Direct Asymmetric Aldol Reactions Catalyzed by Lipase from Porcine Pancreas

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Porcine pancreas lipase type II (PPL II) exhibited unnatural catalytic activity in direct asymmetric aldol reactions between cyclic ketones and aromatic or heteroaromatic aldehydes in acetonitrile in the presence of phosphate buffer. A wide range of substrates was accepted by the enzyme to afford the corresponding aldol products in low to high yields (10 – 98 %), with moderate to excellent enantioselectivities (53 – 94 % ee, for *anti*-isomers) and low to moderate diastereoselectivities (48/52 – 87/13 dr, *anti*/*syn*). This methodology expands the application of PPL II, and it might be developed into a potentially valuable method for sustainable organic synthesis.

Key words: Aldol Reaction, Enantioselectivity, Lipase

Introduction

Enzymes as biocatalysts have attracted more and more attention for their high stereoselectivity and catalytic efficiency. An important feature is that they are environmentally benign and completely biodegradable (Sukumaran and Hanefeld, 2005). In recent years, the concept of enzymatic promiscuity has been regarded as one of the most outstanding concepts in biocatalysis (Bornscheuer and Kazlauskas, 2004; Busto *et al*., 2011; Hult and Berglund, 2007; Humble and Berglund, 2011), which implies that enzymes are not only capable of catalyzing their "natural" reactions but also one or more alternative reactions (O'Brien and Herschlag, 1999). The importance of the promiscuity concept in biocatalysis is noteworthy, since it not only highlights the existing catalysts, but may provide novel and practical synthetic pathways currently not available (Kourist *et al*., 2008; Xu *et al*., 2008). More and more enzymes have been demonstrated to exhibit catalytic promiscuity in synthetic transformations such as Michael additions (Cai *et al*., 2011; Qian *et al*., 2007; Souza *et al*., 2009; Xu *et al*., 2011), Henry reactions (Fuhshuku and Asano, 2011; Gruber-Khadjawi *et al*., 2007; Tang *et al*., 2010; Wang *et al*. 2010a), Mannich reactions (Chai *et al*., 2010; He *et al*., 2010; Xue *et al*., 2012), Markovnikov additions (Lou *et al*., 2008a, b), and tandem reactions (Lai *et al*., 2010; Wang *et al*., 2010b, 2011).

The asymmetric aldol reaction is one of the most powerful carbon-carbon bond-forming reactions and plays an important role in medicinal chemistry and natural products synthesis. The development of catalysts for the asymmetric aldol reaction remains an active area of research. Since the first proline-catalyzed aldol reaction achieved a breakthrough, there has been a blossoming of general asymmetric organocatalyzed reactions (Kanemitsu *et al*., 2011). To date, numerous successful organocatalysts for asymmetric aldol reactions have been described with high efficiency and enantioselectivity (List and Mahrwald, 2004). However, some catalysts have been becoming more and more complicated; as a result, more toxic reagents as well as more synthetic steps are involved. Therefore, simpler, less toxic methods are necessary to complement the chemical approaches to asymmetric catalysis. On the other hand, biocatalysts have often been proven to be more sustainable than current chemical catalysts because of their mild reaction conditions and the potential use of inexpensive regenerable resources (Fess-

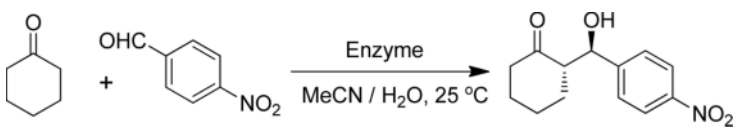
ner and Anthonsen, 2009; Knowles, 1991). Some aldolases (Eyrisch and Fessner, 1995; Heine *et al*., 2001; Kimura *et al*., 1997) and catalytic antibodies (Tanaka *et al*., 2004) have been elegantly used for the asymmetric aldol reactions. However, there are only a few examples of aldol reactions catalyzed by enzymes besides aldolases. Li *et al*. (2008) reported the first enzyme-catalyzed asymmetric aldol addition between acetone and different aromatic aldehydes employing pig pancreas lipase (PPL) as a catalyst in aqueous media, and the best enantioselectivity of 43.6% ee was obtained with a yield of 11.7%. They also found that pepsin could catalyze aldol reactions in aqueous media (Li *et al*., 2010). Thereafter, our laboratory reported that nuclease p1, alkaline protease, chymopapain, and acidic protease were able to catalyze direct asymmetric aldol reactions (Li *et al*., 2011a, b; He *et al*., 2012; Xie *et al*., 2012). Very recently, we reported that type II lipase from porcine pancreas (PPL II) was able to catalyze the aldol reactions of oxygen-, nitrogen-, or sulfur-containing heterocyclic ketones with aldehydes (Guan *et al*., 2012). Because PPL II is readily available and relatively inexpensive, it could be developed into a potentially valuable biocatalyst for organic synthesis. Therefore, it is still necessary to further explore the catalytic promiscuity of this enzyme for asymmetric aldol reactions with a wider substrate range. Herein, we report on the PPL II-catalyzed asymmetric direct aldol reactions of cyclic ketones with aromatic and heteroaromatic aldehydes.

