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Ionic liquid as a recyclable and efficient medium for lipase-catalyzed asymmetric cross aldol reaction

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ABSTRACT

PPL (lipase from porcine pancreas) was found to catalyze asymmetric cross aldol reactions of aromatic and heteroaromatic aldehydes with various ketones in ionic liquid ([BMIM][PF_6]) for the first time. Interestingly, PPL exhibited high catalytic activity and excellent stereoselectivity in this efficient and recyclable room temperature ionic liquid in the presence of moderate water, similar to the results obtained in organic solvents. High yields of up to 99%, excellent enantioselectivities of up to 90% ee, and good diastereoselectivities of up to >99:1 dr were achieved.

1. Introduction

Room temperature ionic liquids (ILs) have been regarded as potentially attractive solvents in modern organic synthesis [1,2]. ILs have low vapour pressure, low flammability, high ionic conductivity and excellent chemical and thermal stability, and they are easily recycled [3,4]. Moreover, the unique and attractive physicochemical properties of ILs, such as viscosity, polarity and solubility, can be designed by selecting the appropriate cation and anion. Thus, ILs have been widely used in synthesis and catalysis owing to these unique properties [5,6]. Many excellent reviews have also been published in the past decade [7,8]. In biocatalysis, ILs have been used as solvents or co-solvents for enzyme-catalyzed reactions in the past decade [9,10], and several biocatalytic reactions, such as oxidation [11], hydrolysis [12], polymerization [13] and transesterification [14,15], have been achieved in ILs. Russell and co-workers first reported the enzyme-catalyzed reaction in IL [BMIM][PF_6] for the synthesis of Z-aspartame in 2000 [16]. With the rapid development of biocatalysis in ILs, Rantwijk and Sheldon published an excellent comprehensive review on the various issues associated with biocatalysis in ILs in 2007 [17]. Recently, Goto and colleagues reviewed the activation and stabilization of enzymes in ILs [18]. Yang et al. researched the specific ion effects of ILs on enzyme activity and stability [19]. Moreover, ILs have been used for the synthesis of sugar derivatives and other special compounds, owing to the strong dissolution power of ILs [20]. In addition to their uses as reaction solvents, ILs also have many other significant applications in biocatalysis and enzyme engineering. ILs can also be utilized as extraction solvents to stabilize and extract enzymes [21] and can incorporate and covalent modify some enzymes or modify the immobilized material of enzymes [22,23]. In fact, as excellent solvents for biocatalysis, ILs provide many advantages over conventional organic solvents, including high activity and selectivity and better stability as well as better recoverability and recyclability [24]. However, few reports regarding the biocatalytic promiscuity of ILs as solvents compare to conventional biocatalysis reactions in ILs exist [25]. Thus, the combination of ILs with more biocatalysis reactions remains an important challenge.

Biocatalysis, as an eco-friendly and sustainable methodology for organic synthesis due to its high efficiency, excellent selectivity and mild reaction conditions, is widely recognized as a practical alternative to traditional organic synthesis [26,27]. However, biocatalysts exhibit specific catalytic activity for distinctly different chemical transformation of natural or non-natural substrates [28,29]. This kind of catalytic promiscuity immensely broadens the application of biocatalysts and provides an available tool for organic synthesis. Recently, several elegant studies on enzymatic promiscuity have been reported, especially with regard to hydrolases that catalyze unconventional reactions, such as aldol reaction [30,31], Mannich reaction [32], Henry reaction [33],

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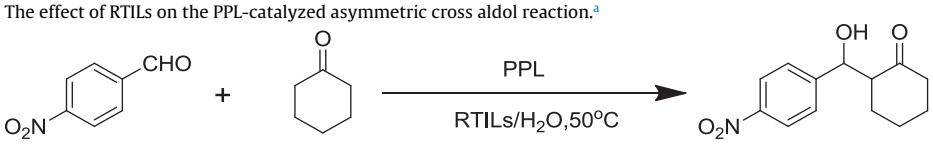
Michael additions [34,35], Ugi reaction [36] and three-component aza-Diels–Alder reaction [37]. Among these reactions, the powerful and atom economical asymmetric aldol reaction was frequently reported. Berglund and co-workers first demonstrated that wild-type lipase B from *Candida antarctica* (CAL-B) and Ser105Ala mutant CAL-B possess catalytic activity for aldol addition in cyclohexane [30]. Guan and co-workers exhibited several asymmetric aldol reactions catalyzed by different hydrolyases [38,39]. Lin and co-workers also reported an aldol condensation reaction catalyzed by acylase [40]. In our previous work, we demonstrated the first lipase-catalyzed asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde greatly promoted by water [31]. However, almost all lipase-catalyzed aldol reactions were performed in the traditional organic solvents in which the enzymes were found to preserve their activities [41,42]. Hence, the search for more efficient media for biocatalytic asymmetric aldol reactions and other chemical transformations has become a considerable challenge. Recently, we found that the lipase-catalyzed stereoselective cross aldol reaction could be performed under solvent-free conditions. However, the enormously excessive ketones used as one of the substrates were not sufficiently cost-efficient [43]. An enzymatic aldol reaction in phosphate–citrate buffer was then attempted, only achieving an optimal enantioselectivity of 66% [44]. Based on these previous works, we continued to seek a more efficient biocatalytic route for asymmetric aldol reactions. We selected lipase from porcine pancreas (PPL) as a catalyst and utilized different ILs as the reaction medium in the presence of water. Surprising, we obtained similar results to those achieved in organic solvents. To the best of our knowledge, no other reports have demonstrated lipase-catalyzed asymmetric cross aldol reaction in ILs.

As a part of our continuing research on enzyme promiscuity, we wish to report a lipase-catalyzed the asymmetric cross aldol reaction between aromatic aldehydes and cyclic ketones under mild conditions using an IL medium. Interestingly, we also observed that the presence of water greatly promoted the catalytic activity and selectivity of PPL, which was consistent with our previous report [31]. High yields and good enantioselectivities and diastereoselectivities were also obtained while simultaneously investigating a wide variety of substrates. Meanwhile, the good reusability of ILs as the medium for PPL was also observed.

2. Results and discussion

It is well known that enzyme activity is strongly affected by reaction medium [42,45]. The feasibility of using ILs as efficient solvents for biocatalysis is well documented. However, ILs are rarely used in enzyme-catalyzed promiscuous reactions, such as aldol reaction. Therefore, we endeavored to find a more efficient medium combination with biocatalysis to meet the criteria of sustainable conscious development. Recently, we reported a lipase-catalyzed asymmetric aldol reaction in cyclohexanone, which is one of the substrates [43]. Although the reaction produced good yield and high selectivity, the excessive ketones were not sufficiently cost-efficient. Furthermore, we continued to attempt this enzymatic aldol reaction in phosphate–citrate buffer considering that lipase could maintain good activity in aqueous solvents [44]. However, the best enantioselectivity achieved was only 66% ee. Thus, we employed efficient ILs as the medium for the asymmetric aldol reaction catalyzed by PPL. The aldol reaction between 4-nitrobenzaldehyde and cyclohexanone was used as a model reaction. The initial experiments were performed in various pure ILs, including five tetrafluoroborate ILs (Table 1, entries 1–5), three hexafluorophosphate ILs (Table 1, entries 6–8) and two chiral ILs (Table 1, entries 9 and 10). Unfortunately, PPL showed extremely poor catalytic activity in all of the tested RTILs, as the products were difficult to detect by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC). The most likely reason for such low yields was that the high viscosity of the IL systems might be limiting the mass transfer of substrates and products to and from the active site of the enzyme [46]. In principle, high viscosity could be overcome through the addition of small amounts of other solvents as additives to the IL medium. According to our previous research, water plays an important role in lipase-catalyzed promiscuous aldol reaction. For most reaction systems, a water content of approximately 10% has produced the best results. Thus, we added 10% water to the ILs to investigate the optimal IL for this reaction. To our surprise, when 10% water was added to the ILs, we detected the aldol product in several ILs. As shown in Table 1, PPL exhibited good catalytic activity in all tested ILs. For the most utilized ILs, both hexafluorophosphate and tetrafluoroborate ILs showed that ILs with shorter alkyl chains on their cations promoted better enzyme activity. The two chiral ILs also induced high activity, and 1-butyl-3-methylimidazolium

