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Different bio/Lewis acid-catalyzed stereoselective aldol reactions in various mediums

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Abstract In this work eight different crude biocatalysts together with six Lewis and three Brønsted acids were used for asymmetric aldol reactions of aromatic, heteroaromatic, cyclic, and acyclic six ketones and eleven aldehydes. Optimum reaction conditions were determined by changing temperature and enzyme, ketone, aldehyde, solvent, cofactors types, amounts, and ratios. PPL (porcine pancreatic lipase) of animal and AL-AN (amano lipase A from *Aspergillus niger*) of fungal origins were the best ones and compared with each other. $CoCl_2$ was the best cofactor and catalyzed the enzymatic aldol reaction better than without cofactor. $CoCl_2$ was not used before for enzymatic aldol reactions. The method in this study, using crude biocatalysts (PPL or AL-AN) and $CoCl_2$ in acetonitrile–water was found as an conventionally useful biocatalytic way for asymmetric aldol reaction and has given better *ee* values than in the literature.
Graphical abstract
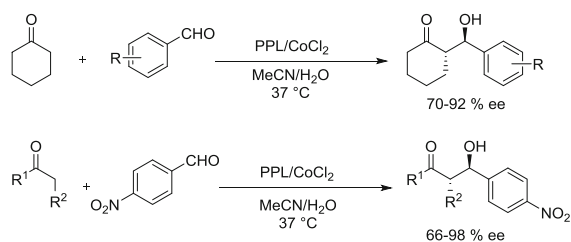
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Introduction

Biocatalyst is considered in catalytic synthetic reactions as a green tool because of its high selectivity, mild conditions, low energy consumption, and side reactions [1–4]. Biocatalytic methods are demanded largely from the pharmaceutical and chemical industries, due to their environmental and green chemistry sensitivity and have been worked widely in recent years [5–7].

Many types of enzyme are used in organic synthesis. Particular lipase are successfully and increasingly applied in the industrial fields such as agrochemicals, pharmaceuticals, and natural compounds [8, 9], because of their good stability, high catalytic effectivity, commercial availability, and broad range of substrate specificity.

Even though lipases can catalyze the hydrolysis of ester bonds, some hydrolytic lipases catalyze non-conventional synthetic organic reactions, which have recently reported, such as Michael additions [10–12], Mannich reactions [13, 14], Markovnikov additions [15], Henry reactions [16, 17], polymerization [18, 19], epoxidation [20–22], and aldol reactions [23–27]. Many asymmetric aldol reactions were achieved using organocatalysis such as proline or proline derivatives [28–31]. Aldolases [32–35] which are costly and unstable catalytic antibodies [36] and small molecules [37] were also not successful in asymmetric aldol reactions. Therefore, developing catalysts for asymmetric aldol reaction has been a subject which continues to attract the attention of researchers.

However, there are a limited number of aldol reactions catalyzed by lipases [38–40]. It was reported that wild CAL-B and Ser105Ala mutant CAL-B have catalytic activity on aldol reaction by Berglund and co-workers [38]. The previous studies showed that some lipases and proteases can catalyze asymmetric aldol additions. One of these is lipase from porcine pancreas (PPL) which was first used in the enzyme-catalyzed asymmetric aldol addition between acetone and different aromatic aldehydes in 2008 [39]. Then Yu and co-workers reported PPL-catalyzed stereoselective cross-aldol reactions between aromatic aldehydes and cyclic ketones in 2013 [41]. Another work about direct asymmetric aldol reactions catalyzed by the lipase from porcine pancreas was reported in 2014 [42].

In this study many asymmetric aldol reactions of various aromatic, heteroaromatic, cyclic, and acyclic ketones and aldehydes were investigated and catalyzed by different crude biocatalysts and Lewis and Brønsted acids via changing solvent, temperature, amounts, ratios, and cofactors. AL-AN and PPL were the best ones. To obtain better yields several Lewis and organic acids were studied together with the crude biocatalysts mentioned for the first time in this paper. $CoCl_2$ was the best appropriated Lewis acid with PPL and AL-AN. The medium $CoCl_2$-MeCN-H_2O (10:1) was not used before for enzymatic aldol reactions. The ketools **3a–3k** and **4b–4g** were not synthesized before by the method in this paper (Tables 8 and 9). **4c** and **4e** are novel keto alcohols.