Results and Discussion

In our initial studies, we chose the aldol reaction of cyclohexanone and 4-nitrobenzaldehyde as a model reaction and a MeCN/H_2O system as the reaction medium. Several commercially available lipases were screened as promiscuous biocatalysts (Table I). Among the tested enzymes, PPL II showed the highest catalytic activity and enantioselectivity providing the aldol product in a good yield of 90% with 73% ee (Table I, entry 1). The other lipases showed low or no catalytic activities in the model aldol reaction (Table I, entries 2 – 6). To verify the specific catalytic effect of PPL II on the aldol reaction, some control experiments were performed under the same conditions. In the absence of enzyme, only a trace amount of product was detected after 168 h (Table I, entry 7). When bovine serum albumin (BSA) was used as a catalyst in the model reaction, product in a moderate yield of 45% was obtained with 49/51 dr and 0% ee (Table I, entry 8), indicating that BSA had the ability to catalyze the aldol reaction, but it did not display any selectivity. Moreover, when urea-denatured PPL II was used as a catalyst, only a trace amount of product was observed (Table I, entry 9), suggesting that the tertiary structure of PPL II is responsible for its activity and selectivity in the aldol reaction. Thus, PPL II was chosen as the catalyst in the following experiments.

The pioneering works of Zaks and Klibanov (1988a) demonstrated that water-soluble enzymes not only

Table I. Catalytic activities of some lipases in the aldol reaction.[a]



Entry	Enzyme	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	Lipase from porcine pancreas, type II (PPL II)	90	67/33	73
2	Lipase Ps Amano SD	5	57/43	48
3	Lipase AK Amano	10	67/33	30
4	Amano lipase A from *Aspergillus niger*	25	57/43	54
5	Lipase from *Candida cylindracea*	10	74/26	40
6	Lipase from *Rhizopus niveus*	Trace	–	–
7	No enzyme	Trace	–	–
8	Bovine serum albumin	45	49/51	0
9	Urea-denatured PPL II[d]	Trace	–	–

[a] Reaction conditions: enzyme lyophilized powder, 100 mg; cyclohexanone, 2.5 mmol; 4-nitrobenzaldehyde, 0.5 mmol; deionized water, 0.10 mL; MeCN, 0.90 mL; 25 °C; 168 h.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H).
[d] PPL II was pre-treated with urea at 100 °C, 24 h.

retain their activity in organic solvents, but show some specific characteristics, such as enhanced stability, altered substrate and enantiomeric specificities, molecular memory, and the ability to catalyze unusual reactions which are impossible in aqueous media (Klibanov, 2001). Therefore, the influence of various solvents on the PPL II-catalyzed aldol reaction was investigated (Table II). The catalytic activity and enantioselectivity of PPL II were remarkably affected by different media. The reaction gave the best yield of 92% with moderate diastereoselectivity and enantioselectivity when extra cyclohexanone was used as a solvent (Table II, entry 2). However, when the reaction was performed in MeCN, it provided the corresponding aldol product in a good yield of 83% with 73% ee for the *anti*-isomer (73/27 dr *anti*/*syn*) (Table II, entry 1). The reaction proceeded smoothly, and moderate to good yields (from 58% to 87%) were obtained in other tested solvents (Table II, entries 3–9). The reaction was also carried out in water, but unfortunately, it provided the product only in a moderate yield with low enantioselectivity (Table II, entry 10). Based on the solvents screened above, MeCN was selected as the reaction medium in the following reactions.

Since enzymes need a certain amount of water to maintain optimal conformation for catalysis (Zaks and Klibanov, 1988b), it is important to determine the proper water content in the reaction system. The effect of water content from 0 vol.-% to 40 vol.-% [deionized water/(deionized water + MeCN)] on the PPL II-catalyzed model aldol reaction was investigated (Table III). The highest yield was obtained at a water content of 20 vol.-% (Table III, entry 4). However, the best enantioselectivity was obtained at a water content of 10 vol.-% (Table III, entry 3). Thus, we chose a water content of 10 vol.-% for the aldol reaction.

To further optimize the PPL II-catalyzed aldol reaction, the influence of the temperature on the model

Table II. Solvent screening for the PPL II-catalyzed aldol reaction.[a]

Entry	Solvent	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	MeCN	83	73/27	73
2	Cyclohexanone[d]	92	71/29	66
3	CH_2Cl_2	59	66/34	65
4	Cyclohexane	87	67/33	55
5	1,4-Dioxane	78	69/31	54
6	THF	82	66/34	53
7	EtOH	58	57/43	26
8	DMF	66	50/50	36
9	DMSO	71	41/59	29
10	H_2O	64	64/36	45

[a] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); cyclohexanone, 2.5 mmol; 4-nitrobenzaldehyde, 0.5 mmol; deionized water, 0.10 mL; organic solvent, 0.90 mL; 25 °C; 150 h.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H).
[d] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); cyclohexanone, 10 mmol; 4-nitrobenzaldehyde, 0.5 mmol; deionized water, 0.10 mL; 25 °C, 150 h. (Using extra cyclohexanone as a solvent.)