Table 1
The effect of RTILs on the PPL-catalyzed asymmetric cross aldol reaction.[a]



Entry	RTILs	Catalyst	Yield%[b]	dr(anti/syn)[b]	ee%(anti)[b]
1	[EMIM][BF_4]	PPL	80	80:20	57
2	[BMIM][BF_4]	PPL	77	83:17	58
3	[HMIM][BF_4]	PPL	71	79:21	53
4	[OMIM][BF_4]	PPL	68	79:21	53
5	[EOMIM][BF_4]	PPL	55	78:22	68
6	[BMIM][PF_6]	PPL	63	86:14	80
7	[HMIM][PF_6]	PPL	45	64:36	38
8	[OMIM][PF_6]	PPL	39	85:15	77
9	[BMIM][LTar]	PPL	84	48:52	8
10	[BMIM][LLac]	PPL	76	47:53	3
11	[BMIM][PF_6]	Bovine serum albumin (BSA)	5	–	–
12	[BMIM][PF_6]	No enzyme	1	–	–
13	[BMIM][PF_6]	Denatured PPL[c]	1	–	–

[a] Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (1.0 mmol), and PPL (10 mg) in RTILs with deionized water (10%, water/([BMIM][PF_6] + water), v/v) at 50 °C for 24 h.
[b] Yield, ee, and dr were determined by HPLC using AD-H chiral column.
[c] Pretreated with guanidine hydrochloride solution (6 mol/L).

L-tartrate ([BMIM][LTar]) showed the best catalytic activity with yield of 84%. The reason for these yields may be that the ILs are highly polar, and the cations with shorter alkyl chains have higher polarity, which increased the solubility of the substrates and led to more efficient and faster reactions [47]. However, 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) exhibited the highest selectivity (80% ee) and a good yield at 63% (Table 1, entry 6). The biphasic (water content of 10% in [BMIM][PF_6] is two phase system) [BMIM][PF_6]/H_2O most likely maintained PPL's conformational flexibility, promoting the reaction. Thus, we chose [BMIM][PF_6]/H_2O as the reaction medium for further reaction investigations.

To demonstrate the specific catalytic effect of PPL on the aldol reaction in the special media, some control experiments were conducted in the same IL system (Table 1, entries 11–13). No product could be detected in the absence of lipase. The non-enzyme proteins bovine serum albumin (BSA) [48,49], which has a surface amino acid distribution similar to that of many soluble enzymes, also demonstrated very low activity with only a trace of product obtained. We obtained a similar result to that of the blank reaction after denatured PPL was employed as the reaction catalyst. Thus, the possibility that the catalysis mainly arose from amino acids of the protein was absolutely excluded. All these facts suggest that the special spatial conformation of the enzyme and the specific catalytic site of PPL are essential in catalyzing the aldol reaction.

The good results obtained with the initial use of [BMIM][PF_6] as an efficient reaction medium for lipase-catalyzed aldol reaction encouraged us to expect better results after optimizing the various reaction conditions. Thus, experiments were performed to screen for the optimal biocatalyst for the aldol reaction between 4-nitrobenzaldehyde and cyclohexanone in the biphasic [BMIM][PF_6]/H_2O medium (Table S1). The results showed that PPL promoted the highest biocatalyst activity among the tested seven lipases, including lipase from porcine pancreas (PPL), lipase from *C. antarctica* B (CAL-B), lipase from *Mucor miehei* (MML), lipase from *Candida cylindracea* (CCL), lipase from *Mucor javanicus* (MJL), lipase from *Penicillium camemberti* (PCL) and lipase from *Rhizopus oryzae* (ROL). The other lipases exhibited very low activities with little yield. The results are consistent with our previous works obtained in organic solvents or buffer. Thus PPL appears to be the best biocatalyst for aldol reaction in the unique [BMIM][PF_6]/H_2O medium.

Water content dramatically influences the activity, stability and probably tertiary structure of the enzymes [50]. Thus, the water content of reaction system was investigated further. Water contents ranging from 0% to 100% (water/([BMIM][PF_6] + water), v/v) in [BMIM][PF_6] were screened, and the results are shown in Fig. 1. The water content was found to greatly influence the activity and selectivity of PPL for the aldol reaction. Both good yields and stereoselectivities were obtained when the water content ranged from 6% to 10%. PPL exhibited the best enantioselectivity and diastereoselectivity with a water content of 8% (water/([BMIM][PF_6] + water), v/v), which produced a 71% yield with 87:13 dr and 80% ee. As shown in Fig. 1, in the absence of water, very little products were obtained. Interestingly, when the water content was greater than 15%, the ee and dr values declined sharply, and the product yield decreased to a low level. However, the enzyme exhibited the highest catalytic activity in the pure water with an 87% yield and poor selectivity. To obtain the best diastereoselectivity and enantioselectivity, 8% water content was chosen as the optimum addition for the PPL-catalyzed aldol reaction. In previous reports concerning enzyme-catalyzed aldol reactions, nearly all reactions were optimized for water content. For example, we reported the first lipase-catalyzed asymmetric aldol reaction between acetone and 4-nitrobenzaldehyde greatly promoted by adding water with an optimum water content of 20% [31]. Guan et al. also found that nuclease-catalyzed aldol

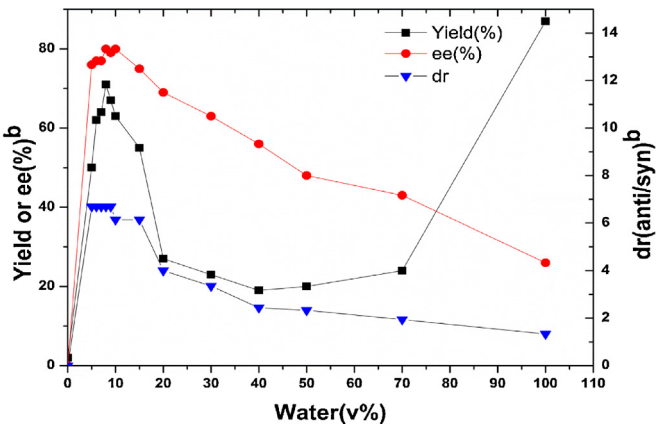


Fig. 1. The effect of water content on the PPL-catalyzed asymmetric cross aldol reaction.[a] [a] Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (1.0 mmol), and PPL (10 mg) in [BMIM][PF_6] with deionized water (0–100%, water/([BMIM][PF_6] + water), v/v) at 50 °C for 24 h. [b] Yield, ee, and dr were determined by HPLC using AD-H chiral column.

reactions required an optimized water content of 15% [38], and the chymopapain-catalyzed direct asymmetric aldol reaction had an optimum water content of 12% [39]. From the discussion above, we may conclude that a reaction system with an optimized water content may maintain the best conformational flexibility of the enzyme and thereby produce the best results.

