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Carica papaya Lipase Catalysed Resolution of β-Amino Esters for the Highly Enantioselective Synthesis of (*S*)-Dapoxetine

Pengyong You,[a] Jian Qiu,[a] Erzheng Su,*[a] and Dongzhi Wei*[a]

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An efficient synthesis of the (*S*)-3-amino-3-phenylpropanoic acid enantiomer has been achieved by *Carica papaya* lipase (CPL) catalysed enantioselective alcoholysis of the corresponding racemic *N*-protected 2,2,2-trifluoroethyl esters in an organic solvent. A high enantioselectivity ($E > 200$) was achieved by two strategies that involved engineering of the substrates and optimization of the reaction conditions. Based on the resolution of a series of amino acids, it was found that the structure of the substrate has a profound effect on the CPL-catalysed resolution. The enantioselectivity and reaction rate were significantly enhanced by switching the conventional methyl ester to an activated trifluoroethyl ester. When considering steric effects, the substituted phenyl and amino groups should not both be large for the CPL-catalysed resolution. The mechanism of the CPL-catalysed enantioselective alcoholysis of the amino acids is discussed to delineate the substrate requirements for CPL-catalysed resolution. Finally, the reaction was scaled up, and the products were separated and obtained in good yields (≥ 80 %). The (*S*)-3-amino-3-phenylpropanoic acid obtained was used as a key chiral intermediate in the synthesis of (*S*)-dapoxetine with very high enantiomeric excess (> 99 %).

Introduction

Chirality is a key feature in the efficiency of many drugs and agrochemicals, and consequently the production of single enantiomers of chiral intermediates has become increasingly important for many industrial applications.[1,2] Enantiopure β-amino acids and their derivatives are important ubiquitous structural motifs in natural products and pharmaceuticals.[3a,3b] In life sciences, chiral β-amino acids have been widely found in biologically active peptides.[3c] Chiral β-amino acids are also widely used as key intermediates or chiral building blocks in the synthesis of pharmaceuticals.[3d] 3-Amino-3-phenylpropionic acid (BPA) is one of the most valuable β-amino acids and a useful compound for the synthesis of pharmaceuticals such as taxol, a complex diterpene isolated from the bark of *Taxus brevifolia* that possesses strong anticancer activity,[4] and (*S*)-dapoxetine [(*S*)-(+)-*N*,*N*-dimethyl-α-[2-(1-naphthalenyloxy)ethyl]benzenemethanamine; Figure 1], which is a potent selective serotonin re-uptake inhibitor (SSRIs) used in the treatment of depression and other disorders such as bulimia or anxiety.[5] (*S*)-Dapoxetine was first launched in 2009 in Europe where it was used for the oral, on-demand treatment of premature ejaculation (PE) in men between 18 and 64 years of age. Nowadays, the asymmetric synthesis of (*S*)-dapoxetine has been intensively investigated because the (*S*) and (*R*) isomers usually display very different pharmacological or physiological properties.[6,7]

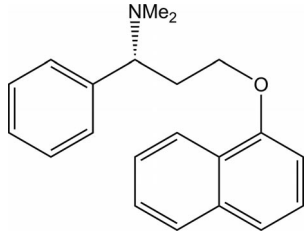

Figure 1. Structure of (*S*)-dapoxetine.

As a key chiral building block for the enantioselective synthesis of (*S*)-dapoxetine, β-amino acids can be obtained in one step by the reaction of readily available aldehydes with malonic acid and ammonium acetate; thus, chiral resolution is one of the most efficient methods for the production of optically pure β-amino acids and their derivatives.[8] Lipase is considered as an ideal tool for the preparation of enantiomerically pure compounds and has been widely exploited for the resolution of racemic mixtures in the preparation of pharmaceuticals. Some commercial lipases such as *Candida antarctica* lipase A (CAL-A), *Candida antarctica* lipase B (CAL-B) and *Pseudomonas cepacia* lipase (PCL) have been used in the kinetic resolution of racemic BPA esters.[6,9] However, the enantioselectivities

[a] State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology,
Shanghai 200237, China
Fax: +86-21-64250068
E-mail: ezhsu@ecust.edu.cn
dzhwei@ecust.edu.cn
Homepage: http://sklbe.ecust.edu.cn/old-2009-2-10/group/wdz.html

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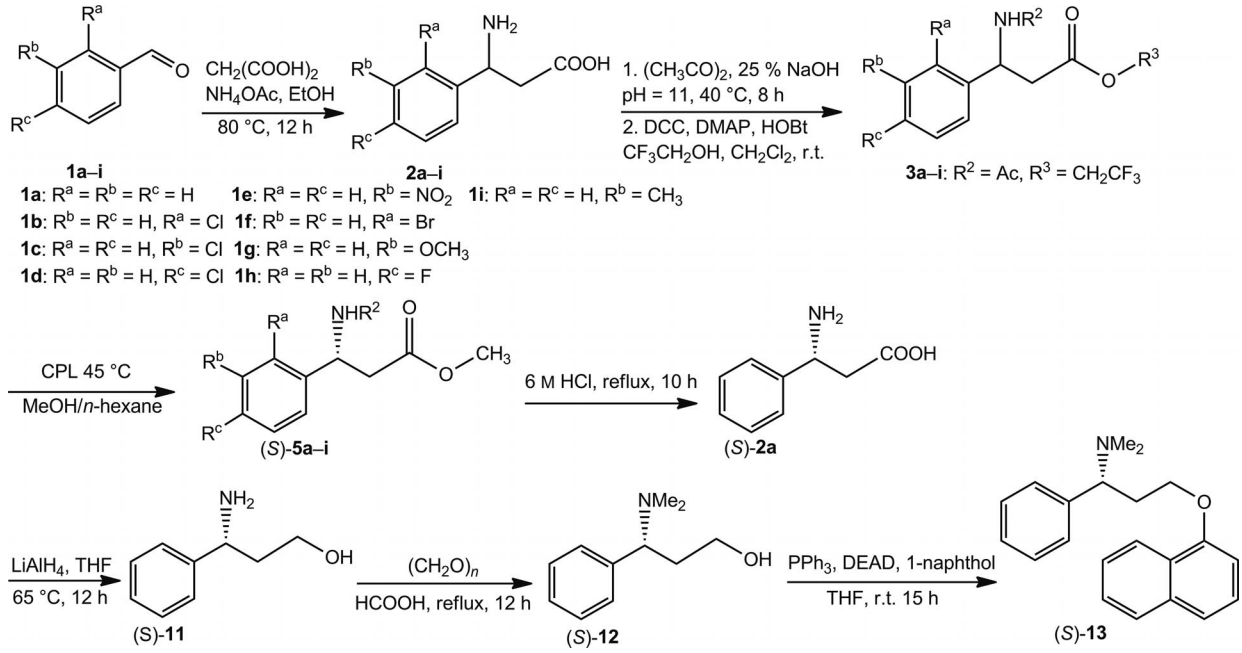