Table III. Influence of the water content on the PPL II-catalyzed aldol reaction.[a]

Entry	Water content (vol.-%)	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	0	54	71/29	59
2	5	60	75/25	59
3	10	85	75/25	66
4	20	86	74/26	62
5	25	80	73/27	56
6	30	76	68/32	54
7	40	72	68/32	51

[a] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); cyclohexanone, 2.5 mmol; 4-nitrobenzaldehyde, 0.5 mmol; water content, 0–40% [deionized water/(deionized water + MeCN)]; deionized + MeCN, 1.0 mL; 25 °C; 144 h.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H).

Table IV. Influence of the temperature on the PPL II-catalyzed aldol reaction.[a]

Entry	Temperature	24 h			144 h		
	[°C]	Yield (%)[b]	dr (%)[c]	ee (%)[c]	Yield (%)[b]	dr (%)[c]	ee (%)[c]
1	15	11	78/22	62	30	79/21	63
2	20	23	78/22	65	85	77/23	67
3	25	26	73/27	60	85	75/25	66
4	30	36	68/32	59	94	67/33	60
5	35	36	62/38	50	88	62/38	54
6	40	35	58/42	42	84	55/45	48
7	50	33	55/45	35	77	51/49	40

[a] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); cyclohexanone, 2.5 mmol; 4-nitrobenzaldehyde, 0.5 mmol; deionized water, 0.10 mL; MeCN, 0.90 mL; temperature, 15 – 50 °C.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H); dr, *anti*/*syn*; ee, *anti*.

reaction was investigated (Table IV). As the temperature was raised from 15 °C to 30 °C, the yield of the product increased from 11% to 36% after 24 h, and from 30% to 94% after 144 h (Table IV, entries 1 – 4). However, once the temperature exceeded 30 °C, yield and selectivity decreased (Table IV, entries 5 – 7). Although yield was highest at 30 °C (Table IV, entry 4), the selectivity was lower than in the reaction performed at 20 °C (Table IV, entry 2). Thus, for best enantioselectivity, we chose 20 °C for the further investigations.

The influence of the enzyme concentration on the model reaction was also investigated (Table V). Below an enzyme concentration of 125 mg (3.0 kU)/mL, the enantioselectivity of the reaction was hardly affected (Table V, entries 1 – 5). When it was 100 mg (2.4 kU)/mL, the model reaction gave the best yield of 85% after 144 h (Table V, entry 4). Thus, we chose an enzyme concentration of 100 mg (2.4 kU)/mL for the following experiments.

Next, the influence of the molar ratio of substrates on the model reaction was investigated (Table VI). When the molar ratio of cyclohexanone to 4-nitrobenzaldehyde was increased from 1:1 to 25:1, both diastereoselectivity and enantioselectivity increased (Table VI, entries 1 – 6). When the molar ratio was 20:1, the reaction gave the best enantioselectivity (Table VI, entry 5). Thus, a molar ratio of ketone to aldehyde of 20:1 was chosen as the optimal condition for the following experiments.

It is known that the variation of the pH value strongly influences the ionic environment of an enzyme, thus affecting its interaction with the substrates and hence its activity (Li *et al*., 2011c). Each enzyme has its specific optimum pH at which the reaction rate is maximized. Thus, we used phosphate buffer (0.2 M, pH from 4.62 to 6.86) to replace the optimized water content in the reaction system [buffer/(MeCN + buffer) = 1:10, v/v] to determine the optimum reaction conditions (Table VII). The yield could be improved with the pH value increasing from 4.62 to 5.60 (Table VII, entries 1 – 4). When the reaction was performed in the buffer of pH 5.41, the aldol product was obtained in the best

Table V. Influence of the enzyme concentration on the PPL II-catalyzed aldol reaction.[a]

Entry	Enzyme concentration [mg/mL]	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	25 (0.6 kU)	41	83/17	67
2	50 (1.2 kU)	65	81/19	67
3	75 (1.8 kU)	78	80/20	67
4	100 (2.4 kU)	85	77/23	67
5	125 (3.0 kU)	77	75/25	66
6	150 (3.6 kU)	73	78/22	61

[a] Reaction conditions: PPL II lyophilized powder, 25 – 150 mg (0.6 – 3.6 kU)/mL; cyclohexanone, 2.5 mmol; 4-nitrobenzaldehyde, 0.5 mmol; deionized water, 0.10 mL; MeCN, 0.90 mL; 20 °C; 144 h.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H).

Table VI. Influence of the molar ratio of aldehyde to ketone on the PPL II-catalyzed aldol reaction.[a]

Entry	**1**:**2a**	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	1:1	36	74/26	60
2	5:1	85	77/23	67
3	10:1	73	75/25	67
4	15:1	87	80/20	71
5	20:1	88	82/18	74
6	25:1	90	83/17	74
7	30:1	88	80/20	70

[a] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); cyclohexanone (**1**), 0.5 – 15 mmol; 4-nitrobenzaldehyde (**2a**), 0.5 mmol; deionized water, 0.10 mL; MeCN, 0.90 mL; 20 °C; 144 h.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H).