Results and discussion

Different biocatalysts demonstrated encouraging results with good diastereoselectiviies and enantioselectivities under solvent-free and no additive conditions in case of using cyclohexanone (**1a**) and 4-nitrobenzaldehyde (**2a**) over 6 days (Table 1). Six commercially available lipase enzymes and two liver acetone powders which provide low-cost crude lipase sources were used in aldolisation reaction as crude biocatalysts. These are lipase from *Candida cylindracea* (CCL), lipase from *Candida rugosa* (CRL), porcine pancreatic lipase (PPL), amano lipase from *Burkholderica cepacia* (*Pseudomonas cepacia*) (AL-PS), amano lipase A from *Aspergillus niger* (AL-AN), amano lipase from *Pseudomonas fluorescens* (AL-PF), liver acetone powder-horse (LAP-H), and liver acetone powder-guinea pig (LAP-GP). AL-PS, AL-PF, LAP-H and LAP-GP were used as biocatalysts for the first time in this enzymatic aldol reaction. It is shown in Table 1, entries 4 and 6, that PPL and AL-AN gave the best promiscuous activity. Comparing with other lipases, PPL with nearly 85% yield and 75% *ee* was the best and AL-AN with 84% yield and 74% *ee* was the second best one. Interestingly,

although AL-PF gave the best yield result, its *ee* was only 44%. Based on these results, PPL and AL-AN were selected as biocatalyst for our enantioselective aldol reaction.

Time is also an important parameter of the enzymatic aldol reaction. We also checked the reaction time at regular intervals, we observed that the yield was increased pleasingly when the reaction period was raised, although the selectivity remained nearly unchanged. The best yield and selectivity was obtained for 4 days, in this study.

In the next step, to improve the *ee* results, some organic solvents with a small amount of water were tried in the same reaction conditions and same substrates (Table 2). Although high selectivity we achieved as *dr* 90:10, *ee* 79% for PPL, *dr* 89:11, *ee* 75% for AL-AN in MeCN (Table 2, entry 3), the yield decreased to 65% and 63%. As seen from the Table 2, the reaction between **1a** and **2a** with PPL or AL-AN as catalyst kept on with substantially lower results in non-polar solvents such as *n*-hexane and toluene. Ethanol as a polar protic solvent was also not sufficient. Polar aprotic solvents such as MeCN, DMSO, THF, and DMF have given much better results than the others.

In consistence with previous reports [39, 40] and also our experiments (Table 2, entry 9 and Table 3, entry 1) it proved that water is an important factor in this enzymatic reaction. Therefore, varying the ratio between solvent and water revealed that 10:1 was the optimum ratio (Table 3). However, the best yield was obtained for PPL when the ratio of MeCN/H_2O was 5:1 which gave the product in 68% yield (74% *ee*, 86:14 *dr*) (Table 3, entry 4). The best selectivity was obtained with 10:1 ratio of MeCN/H_2O as the optimal ratio for the PPL or AL-AN-catalyzed aldol reactions.

Moreover, we examined the effect of lipase concentration on the enzyme-catalyzed enzymatic aldol reaction (Table 4). We found that the yield had a nice improvement when the lipase concentration was increased from 10 to 200 mg/cm^3. However, using much more enzyme could not cause better selectivity. 50 and 100 mg/cm^3 enzyme concentration gave almost the same results. Based on the selectivity, 50 mg/cm^3 was chosen as the optimum enzyme concentration (Table 4, entry 3). Although selectivity was not raised too much, at least we achieved 80% for PPL and 76% AL-AN yield using 50 mg/cm^3 enzyme amount.