Scheme 1. Chemoenzymatic synthesis of (*S*)-dapoxetine by CPL-catalysed enantioselective resolution of BPA and its derivatives.

and production yields were not very satisfactory, and furthermore these lipases are expensive.

Carica papaya lipase (CPL), found in crude papain, is available as a "natural immobilized" biocatalyst at a competitive price, and is an emerging and promising biocatalyst.[10] It is more active, enantioselective and thermally more stable than *Candida rugosa* lipase, and is known to show high enantioselectivity towards carboxylic acids.[11] In recent years it has been used as an excellent biocatalyst in asymmetric synthesis, including in the kinetic resolution of racemic secondary alcohols,[12a] non-steroidal anti-inflammatory drugs,[12b] mandelate esters[12c] and α-amino acids.[12d]

Herein we report our work on the CPL-catalysed kinetic resolution of 3-amino-3-phenylpropionic acid (BPA) and its derivatives in terms of substrate structure, acyl donor, solvent and other parameters that have an influence on biocatalysis processes. The CPL enantiorecognition mechanism was investigated from an experimental point of view to gain an understanding of the results obtained during these investigations. Finally, one of the valuable intermediates obtained by this methodology was employed as the starting material for the synthesis of (*S*)-dapoxetine with high enantiomeric purity, highlighting the importance and potential of the methodology described in this work (Scheme 1).

Results and Discussion

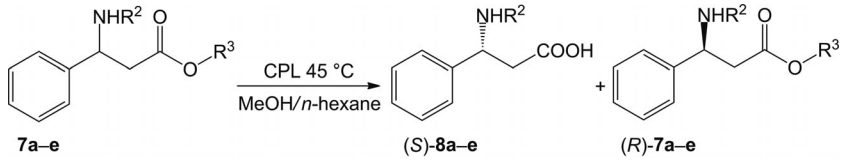
Effect of Substrate Structure on CPL-Catalysed Enantioselective Resolution of β-Amino Esters

The CPL-catalysed resolution of *N*-protected BPA conventional aliphatic esters showed moderate enantioselectivity (Table 1). For example, when *N*-acetyl-BPA methyl ester **7b** was used as the substrate, moderate selectivity was observed ($E = 14$). A number of strategies (engineering of the reaction medium, substrate molecule or enzyme) for enhancing the enantioselectivity have been reported.[13] Among these strategies, engineering of the substrate is a useful and simple method, as reported by Miyazawa et al.[14] In their work, non-protein amino acid esters were enantioselectively hydrolysed by microbial protease, and a significant increase in enantioselectivity was obtained by using esters with longer alkyl chains. Thus, to improve the enantioselectivity of the CPL-catalysed resolution, similar reactions were performed by using esters with longer alkyl chains. However, no marked increase in enantioselectivity was obtained, as shown in Table 1 (Entries 1–4). Analysis of the reaction parameters shows that the moderate enantioselectivities may be caused by the low reaction rates of the (*S*) substrates, which contrast the normally accepted results.[15] As the enantioselectivity E is the quotient of V_S and V_R [Equation (1) in which V_S and V_R are the reaction rates of the (*S*)- and (*R*)-esters, respectively, and k_{2R} and k_{2S} are the kinetic constants for the (*S*) and (*R*) substrates, respectively], increasing the (*S*) substrate reaction rate or decreasing the (*R*) substrate reaction rate can both result in an increase in the enantioselectivity. As the reaction rate of (*R*)-**7b** is low ($V_R = 1.03 \times 10^{-6}$ mM/h), the key point was to find a way to increase the reaction rate of (*S*)-**7b** effectively. In the following experiments, we substituted trifluoroethyl ester for longer alkyl esters.

$$E = V_S/V_R = k_{2S}K_{MR}/k_{2R}K_{MS} \quad (1)$$

Initially, β-phenylalanine (3-amino-3-phenylpropionic acid, BPA) was chosen as a model amino acid. In a general experimental procedure, the 2,2,2-trifluoroethyl ester of *N*-protected BPA [(*RS*)-**3a**] was allowed to react with an

Table 1. CPL-catalysed resolution of BPA alkyl esters (*RS*)-**7a**–**e** with different alkyl chain lengths.



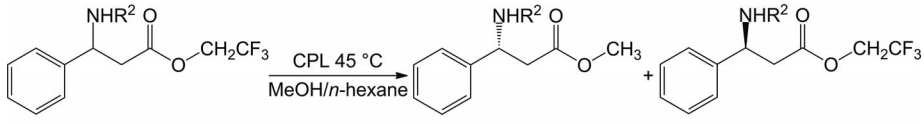
7a–e (*S*)-**8a–e** (*R*)-**7a–e**

7a: R^2 = H, R^3 = Me **7d**: R^2 = Ac, R^3 = $CH_2CH_2CH_3$
7b: R^2 = Ac, R^3 = Me **7e**: R^2 = Ac, R^3 = $CH_2CH_2CH_2CH_3$
7c: R^2 = Ac, R^3 = Et

Entry	Compound	V_S [10^{-5} mM/h][a]	V_R [10^{-6} mM/h]	E[b]
1	**7b**	1.44	1.03	14
2	**7c**	1.32	1.10	12
3	**7d**	1.30	1.08	12
4	**7e**	1.21	1.01	12

[a] Reaction rate of the (*S*) isomer. [b] $E = \ln[(1 - C)(1 - ee_s)]/\ln[(1 - C)(1 + ee_s)]$. C is the reaction conversion and ee_s is the *ee* of the substrate.