Next, we examined the influence of enzyme loading on the model reaction. As shown in Fig. 2, the yield and the stereoselectivity were significantly influenced by different enzyme loading that ranged from 2 mg to 80 mg. The best yields were obtained at 25 mg or 30 mg (74%). However, low stereoselectivity was observed. Thus, 10 mg of PPL was selected as the optimal quantity for the model reaction of 4-nitrobenzaldehyde and cyclohexanone, having better stereoselectivity and approximate yield, considering stereoselectivity and cost-efficient amount of enzyme. As shown, a small decline in yield and diastereoselectivity was observed, and the enantioselectivity changed very little when the enzyme loading

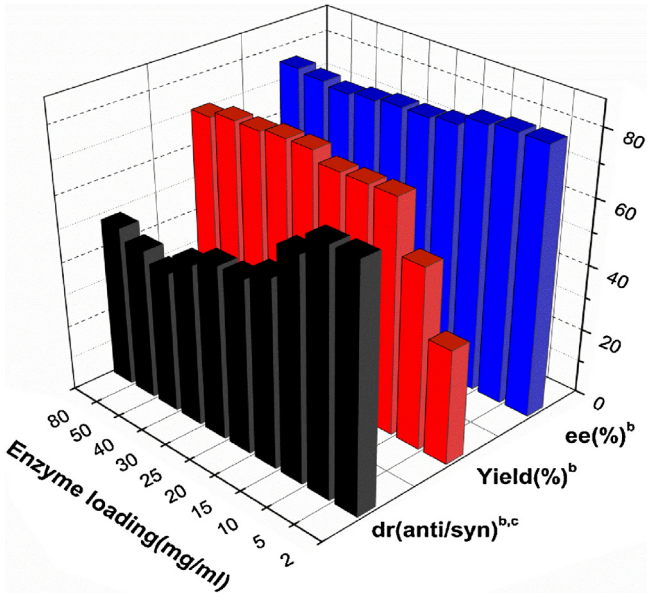
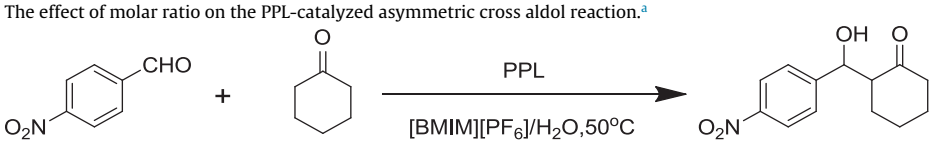


Fig. 2. The effect of enzyme loading on the PPL-catalyzed asymmetric cross aldol reaction.[a] [a] Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (1.0 mmol), and PPL (2, 5, 10, 15, 20, 25, 30, 40, 50, 80 mg/mL) in [BMIM][PF_6] (920 μL) with deionized water (80 μL) at 50 °C for 24 h. [b] Yield, ee, and dr were determined by HPLC using AD-H chiral column. [c] dr = 10 × (anti/syn).

Table 2
The effect of molar ratio on the PPL-catalyzed asymmetric cross aldol reaction.[a]



Entry	Molar ratio[b]	Yield%[c]	dr(anti/syn)[c]	ee%(anti)[c]
1	1:1	10	87:13	79
2	1:5	42	87:13	80
3	1:10	70	87:13	80
4	1:15	81	87:13	80
5	1:20	90	87:13	80
6	1:25	89	87:13	79
7	1:30	91	87:13	80
8	1:40	92	87:13	78
9	1:50	91	87:13	78

[a] Conditions:4-nitrobenzaldehyde(0.1 mmol), cyclohexanone (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 mmol), and PPL (10 mg) in [BMIM][PF_6] (920 μL) with deionized water (80 μL) at 50 °C for 24 h.
[b] Molar ratio = 4-nitrobenzaldehyde/cyclohexanone.
[c] Yield, ee, and dr were determined by HPLC using AD-H chiral column.

was largely increased. During the reaction process, when a large amount of PPL was added to the system, the aggregation of some of the enzyme was clearly observed. This aggregation behavior may be due to the viscosity of the IL, preventing the large enzyme from dispersing efficiently in the reaction system. On the other hand, due to the IL's viscosity, the enzyme activity was controlled by affecting the mass-transfer limitations [46]. Consequently, more enzyme loading did not favor the lipase-catalyzed reaction.

Encouraged by the above results, other influencing factors were screened to obtain higher yields and stereoselectivities. The effect of the molar ratio of the substrates on the PPL-catalyzed aldol reaction was investigated. As shown in Table 2, the molar ratio substantially influenced the reaction yield. When the molar ratio of 4-nitrobenzaldehyde to cyclohexanone was varied from 1:1 to 1:20, the yield was enormously improved from 10% to 90%. However, no obvious difference was observed in the yield after increasing the mole ratio from 1:20 to 1:50. The diastereoselectivity and enantioselectivity were maintained throughout the process of testing the effect of the substrate molar ratio on the reaction between 4-nitrobenzaldehyde and cyclohexanone. The probable reason for these observations is that the reaction reached equilibrium when the molar ratio of 4-nitrobenzaldehyde to cyclohexanone was 1:20, therefore, no obvious improvements were observed when the molar ratio was increased. Thus, 1:20 was selected as the optimal molar ratio for the aldol reaction.

To obtain better results from this lipase-catalyzed asymmetric cross aldol reaction, we also investigated the reaction temperature and duration combinations. We performed the model reaction at five different temperatures that ranged from 20 °C to 70 °C for 6 h, 12 h, 24 h, 48 h, 72 h and 120 h. As shown in Table S2, the reaction at 37 °C for 72 h offered the best result with yields of up to 93% and the highest enantioselectivity (85% ee) and diastereoselectivity (91:9 dr). At the higher temperature (50 °C and 70 °C), and by prolonging the reaction time, both the enzyme activity and the stereoselectivity decreased, in particular, the stereoselectivity dropped sharply. It was most likely that the optimum conformation of the enzyme appeared at the optimum temperature, and unfavorable conformation occurred at higher temperature. However, at the lower temperature (20 °C and 30 °C), the enzyme activity was too low. To obtain the best yield, diastereoselectivity and enantioselectivity, the reaction temperature of 37 °C and time of 72 h were chosen for the PPL-catalyzed aldol reaction.

To further demonstrate the advantage of ILs as the medium for this enzymatic reaction, we recycled the biocatalyst and the media together. After some recycling experiments, we found that the free enzyme was not easy to recycle and recover because a portion of the enzyme usually collected on the wall of the round-bottom flask or aggregated or dispersed into the IL. Thus, recycling and recovering free PPL was difficult. However, the further research on using immobilized PPL for recycling is in progress. We then recovered and recycled only the IL [BMIM][PF_6] using the model reaction. Amazingly, an unexpected result was observed. As shown in Table 3, the recovered IL was recycled for five runs with no loss in enzyme activity or stereoselectivity under the same reaction conditions. [BMIM][PF_6], itself is a widely used IL in biocatalysis, can undoubtedly broaden its applications in many aspects due to its excellent recyclability and recoverability. Over the past decade, the field of ionic liquids has grown at an unpredictable rate. Due to their unique and tunable physicochemical properties, many applications of ILs have been reported, such as the application in chemical industry and pharmaceutical industry [7,51]. At the same time, we are confident that ILs will enable more novel applications that are not possible in conventional solvents in the future and will be widely used in numerous fields including biocatalysis, electrochemistry, physical chemistry, engineering and so on.