Table VII. Effect of the pH value on the PPL II-catalyzed aldol reaction.[a]

Entry	pH	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	4.62	90	82/18	72
2	4.91	94	83/17	72
3	5.41	98	82/18	72
4	5.60	97	81/19	71
5	6.86	89	80/20	70
6[d]	4.62 (no enzyme)	15	77/23	0
7[d]	5.41 (no enzyme)	9	78/22	0
8[d]	6.86 (no enzyme)	Trace	–	–

[a] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); cyclohexanone, 10 mmol; 4-nitrobenzaldehyde, 0.5 mmol; phosphate buffer, 0.2 M, 0.10 mL; MeCN, 0.90 mL; 20 °C; 144 h.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H).
[d] Reaction conditions: as in a, without addition of PPL II.

yield of 98%. Within the pH range from 4.62 to 6.86, there was no obvious effect on the diastereoselectivity and enantioselectivity of the reaction. Moreover, to exclude the effects of the acidic environment provided by the phosphate buffer, some control experiments were carried out (Table VII, entries 6 – 8). In the absence of enzyme, the product yields were 15% and 9% at pH 4.62 and 5.41, respectively, indicating that an acidic environment has some effect on the reaction. Nevertheless, in comparison with the yields obtained in the presence of enzyme (Table VII, entries 1 and 3), which were 90% at pH 4.62 and 98% at pH 5.41, respectively, this background is still acceptable. The control experiment in the neutral buffer (pH 6.86) only gave a trace amount of product, which could be observed by TLC but was not sufficient for isolation (Table VII, entry 8). Based on the above experiments, we chose the phosphate buffer of pH 5.41 [buffer/(MeCN + buffer) = 0.10, v/v] for further investigations.

Based on the above results, the time course of the enzymatic aldol reaction was investigated under the optimal conditions (Table VIII). Equilibrium was reached after 6 days with the excellent yield of 98%, and the diastereoselectivity and enantioselectivity were slightly increased with time. In order to check whether the reversibility of the aldol reaction could be responsible for the increasing selectivity over time, an appropriate experiment was performed. The racemic product (*syn* + *anti*) prepared in a $NaHCO_3$-catalyzed aldol reaction was incubated with PPL II under the optimized conditions for 120 h, and a trace amount of aldehyde was observed by HPLC analysis (data not shown), indicating that the aldol reaction was reversible.

With the optimal reaction conditions in hand, aldol reactions of substituted aromatic and heteroaromatic aldehydes with cyclic ketones were tested to gain information about the generality and extent of this enzymatic promiscuity. In general, the PPL II-catalyzed aldol reaction proceeded smoothly with a wide range of substrates (Table IX). The electronic features of the substituents on the phenyl ring of the aldehydes had a strong effect on the yields. When reacting with cyclohexanone, the benzaldehydes with strong electron-

Table VIII. Time course of the PPL II-catalyzed aldol reaction.[a]

Entry	Time [d]	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	0.5	19	75/25	67
2	1	26	77/23	68
3	2	52	87/13	74
4	3	65	82/18	74
5	4	80	83/17	75
6	5	94	83/17	74
7	6	98	82/18	72
8	7	98	83/17	76
9	8	97	83/17	76

[a] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); cyclohexanone, 10 mmol; 4-nitrobenzaldehyde, 0.5 mmol; phosphate buffer, 0.2 M, pH 5.41, 0.10 mL; MeCN, 0.9 mL; 20 °C; 1 – 8 d.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H).

Table IX. Scope of the PPL II-catalyzed direct aldol reaction.[a]

1 **2** **3**

Entry	R	*n*	Compound	Time [h]	Yield (%)[b]	dr (*anti*/*syn*) (%)[c]	ee (*anti*) (%)[c]
1	4-$NO_2C_6H_4$	2	**3a**	144	98	82/18	74
2	2-$NO_2C_6H_4$	2	**3b**	168	53	82/18	82
3	3-$NO_2C_6H_4$	2	**3c**	144	94	83/17	82
4	4-$CF_3C_6H_4$	2	**3d**	144	90	87/13	85
5	4-CNC_6H_4	2	**3e**	168	95	73/27	75
6	4-BrC_6H_4	2	**3f**	144	40	85/15	84
7	4-ClC_6H_4	2	**3g**	168	40	83/17	84
8	3-ClC_6H_4	2	**3h**	168	15	78/22	84
9	4-FC_6H_4	2	**3i**	168	20	67/33	74
10	2,6-$Cl_2C_6H_3$	2	**3j**	168	60	80/20	94
11	C_6H_5	2	**3k**	192	24	48/52	66/66[d]
12	4-$OCH_3C_6H_4$	2	**3l**	192	10	77/23	86
13	2-Furanyl	2	**3m**	168	20	67/33	70
14	2-Thienyl	2	**3n**	168	17	74/26	80
15	4-$NO_2C_6H_4$	1	**3o**	144	20	62/38	53