Table 2. CPL-catalysed resolution of BPA alkyl esters (*RS*)-**3a**, **4a**–**d** and **7a** with different protecting groups.



3a, **4a–d** (*S*)-**5a**, **6a–d** (*R*)-**3a**, **4a–d**

3a: R^2 = Ac **4c**: R^2 = Pac (phenylacetyl) **5a**: R^2 = Ac **6c**: R^2 = Pac
4a: R^2 = Boc **4d**: R^2 = Fmoc **6a**: R^2 = Boc **6d**: R^2 = Fmoc
4b: R^2 = Z **6b**: R^2 = Z

Entry	Compound	V_S [mM/h]	V_R [10^{-5} mM/h]	E
1	**3a**	6.40×10^{-3}	2.80	230
2	**4a**	4.15×10^{-3}	2.02	205
3	**4b**	4.60×10^{-4}	1.15	40
4	**4c**	2.58×10^{-4}	1.03	25
5	**4d**	n.d.[a]	n.d.	n.d.
6	**7a**[b]	1.01×10^{-2}	5.36	189

[a] n.d. = no reaction detected. [b] For the structure of **7a**, see Table 1.

alcohol (10 equiv.) in an organic solvent in the presence of CPL at a constant temperature (Table 2). The reaction conversion and enantiomeric excess were monitored by HPLC on a chiral column. As shown in Table 2 (Entry 1), the reaction rate V_S and enantioselectivity E for compound **3a** were greatly improved to 6.40×10^{-3} mM/h and 230, respectively. Compared with the methyl ester **7b**, the reaction rate for (*S*)-**3a** was enhanced 440 times. Switching the conventional methyl ester to activated esters such as the trifluoroethyl ester not only changed the enantioselectivity of the CPL, but also improved the reaction rate of the (*S*) isomer significantly, which resulted in excellent enantioselectivity. From the above results it can be concluded that the R^3 group, that is, the ester group, has a profound effect on CPL-catalysed resolution (Figure 2). In the following experiments the effect of the other two groups R^1 (substituents on the aromatic ring and size of the R^1 group) and R^2 (the size of the protecting group and the relative position of the amino group with respect to the R^1 group) was systematically investigated.

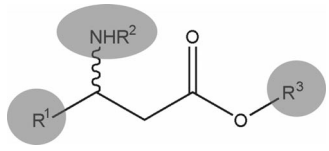


Figure 2. Structure of the substrate.

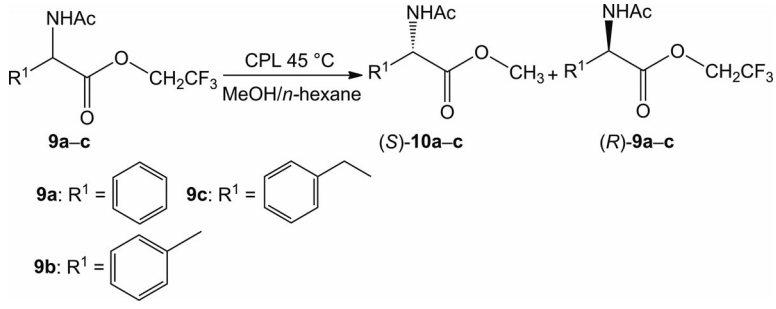
Protecting groups play a pivotal role in organic chemistry, and especially in peptide, carbohydrate and nucleic acid synthesis. In peptide synthesis, amine functionalities are usually protected as acid amides.[16] *N*-Protected esters have the advantage of affording compounds ready for further synthetic manipulation and improved solvent solubility. The substituents at the stereocentre have a very significant influence on the lipase-catalysed resolution of carboxylic acids.[17] However, to the best of our knowledge, there are no reports on the influence of different *N*-protecting groups on enantioselectivity. This led us to investigate the influence of the *N*-protecting groups (R^2 groups) on the CPL-cata-

lysed resolution of β-BPA esters. As shown in Table 2 (Entries 1–5), the reaction rate and enantioselectivity increased with decreasing size of the *N*-protecting group. Note that CPL could not catalyse the alcoholysis of the 2,2,2-trifluoroethyl ester of *N*-Fmoc-β-BPA (**4d**). When switching the large *N*-protecting group Fmoc to the relatively small protecting group acetyl (**3a**), it was found that the acetyl group increased the alcoholysis rate (6.4×10^{-3} mM/h) and gave excellent enantioselectivity (Table 2, Entries 1 and 5). Alcoholysis of the *N*-Boc, Pac (phenylacetyl), and Z-β-BPA 2,2,2-trifluoroethyl esters (**4a**, **4c**, **4b**) also gave moderate reaction rates and good to excellent enantioselectivities (Table 2, Entries 2–4). This phenomenon can be explained by the fact that the reaction rate is considerably influenced by the size of the substituents at the stereocentre. Large substituents, for example, the Fmoc group, hindered the formation of the substrate–enzyme (CPL) intermediate and resulted in either no reaction or a low reaction rate. This phenomenon was also reported for lipase CAL-B, for which catalysed alcoholysis of *N*-Bz-, Cbz-, and Fmoc-protected β-BPA methyl esters occurred with very low reaction rates.[12d] The decrease in the reaction rate was caused by steric effects exerted by the Pac or Fmoc group. It can be concluded that the size of the protecting group has an important effect on the CPL-catalysed resolution of β-BPA, not only on the reaction rate, but also on the enantioselectivity. From the point of view of steric effects, the R^1 and R^2 groups should not both be large groups for CPL-catalysed resolution. Janes and Kazlauskas reported that high enantioselectivity required a protonated amino group (NH_3^+)[18] for lipase-catalysed resolution of amino acids. In their research, by changing the pH of the reaction solvent, the enantioselectivity was greatly changed; but in our work, CPL-catalysed resolution of the *N*-protected BPA ester (NH) **7a** and *N*-unprotected BPA ester (NH_2) **3a** both gave good enantioselectivities (Table 2, Entries 1 and 6), which indicates that a protonated amino group may not be needed for CPL-catalysed resolution of amino acids.