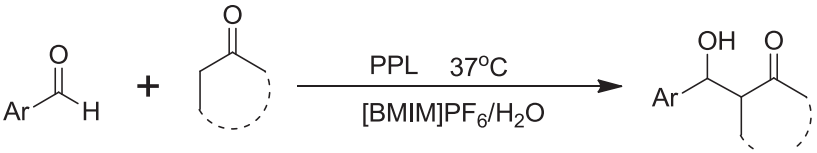
With the optimal reaction conditions in hand, we further studied the scope and the generality of this biocatalytic promiscuity. Several substrates were used to expand the PPL-catalyzed aldol reaction. As shown in Table 4, both aromatic and heteroaromatic aldehydes with cyclic or heterocyclic ketones or with acetone gave moderate to high yields, good enantioselectivities and diastereoselectivities in the biphasic [BMIM][PF_6]/H_2O medium (H_2O/([BMIM][PF_6] + H_2O) = 8%, v/v). A maximum yield of 99%, optimal diastereoselectivity of >99:1 dr and best enantioselectivity of 90% ee were achieved using this medium. Generally, benzaldehydes with strong electron-withdrawing substituents served as better

Table 3
Recycling studies for the aldol reaction of the model reaction.[a]

Number of cycles	Yield%[b]	dr(anti/syn)[c]	ee%[c]
1	92	92:8	86
2	93	91:9	85
3	93	91:9	85
4	93	90:10	84
5	92	92:8	86

[a] At the beginning, the reaction was carried out using 4-nitrobenzaldehyde (0.3 mmol), cyclohexanone (6.0 mmol), and PPL (30 mg) in [BMIM][PF_6] (2760 μL) with deionized water (240 μL) at 37 °C for 72 h.
[b] Yield of the isolated product after chromatography on silica gel.
[c] ee, and dr were determined by HPLC using AD-H chiral column.

Table 4
Substrate scope of the PPL-catalyzed asymmetric cross aldol reaction.[a]



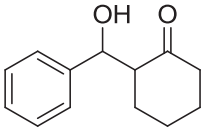
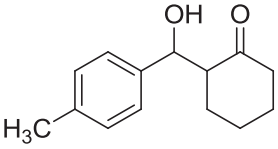
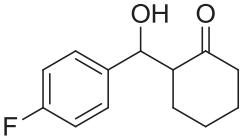
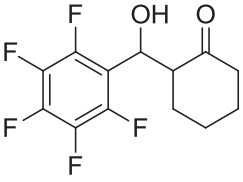
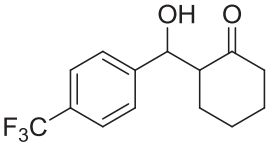
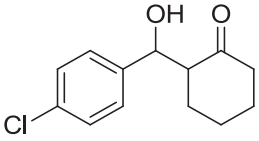
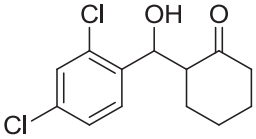
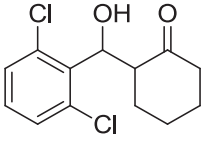
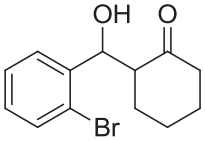
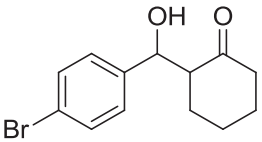
Entry	Product	Time (h)	Yield%[b]	dr(anti:syn)[c]	ee%(anti)[c]
1		240	37	76:24	80
2		240	45	84:16	77
3		192	38	83:17	89
4		72	99	94:6	69
5		168	84	87:13	87
6		192	60	86:14	82
7		96	72	99:1	88
8		100	96	>99:1	67
9		84	48	87:13	90
10		192	74	87:13	84

Table 4 (*Continued*)

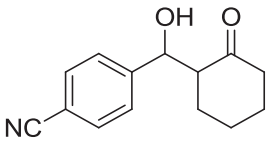
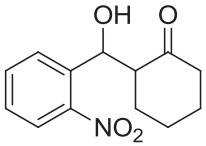
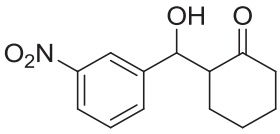
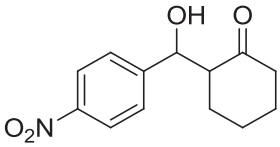
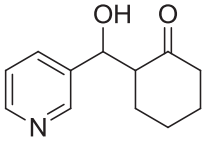
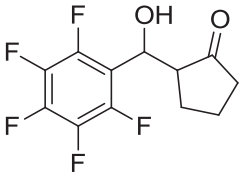
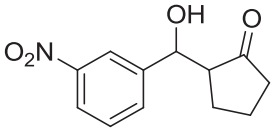
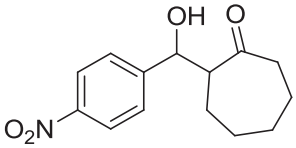
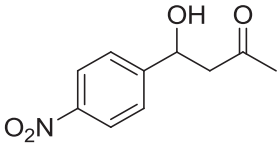
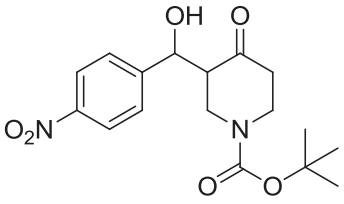
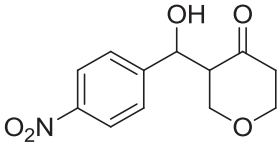
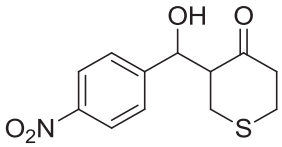
Entry	Product	Time (h)	Yield%[b]	dr(anti:syn)[c]	ee%(anti)[c]
11		168	88	85:15	80
12		120	83	93:7	80
13		72	83	91:9	88
14		72	93	91:9	85
15		192	89	84:16	85
16		72	92	85:15	66
17		72	85	70:30	75
18		240	35	50:50	25/8[d]
19		288	41	–	35
20		170	88	71:29	74
21		192	85	72:28	74

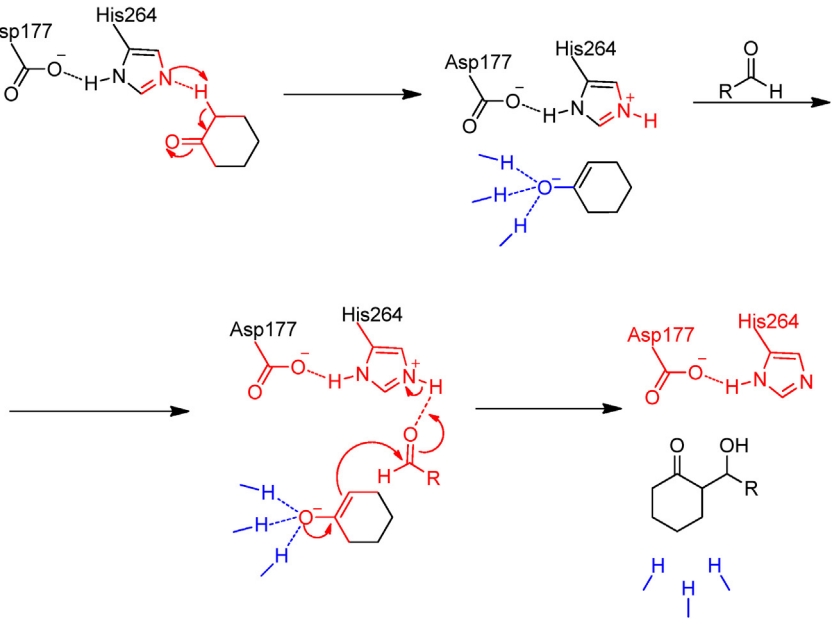
Table 4 (*Continued*)

