel 60 (230–240 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on using NaCl plates or KBr pellets in a Perkin–Elmer 1720-X F7. ^{1}H, ^{13}C NMR, DEPT, and ^{1}H-^{13}C heteronuclear experiments were obtained using Bruker AV-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz), DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz), or NAV-400 (^{1}H, 400.13 MHz and ^{13}C, 100.6 MHz) spectrometers. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). ESI^+, EI^+ or $APCI^+$ employing an HP1100 chromatograph mass detector were used to record mass spectra (MS). Measurement of the optical rotation was done in a Perkin–Elmer 241 polarimeter. High performance liquid chromatography (HPLC) analyses were carried out in a Hewlett-Packard 1100 chromatograph UV detector at 210 nm using a Daicel CHIRALCEL OJ-H, OD or a CHIRALPAK AS column (25 cm × 4.6 mm I.D.), conditions and retention times are given in the Supporting Information. Spectroscopic data of β-amino esters **4a**–**k**, methoxyacetamides **5b**–**c**, **h**–**j** and benzylcarbamates **6a**, **d**–**g** are given in the Supporting Information.

Experimental conditions for HPLC chiral analyses, characterisation data for new compounds and copies of ^{1}H, ^{13}C and DEPT NMR experiments are also available in the Supporting Information.

Synthesis of β-Amino Acids 2b–k

Aldehyde (30.0 mmol), ammonium acetate (4.65 g, 60.3 mmol) and malonic acid (3.15 g, 30.3 mmol) were refluxed in EtOH (75 mL) for 20 h. The reaction mixture was cooled to room temperature and the white solid was collected by filtration, washing with EtOH (25 mL) and Et_2O (25 mL). Solid was recrystallised in mixtures EtOH:H_2O depending on the corresponding substrate, obtaining the β-aminoacid **2b**–**k** as white solid crystals; yield: 40–84%.

Synthesis of β-Amino Esters 4a–k

To a suspension of the corresponding β-amino acid **2a**–**k** (5.5 mmol) in MeOH (91 mL) a solution of concentrated aqueous HCl (1 mL) was added dropwise and the clear solution refluxed for 12 h. The solution was cooled to room temperature and the solvent evaporated under reduced pressure. The resulting mixture was purified by flash chromatography on silica gel (10% MeOH/CH_2Cl_2 or 30% MeOH/EtOAc), obtaining the β-amino ester **4a–k** as a yellow oil or a pale yellow solid; yield: 24–98%.

Typical Procedure for the Enzymatic Kinetic Resolution of 3-Amino-3-phenylpropionic Methyl Ester (±)-4a by Lipase-Mediated Acylation

To a suspension of racemic amino ester **4a** (30 mg, 0.17 mmol) and enzyme (CAL-B, PCL-C I or CAL-A, 30 mg) in dry TBME (1.7 mL), ethyl methoxyacetate (99 μL, 0.84 mmol) was added under nitrogen atmosphere. The reaction was shaken at 30 or 45 °C and 250 rpm during 27 h. Then the reaction was stopped, and the enzyme filtered with CH_2Cl_2 (5 × 1 mL). The solvent was evaporated and the crude of the reaction purified by flash chromatography (10–30% MeOH/EtOAc) affording optically active methoxyacetamide **5a** and β-amino ester **4a**. The latter was transformed into the corresponding methoxyacetamide in order to measure both enantiomeric excesses by HPLC methods (see Table 1).

Typical Procedure for the Enzymatic Hydrolysis of 4a–k

To a solution of β-amino ester **4a–k** (0.28 mmol) in 1,4-dioxane (2.8 mL) were successively added distilled water (25 μL, 1.40 mmol) and PCL (ratio 2:1 in weight respect to the β-amino ester). Then the mixture was shaken at 45 °C and 250 rpm. Reaction time course (β-amino ester enantiomeric excess) was followed by HPLC (see Table 3). The β-amino acid precipitated in the reaction media was filtered with the enzyme while the amino ester remained in the solution, so the organic solvent was then evaporated under reduced pressure. For the measurement of the enantiomeric excesses: a) the β-amino ester was transformed into the methoxyacetamide (*R*)-**5b, c**, **h–k** or benzyl carbamate (*R*)-**6a**, **d**–**g**; b) the β-amino acid was esterified by the experimental procedure already described and then transformed into the corresponding methoxyacetamide (*S*)-**5b, c, h–k** or benzyl carbamate (*S*)-**6a**, **d**–**g** (see Scheme 3).