[a] Reaction conditions: PPL II lyophilized powder, 100 mg (2.4 kU); ketone **1**, 10 mmol; aldehyde **2**, 0.5 mmol; phosphate buffer, 0.2 M, pH 5.41, 0.10 mL; MeCN, 0.90 mL; 20 °C.
[b] Yield of the isolated product after silica gel chromatography.
[c] Determined by chiral HPLC analysis (AD-H, OD-H, AS-H).
[d] *anti* (66% ee) and *syn* (66% ee).

withdrawing groups, such as nitro, trifluoromethyl, and cyano (except 2-nitrobenzaldehyde), gave the corresponding aldol products in high yields of 90 – 98% (Table IX, entries 1 and 3 – 5), while a benzaldehyde with a strong electron-donating group, such as the methoxy group, gave the product only in a poor yield of 10% (Table IX, entry 12). The reactions with halogen atom-substituted benzaldehydes provided products in low to moderate yields of 15 – 60% (Table IX, entries 6 – 10). Moreover, steric hindrance of substituents on benzaldehydes had a great impact on the selectivity and the yield of the reaction. The reaction with 2-nitrobenzaldehyde gave a much lower yield compared to those obtained with 3- or 4-nitrobenzaldehydes (Table IX, entries 1 – 3), and the most sterically hindered substrate 2,6-dichlorobenzaldehyde gave the highest

enantioselectivity of 94% ee (Table IX, entry 10). In addition, the heteroaromatic aldehydes 2-furaldehyde and 2-thiophenaldehyde could react with cyclohexanone under optimal conditions giving products in low yields (Table IX, entries 13 and 14). Cyclopentanone also could be used as an aldol donor in this enzymatic reaction, which gave a low yield and low selectivity when reacting with 4-nitrobenzaldehyde (Table IX, entry 15). The *anti*-isomers were obtained as the major products in almost all the investigated reactions except in the reaction between benzaldehyde and cyclohexanone. PPL II showed moderate to high enantioselectivities for *anti*-isomers, but low or no enantioselectivities for *syn*-isomers.

Conclusion

PPL II-catalyzed direct asymmetric aldol reaction was established in a phosphate buffer (pH 5.41)/MeCN system. A wide range of aromatic and heteroaromatic aldehydes with cyclic ketones could participate in the reaction. Mainly *anti*-aldol products were obtained in low to excellent yields (10–98%), and low to moderate diastereoselectivities (48/52 to 87/1 dr, *anti*/*syn*) and moderate to good enantioselectivities (53–94% ee) were observed. PPL II accepted different substrates in the reaction without the need for additional cofactors or special equipment. This methodology expands the application of PPL II in asymmetric syntheses. As a readily available and relatively inexpensive hydrolase, PPL II has the potential for development into a valuable biocatalyst for organic syntheses.

Experimental

Materials

Lipase from porcine pancreas, type II (42 U/mg protein; protein by biuret: 56%; one unit will hydrolyze 1.0 microequivalent of fatty acid from triacetin within 1 h at pH 7.4 and 37 °C), lipase from *Rhizopus niveus* (3,600 U/g; one unit corresponds to the amount of enzyme producing 1 μmol acid per min at pH 8.0 and 40 °C), Amano lipase A from *Aspergillus niger* (12,000 U/g; one unit is defined as the amount of enzyme to liberate 0.1 μmol fatty acid from olive oil per min at pH 6.0 and 37 °C), and lipase from *Candida cylindracea* (4.28 U/mg; one unit corresponds to the amount of enzyme which liberates 1 μmol oleic acid from triolein per min at pH 8.0 and 40 °C) were purchased from Sigma-Aldrich (Shanghai, China). Lipase Ps Amano SD (23,000 U/g; one unit is defined as the amount of enzyme to liberate 1 μmol fatty acid from olive oil per min at pH 7.0 and 37 °C) and lipase AK Amano (20,000 U/g; one unit is defined as the amount of enzyme to liberate 1 μmol fatty acid from olive oil per min at pH 8.0 and 60 °C) were gifts from Amano Enzyme Inc. (Shanghai, China). Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification.

Analysis methods

The NMR spectra were recorded on a Bruker AMX-300 MHz spectrometer (Bruker, Fällanden, Switzerland). Chemical shifts (δ) were expressed in ppm, and coupling constants (J) were reported in Hz. Routine monitoring of the reactions was performed by thin-layer chromatography (TLC) using precoated Haiyang GF254 silica gel TLC plates (Qingdao, China). All column chromatography separations were done using silica gel (100–200 mesh) at increased pressure. Petroleum ether used was of boiling range 60–80 °C. The enantiomeric excess (ee) of aldol products was determined by chiral high-performance liquid chromatography (HPLC) analysis (Shimadzu LC-20AT; Kyoto, Japan) on Chiralpak AD-H and AS-H, and Chiralcel OD-H columns (Daicel Chemical Industries, Tokyo, Japan). Relative and absolute configurations of the products were determined by comparison with known ^{1}H NMR and chiral HPLC analyses.