Based on the above results, 2,2,2-trifluoroethyl esters of *N*-Ac-(*RS*)-β-BPA and its analogues **9a**–**9c** were resolved by CPL to investigate the steric effect between the amino and R^1 groups (Table 3). The distance between the amino and R^1 groups increases in the order **9a** < **9b** < **9c**. Thus, for the CPL-catalysed resolution of compounds **9a**–**c**, steric hindrance decreases in the order **9a** > **9b** > **9c**. The reaction rate was observed to increase with decreasing steric hindrance, which indicates that the relative distance between the amino and R^1 groups has a profound effect on the reaction rate. In comparison with **9a**–**c**, CPL also shows excellent enantioselectivity towards **3a**, which is a β-amino acid. Thus, it can be concluded that CPL shows high specific activity towards this kind of amino acid with a phenyl sidechain, no matter whether it is an α- or β-amino acid.

Next, the scope of the reaction was examined with respect to the tolerance of substituents on the aromatic ring. *N*-Ac-BPA 2,2,2-trifluoroethyl esters with different substituents on the benzene ring were subjected to CPL-catalysed alcoholysis. The alcoholysis of the 2,2,2-trifluoroethyl esters of *N*-protected, halogenated β-BPA proceeded about 10 times more slowly than that of the corresponding non-halogenated esters (Table 4, Entry 1). In the case of the Cl-substituted β-BPAs, the *ortho*-substituted β-BPA reacted faster than the corresponding *meta*- and *para*-substituted esters. Substitution of the Cl with more or less electron-donating groups such as NO_2 and Br or with large or small groups such as OCH_3 and F had no significant effect on the reaction rate. One explanation for this might be that the presence of substituent groups on the benzene ring makes the substrates too large to be accumulated at the active site, and this steric effect reduces the reaction rate markedly. In addition to steric effects, the non-steric properties (e.g., charge distribution, polarizability) of the substituent group also have an important effect on the reaction rate, because CPL-catalysed alcoholysis exhibits different reaction rates with bromo, methyl and methoxy substituents, which are of a similar size (Table 4, Entries 6, 7 and 9). Thus, it is reason-

Table 3. CPL-catalysed resolution of the 2,2,2-trifluoroethyl ester of *N*-Ac-BPA (*RS*)-**3a** and its analogues (*RS*)-**9a**–**c**.



Entry	Compound	V_S [10^{-3} mM/h]	V_R [10^{-5} mM/h]	*E*
1	**3a**[a]	6.40	2.80	230
2	**9a**	5.46	2.60	210
3	**9b**	6.56	2.70	243
4	**9c**	8.01	3.00	267

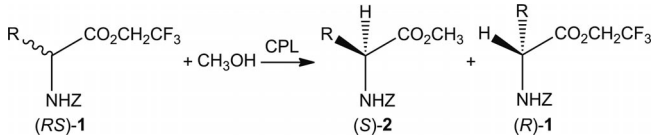
[a] For the structure of **3a**, see Table 2.

Table 4. CPL-catalysed resolution of 2,2,2-trifluoroethyl esters of *N*-Ac-BPA with different substituents on the aromatic ring (*RS*)-**3a**–**i**.[a]

Entry	Compound	Aromatic substituent	V_S [10^{-4} mM/h]	V_R [10^{-6} mM/h]	Conversion [%]	Reaction time [h]	*E*
1	**3a**	H	64.0	28.0	50.0	10	230
2	**3b**	2-Cl	6.80	3.05	46.5	90	223
3	**3c**	3-Cl	5.83	2.75	45.0	90	212
4	**3d**	4-Cl	5.54	2.70	44.5	90	205
5	**3e**	3-NO_2	5.64	2.30	40.5	120	245
6	**3f**	2-Br	6.32	2.60	44.0	90	243
7	**3g**	3-CH_3O	5.95	2.50	43.0	90	238
8	**3h**	4-F	5.04	2.40	43.6	120	210
9	**3i**	3-CH_3	19.4	6.70	47.5	40	290

[a] Reaction conditions: 45 °C in *n*-hexane with methanol as nucleophile.

Table 5. CPL-catalysed enantioselective alcoholysis of (*RS*)-*N*-*Z*-amino acid 2,2,2-trifluoroethyl ester (*RS*)-**1** and methanol.



Compound	R	Conversion [%]	ee_P [%]	*E*
1a	CH_3	55.6	78.4	38
1b	CH_3CH_2	50.8	95.6	>200
1c	$CH_3(CH_2)_2$	46.3	>99.8	>200
1d	$CH_3(CH_2)_3$	50.2	99.2	>200
1e	$(CH_3)_2CHCH_2$	43.6	>99.8	>200
1f	$CH_3(CH_2)_4$	46.8	>99.8	>200
1g	$(CH_3)_2CH(CH_2)_2$	48.0	>99.8	>200
1h	*cyclo*-$C_6H_{11}CH_2$	26.0	>99.8	>200
1i	$CH_3SCH_2CH_2$	50.2	99.2	>200
1j	$CH_3CH_2SCH_2CH_2$	47.7	>99.8	>200

able to assume that non-steric interactions between CPL and the substrate play an important role.[19] Alcoholysis of the CH_3-substituted 2,2,2-trifluoroethyl ester of *N*-Ac-BPA (**3i**) was conducted to verify this. The reaction rate and enantioselectivity are in accord with our explanation; compared with compound **3b**, the reaction rate is enhanced nearly three-fold and gives excellent enantioselectivity.

CPL did not only show excellent enantioselectivity towards aromatic amino acids, but also towards aliphatic amino acids.[20] As shown in Table 5, the R group of these amino acids are C_2 to C_5 aliphatic chains (the one exception is **1h** with a cyclohexyl group; Z = benzyloxycarbonyl). In almost all the cases examined, the reactions were almost completely enantioselective ($E > 200$). Only slight differences were observed in terms of enantioselectivity for compounds **1a**–**1j** with increasing length of the side-chain. One exception is the result for compound **1h**; the conversion after incubation for 24 h was sharply reduced to 26.0%, but a high enantioselectivity was still obtained. In compound **1h**, the R group is cyclohexyl and therefore, combined with the Z group, compound **1h** has two large groups. Thus, this lower conversion could be ascribed to steric hindrance. As stated above, the R and amino substituent should not both be large for CPL-catalysed resolution.