Synthesis of Methoxyacetamides 5b, c, h–k

To a solution of β-amino ester **4b**, **c**, **h**–**k** (0.28 mmol) in dry CH_2Cl_2 (1.9 mL) pyridine (28 μL, 0.35 mmol) was added under an inert atmosphere and the resulting solution cooled at 0 °C. Then methoxyacetyl chloride (64 μL, 0.70 mmol) was carefully added. The reaction mixture was stirred for 4 h at room temperature until complete consumption of the starting material (analysis by TLC). The solvent was evaporated and the reaction crude purified by flash chromatography on silica gel (10% MeOH/CH_2Cl_2 or 70% EtOAc/hexane), obtaining the corresponding methoxyacetamide **5b, c**, **h–k** as a pale yellow solid.

Synthesis of Benzyl Carbamates 6a, d–g

To a solution of β-amino ester **4a**, **d**–**g** (0.28 mmol) in dry CH_2Cl_2 (1.9 mL) pyridine (25 μL, 0.31 mmol) was added under inert atmosphere and the resulting solution cooled at 0 °C. Then benzyl chloroformate (44 μL, 0.31 mmol) was carefully added. The reaction mixture was stirred for 4 h at room temperature until complete consumption of the starting material (analysis by TLC). The solvent was evaporated and the reaction crude purified by flash chromatography on silica gel (10% MeOH/CH_2Cl_2 or 70% EtOAc/hexane), obtaining the corresponding benzyl carbamate **6a**, **d–g** as a pale yellow solid.

Synthesis of β-Amino Alcohol (*S*)-7

A solution of β-amino acid (*S*)-**4a** (1.00 g, 6.05 mmol) in dry THF (21.6 mL) was cooled to 0 °C and $LiAlH_4$ (460 mg, 12.11 mmol) added in small portions. The reaction mixture was refluxed for 12 h and then the reaction mixture cooled to 0 °C, and hydride excess destroyed by addition of H_2O dropwise. The mixture was extracted in EtOAc (4×20 mL) and the organic phases combined, dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (100% MeOH) isolating the β-amino alcohol **7** as a white solid; yield: 81%. R_f (100% MeOH): 0.17; mp 68–69 °C; IR (KBr): ν = 3347, 3280, 3130, 2940, 2922, 2846, 1600, 1493, 1457, 1430, 1371, 1154, 1081, 1062, 1050, 961, 762, 702 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ = 7.38–7.23 (m, 5H, Ar), 4.13 (dd, 1H, H-1, $^3J_{H,H}$ = 5.2 Hz, $^3J_{H,H}$ = 7.9 Hz), 3.87–3.75 (m, 2H, H-3), 2.72 (br s, 3H, NH_2 and OH), 1.98–1.82 (m, 2H, H-2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ = 146.0 (C-4), 128.6 (2C, C-5), 127.1 (C-7), 125.6 (2C, C-6), 62.1 (C-3), 56.5 (C-1), 39.4 (C-2); MS (ESI^+): *m/z* = 152 [$(M+H)^+$, 100%], 174 [$(M+Na)^+$, 5%)], 285 [$(2M-H_2O+H)^+$, 45%)]; $[\alpha]_D^{20}$: 21.8 (*c* 1.0, $CHCl_3$) for 98% *ee*.

Synthesis of O-Protected β-Amino Alcohol (*S*)-8

To a solution of amino alcohol (*S*)-**7** (200 mg, 1.32 mmol) in dry CH_2Cl_2 (9.5 mL) imidazole (225.1 mg, 3.31 mmol), 4-DMAP (16.0 mg, 0.13 mmol) and TBDMSCl (399.0 mg, 2.65 mmol) were successively added under a nitrogen atmosphere. The mixture was stirred overnight and the disappearance of the starting material followed by TLC analysis (100% MeOH). The reaction was stopped by adding an aqueous saturated NH_4Cl solution (10 mL) and extracted with CH_2Cl_2 (8×10 mL). The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (100% EtOAc) affording compound (*S*)-**7** as a clear oil; yield: 91%. R_f (20% MeOH/EtOAc): 0.31; IR (NaCl): ν = 775, 835, 938, 1099, 1256, 1360, 1388, 1454, 1463, 1472, 1492, 1602, 2856, 2928, 29543027, 3062, 3297, 3377 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ = 7.35–7.20 (m, 5H, Ar), 4.10 (t, 1H, H-1, $^3J_{H,H}$ = 6.1 Hz), 3.73–3.56 (m, 2H, H-3), 1.91–1.84 + 1.76 (m + br s, 4H, H-2, NH_2), 0.90 (s, 9H, H-6), 0.04 (s, 3H, H-4), 0.03 (s, 3H, H-4); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ = 146.3 (C-7), 128.3, 126.3 (4C, C-8 + C-9), 126.8 (C-10), 60.7 (C-3), 53.4 (C-1), 42.0 (C-2), 25.8 (3C, C-6), 18.2 (C-5), −5.5 (2C, C-4); MS (ESI^+): *m/z* = 266 [$(M+H)^+$, 100%], 145 [$(CH_2OTBDMS)^+$, 20%]; $[\alpha]_D^{20}$: +0.8 (*c* 1.0, $CHCl_3$) for > 99% *ee*.