Entry	Product	Time (h)	Yield%[b]	dr(anti:syn)[c]	ee%(anti)[c]
22		192	80	80:20	79

[a] Reaction conditions: aromatic aldehyde (0.3 mmol), ketone (6 mmol) and PPL (30 mg) in [BMIM][PF_6] (2760 μL) with deionized water (240 μL) at 37 °C.
[b] Yield of the isolated product after chromatography on silica gel.
[c] dr and ee were determined by HPLC using a chiral column.
[d] ee of anti/ee of syn.

acceptors, producing better yields (Table 4, entries 3–14). The reactions of aromatic aldehydes with strong electron-withdrawing substituents provided products in the highest yield of 99%, the best diastereoselectivity of >99:1 dr and the best enantioselectivity of 90% ee (Table 4, entries 4, 8 and 9). However, for aromatic aldehydes with electron-donating substituents or no substituents, such as 4-methyl benzaldehyde and benzaldehyde, the yields were very low (Table 4, entries 1 and 2). Furthermore, we examined the effect of various ketones. Cyclopentanone afforded the products in good yields and moderate stereoselectivity (Table 4, entries 16 and 17). However, both cycloheptanone and acetone produced poor yields and stereoselectivities (Table 4, entries 18 and 19). In terms of yield and stereoselectivity, cyclohexanone promoted the best results. Moreover, the substituent positions on the benzene on benzaldehydes had a great impact on the yield of the reaction. For instance, the aldol reaction of 4-nitrobenzaldehyde with cyclohexanone generated the aldol product with a higher yield than that of 2-nitrobenzaldehyde and 3-nitrobenzaldehyde. However, 3-nitrobenzaldehyde had the best enantioselectivity of 88% ee (Table 4, entries 12–14). Meanwhile, the steric hindrance of substituents on benzaldehyde had a great influence on the yield and stereoselectivity of the reaction. For example, among 2,6-dichlorobenzaldehyde, 2,4-dichlorobenzaldehyde, 4-chlorobenzaldehyde, the most hindered substrate 2,6-dichlorobenzaldehyde promoted the highest diastereoselectivity (>99:1 dr) and yield (96%) with a moderate enantioselectivity of 67% ee (Table 4, entry 8). Additionally, 2,4-dichlorobenzaldehyde promoted the highest enantioselectivity of 88% with high diastereoselectivity of 99:1 dr (Table 4, entry 7). While, the least sterically hindered compound 4-chlorobenzaldehyde did not promote good results (Table 4, entry 6). In addition, to further examine the generality of this catalytic system, a heteroaromatic aldehyde, 3-pyridylbenzaldehyde was accepted by PPL as a substrate giving a good yield of 89% with moderate diastereoselectivity and enantioselectivity (Table 4, entry 15). Heterocyclic ketones were also accepted by PPL as substrates, Affording good yields and moderate stereoselectivities (Table 4, entries 20–22). Furthermore, in order to demonstrate advantages of this approach we have scaled up this reaction to the industrial scale. The reaction was scaled up using 1.057 g of 4-nitrobenzaldehyde (7 mmol), obtaining a fine results with yields of up to 80% and almost no changed enantioselectivity (83% ee) and diastereoselectivity (90:10 dr) determined by HPLC using a chiral column. All of these results may illustrate that PPL in [BMIM][PF_6] with 8% water content exhibited outstanding activity and stereoselectivity.

Finally, we proposed a possible mechanism for the lipase-catalyzed asymmetric cross aldol reaction using the biphasic [BMIM][PF_6]/H_2O medium. PPL is a small globular protein composed of a single chain of 449 amino acids and contains a catalytic triad of Ser153, Asp177 and His264 in its active site [52]. On the basis of the previously proposed mechanism of promiscuous hydrolase-catalyzed aldol reaction [30,31,43], we hypothesized a possible mechanism for the PPL-catalyzed aldol reaction, as shown in Scheme 1. First, substrate cyclohexanone is stabilized by the



Scheme 1. Proposed mechanism for PPL-catalyzed aldol reaction.

Asp-His dyad and the oxyanion. Second, a proton is transferred from cyclohexanone to the His residue, forming an enolate ion. Third, another substrate aldehyde accepts the proton from the His264 imidazolyl and forms a new C—C bond with cyclohexanone. Finally, the aldol adduct is released from the oxyanion hole and separates from the active site. A further mechanism study about this reaction is currently in progress.

3. Conclusions

In summary, we develop a new efficient biocatalytic route for the PPL-catalyzed asymmetric aldol reaction in the biphasic [BMIM][PF_6]/H_2O medium for the first time. PPL exhibited high catalytic activity and excellent stereoselectivity in this efficient and recyclable room temperature ionic liquid in the presence of moderate water, similar to the results obtained in organic solvents. To optimize the PPL-catalyzed asymmetric aldol reaction, some important factors, including solvent, enzyme, water content, temperature and reaction duration were investigated. This catalytic system provides a wide reaction scope and is general. Compared to benzaldehydes with electron-donating groups or no groups, benzaldehydes with electron-withdrawing groups displayed better activity and stereoselectivity. Cyclohexanone could act as a more suitable donor accepted by the enzyme to react with different aromatic or heteroaromatic aldehydes. Heteroaromatic aldehyde with cyclohexanone and the heterocyclic ketones with aromatic aldehydes also promoted good reaction results. Excitingly, [BMIM][PF_6] exhibited excellent recyclability and recoverability, as the recovered IL was recycled for five runs with no loss in PPL's activity or stereoselectivity. As an effective and efficient synthetic method, the PPL-catalyzed asymmetric aldol reaction in [BMIM][PF_6]/H_2O provides a novel case of catalytic promiscuity and might be a useful strategy for organic synthesis and even industrial applications.

4. Experimental

4.1. Materials and analytical methods

All enzymes were purchased from Sigma–Aldrich Co. LLC (US), BSA was purchased from Aladdin Co., Ltd. (Shanghai, China). 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-hexyl-3-methylimidazolium hexafluorophosphate ([HMIM][PF_6]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), 1-hexyl-3-methylimidazolium tetrafluoroborate ([HMIM][BF_4]), 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF_6]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIM][BF_4]), 1-octyl-3-methylimidazolium tetrafluoroborate ([OMIM][BF_4]), 1-butyl-3-methylimidazolim L-tartrate ([BMIM][LTar]), 1-butyl-3-methylimidazolium L-lactate ([BMIM][LLac]), 1-hydroxyethyl-3-methylimidazolium tetrafluoroborate ([EOMIM][BF_4]) were purchased from ShangHai Cheng Jie Chemical Co. Ltd. (China). Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification.

The NMR spectra were recorded on a Bruker 400 MHz instrument using $CDCl_3$ as solvent. Chemical shifts (δ) were expressed in ppm with TMS as internal standard, and coupling constants (J) were reported in Hz. HPLC was carried out on SHIMADZU instrument (LC-2010A HT, UV/VIS Detector) using a chiral column (4.6 mm, 250 mm). The melting points were measured by Microcomputer melting point meter (WRS-2) which was produced by ShangHai Shenguang Instrument Co. Ltd. (China).