Optimization of the Reaction Conditions

As reported previously, the enantioselectivity of the lipase-catalysed resolution is greatly affected by the alcohol nucleophile.[21] The effect of the alcohol nucleophile on the CPL-catalysed alcoholysis of (*RS*)-**3a** was investigated in *n*-hexane. The reaction proceeded in an almost completely enantioselective manner (Table 6). The (*S*)-methyl ester (*S*)-**5a** was solely formed until the reaction reached 50% conversion, the (*R*)-trifluoroethyl ester (*R*)-**3a** remaining nearly intact. In this case, the enantiomeric ratio *E* was evaluated to be more than 200. There was almost no difference observed between methanol, ethanol, propanol and butanol with regard to reaction rate or enantioselectivity. When the

Table 6. CPL-catalysed resolution of (*RS*)-**3a** using different chain length alcohols as the nucleophile.[a]

Entry	Alcohol	Conversion [%]	ee_P [%]	*E*
1	methanol	50.0	99.2	>200
2	ethanol	50.5	98.5	>200
3	propanol	45.0	98.0	>200
4	butanol	44.5	98.0	>200
5	hexanol	50.2	96.0	161

[a] Reaction conditions: 45 °C in *n*-hexane.

Table 7. Solvent effect on the CPL-catalysed resolution of (*RS*)-**3a**.[a]

Entry	Solvent	ee_p [%]	Conversion [%]	Reaction time [h]	*E*
1	*n*-hexane	>99	50	10	>200
2	cyclohexane	>99	49	10	>200
3	isooctane	94.5	51	10	45
4	*tert*-amyl alcohol	>99	12	40	>200
5	tetrahydrofuran	>99	10	40	>200
6	diethyl ether	72	44	10	11
7	toluene	>99	23	40	>200

[a] Reaction conditions: 45 °C with methanol as nucleophile.

chain length of the alcohol became longer, the conversion decreased, with hexanol serving as the poorest nucleophile. On the basis of these results, methanol was chosen as the best nucleophile for CPL-catalysed resolution.

We next investigated the CPL-catalysed resolution of (*RS*)-**3a** in different solvents, the most commonly used organic solvents for lipase-catalysed resolution. Both the reaction rate and enantioselectivity were significantly affected by the solvent employed. The reactions were highly enantioselective ($E > 200$) but extremely slow in relatively polar solvents (Table 7, Entries 4, 5 and 7). The use of non-polar solvents such as *n*-hexane and cyclohexane resulted in a marked increase in the reaction rate and excellent enantioselectivities (Entries 1 and 2). In the case of isooctane, the reaction rate also increased significantly, but the value of *E* decreased to 45 (Entry 3). The use of Et_2O proved to be useless due to its low enantioselectivity, its *E* value sharply dropping to 11. In conclusion, *n*-hexane and cyclohexane proved to be the most efficient solvents.

In an attempt to shorten the reaction time, reactions were performed at different temperatures. The initial reaction rates of the two enantiomers and the *E* value at each temperature were estimated and are summarized in Table 8. CPL showed excellent enantioselectivity with values of *E* more than 200 below 50 °C. CPL also showed good thermal stability, and this can be attributed to the lipase being naturally bound to the non-soluble matrix of the *papaya* latex.[22] Increasing the temperature to 70 or 75 °C resulted in a clear decrease in the value of *E* (*E* = 32 and 26). When the reaction was carried out at these temperatures (70 or 75 °C), the colour of CPL gradually changed from pale yellow to dark brown, which implies that the CPL is deactivated above 50 °C.

Table 8. CPL-catalysed resolution of (*RS*)-**3a** at different temperatures.[a]

Entry	Temperature [°C]	V_S [10^{-3} mM/h]	V_R [10^{-5} mM/h]	*E*
1	35	5.80	2.15	270
2	45	6.40	2.80	230
3	50	6.75	3.29	205
4	55	7.05	4.15	170
5	65	7.60	6.90	110
6	70	7.90	24.7	32
7	75	8.35	32.1	26

[a] Reaction conditions: in *n*-hexane with methanol as nucleophile.

Mechanistic Analysis

In this work we found that CPL shows moderate enantioselectivity with *N*-Ac-β-BPA methyl ester **7b**, and no enhancement of enantioselectivity was obtained when using esters with longer alkyl chains (**7c**–**e**). Changing the conventional methyl ester to the trifluoroethyl ester **3a** gave excellent enantioselectivity. The *N*-protecting group (R^2 group) also plays an important role in the CPL-catalysed resolution of *N*-protected β-BPA esters. The value of *E* increases in the order of Fmoc < Pac < Cbz < Boc < Ac. Thus, large protecting groups have a negative effect on the enantioselectivity and reaction rate. For example, CPL showed no activity towards the Fmoc-protected β-BPA ester **4d**. When considering the effect of the size of the protecting group, Kazlauskas' rule seemed valid for CPL-catalysed resolution. The lipase CPL contains a large hydrophobic binding hole, which is open to the solvent, and this hole can accommodate a large substituent. However, it is difficult to explain why protecting groups with a size similar to the benzene ring, for example Cbz (**4b**) and Pac (**4c**), also gave good enantioselectivities. Furthermore, very similar enantioselectivities were obtained when the resolution of BPA methyl ester **7a** and *N*-Ac- and *N*-Boc-β-BPA 2,2,2-trifluoroethyl esters **3a** and **4a**, respectively, were conducted in *n*-hexane (Table 2, Entries 1, 2 and 6). The above results imply that within a certain size range the enantioselectivity is not determined by the relative size of the substituents. When attention was paid to the phenyl substituent (R^1), similar results were obtained. For the CPL-catalysed resolution of *N*-protected α-amino acids (Table 5), the R^1 groups were C_2 to C_5 aliphatic chains and showed no difference in enantioselectivity, as judged by the *E* values.[20] These results are in accord with the work of Janes and Kazlauskas;[18] they reported that several carboxylic acids containing similarly sized substituents also gave good to excellent enantioselectivity, the enantioselectivity of ANL-catalysed (ANL = *Aspergillus niger* lipase) resolution of carboxylic acids being independent of the size of the substituents. Instead, they found that steric effects and the charge of the amino acid had a profound effect on the ANL-catalysed resolution. Similar results have also been reported for other lipases,[17,23] for example, the CAL-B-catalysed resolution of *N*-protected β-amino acid methyl esters. Thus, we have concluded that it is difficult to ascribe the enantioselectivity to the relative size of the substituents at the stereocentre for

CPL-catalysed resolution of amino acids. The general rule used to predict the preferred enantiomers of secondary alcohols or carboxylic acids might not be valid for amino acids. Thus, there might be a different mechanism to account for their behaviour. As the amino acid sequence and structure of CPL remain unknown, it is difficult to employ molecular modelling techniques to qualitatively or even quantitatively elucidate the lipase activity and enantioselectivity.