*General procedure for the synthesis of **3a–3o***

A 10-mL round-bottom flask was charged with 100 mg (2.4 kU) PPL II lyophilized powder, 0.90 mL MeCN, and 0.5 mmol aldehyde, to which the phosphate buffer (0.2 M, pH 5.41, 0.10 mL) and 10 mmol ketone were introduced. The resultant mixture was stirred at 20 °C for a specified time. The reaction was terminated by filtering off the enzyme, and ethyl acetate was used to wash the filter paper and the residue to assure that the products were dissolved in the filtrate. The solvents were then evaporated under reduced pressure. The crude products were purified by column chromatography with petroleum ether/ethyl acetate as eluent. All products are known compounds (Li *et al*., 2011b; Qian *et al*., 2010; Wu *et al*., 2006; Yang *et al*., 2010).

2-[Hydroxy-(p-nitrophenyl)methyl]cyclohexan-1-one (**3a**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.21 (d, J = 8.1 Hz, 2H), 7.51 (d, J = 7.4 Hz, 2H), 5.49 (s, 0.25H), 4.90 (d, J = 7.9 Hz, 0.75H), 4.09 (s, 0.67H), 3.20 (s, 0.28H), 2.66 – 2.44 (m, 2H), 2.40 – 2.31 (m, 1H), 2.12 (d, J = 11.3 Hz, 1H), 1.85 – 1.31 (m, 5H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 214.7, 214.0, 149.0, 148.3, 147.5, 127.8, 126.5, 123.5, 123.4, 73.9, 70.0, 57.1, 56.7, 42.6, 30.7, 27.8, 27.5, 25.8, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, t_R = 35.976 min; minor enantiomer, t_R = 27.339 min.

2-[Hydroxy-(o-nitrophenyl)methyl]cyclohexan-1-one (**3b**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 7.61 (t, J = 7.4 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 5.93 (s, 0.09H), 5.42 (d, J = 7.0 Hz, 0.89H), 4.05 (s, 0.89H), 2.85 – 2.71 (m, 1H), 2.46 – 2.27 (m, 2H), 2.05 (s, 1H), 1.86 – 1.54 (m, 5H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 214.8, 148.6, 136.4, 133.0, 129.5, 128.9, 128.3, 127.8, 124.5, 123.9, 69.5, 66.4, 57.2, 54.8, 42.7, 42.4, 30.9, 29.6, 27.7, 26.3, 24.8. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 0.5 mL/min]: major enantiomer, t_R = 21.393 min; minor enantiomer, t_R = 23.025 min.

2-[Hydroxy-(m-nitrophenyl)methyl]cyclohexan-1-one (**3c**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.19 – 8.07 (m, 2H), 7.65 (d, J = 7.4 Hz, 1H), 7.51 (t, J = 7.7 Hz, 1H), 5.45 (s, 0.13H), 4.88 (d, J = 8.3 Hz, 0.91H), 4.14 (s, 0.88H), 3.29 (s, 0.12H), 2.68 – 2.58 (m, 1H), 2.54 – 2.30 (m, 2H), 2.09 (d, J = 11.6 Hz, 1H), 1.81 (d, J = 9.3 Hz, 1H), 1.72 – 1.49 (m, 3H), 1.42 – 1.31 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 214.7, 148.1, 143.2, 133.1, 131.9, 129.2, 129.0, 122.7, 121.9, 120.8, 73.8, 69.7, 57.0, 56.6, 42.5, 30.6, 27.7, 27.5, 25.8, 24.5. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, t_R = 41.471 min; minor enantiomer, t_R = 52.417 min.

2-[Hydroxy-(p-(trifluoromethyl)phenyl)methyl]cyclohexan-1-one (**3d**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.61 (d, J = 7.7 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 5.43 (s, 0.12H), 4.85 (d, J = 8.4 Hz, 0.88H), 4.07 (s, 0.79H), 3.19 (s, 0.09H), 2.65 – 2.54 (m, 1H), 2.51 – 2.39 (m, 2H), 2.14 – 2.08 (m, 1H), 1.85 – 1.49 (m, 4H), 1.39 – 1.31 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 215.0, 144.9, 130.2, 129.7, 127.3, 126.0, 125.2, 122.2, 74.1, 57.1, 42.5, 30.6, 27.6, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 0.5 mL/min]: major enantiomer, t_R = 25.166 min; minor enantiomer, t_R = 20.719 min.

2-[Hydroxy-(p-cyanophenyl)methyl]cyclohexan-1-one (**3e**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.55 (d, J = 7.7 Hz, 2H), 7.38 (d, J = 7.5 Hz, 2H), 5.33 (s, 0.17H), 4.79 (d, J = 8.1 Hz, 0.78H), 4.11 (s, 0.62H), 3.40 (s, 0.09H), 2.60 – 2.44 (m, 2H), 2.36 – 2.23 (m, 1H), 1.99 (s, 1H), 1.73 (d, J = 9.8 Hz, 1H), 1.65 – 1.38 (m, 3H), 1.36 – 1.28 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 214.5, 213.6, 147.5, 146.5, 132.1, 131.8, 127.7, 126.5, 118.6, 111.3, 110.5, 73.7, 69.9, 56.9, 56.6, 42.4, 30.5, 27.5, 25.7, 24.4. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 0.5 mL/min]: major enantiomer, t_R = 31.162 min; minor enantiomer, t_R = 24.728 min.