4.2. General procedure for aldol reaction

Aldehyde (0.3 mmol), PPL (30 mg), ketone (6.0 mmol), [BMIM][PF_6] (2.76 mL) and deionized water (0.24 mL) were added in a 25 mL round-bottom flask. The mixture was stirred at 1000 rpm at 37 °C. After completion of the reaction, the product was directly analyzed by HPLC in comparison with a standard sample. Then, the solution was extracted with diethyl ether (10 mL) for eight times. The organic phase was combined and evaporated in vacuum. The residue was purified by flash column chromatography using ethyl acetate–petroleum ether as mobile phase.

4.3. General procedure for recovering and recycling [BMIM][PF_6]

4-Nitrobenzaldehyde (0.3 mmol), PPL (30 mg), cyclohexanone (6.0 mmol), [BMIM][PF_6] (2.76 mL) and deionized water (0.24 mL) were combined in a 25 mL round-bottom flask. The mixture was stirred at 1000 rpm at 37 °C for 72 h. The solution was then extracted eight times with diethyl ether (10 mL) until the reaction substrates and products had been completely extracted, as monitored via TLC and visualized using UV light or phosphomolybdic acid ethanol solution. Afterwards, the IL layer was dissolved in dichloromethane, filtered and evaporated under vacuum. After 12 h in the vacuum drying oven, the recovered and recycled ionic liquid was used in the next reaction.

4.3.1. 2-(Hydroxy(phenyl)methyl)cyclohexanone (**a**)

White solid; mp: 100–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.4–7.24 (m, 5H), 5.41 (d, J = 2.2 Hz, 0.24H), 4.81 (d, J = 8.8 Hz, 0.74H), 4.00 (s, 1H), 2.65–2.61 (m, 1H), 2.52–2.49 (m, 1H), 2.42–2.34 (m, 1H), 2.15–2.05 (m, 1H), 1.84–1.76 (m, 1H), 1.72–1.51 (m, 3H), 1.36–1.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.55, 214.80, 140.94, 128.38, 127.90, 127.03, 74.76, 57.44, 42.68, 30.86, 27.82, 24.73. Enantiomeric excess was determined by HPLC with a CHIRALCEL OD-H column (90:10 hexane:2-propanol), 25 °C, 220 nm, 0.5 mL/min; major enantiomer tr = 16.4 min, minor enantiomer tr = 22.7 min.

4.3.2. 2-(Hydroxy(4-tolyl)methyl)cyclohexanone (**b**)

Yellow solid; mp: 75–77 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (d, J = 7.5 Hz, 2H), 7.15 (d, J = 7.5 Hz, 2H), 5.35 (s, 0.15H), 4.75 (d, J = 8.8 Hz, 0.95H), 3.91 (s, 1H), 2.64–2.57 (m, 1H), 2.50–2.46 (m, 1H), 2.42–2.30 (m, 4H), 2.10–2.06 (m, 1H), 1.80–1.49 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.62, 214.85, 138.49, 137.98, 137.55, 136.54, 129.05, 126.93, 74.54, 70.56, 57.45, 42.69, 30.89, 27.84, 24.74, 21.16. Enantiomeric excess was determined by HPLC with a CHIRALPAK AS-H column (95:5 hexane:2-propanol), 25 °C, 221 nm, 0.8 mL/min; major enantiomer tr = 23.4 min, minor enantiomer tr = 30.3 min.

4.3.3. 2-((4-Fluorophenyl)(hydroxy)methyl)cyclohexanone (**c**)

White solid; mp: 74–76 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.25 (m, 2H), 7.05 (t, J = 8.7 Hz, 2H), 5.37 (s, 0.18H), 4.79 (d, J = 8.8 Hz, 0.85H), 4.04 (s, 1H), 2.62–2.58 (m, 1H), 2.52–2.48 (m, 1H), 2.43–2.32 (m, 1H), 2.13–2.09 (m, 1H), 1.83–1.80 (m, 1H), 1.73–1.51 (m, 3H), 1.35–1.27 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.46, 214.76, 163.59, 161.15, 136.75, 128.63, 115.34, 115.13, 74.12, 57.46, 42.67, 30.76, 27.75, 24.71. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (95:5 hexane:2-propanol), 25 °C, 210 nm, 1.0 mL/min; major enantiomer tr = 22.2 min, minor enantiomer tr = 19.7 min.

4.3.4. 2-(Hydroxy(perfluorophenyl)methyl)cyclohexanone (**d**)

Yellow solid; mp: 81–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.33 (d, J = 9.6, 2.9 Hz, 1H), 3.96 (d, J = 3.3 Hz, 1H), 3.05–2.98 (m, 1H), 2.56–2.51 (m, 1H), 2.44–2.39 (m, 1H), 2.18–2.13 (m, 1H), 1.90–1.86

(m, 1H), 1.71–1.60 (m, 3H), 1.39–1.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.12, 211.81, 146.50, 143.98, 142.05, 139.58, 138.78, 136.28, 65.96, 54.20, 42.35, 30.16, 27.49, 24.49. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:2-propanol), 25 °C, 215 nm, 0.5 mL/min; major enantiomer tr = 14.0 min, minor enantiomer tr = 17.1 min.

*4.3.5. 2-(Hydroxy(4-(trifluoromethyl)phenyl)methyl)cyclohexanone (**e**)*

White solid; mp: 79–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 5.46 (s, 0.13H), 4.87 (d, *J* = 8.6 Hz, 0.87H), 4.08 (s, 1H), 2.65–2.58 (m, 1H), 2.52–2.49 (m, 1H), 2.41–2.34 (m, 1H), 2.14–2.09 (m, 1H), 1.84–1.81 (m, 1H), 1.73–1.67 (m, 1H), 1.61–1.55 (m, 2H), 1.40–1.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.12, 214.41, 144.96, 130.22, 129.89, 127.37, 126.08, 125.31, 74.26, 57.26, 42.67, 30.76, 27.71, 24.70. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (98:2 hexane:2-propanol), 25 °C, 220 nm, 1.0 mL/min; major enantiomer tr = 39.2 min, minor enantiomer tr = 27.7 min.

*4.3.6. 2-((4-Chlorophenyl)(hydroxy)methyl)cyclohexanone (**f**)*

Yellow solid; mp: 91–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$):δ 7.35–7.32 (t, *J* = 6.2 Hz, 2H), 7.28 (d, *J* = 8.4 Hz, 2H), 5.38 (s, 0.19H), 4.79 (d, *J* = 8.7 Hz, 0.86H), 4.01 (s, 1H), 2.64–2.55 (m, 1H), 2.53–2.48 (m, 1H), 2.42–2.33 (m, 1H), 2.19–2.04 (m, 1H), 1.91–1.77 (m, 1H), 1.70–1.56 (m, 3H), 1.35–1.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.23, 214.52, 139.51, 133.57, 128.52, 128.38, 74.11, 57.36, 42.65, 30.75, 27.72, 24.70. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:2-propanol), 25 °C, 221 nm, 0.5 mL/min; major enantiomer tr = 27.9 min, minor enantiomer tr = 24.4 min.