Then, the efficiency of the molar ratio between aldehyde and ketone (**1a** and **2a**) was examined (Table 5) to increase the yield and selectivity. Several molar ratios of **1a** and **2a** from 1:1 to 20:1 were tested. The best enantioselectivity was obtained as 20:1 for the ratio of **1a** and **2a** with 82% *ee* for PPL (Table 5, entry 5). However, increasing the proportion of ketone could not show a remarkable influence on the enantioselectivity of the reaction, the yield was clearly improved up to 87 and 78% for PPL and AL-AN,

Table 1 Screening of different crude biocatalysts for the asymmetric aldol reactions
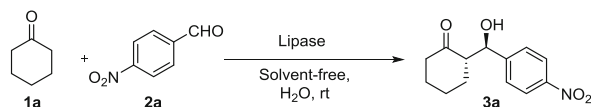
Entry	Lipase	Yield/%	dr (anti:syn)[a]	ee/%[a]
1	No enzyme	0	–	–
2	CCL	45	67:33	50
3	CCR	36	68:32	42
4	PPL	85	85:15	75
5	AL-PS	81	63:37	20
6	AL-AN	84	76:24	74
7	AL-PF	92	70:30	44
8	LAP-H	20	52:48	20
9	LAP-GP	0	–	–

Conditions: 4-nitrobenzaldehyde (0.1 mmol), 2 cm^3 cyclohexanone, and 20 mg lipase in 0.1 cm^3 deionized water at room temperature for 4 days

[a] ee and dr were determined by HPLC analysis using a chiral column

Table 2 Investigation of solvents for the enzymatic aldol reactions
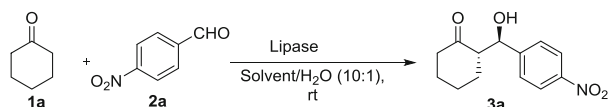
Entry	Solvent	Yield/% AL-AN	Yield/% PPL	dr (anti:syn)[a] AL-AN	dr (anti:syn)[a] PPL	ee/%[a] AL-AN	ee/%[a] PPL
1	DMSO	81	82	75:25	77:23	56	57
2	EtOH	42	46	65:35	66:34	25	28
3	MeCN	63	65	89:11	90:10	75	79
4	Hexane	12	10	54:46	57:43	15	20
5	THF	18	22	75:25	84:16	65	72
6	Toluene	15	12	61:39	62:38	18	20
7	DMF	75	76	80:20	81:19	66	68
8	Cyclohexanone	84	85	76:24	85:15	74	75
9	Cyclohexanone[b]	45	75	72:28	80:10	42	57

Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (1 mmol), and enzyme [PPL: 20 mg (0.48 kU), AL-AN: 20 mg (2.4 kU)] in organic solvent and deionized water (10:1, 1 cm^3) at room temperature for 4 days

[a] ee and dr were determined by HPLC analysis using a chiral column

[b] No water

respectively (Table 5, entry 5). Therefore, for further studies, the molar ratio of cyclohexanone to 4-nitrobenzaldehyde was determined 20:1 as the optimal ratio.

A large factor of their convenient suitability in living systems is that an important fraction of enzymes needs metals for their catalytic activity. Many metal-bonded enzymes are found in nature which act in fundamental biological processes, including photosynthesis, respiration, and nitrogen fixation [43].

The system of Lewis-acid and organocatalytic activation is inspired from enzymatic mechanism in the nature [44, 45]. Herein we tried for the first time the Lewis acid or Brønsted acid/bio-catalyzed aldol reactions. For this, we tested a series of metal salts or some natural acids as cofactor (Table 6).

We had very interesting results in this step using Lewis-acid as cofactor in enzymatic aldol reactions. Based on Table 6, the best enantioselectivity was obtained by CoCl$_2$

Table 3 Effect of MeCN/water ratio for the enzymatic aldol reactions
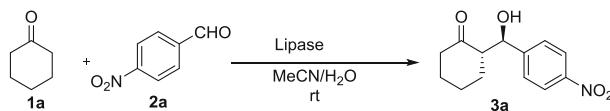
Entry	MeCN/H$_2$O	Yield/% AL-AN	Yield/% PPL	dr (anti:syn)^a AL-AN	dr (anti:syn)^a PPL	ee/%^{a} AL-AN	ee/%^{a} PPL
1	No water	15	27	75:25	83:17	60	62
2	1:1	60	62	76:24	76:24	51	50
3	2:1	61	65	83:17	85:15	68	70
4	5:1	65	68	85:15	86:14	67	74
5	10:1	63	65	89:11	90:10	75	79
7	20:1	62	64	88:12	90:10	71	77

Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (1 mmol), and enzyme [PPL: 20 mg (0.48 kU), AL-AN: 20 mg (2.4 kU)] in MeCN and deionized water (1 cm^3) at room temperature for 4 days

^{a} ee and dr were determined by HPLC analysis using a chiral column

Table 4 Investigation of enzyme concentration on the lipase-catalyzed enzymatic aldol reactions
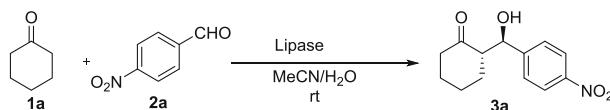
Entry	Enzyme conc./mg cm^{-3}	Yield/% AL-AN	Yield/% PPL	dr (anti:syn)^a AL-AN	dr (anti:syn)^a PPL	ee/%^{a} AL-AN	ee/%^{a} PPL
1	10	34	45	85:15	87:13	64	75
2	20	63	65	89:11	90:10	75	79
3	50	75	80	89:11	90:10	76	80
4	100	76	81	88:12	89:11	74	80
5	200	72	75	86:14	85:15	65	78

Condition: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (1 mmol), and enzyme in MeCN and deionized water (10:1, 1 cm^3) at room temperature for 4 days

^{a} ee and dr were determined by HPLC analysis using a chiral column

as cofactor with 88% ee for PPL and 79% ee for AL-AN at room temperature. Their yields also were lower as 75 and 60% (Table 6, entry 5). To increase the yield we studied the reaction at 37 °C (Table 6, entry 6). Surprisingly the yields increased to 95% for PPL and 82% for AL-AN, the enantioselectivity of PPL stayed at the same value with 88% and the enantioselectivity of AL-AN was increased slightly to 80% at 37 °C.

Further investigation about the amount of cobalt salt in the enzymatic aldol reaction was carried out with PPL (Table 7). Various amounts were studied at this point and it was seen that the increasing amount of cobalt decreased the yield and selectivity (Table 7). Increasing in the amount of cobalt showed a deactivating effect on the enzyme. Therefore, the best amount was determined with 20% mol. Also when the enzyme (PPL) was not used in the reaction

with CoCl$_2$, it was found that the yield was almost none and there was no selectivity (Table 7, entry 4).

Various aromatic aldehydes were tested in the reaction (Table 8). Also a wide range of ketones having different structures were explored in the lipase/CoCl$_2$-catalyzed aldol reaction. As seen from Table 8, the steric hindrance had no significant effect on aldehydes of different structures. Good selectivities were achieved when using benzaldehyde having either electron-withdrawing or electron-donating substituents. PPL or AL-AN/CoCl$_2$ combination gave almost equal enantioselectivity for aldol reaction of aromatic aldehydes and cyclohexanone. However, yields were obtained with good values of 67–95% for PPL, 50–92% for AL-AN. While the best enantioselectivity with PPL was achieved by 2-naphthaldehyde and 4-methoxybenzaldehyde with 92% ee

Table 5 Influence of ketone/aldehyde molar ratio for the enzymatic aldol reactions
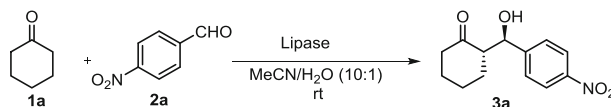
Entry	1a/2a	Yield/% AL-AN	Yield/% PPL	dr (anti:syn)[a] AL-AN	dr (anti:syn)[a] PPL	ee/%[a] AL-AN	ee/%[a] PPL
1	1:1	51	60	74:26	86:14	71	76
2	2:1	58	62	75:25	81:19	72	76
3	5:1	61	65	82:18	86:14	73	74
4	10:1	75	80	89:11	90:10	76	80
5	20:1	78	87	88:12	89:11	76	82

Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone, and enzyme [PPL: 50 mg (1.2 kU), AL-AN: 50 mg (6 kU)] in MeCN and deionized water (10:1, 1 cm^3) at room temperature for 4 days