Synthesis of Alcohol (*S*)-9 from Compound (*S*)-7

To a solution of amino alcohol (*S*)-**7** (100 mg, 0.66 mmol) in CH_2Cl_2 (8.3 mL) placed in a sealed tube, a 37% aqueous formaldehyde solution (149 μL, 1.98 mmol), Na_2SO_4 to capture water (15 mg, 0.11 mmol) and $NaBH(OAc)_3$ (561 mg, 2.65 mmol) were successively added. The mixture was stirred overnight at 40 °C and the disappearance of the starting material followed by TLC analysis (100% MeOH). The reaction was stopped by adding an aqueous $NaHCO_3$ solution until pH 8–9 and extracted with CH_2Cl_2 (5×10 mL). The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The reaction crude was finally purified by flash chromatography on silica gel (gradient eluent 50–60% MeOH/EtOAc) affording compound (*S*)-**9** as a clear oil; yield: 60%. R_f (60% MeOH/EtOAc): 0.20; IR (NaCl): ν = 704, 737, 772, 922, 1049, 1163, 1267, 1363, 1456, 1598, 2715, 2782, 2830, 2869, 2949, 3031, 3061, 3370 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ = 7.39–7.31 (m, 3H, Ar), 7.27–7.20 (m, 2H, Ar), 6.41 (br s, 1H, OH), 3.94 (dd, 1H, H-1, $^3J_{H,H}$ = 8.5 Hz, $^3J_{H,H}$ = 5.9 Hz), 3.80–3.73 (m, 1H, H-3), 3.70–3–60 (m, 1H, H-3), 2.47–2.34 (m, 1H, H-2), 2.29 (s, 6H, H-4), 1.89–1.80 (m, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ = 134.7 (C-5), 129.1 (2C, C-7), 128.2 (2C, C-6), 128.2 (C-8), 68.6 (C-1), 61.2 (C-3), 40.6 (C-4), 32.7 (C-2); MS (ESI^+): *m/z* = 180 [$(M+H)^+$, 100%], 202 [$(M+Na)^+$, 5%)]; $[\alpha]_D^{20}$: +36.8 (*c* 1.0, $CHCl_3$) for 99% *ee*.

Synthesis of Alcohol (*S*)-9 from Compound (*S*)-8

To a solution of (*S*)-**8** (150 mg, 0.56 mmol) in formic acid (286 μL, 7.46 mmol) was added a 37% aqueous solution of formaldehyde (559 μL, 7.46 mmol) and the mixture refluxed during 12 h. After this time the solution was evaporated under reduced pressure and the resulting crude purified by flash chromatography on silica gel (gradient eluent 40–80% MeOH/EtOAc) affording compound (*S*)-**9** as a clear oil; isolated yield: 74%.

Synthesis of (*S*)-Dapoxetine (1)

To a solution of (*S*)-**9** (46 mg, 0.26 mmol) in dry THF (3.8 mL) was added 1-naphthol (75.9 mg, 0.53 mmol) under a nitrogen atmosphere. The mixture was cooled to 0 °C and PPh_3 (139.3 mg, 0.53 mmol) and DEAD (84.0 μL, 0.53 mmol) were successively added. The solution was allowed to warm until room temperature and stirred during 15 h. The solution was evaporated and the crude purified by flash chromatography on silica gel (100% EtOAc) isolating (*S*)-**1** as an orangish oil; yield: 72%. R_f (20% MeOH/EtOAc): 0.46; IR (NaCl): ν = 770, 792, 1021, 1068, 1100, 1156, 1178, 1239, 1270, 1389, 1404, 1460, 1508, 1580, 1595, 1724, 2776, 2820, 2955, 3055 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ = 8.28–8.23 (m, 1H, H-11), 7.81–7.78 (m, 1H, H-14), 7.51–7.47 (m, 2H, H-12 + H-13), 7.34–7.26 (m, 7H, 2H-6 + 2H-7 + H-8 + H-16 + H-17), 6.66 (d, 1H, H-18, $^3J_{H,H}$ = 7.6 Hz), 4.12–4.05 (m, 1H, H-3), 3.95–3.87 (m, 1H, H-3), 3.79 (dd, 1H, H-1, $^3J_{H,H}$ = 5.4 Hz, $^3J_{H,H}$ = 9.3 Hz), 2.71–2.60 (m, 1H, H-2), 2.36–2.24 (m, 1H, H-2), 2.27 (s, 6H, H-4); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ = 154.5 (C-9), 139.4 (C-5), 134.4 (C-15), 128,5 (2C, C-7), 128.1 (2C, C-6), 127.3, 127.2, 126.2, 125.8, 125.6, 125.0 (C-8 + C-10 + C-12 + C-13 + C-14 + C-17), 121.9 (C-11), 119.9 (C-16), 104.5 (C-18), 67.6 (C-1), 65.5 (C-3), 42.7 (2C, C-4), 32.9 (C-2); MS (ESI^+): *m/z* = 306 [$(M+H)^+$, 100%)], 261 [$(M-NMe_2)^+$, 35%]; $[\alpha]_D^{20}$: +71.4 (*c* 1.0, $CHCl_3$) for 98% *ee*.