*4.3.7. 2-((2,4-Dichlorophenyl)(hydroxy)methyl)cyclohexanone (**g**)*

White solid; mp: 89–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 8.4 Hz, 1H), 7.37 (d, *J* = 2.1 Hz, 1H), 7.32–7.28 (m, 1H), 5.31 (dd, *J* = 8.0, 3.6 Hz, 1H), 4.08 (d, *J* = 3.9 Hz, 1H), 2.67–2.61 (m, 1H), 2.50–2.47 (m, 1H), 2.41–2.27 (m, 1H), 2.13–2.09 (m, 1H), 1.85 (d, *J* = 6.0 Hz, 1H), 1.71–1.55 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.10, 137.82, 133.79, 133.50, 129.26, 128.90, 127.63, 70.09, 57.51, 42.71, 30.37, 27.76, 24.89. Enantiomeric excess was determined by HPLC with a CHIRALPAK AS-H column (90:10 hexane:2-propanol), 25 °C, 220 nm, 1.0 mL/min; major enantiomer tr = 11.0 min, minor enantiomer tr = 9.4 min.

*4.3.8. 2-((2,6-Dichlorophenyl)(hydroxy)methyl)cyclohexanone (**h**)*

Yellow solid; mp: 128–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (d, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 7.9 Hz, 1H), 5.86 (dd, *J* = 9.5, 2.9 Hz, 1H), 3.68 (d, *J* = 3.1 Hz, 1H), 3.60–3.44 (m, 1H), 2.54 (d, *J* = 13.7 Hz, 1H), 2.49–2.35 (m, 1H), 2.12 (d, *J* = 9.5 Hz, 1H), 1.85 (d, *J* = 11.5 Hz, 1H), 1.79–1.61 (m, 2H), 1.44–1.35 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.36, 135.68, 134.73, 129.33, 70.55, 53.65, 42.43, 29.87, 27.63, 24.69. Enantiomeric excess was determined by HPLC with a CHIRALPAK AS-H column (98:2 hexane:2-propanol), 25 °C, 220 nm, 0.5 mL/min; major enantiomer tr = 53.0 min, minor enantiomer tr = 45.2 min.

*4.3.9. 2-((2-Bromophenyl)(hydroxy)methyl)cyclohexanone (**i**)*

Yellow solid; mp: 93–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56–7.52 (m, 2H), 7.39–7.35 (m, 1H), 7.17–7.06 (m, 1H), 5.67 (s, 0.04H), 5.32 (d, *J* = 7.9 Hz, 0.97H), 4.09 (s, 1H), 2.74–2.67 (m, 1H), 2.50–2.45 (m, 1H), 2.41–2.28 (m, 1H), 2.18–2.03 (m, 1H), 1.93–1.80 (m, 1H), 1.77–1.51 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 215.25, 214.71, 140.73, 132.50, 129.11, 128.47, 127.87, 123.37, 72.85, 57.67, 42.75, 30.56, 27.83, 24.96. Enantiomeric excess was determined by HPLC with a CHIR-ALPAK AD-H column (98:2 hexane:2-propanol), 25 °C, 220 nm, 1.0 mL/min; major enantiomer tr = 28.6 min, minor enantiomer tr = 32.8 min.

*4.3.10. 2-((4-Bromophenyl)(hydroxy)methyl)cyclohexanone (**j**)*

Yellow solid; mp: 114–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.3 Hz, 2H), 5.34 (s, 0.05H), 4.75 (dd, *J* = 8.7, 2.7 Hz, 0.99H), 3.99 (d, *J* = 2.7 Hz, 1H), 2.62–2.51 (m, 1H), 2.49–2.45 (m, 1H), 2.39–2.34 (m, 1H), 2.11–2.07 (m, 1H), 1.88–1.74 (m, 1H), 1.71–1.50 (m, 3H), 1.31–1.27 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 215.22, 214.52, 140.03, 131.47, 128.73, 121.71, 74.17, 57.32, 42.65, 30.75, 27.72, 24.70. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:2-propanol), 25 °C, 220 nm, 0.5 mL/min; major enantiomer tr = 30.3 min, minor enantiomer tr = 26.1 min.

*4.3.11. 4-(Hydroxy(2-oxocyclohexyl)methyl)benzonitrile (**k**)*

Yellow solid; mp: 69–70 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 6.9 Hz, 2H), 7.46–7.42 (m, 2H), 5.43 (s, 0.17H), 4.84 (d, *J* = 8.4 Hz, 0.84H), 4.07 (s, 1H), 2.61–2.54 (m, 1H), 2.51–2.47 (m, 1H), 2.40–2.34 (m, 1H), 2.13–2.10 (m, 1H), 1.82 (d, *J* = 12.9 Hz, 1H), 1.71–1.50 (m, 3H), 1.40–1.25 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.75, 214.03, 146.40, 132.09, 127.77, 118.77, 111.68, 74.19, 57.13, 42.62, 30.72, 27.73, 24.72. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (80:20 hexane:2-propanol), 25 °C, 220 nm, 0.5 mL/min; major enantiomer tr = 26.2 min, minor enantiomer tr = 21.7 min.

*4.3.12. 2-(Hydroxy(2-nitrophenyl)methyl)cyclohexanone (**l**)*

Yellow solid; mp: 97–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 5.96 (s, 0.08H), 5.45 (d, *J* = 7.0 Hz, 0.94H), 4.19 (s, 1H), 2.84–2.70 (m, 1H), 2.46 (d, *J* = 12.9 Hz, 1H), 2.40–2.28 (m, 1H), 2.11–2.05 (m, 1H), 1.86–1.58 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.89, 213.95, 148.75, 136.60, 133.04, 129.00, 128.39, 124.06, 69.76, 57.31, 42.82, 31.11, 27.75, 24.97. Enantiomeric excess was determined by HPLC with a CHIRALCEL OD-H column (95:5 hexane:2-propanol), 25 °C, 254 nm, 0.5 mL/min; major enantiomer tr = 30.6 min, minor enantiomer tr = 35.8 min.

*4.3.13. 2-(Hydroxy(3-nitrophenyl)methyl)cyclohexanone (**m**)*

Yellow solid; mp: 110–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31–8.06 (m, 2H), 7.68 (d, *J* = 7.5 Hz, 1H), 7.53 (t, *J* = 7.9 Hz, 1H), 5.48 (s, 0.08H), 4.90 (d, *J* = 8.4 Hz, 0.93H), 4.13 (s, 1H), 2.72–2.57 (m, 1H), 2.51 (d, *J* = 13.8 Hz, 1H), 2.45–2.31 (m, 1H), 2.13 (d, *J* = 10.9 Hz, 1H), 1.84 (d, *J* = 12.7 Hz, 1H), 1.73–1.34 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.81, 214.03, 148.28, 143.30, 133.20, 129.21, 122.84, 122.03, 73.99, 57.12, 42.62, 30.73, 27.62,, 24.69. Enantiomeric excess determined by HPLC with a CHIRALPAK AD-H column (95:5 hexane:2-propanol), 25 °C, 254 nm, 0.7 mL/min; major enantiomer tr = 44.0 min, minor enantiomer tr = 56.0 min.

*4.3.14. 2-(Hydroxy(4-nitrophenyl)methyl)cyclohexanone (**n**)*

Yellow solid; mp: 96–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 7.6 Hz, 2H), 7.53 (d, *J* = 7.7 Hz, 2H), 5.51 (s, 0.19H), 4.92 (d, *J* = 8.2 Hz, 0.88H), 4.09 (s, 1H), 2.75–2.57 (m, 1H), 2.52 (d, *J* = 13.7 Hz, 1H), 2.43–2.35 (m, 1H), 2.14 (d, *J* = 10.6 Hz, 1H), 1.85 (d, *J* = 12.4 Hz, 1H), 1.73–1.51 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.68, 213.96, 148.40, 127.87, 123.48, 73.97, 57.17, 42.62, 30.75, 27.72, 25.90, 24.72. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (93:7 hexane:2-propanol), 25 °C, 254 nm, 1.0 mL/min; major enantiomer tr = 39.8 min, minor enantiomer tr = 29.1 min.