[a] ee and dr were determined by HPLC analysis using a chiral column

Table 6 Screening of cofactor for the enzymatic aldol reactions
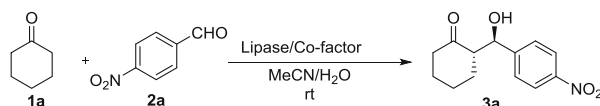
Entry	Cofactor (20%)	Yield/% AL-AN	Yield/% PPL	dr (anti:syn)[a] AL-AN	dr (anti:syn)[a] PPL	ee/%[a] AL-AN	ee/%[a] PPL
1	No	78	87	88:12	89:11	76	82
2	Cu(OTf)$_2$	45	53	86:14	90:10	76	82
3	FeCl$_3$	51	56	90:10	92:8	78	86
4	MnCl$_2$	35	42	85:15	88:12	78	86
5	CoCl$_2$	60	75	89:11	93:7	79	88
6	CoCl$_2$[b]	82	95	88:12	90:10	80	88
7	ZnCl$_2$	25	35	61:39	66:34	65	70
8	Mandalic acid	53	60	83:17	87:13	73	84
9	Lactic acid	52	56	80:20	85:15	74	85
10	Benzoic acid	94	95	53:47	58:42	50	45

Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (2 mmol), cofactor (20%), and enzyme [PPL: 50 mg (1.2 kU), AL-AN: 50 mg (6 kU)] in MeCN and deionized water (10:1, 1 cm^3) at room temperature for 4 days

[a] ee and dr were determined by HPLC analysis using a chiral column

[b] At 37 °C

(Table 8, entry 6 and 9), the best enantioselectivity with AL-AN was obtained by 2-furylaldehyde with 85% *ee* (Table 8, entry 7). We used also different ketones as aldol donors with 4-nitrobenzaldehyde, and we observed interesting results (Table 9). At this stage, we tried some ketones (1c, 1d and 1f) for the first time on the enzymatic direct aldol reaction.

In the absence of crude biocatalyst, the mentioned aldol reactions did not occur in this study (Table 1, entry 1). If CoCl$_2$ was used alone, it almost did not give any aldolisation reaction (Table 7, entry 4). The best biocatalyst PPL without CoCl$_2$ gave the aldol reaction with lower *ee* value

(Table 6, entry 1) than with CoCl$_2$ (Table 6, entry 6). Consequently, the best biocatalyst PPL with CoCl$_2$ in acetonitrile increased the enantioselectivity from 82 to 88%.

According to the literature [38, 41, 46] lipase sorts are described as having three amino acids residues Asp, His, Ser in their active sites. Due to these literature studies, histidine may be the most active edge of PPL and AL-AN in aldolisation. In this work is suggested that CoCl$_2$ has activated histidine residue more than the other active centers, so that better *ee* values were achieved in this aldolisation study.

Table 7 Effect of CoCl$_2$ amount for the enzymatic aldol reactions with PPL

Entry	CoCl$_2$/%mol	Yield/%	dr (anti:syn)[a]	ee/%[a]
1	No	97	88:12	82
2	10	94	89:11	87
3	20	95	90:10	88
4	20[b]	2	60:40	0
5	50	90	87:13	86
6	75	85	86:14	84
7	100	55	82:18	83
8	150	43	80:20	75

Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (2 mmol), CoCl$_2$, and PPL [50 mg (1.2 kU)] in MeCN and deionized water (10:1, 1 cm^3) at 37 °C for 4 days