Synthesis of (*S*)-Dapoxetine

Following the preliminary results, the gram-scale resolution of (*RS*)-**3a** was performed in *n*-hexane with 10 mmol of *N*-Ac-BPA 2,2,2-trifluoroethyl ester **3a** and 10 equiv. of methanol in the presence of 3.0 g of CPL preparation at 50 °C. The faster-reacting enantiomer (*S*)-**3a** was transformed into its *N*-Ac-protected derivative (*S*)-**5a**. When the reaction conversion reached 50%, CPL was separated by centrifugation, and the solvent was evaporated. Esters (*S*)-**5a** and (*R*)-**3a** were separated by column chromatography. (*S*)-Dapoxetine was synthesized with (*S*)-*N*-Ac-BPA methyl ester (*S*)-**5a** in 99% *ee* as the intermediate, which was then hydrolysed with 6 M HCl to the corresponding amino acid (*S*)-**2a** (72%; Scheme 1). Initially, we attempted to obtain (*S*)-**11** by reducing (*S*)-**5a** with $NaBH_4$, as the methyl ester is more susceptible to reduction than the acid. Unfortunately, this failed to yield the desired compound (*S*)-**11** as the protecting group was also reduced. Consequently, the methyl ester and *N*-protecting group were deprotected in HCl solution. Then (*S*)-**2a** was chemically reduced with lithium aluminium hydride ($LiAlH_4$) in dry THF at 65 °C to the amino alcohol (*S*)-**11** followed by Eschweiler–Clarke methylation, leading to the formation of (*S*)-**12** in high yield (87%). (*S*)-Dapoxetine (*S*)-**13** was synthesized by treating the intermediate (*S*)-**12** with 1-fluoronaphthalene, pretreated with HCl/EtOAc, in dry THF under nitrogen to yield (*S*)-dapoxetine [(*S*)-**13**]. One clear advantage of this chemoenzymatic method is that (*S*)-dapoxetine could be obtained with higher enantiopurity (nearly enantiomerically pure, 99%).

Conclusions

We have chemically synthesized a family of racemic β-amino esters in good overall yields, which were then subjected to CPL-catalysed kinetic resolution. CPL showed moderate enantioselectivity towards the *N*-Ac-BPA-methyl ester. The enantioselectivity was not enhanced by switching the conventional methyl ester to long-chain alkyl esters. However, the enantioselectivity and reaction rate were greatly enhanced by employing 2,2,2-trifluoroethyl esters. This indicates that the ester group has a profound effect on the CPL-catalysed resolution. The effect of the other two groups, the phenyl (R^1) and nitrogen (R^2) substituents, was also systematically investigated. It was found that the size of the R^1 and R^2 groups is important for CPL-catalysed resolution. To obtain excellent enantioselectivity, the R^1 and R^2 groups should not both be large. It is the actual size of the substituents R^1 and R^2 that is important to the enantioselectivity of CPL-catalysed resolution, rather than their relative size. By optimizing the reaction conditions, high enantioselectivity ($E > 200$) was observed when the CPL-catalysed reactions were performed with methanol as the nucleophile in *n*-hexane at 50 °C. The products were separated and obtained in good yields (≥80%). The (*S*)-3-amino-3-phenylpropanoic acid [(*S*)-**2**] obtained was the key chiral intermediate for the synthesis of (*S*)-dapoxetine [(*S*)-**13**] with very high enantiomeric excess (>99%).

Experimental Section

General: Crude *Carica papaya* lipase was obtained from Shanghai Bairui Biotech Co., Ltd. Before use it was purified by the following procedure: deionized water (200 mL) was added to the crude *Carica papaya* lipase (20 g) at 4 °C with gentle stirring for 30 min. The resultant solution was centrifuged at 10000 rpm for 15 min, and the supernatant was discarded. This procedure was repeated three times. The remaining precipitate was then collected, fast-frozen by using liquid nitrogen and then lyophilized for 8 h. This preparation was used as CPL in this work. All other chemicals and reagents were obtained commercially and were of analytical grade. NMR spectra were obtained at 300 MHz with a Varian Unity 300 spectrometer by using [D]chloroform as the solvent with TMS as the internal standard.

General Procedure for the Alcoholysis Reactions: A solution of *N*-protected amino acid 2,2,2-trifluoroethyl ester (0.1 mmol) and an alcohol (1 mmol) in an organic solvent (5 mL) was mixed with the CPL preparation (10 mg) in a 25-mL screw-capped vial in a thermostatted shaker. The reaction was monitored and the *ee* of the newly formed ester assessed by HPLC analysis on the chiral columns as stated in the HPLC Analysis section. Aliquots (100 μL) of the reaction mixture were withdrawn at frequent intervals, diluted with hexane/2-propanol (9:1, 900 μL), centrifuged and the supernatant was then injected onto the column.

General Procedure for the Preparative-Scale Resolution of 3a: Racemic compound **3a** (10 mmol) was dissolved in methanol (5 mL). Lipase CPL (1.0 g) and *n*-hexane (100 mL) were added, and the mixture was shaken in an incubator shaker at 45 °C for 24–36 h. The reaction was stopped by filtering off the CPL at 50% conversion. The solvent was removed by evaporation, and the residue was purified by column chromatography on silica gel with petroleum ether/ethyl acetate (4:1→8:1) as eluent to obtain **5a** and **6a**.