*4.3.15. 2-(Hydroxy(pyridin-3-yl)methyl)cyclohexanone (**o**)*

Yellow liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51–8.45 (m, 2H), 7.69 (d, J = 6.3 Hz, 1H), 7.33–7.18 (m, 1H), 5.39 (s, 0.52H), 4.84 (d, J = 8.6 Hz, 0.49H), 3.88 (s, 1H), 2.69–2.52 (m, 1H), 2.49–2.42 (m, 1H), 2.40–2.28 (m, 1H), 2.15–2.01 (m, 1H), 1.88–1.24 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 214.76, 213.79, 149.12, 148.67, 148.18, 147.49, 137.49, 136.68, 134.64, 133.98, 123.51, 123.18, 72.37, 57.15, 56.89, 42.54, 30.77, 27.68, 24.71. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:2-propanol), 25 °C, 254 nm, 0.8 mL/min; major enantiomer tr = 29.8 min, minor enantiomer tr = 35.9 min.

*4.3.16. 2-(Hydroxyl(perfluorophenyl)methyl)cyclopentanone (**p**)*

White solid; mp: 74–75 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (d, J = 5.8 Hz, 0.30H), 5.15 (d, J = 10.1 Hz, 0.86H), 4.53 (s, 1H), 2.93–2.85 (m, 1H), 2.67–2.47 (m, 1H), 2.37–2.06 (m, 2H), 1.92–1.76 (m, 2H), 1.50–1.46 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 217.44, 146.62, 144.10, 142.27, 139.74, 138.84, 136.39, 66.25, 52.02, 38.25, 26.48, 20.31. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:2-propanol), 25 °C, 215 nm, 0.5 mL/min; major enantiomer tr = 17.0 min, minor enantiomer tr = 16.4 min.

*4.3.17. 2-(Hydroxy(3-nitrophenyl)methyl)cyclopentanone (**q**)*

Yellow solid; mp: 79–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, J = 1.6 Hz, 1H), 8.13 (dd, J = 17.5, 8.1 Hz, 1H), 7.69 (t, J = 8.1 Hz, 1H), 7.63–7.48 (m, 1H), 5.41 (s, 0.37H), 4.84 (m, 1.30H), 2.57–2.38 (m, 2H), 2.36–2.12 (m, 1H), 2.07–1.97 (m, 1H), 1.78–1.70 (m, 2H), 1.59–1.54 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.35, 145.06, 143.68, 132.67, 131.63, 129.40, 122.98, 122.26, 121.60, 120.61, 74.41, 70.27, 56.07, 55.08, 38.97, 38.60, 29.64, 26.86, 22.43, 20.34. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (95:5 hexane:2-propanol), 25 °C, 254 nm, 1.0 mL/min; major enantiomer tr = 32.7 min, minor enantiomer tr = 49.0 min.

*4.3.18. 2-(Hydroxy(4-nitrophenyl)methyl)cycloheptanone (**r**)*

Yellow solid; mp: 89–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H), 5.31 (s, 0.47H), 4.94 (d, J = 7.5 Hz, 0.55H), 2.99–2.86 (m, 1H), 2.72–2.43 (m, 2H), 1.89–1.63 (m, 4H), 1.52–1.22 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 217.55, 216.99, 149.48, 149.25, 147.47, 147.08, 127.76, 126.77, 123.56, 74.77, 72.41, 57.86, 57.12, 44.13, 43.89, 29.09, 28.63, 28.21, 23.91, 23.48. Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:2-propanol), 25 °C, 254 nm, 1.0 mL/min; anti-diastereomer: major enantiomer tr = 43.8 min, minor enantiomer tr = 19.1 min; syn-diastereomer: major enantiomer tr = 12.6 min, minor enantiomer tr = 15.5 min.

*4.3.19. 4-Hydroxy-4-(3-nitrophenyl)butan-2-one (**s**)*

Yellow solid; mp: 60–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 8.6 Hz, 2H), 5.32–5.25 (m, 1H), 3.59 (s, 1H), 2.94–2.81 (m, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 208.60, 148.39, 144.90, 131.84, 129.52, 122.57, 120.72, 68.79, 51.53, 30.72. Enantiomeric excess was determined by HPLC with a CHIRALCEL AS-H column (70:30 hexane:2-propanol), 25 °C, 254 nm, 1.0 mL/min; major enantiomer tr = 11.9 min, minor enantiomer tr =14.2 min.

*4.3.20. tert-Butyl 3-(hydroxyl(4-nitrophenyl)methyl)-4-oxopiperidine-1-carboxylate (**t**)*

Yellow solid; mp: 99–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (dd, J = 8.6, 3.5 Hz, 2H), 7.55 (dd, J = 11.8, 8.7 Hz, 2H), 5.49 (s, 0.53H), 4.99 (dd, J = 8.0, 3.2 Hz, 0.42H), 4.24 (d, J = 39.1 Hz, 1H), 3.99–3.57 (m, 2H), 3.30 (d, J = 15.7 Hz, 2H), 2.97–2.73 (m, 1H), 2.63–2.40 (m, 2H), 1.51–1.28 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 210.57, 209.16, 154.32, 147.74, 147.47, 127.67, 123.82, 80.98, 71.81, 56.20, 45.55, 43.70, 41.54, 28.44 (3C). Enantiomeric excess was determined by HPLC with a CHIRALCEL AD-H column (96:4 hexane:2-propanol), 25 °C, 254 nm, 1.0 mL/min; major enantiomer tr = 48.5 min, minor enantiomer tr = 55.3 min.

*4.3.21. 3-(Hydroxyl(4-nitrophenyl)methyl)dihydro-2H-pyran-4(3H)-one (**u**)*

Yellow solid; mp: 117–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 8.8 Hz, 2H), 5.57 (s, 0.64H), 5.01 (d, J = 11.1 Hz, 0.54H), 4.35–4.15 (m, 1H), 3.93–3.81 (m, 1H), 3.82–3.70 (m, 2H), 3.55–3.40 (m, 1H), 3.00–2.90 (m, 1H), 2.81–2.63 (m, 1H), 2.60–2.42 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 209.24, 208.28, 148.17, 147.35, 127.48, 123.79, 71.33, 69.78, 68.33, 57.64, 42.85. Enantiomeric excess was determined by HPLC with a CHIRALCEL AD-H column (90:10 hexane:2-propanol), 25 °C, 254 nm, 1.0 mL/min; major enantiomer tr = 51.0 min, minor enantiomer tr =42.2 min.

*4.3.22. 3-(Hydroxyl(4-nitrophenyl)methyl)dihydro-2H-thiopyran-4(3H)-one (**v**)*

Yellow solid; mp: 120–121 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 8.7 Hz, 2H), 5.54 (s, 0.32H), 5.08 (dd, J = 8.0, 4.0 Hz, 0.71H), 3.64 (d, J = 4.1 Hz, 1H), 3.09–2.97 (m, 3H), 2.90–2.76 (m, 2H), 2.70 (dd, J = 13.6, 10.9 Hz, 1H), 2.57–2.52 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 211.14, 210.83, 147.72, 147.29, 127.80, 123.79, 73.15, 59.46, 44.70, 32.81, 30.80. Enantiomeric excess was determined by HPLC with a CHIRALCEL AD-H column (90:10 hexane:2-propanol), 25 °C, 254 nm, 1.0 mL/min; major enantiomer tr = 62.1 min, minor enantiomer tr = 34.3 min.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2014.10.008.

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