General Computational Methods

All computations were performed in an iMac using the molecular modelling package Molecular Operating Environment (MOE) 2008.10 (Chemical Computing Group, Inc.).[25] In all cases the Amber99 force field[26] and the corresponding dictionary charges were used as implemented in MOE. Due to the lack of parameters for 1,4-dioxane compatible

with the force field used we opted for a continuum solvation method. In particular, a distance-dependent relative dielectric constant of 2 was selected as a good compromise between the dielectric constant of 1,4-dioxane[27] and the one of a globular protein matrix.[28] A non-bonded cut-off of 8 Å with a smoothing function acting in the range 8–10 Å was used. In all MDS the NVT ensemble and the Nosé–Poincaré–Anderson (NPA) equations were selected. The initial and simulation temperatures were set to 0 K and 300 K, respectively. No constraints were imposed on any bond and a step size of 1 fs was used. The heating period duration and the temperature relaxation time were set to 1 ps and 10 fs, respectively. The convergence criterion of the energy minimisations (EM) was set to a gradient value of 0.01 kcal mol^{-1} when only parts of the protein-ligand complex were minimised; if the whole enzyme-substrate complex was minimised it was lowered to 0.001 kcal mol^{-1}. High quality pictures of representative structures were generated with PyMOL 0.99.[29]

Preparation of the Enzyme Model

The coordinates of PCL were retrieved from the Brookhaven Protein Data Bank[30] (entry number 3 LIP[31]). The carboxy and amino terminal groups of the protein backbone were changed by carboxylate and ammonium, respectively. The catalytic histidine was kept neutral but analysis of the hydrogen bonding network of the protein suggested a protonated state for Glu289.[32] Next, the enzyme starting structure to be used in all computations was prepared following a previously described protocol.[21g,33] Hydrogens were subsequently added and in order to improve their initial orientation, a series of 1 ps MDS (heating period: 1 ps) followed by EM were carried out as follows: 1) first only water hydrogens, and then hydrogens of the side chains of certain amino acids (Ala, Cys, Ile, Leu, Lys, Met, Ser, Thr, Tyr, Val) and the ligand were allowed to move. Next, subsequent EMs were carried out over the water molecules, and the side chains of all the amino acids and the ligand. At this stage, any water molecule not hydrogen-bonded to any atom of the system was removed from the protein structure by means of the LigX tool implemented in MOE. Finally, subsequent EM over the remaining water molecules, the side chains of the amino acids and the ligand, and the whole protein-ligand complex were carried out.

MultiFragment Search Simulations (MFSS)

The phenyl group present in the amino ester **4a** was sketched with the Build panel implemented in MOE, subjected to EM and used as input for an MFSS run in the active site of the previously prepared structure of PCL. The residues lining the active site were allowed to move by setting a belly distance of 4.5 Å. The rest of parameters were set to their default values.

Preparation of the Tetrahedral Intermediate of the 3-Aminopropanoate Core

The transition state of the PCL-catalysed hydrolysis of methyl 3-aminopropanoate selected for visualisation was that corresponding to the deacylation step of the catalytic serine.[34] It was built manually by modification of the side chain of Ser87 in the enzyme model as follows: (i) first, a tetrahedral carbon covalently bonded to the Oγ of Ser87, and to aliphatic C, and O atoms was built. In all cases a formal charge of −1 was set on the oxyanion atom, the catalytic His (His286) was kept neutral and the nucleophilic oxygen was assigned a formal charge of +1; (ii) torsions were modified so that each substituent was placed in the corresponding pocket; (iii) an EM of the so-created atoms was carried out; (iv) the rest of the acyl chain was built atom by atom and we used 1 ps MDS to explore the preferred location of each "new" atom in the acyl binding pocket of PCL; (v) the whole side chain of the so-created amino acid and (vi) finally, the whole tetrahedral intermediate was energy minimised. A 100 ps MDS was run over the side chain of the newly created amino acid and the snapshots were ranked according to the internal and interaction energy of the atoms of the side chain allowed to move. The lowest in energy one was EM and used for visualisation purposes.

Acknowledgements

Financial support by MEC (CTQ 2007-61126) is acknowledged. V. G.-F. (Ramón y Cajal Program), and M. R.-M. (FPU fellowship) thank MICINN for personal funding.

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