HPLC Analysis: Alcoholysis reactions were monitored at 254 nm by chiral HPLC on a Chiralcel OD column (4.6 mm i.d. × 250 mm) or AD column (Daicel Chemical Industries) by using hexane/2-propanol as eluent. The liquid chromatograph employed was an Agilent 1100 instrument equipped with a DAD detector. The temperature of the column was maintained at 20 °C. The enantiomers of the methyl esters of *N*-Z-amino acids or the 2,2,2-trifluoroethyl esters were separated well enough for the accurate determination of the *ee* values on either of the columns by choosing an appropriate proportion of hexane/2-propanol for each compound. In general, the enantiomeric separations of the corresponding 2,2,2-trifluoroethyl esters were inferior to those of the methyl esters on either column. The separations of the enantiomers of the *N*-Z-amino acid 2,2,2-trifluoroethyl esters and methyl esters on the columns are pre-

sented in Table S1 in the Supporting Information. The *ee* values of the amino acids were determined on a Chirobiotic T column (4.6 mm i.d. × 250 mm) (Advanced Separation Technologies Inc). Diluted samples were subjected to HPLC analysis, eluted at 20 °C with 60% (v/v) phosphate sodium buffer (20 mM, pH = 4.0) and 40% (v/v) methanol at 1 mL/min, and monitored at 210 and 254 nm.

Preparation of Substrate: Benzaldehyde was purchased from Sinopharm Chemical Reagent Co., Ltd. (*RS*)-Phenylalanine, (*RS*)-homophenylalanine, (*RS*)-phenylglycine, 3-Cl-BPA and *N*-Fmoc-BPA were purchased from Shanghai Hengbai Biotech Co., Ltd.

Synthesis of BPA and Its Derivatives: Compounds **2a**–**i** were prepared by a modification of the Rodionov synthesis from corresponding **1a**–**i** (5 mmol) by condensation with malonic acid (1.36 g, 10 mmol, 2 equiv.) in the presence of NH_4Ac (0.77 g, 10 mmol, 2 equiv.) in EtOH at reflux for 12 h.[24] The reaction mixture was allowed to cool to room temperature before the white solid was collected by filtration. This white solid was then recrystallized twice from hot MeOH to afford **2a**–**i** as white crystals (yield: 25–85%).

Synthesis of *N*-Protected BPA and Its Derivatives: *N*-Protected BPA and its derivatives were prepared under Schotten–Baumann conditions.[25]

Synthesis of *N*-Pac-BPA and *N*-Z-BPA: Phenylacetyl chloride or benzyloxycarbonyl chloride (1.72 mL, 13 mmol) in acetone (5 mL) was added dropwise to a stirred solution of 3-amino-3-phenylpropanoic acid (**2a**; 1.65 g, 10 mmol) and Et_3N (3.35 mL, 24 mmol) in water (15 mL) and acetone (5 mL) at –5 °C. The mixture was stirred at –5 °C for 2 h and then at room temperature for 3 h. Then the precipitate was filtered off, the acetone was removed under reduced pressure, and the residue was extracted twice with ethyl acetate. A 2 M HCl solution was added to the aqueous layer until pH = 2 was reached, and then the solution was extracted with ethyl acetate (3 × 10 mL), dried with Na_2SO_4 and concentrated to give compound *N*-Pac-BPA (2.09 g,78% yield) or *N*-Z-BPA (2.34 g,78% yield).

Synthesis of *N*-Boc-BPA: A 100ml flask was charged with a solution of sodium hydroxide (0.44 g, 11 mmol) in water (10 mL). Stirring was initiated and (*RS*)-β-phenylalanine (**2a**; 1.65 g, 10 mol) was added at ambient temperature. The mixture was then diluted with *tert*-butyl alcohol (7.5 mL). Di-*tert*-butyl dicarbonate (2.23 g, 10 mol) was added dropwise to the well-stirred, clear solution. A white precipitate appeared during the addition of the di-*tert*-butyl dicarbonate. After a short induction period, the temperature rose to about 30–35 °C. The reaction was brought to completion by stirring at room temperature overnight. The reaction mixture was extracted with *n*-hexane (2 × 5 mL), and the *n*-hexane phases were then discarded. The aqueous layer was acidified to pH = 1.0–1.5 by careful addition of a solution of potassium hydrogen sulfate (2.24 g, 16.5 mmol) in water (15 mL). The turbid reaction mixture was then extracted with diethyl ether (3 × 10 mL). The combined organic layers were washed with water (2 × 5 mL), dried with anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure. The white precipitate *N*-Boc-BPA (2.24 g, 85% yield) was collected.

Synthesis of *N*-Ac-BPA: A solution of **2a** (4.53 g, 30 mmol) in water (18.2 ml) was basified with 25% sodium hydroxide solution to raise the pH to 11. Acetic anhydride (5.8 ml) and 25% sodium hydroxide solution were added while adjusting the pH to 11–12. The reaction mixture was then heated to 40 °C and stirred for 8 h, adjusting the pH to 11–12. Insoluble matter was removed by filtration, and the mother liquor was cooled in an ice bath. Concentrated HCl (10.5 ml) was added to adjust the pH to 2, and the reaction mixture was crystallized for 2 h. The crystallized solid was separated and washed under vacuum to obtain *N*-Ac-β-BPA (6.2 g, 95% yield).

Synthesis of *N*-Protected BPA Esters and Its Derivatives: The amine group in compounds **2b**–**i** was protected with acetic anhydride. DCC (5 mmol) and 1-hydroxybenzotriazole (HOBt; 2.5 mmol) were then added to an ice-cooled solution of the *N*-protected amino acid (5 mmol) and trifluoroethanol (5 mmol) in anhydrous CH_2Cl_2 (10 mL). 4-(Dimethylamino)pyridine (DMAP; 0.8 mmol) was then added dropwise.[26] The mixture was stirred at room temperature overnight, and CH_2Cl_2 was removed in vacuo. The residue was dissolved in EtOAc (30 mL), and then the precipitate was filtered off. EtOAc was washed with 10% HCl (in the case of *N*-Boc-BPA, 5% citric acid was used instead of 10% HCl), 5% $NaHCO_3$ and saturated NaCl, and dried with $MgSO_4$. The solvent was removed, and the residue was recrystallized from EtOAc/hexane to give compounds **3a**–**i** in yields of 90%. The 2,2,2-trifluoroethyl esters of phenylglycine, phenylalanine, homophenylalanine and *N*-Fmoc-, Pac-, and Z-BPA were prepared by a similar procedure.