2-[Hydroxy-(p-bromophenyl)methyl]cyclohexan-1-one (**3f**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47 (d, J = 7.5 Hz, 2H), 7.20 (d, J = 7.4 Hz, 2H), 5.32 (s, 0.13H), 4.75 (d, J = 8.4 Hz, 0.86H), 4.02 (s, 0.85H), 3.14 (s, 0.11H), 2.55 – 2.45 (m, 2H), 2.34 (td, J = 12.9, 6.1 Hz, 1H), 2.06 (s, 1H), 1.79 (d, J = 10.8 Hz, 1H), 1.72 – 1.47 (m, 3H), 1.37 – 1.26 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 215.1, 140.6, 139.9, 131.4, 131.1, 128.7, 127.5, 121.6, 120.6, 74.0, 70.0, 57.2, 42.5, 30.7, 27.8, 27.6, 25.8, 24.7, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, t_R = 17.611 min; minor enantiomer, t_R = 15.143 min.

2-[Hydroxy-(p-chlorophenyl)methyl]cyclohexan-1-one (**3g**): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28 (dd, J = 18.4, 7.7 Hz, 4H), 5.34 (s, 0.16H), 4.76 (d, J = 8.6 Hz, 0.87H), 4.03 (s, 0.76H), 0.15 (s, 0.16H), 2.62 – 2.42 (m, 2H), 2.34 (td, J = 13.0, 5.5 Hz, 1H), 2.08 (d, J = 12.3 Hz, 1H), 1.81 (t, J = 14.2 Hz, 1H), 1.69 – 1.46 (m, 3H), 1.36 – 1.24 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 215.2, 214.4, 140.0, 139.4, 133.4, 132.5, 128.4, 128.3, 128.2, 127.1, 74.0, 70.0, 57.2, 56.9, 42.5, 30.7, 27.8, 27.6, 25.9, 24.7, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, t_R = 16.208 min; minor enantiomer, t_R = 14.055 min.

2-[Hydroxy-(m-chlorophenyl)methyl]cyclohexan-1-one (**3h**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.26$ (t, $J = 22.8$ Hz, 4H), 5.35 (s, 0.19H), 4.75 (d, $J = 8.6$ Hz, 0.89H), 4.06 (s, 0.73H), 3.11 (s, 0.15H), 2.60 – 2.54 (m, 1H), 2.47 (d, $J = 13.8$ Hz, 1H), 2.40 – 2.30 (m, 1H), 2.07 (s, 1H), 1.87 – 1.48 (m, 4H), 1.36 – 1.26 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 215.1$, 143.0, 134.2, 129.5, 127.9, 127.0, 125.9, 125.2, 123.8, 74.1, 69.9, 57.1, 42.5, 30.7, 27.7, 25.8, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralcel OD-H column [*n*-hexane/2-propanol (95:5, v/v), 210 nm, 1.0 mL/min]: major enantiomer, $t_R = 8.547$ min; minor enantiomer, $t_R = 10.010$ min.

2-[Hydroxy-(p-fluorophenyl)methyl]cyclohexan-1-one (**3i**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.32 - 7.21$ (m, 2H), 7.03 (t, $J = 7.8$ Hz, 2H), 5.36 (s, 0.31H), 4.78 (d, $J = 8.7$ Hz, 0.62H), 4.05 (s, 0.51H), 2.61 – 2.47 (m, 2H), 2.42 – 2.31 (m, 1H), 2.18 – 2.01 (m, 1H), 1.88 – 1.76 (m, 1H), 1.74 – 1.49 (m, 3H), 1.26 (s, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 215.3$, 214.6, 136.7, 136.6, 128.6, 128.5, 127.3, 127.2, 115.3, 115.0, 114.7, 74.0, 70.1, 57.3, 42.5, 30.7, 29.6, 27.8, 27.6, 25.9, 24.7, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, $t_R = 14.846$ min; minor enantiomer, $t_R = 13.516$ min.

2-[Hydroxy-(2,6-dichlorophenyl)methyl]cyclohexan-1-one (**3j**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.30$ (d, $J = 7.9$ Hz, 2H), 7.15 (t, $J = 7.9$ Hz, 1H), 5.85 – 5.82 (m, 1H), 3.74 (s, 1H), 3.49 (td, $J = 12.4$, 6.2 Hz, 1H), 2.51 (d, $J = 13.4$ Hz, 1H), 2.46 – 2.35 (m, 1H), 2.07 (s, 1H), 1.85 – 1.74 (m, 1H), 1.73 – 1.50 (m, 3H), 1.44 – 1.31 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 214.3$, 135.5, 134.6, 129.2, 70.4, 53.5, 42.3, 29.8, 27.5, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AS-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 0.5 mL/min]: major enantiomer, $t_R = 25.730$ min; minor enantiomer, $t_R = 19.690$ min.