[a] ee and dr were determined by HPLC analysis using a chiral column

[b] No enzyme

Table 8 Scope of aromatic aldehydes for the enzymatic aldol reactions of cyclohexanone by lipase/CoCl$_2$
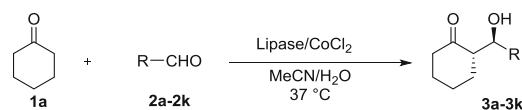
Entry	Aldehyde	R	Yield/%[a] AL-AN	Yield/%[a] PPL	dr (anti:syn)[b] AL-AN	dr (anti:syn)[b] PPL	ee/%[b] AL-AN	ee/%[b] PPL
1	**2a**	4-NO$_2$C$_6$H$_4$	82	95	88:12	90:10	80	88
2	**2b**	C$_6$H$_5$	76	92	82:18	83:17	79	86
3	**2c**	2-CH$_3$C$_6$H$_4$	62	68	86:14	88:12	81	88
4	**2d**	3-CH$_3$C$_6$H$_4$	71	76	83:17	85:15	80	88
5	**2e**	2-CH$_3$OC$_6$H$_4$	85	90	90:10	96:4	66	70
6	**2f**	4-CH$_3$OC$_6$H$_4$	50	67	90:10	89:11	84	92
7	**2g**	2-Furyl	76	88	75:25	70:30	85	90
8	**2h**	2-Thienyl	85	92	85:15	88:12	81	88
9	**2i**	2-Naphthyl	92	95	89:11	92:8	86	92
10	**2j**	2-FC$_6$H$_4$	75	81	88:12	92:8	82	86
11	**2k**	2-BrC$_6$H$_4$	68	74	88:12	87:13	81	88

Conditions: aldehyde (0.1 mmol), cyclohexanone (2 mmol), CoCl$_2$ (20%), and enzyme [PPL: 50 mg (1.2 kU), AL-AN: 50 mg (6 kU)] in MeCN and deionized water (10:1, 1 cm^3) at 37 °C for 4–6 days
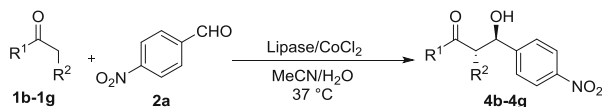
[a] Isolated yield of products

[b] ee and dr were determined by HPLC analysis using a chiral column

Conclusion

In summary, the study of developing new crude bio-Lewis acid-catalyzed aldol reaction between aromatic aldehydes and cyclic-acyclic ketones led us to find some innovations. In this context, we investigated so many effects such as lipase, solvents, cofactor types, water contents, temperature, molar ratio of substrates and enzyme concentrations on the enzyme-catalyzed aldol reaction to find the best enantioselectivity. After a broad screening of lipases, PPL and AL-AN gave the highest stereoselectivities and yields.

Therefore, we used both lipases in all reactions and compared the animal-derived (PPL) with fungal-derived (AL-AN). As seen from the results, PPL gave slightly better yields and enantioselectivities than AL-AN.

The optimized PPL or AL-AN/CoCl$_2$-catalyzed reaction conditions gave *anti*-products with better enantioselectivities between 80 and 100% *ee* when compared with other enzyme-catalyzed procedures. Finally a green, cheap, and novel protocol was developed as an example of enzymatic promiscuity using a combination of PPL or AL-AN/CoCl$_2$ as biocatalyst.

Table 9 Screening of various structured ketones for the enzymatic aldol reactions with 4-nitrobenzaldehyde by lipase/CoCl$_2$

Entry	Ketone	Product	Yield/%[a] AL-AN	Yield/%[a] PPL	dr (anti:syn)[b] AL-AN	dr (anti:syn)[b] PPL	ee/%[b] AL-AN	ee/%[b] PPL
1	1b	4b	80	85	–	–	62	66
2	1c	4c	60	64	–	–	48	51
3	1d	4d	42	45	–	–	37	38
4	1d	4e	30	25	55:45	53:47	78/76[c]	99/96[c]
5	1f	4f	66	69	56:44	62:38	37	42
6	1g	4g	90	95	90:10	93:7	87	98

Conditions: 4-nitrobenzaldehyde (0.1 mmol), ketone (2 mmol), CoCl$_2$ (20%), and enzyme [PPL: 50 mg (1.2 kU), AL-AN: 50 mg (6 kU)] in MeCN and deionized water (10:1, 1 cm^3) at 37 °C for 4–6 days

[a] Isolated yield of products

[b] ee and dr were determined by HPLC analysis using a chiral column

[c] The ee values for anti/syn

Experimental

The majority of the chemicals and all enzymes used in this work were commercially available from Merck or Sigma-Aldrich. All reagents were obtained from commercial suppliers and were used without further purification unless otherwise noted. 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), amano lipase A from *A. niger* (120,000 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). The reactions were monitored by TLC using silica gel plates and the products were purified by flash column chromatography on silica gel (Merck; 230–400 mesh).

The NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C using Me$_4$Si as the internal standard in CDCl$_3$. HPLC was performed on a Shimadzu/DGU-20A$_5$ HPLC apparatus fitted with a 25 cm Chiralcel OD and Chiralpac AD-H chiral columns. Optical rotations were measured with Optical Activity AA-55 digital polarimeter at room temperature.

General procedure for the aldol reaction

Aromatic aldehyde (0.1 mmol), ketone (2 mmol), CoCl$_2$ (20%), and PPL (50 mg) in MeCN and deionized water (10:1, 1 cm^3) were added to a flask then the mixture was stirred at 37 °C for 4–6 days. The reaction extracted with ethyl acetate three times. The combined extracts were dried over anhydrous Na$_2$SO$_4$, and the solvents were then removed under reduced pressure. Reactions were monitored by thin-layer chromatography and visualized using UV light. After finishing of the reaction, the product was first analyzed by HPLC. For determination of the isolated yield, flash chromatography was performed on silica gel (Merck; 230–400 mesh) with hexane–ethyl acetate (v/v 9:1) as the mobile phase. Racemic compounds of all aldol products were synthesized by known methods as references for HPLC.

Characterization data for compounds already described in the literature can be found in the Supplementary Material.

1-Hydroxy-1-(4-nitrophenyl)-octan-3-one (4c, C$_{14}$H$_{19}$NO$_4$)

Yield 64%; enantiomeric excess 51%; $[\alpha]_D^{25} = +18.5$ (c = 0.9, CHCl$_3$); IR (neat): $\bar{v}$ = 3469, 3069, 2938, 2892, 1679, 1600, 1492, 1292, 1253, 984, 761 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.14 (d, J = 9 Hz, 2H), 7.46 (d, J = 8.5 Hz, 2H), 5.19 (dd, J_1 = 4 Hz, J_2 = 8.5 Hz, 1H), 3.56 (brs, 1H), 2.73–2.76 (m, 2H), 2.37 (t, J = 7 Hz, 2H), 1.49–1.55 (m, 2H), 1.15–1.25 (m, 4H), 0.82 (t, J = 7 Hz, 3H) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 211.3, 150.3, 147.5, 126.7, 126.5, 124.0, 123.8, 69.2, 50.47, 43.8, 31.4, 23.4, 22.5, 14.0 ppm.

Enantiomeric excess of *anti*-diastereomer was determined by HPLC with a CHIRALPAK AD-H column (95:5 hexane:2-propanol), 25 °C, 263 nm, 1.0 cm^3/min; major enantiomer $t_R = 37.5$ min, minor enantiomer $t_R = 35.4$ min.

4-Hydroxy-4-(4-nitrophenyl)-3-phenylbutan-2-one (**4e**, C$_{16}$H$_{15}$NO$_4$)

Yield 25%; dr (*anti*:*syn*) 54:47; enantiomeric excess 99% for *anti*, 96% for *syn*; IR (neat): = 3472, 3065, 2946, 2889, 1685, 1606, 1499, 1292, 1242, 988, 763 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 8.00$ (d, $J = 9$ Hz, 2H), 7.19–7.23 (m, 5H), 6.96–6.97 (m, 2H), 5.44 (dd, $J_1 = 2$ Hz, $J_2 = 4.5$ Hz, 1H), 3.82 (d, $J = 4.5$ Hz, 1H), 3.36 (d, $J = 2$ Hz, 1H), 1.99 (s, 3H), 1.18 (brs, 1H) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): $\delta = 209.3, 148.7, 147.4, 132.7, 130.0, 129.9, 129.1, 128.9, 127.5, 123.5, 124.4, 72.9, 72.9, 65.9, 65.8, 30.1$ ppm.

Enantiomeric excess of diastereomers was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:2-propanol), 25 °C, 210 nm, 1.0 cm^3/min; for *anti*-diastereomer major enantiomer $t_R = 37.9$ min, minor enantiomer $t_R = 23.2$ min; for *syn* diastereomer major enantiomer $t_R = 24.6$ min, minor enantiomer $t_R = 30.1$ min.

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