Synthesis of Compounds 7a–e: For compound **7a**, (*RS*)-β-phenylalanine (**2a**; 3 g, 18 mmol) was added with stirring at 0 °C in portions to methanol (100 mL), to which had previously been added $SOCl_2$ (8 mL, 22 mmol). In the cases of compounds **7b**–**e**, **3a** was used instead of **2a** with methanol, ethanol, propanol and butanol. The mixture was kept at room temperature for 24 h and then concentrated to dryness under vacuum. The residue was taken up in methanol and the mixture concentrated, repeating the operation three times. The solid residue was suspended in CH_2Cl_2 (100 mL) and treated with acetic anhydride (10 g, 0.098 mol) followed by pyridine (15 g; 0.189 mol) whilst stirring at 0 °C. After 24 h at room temperature, the reaction mixture was poured onto crushed ice containing $NaHCO_3$ (5 g, 0.059 mol). The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 100ml). The combined organic layers were washed with cold 5% HCl (2 × 100 mL), saturated aqueous $NaHCO_3$ and water (100 mL each), dried (Na_2SO_4), and the solvents were evaporated. The residue was crystallized from hexane/ethyl acetate to give the desired **7a** (3 g, 75%).

Synthesis of Compound (*S*)-2a: In a typical experiment, (*S*)-*N*-Ac-BPA methyl ester **5a** (1.11 g, 5 mmol) was added to 6 M HCl solution (20 mL). The reaction mixture was heated at reflux for 10 h and then concentrated to dryness under vacuum. The residue was taken up in water at 80 °C, and Et_3N was added to give pH = 5.0. A four-fold volume of anhydrous alcohol was then added to the solution. The reaction mixture was cooled to room temperature, and the solid was collected by filtration and washing with EtOH to afford (*S*)-**2a** as a white solid (0.82 g, 72%). The *ee* was determined by HPLC (*ee* 99%).

Synthesis of β-Amino Alcohol (*S*)-11: A solution of β-amino acid (*S*)-**2a** (1.00 g, 6.0 mmol) in dry THF (21.6 mL) was cooled to 0 °C, and $LiAlH_4$ (460 mg, 12.11 mmol) was added in small portions. The reaction mixture was heated at reflux for 12 h, and then the reaction mixture was cooled to 0 °C, and excess hydride was destroyed by dropwise addition of H_2O. The mixture was extracted with EtOAc (3 × 10 mL), and the organic phases were combined, dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (100% MeOH), isolating the β-amino alcohol (*S*)-**11** as a white solid (yield 87%). R_f = 0.16 (100% MeOH); m.p. 76–77 °C. $[\alpha]_D^{20}$ = 21.9 (*c* = 1.0, $CHCl_3$) for 99% *ee*. ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.38–7.23 (m, 5 H, Ar), 4.13 [dd, $^3J(H,H)$ = 5.2, $^3J_{H,H}$ = 7.9 Hz, 1-H], 3.87–3.78 (m, 2 H, 3-H), 2.72 (br. s, 3 H, NH_2, OH), 1.98–

1.82 (m, 2 H, 2-H) ppm. MS (ESI+): *m*/*z* (%) = 152 (100) $[M + H]^+$, 174 (5) $[M + Na]^+$, 285 (45) $[2 M – H_2O + H]^+$.

Synthesis of (*S*)-3-(Dimethylamino)-3-phenylpropan-1-ol (12): A 30% aqueous solution of formaldehyde (1.5 mL, 16 mmol) was added to a solution of (*S*)-**11** (755 mg, 3 mmol) in formic acid (0.6 ml), and the mixture was heated at reflux for 8 h. After this time, the solution was basified with 3 M NaOH solution until pH = 12. The mixture was extracted with EtOAc (3 × 15 mL), and the organic phases were combined, dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (65% MeOH/EtOAc) to afford a hygroscopic solid (0.47 g, 84% isolated yield). R_f = 0.21 (60% MeOH/EtOAc). $[a]_D^{20}$ = +36.5 (*c* = 0.5, $CHCl_3$) for 99% *ee*. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.38–7.15 (m, 5 H, Ar), 3.86–3.75 (m, 2 H, 1-H), 3.75 (dd, $^3J_{HH}$ = 10.50, $^3J_{HH}$ = 3.75 Hz, 1 H, 3-H), 2.50–2.38 (m, 1 H, 2-H), 2.18 (s, 6 H, CH_3), 1.70–1.60 (m, 1 H, 2-H) ppm. MS (ESI+): *m*/*z* (%) = 180 (100) $[M + H]^+$, 202 (12) $[M + Na]^+$.

Synthesis of (*S*)-Dapoxetine (13): 1-Naphthol (0.6 mL, 5 mmol) was added to a solution of (*S*)-**12** (0.9 g, 5 mmol) in dry THF (5.0 mL) under nitrogen. The mixture was cooled to 0 °C, and PPh_3 (1.43 g, 5.5 mmol) and DEAD (0.87 mL, 5.5 mmol) were successively added. The solution was warmed to room temperature and stirred for 15 h. The solution was concentrated and the crude product purified by flash chromatography (gradient eluent 100% EtOAc to 10% MeOH/EtOAc) to yield colourless (S)-**13** (1.07 g, 73% isolated yield). R_f = 0.35 (20% MeOH/EtOAc). $[a]_D^{20}$ = +59.5 (*c* = 0.5, $CHCl_3$) for 99% *ee*. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.28–8.25 (m, 1 H, Ar), 7.80–7.77 (m, 1 H, Ar), 7.59–7.50 (m, 2 H, Ar), 7.46–7.30 (m, 7 H, Ar), 6.72–6.71 (m, 1 H, Ar), 4.17–4.02 (m, 1 H, 1-H), 3.98–3.92 (m, 1 H, 1-H), 3.70–3.65 (m, 1 H, 3-H), 2.75–2.63 (m, 1 H, 2-H), 2.39–2.310 (m, 1 H, 2-H), 2.33 (s, 6 H, CH_3) ppm. MS (ESI+): *m*/*z* (%) = 306 (100) $[M + H]^+$, 261 (35) $[M – NMe_2]^+$.

Supporting Information (see footnote on the first page of this article): Seperation conditions of the substrates by chiral column and NMR data for key intermediates.

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