2-[Hydroxy(phenyl)methyl]cyclohexan-1-one (**3k**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.32$ (m, 5H), 5.39 (s, 0.35H), 4.79 (d, $J = 8.7$ Hz, 0.70H), 3.96 (s, 0.68H), 3.03 (s, 0.33H), 2.69 – 2.55 (m, 1H), 2.53 – 2.28 (m, 2H), 2.07 (m, 1H), 1.89 – 1.67 (m, 1H), 1.67 – 1.47 (m, 3H), 1.38 – 1.18 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 215.6$, 214.8, 140.8, 133.6, 130.1, 128.4, 128.1, 127.8, 126.9, 125.7, 77.4, 76.9, 76.5, 74.7, 70.5, 57.3, 42.6, 30.8, 29.6, 27.8, 27.7, 25.9, 24.8, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralcel OD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, $t_R = 17.304$ min; minor enantiomer, $t_R = 21.117$ min.

2-[Hydroxy-(p-methoxyphenyl)methyl]cyclohexan-1-one (**3l**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.24$ (d, $J = 7.9$ Hz, 2H), 6.88 (d, $J = 8.0$ Hz, 2H), 5.33 (d, $J = 6.4$ Hz, 0.39H), 4.75 (d, $J = 8.8$ Hz, 0.68H), 3.97 (s, 0.33H), 3.80 (s, 3H), 2.65 – 2.53 (m, 1H), 2.46 (t, $J = 14.2$ Hz, 1H), 2.39 – 2.29 (m, 1H), 2.06 (s, 1H), 1.78 (d, $J = 9.3$ Hz, 1H), 1.70 – 1.50 (m, 3H), 1.31 (s, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 215.6$, 159.2, 133.0, 128.1, 126.8, 113.7, 74.2, 57.4, 55.2, 42.5, 30.7, 27.7, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, $t_R = 21.972$ min; minor enantiomer, $t_R = 21.242$ min.

2-[Hydroxy-(furan-2-yl)methyl]cyclohexan-1-one (**3m**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.36$ (d, $J = 13.5$ Hz, 1H), 6.31 (d, $J = 14.5$ Hz, 2H), 5.35 (s, 0.29H), 5.27 (s, 0.37H), 4.84 (d, $J = 8.4$ Hz, 0.82H), 3.97 (s, 0.48H), 2.99 – 2.83 (m, 1H), 2.39 (dt, $J = 15.0$, 10.2 Hz, 2H), 2.10 (s, 1H), 1.92 – 1.84 (m, 1H), 1.64 (d, $J = 9.2$ Hz, 3H), 1.31 (s, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 214.9$, 213.6, 153.5, 142.4, 141.4, 110.2, 110.3, 107.9, 106.4, 68.1, 66.5, 54.7, 54.6, 42.5, 42.4, 30.6, 29.6, 27.7, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 220 nm, 0.5 mL/min]: major enantiomer, $t_R = 29.515$ min; minor enantiomer, $t_R = 31.813$ min.

2-[Hydroxy-(thien-2-yl)methyl]cyclohexan-1-one (**3n**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.35 - 7.11$ (m, 1H), 6.94 (d, $J = 8.8$ Hz, 2H), 5.56 (s, 0.21H), 5.08 (d, $J = 8.3$ Hz, 0.75H), 4.10 (s, 0.72H), 3.18 (s, 0.18H), 2.71 – 2.62 (m, 1H), 2.50 – 2.31 (m, 2H), 2.16 – 1.99 (m, 1H), 1.85 – 1.59 (m, 4H), 1.35 (m, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 215.0$, 144.5, 126.2, 125.1, 125.0, 124.1, 123.1, 70.7, 68.4, 57.8, 42.5, 30.8, 29.6, 27.7, 24.6. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 254 nm, 1.0 mL/min]: major enantiomer, $t_R = 14.945$ min; minor enantiomer, $t_R = 16.560$ min.

2-[Hydroxy-(p-nitrophenyl)methyl]cyclopentan-1-one (**3o**): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.19$ (d,

J = 7.7 Hz, 2H), 7.54 (d, J = 7.6 Hz, 2H), 5.41 (s, 0.41H), 4.87 (d, J = 8.8 Hz, 0.67H), 4.79 (s, 0.63H), 3.29 (s, 0.37H), 2.52 – 2.17 (m, 3H), 2.02 (s, 1H), 1.86 – 1.50 (m, 3H). – ^{13}C NMR (75 MHz, $CDCl_3$): δ = 222.1, 219.5, 150.5, 148.6, 147.5, 147.0, 127.3, 126.3, 123.5, 74.2, 70.2, 55.9, 55.0, 38.9, 38.5, 26.6, 22.2, 20.2. – Enantiomeric excess was determined by HPLC with a Chiralpak AD-H column [*n*-hexane/2-propanol (9:1, v/v), 220 nm, 0.5 mL/min]: major enantiomer, t_R = 23.546 min; minor enantiomer, t_R = 22.937 min.

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