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Pepsin-Catalyzed Asymmetric Cross Aldol Reaction Promoted by Ionic Liquids and Deep Eutectic Solvents

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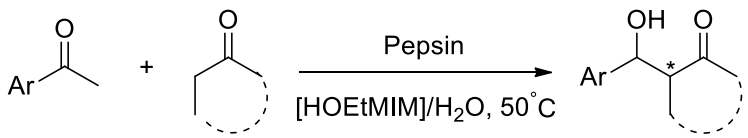
Abstract
Pepsin was found to catalyze asymmetric cross aldol reactions of aromatic and polycyclicaromatic aldehydes with cyclic ketones in ionic liquids and deep eutectic solvents for the first time. Pepsin exhibited high catalytic activity and excellent stereoselectivity in ionic liquids and deep eutectic solvents in the presence of moderate water. High yields of up to 93.1%, excellent enantioselectivities of up to 96% ee, and good diastereoselectivities of up to 5:95 dr were achieved.

Graphic Abstract



Keywords Biocatalytic promiscuity · Pepsin · Aldol reaction · Ionic liquid · Deep eutectic solvent

1 Introduction

Biocatalysis has high selectivity, mild reaction conditions and potential utilization of cheap renewable resources. It is an efficient and green tool for modern organic synthesis [1]. However, biocatalysis has some disadvantages. For example, the effectiveness of the enzyme is limited, and the substrate specificity of the enzyme is limited [2]. Therefore, it is important to find new unnatural activities((i.e.promiscuity) of existing enzymes to broaden the applicability of existing enzymes [3]. Recently, the study of biocatalytic promiscuity has attracted significant attention from chemists and biochemists [4, 5]. These enzymes display significant levels of activity in a variety of nonconventional reactions such as Michael addition [6–8], Mannich reaction [9, 10], aldol reaction [11, 12], Henry reaction [13, 14] and epoxidation reaction [15]. Among these reactions, asymmetric aldol condensation with strong atom economy is often reported. Berglund and co-workers firstly reported that wild CAL-B (*lipase B from Candida antarctica*) and Ser105Ala mutant CAL-B have the catalytic activity for aldol reactions in 2003 [16]. Guan and co-workers used different hydrolyases to catalyze several asymmetric aldol reactions [17]. Lin and co-workers also reported an application of acylase in aldol reaction [18]. Wang and co-workers demonstrated the lipase-catalyzed asymmetric aldol reaction in organic solvents greatly promoted by water [19]. These lipase catalyzed aldol reactions were carried out in organic solvents, and it is found that the enzyme can maintain its activity in organic solvents [20, 21]. However, the harm of organic solvents to the environment is inevitable. In addition, there are more or less problems such as long reaction time, high reaction temperature and low enzyme activity. Hence, the search for environmentally friendly media for biocatalytic asymmetric aldol reactions and other chemical transformations has become a considerable challenge. In 2011 and 2013, Guan group [22] and Wang group [23] respectively found that the

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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 or eco-friendly. In 2013, an enzymatic aldol reaction was then attempted in phosphate-citrate buffer by Wang and co-workers, only achieving an optimal enantioselectivity of 66% [24]. Therefore, it is urgent to find better reaction media for enzyme catalysis. In the search for sustainable conditions for the development of chemical reactions, ionic liquids (ILs) and deep eutectic solvents (DESs) have appeared in recent years as promising biodegradable and environmentally friendly solvents.

Ionic liquids (ILs) are the first non-traditional media compatible with enzymes, developed from the concept of green and sustainable (considering their low vapor pressure). Many reactions, such as hydrolysis and redox reactions, and the formation of C–C bonds, have been successfully carried out in ILs-containing media [25]. Wang et al. reported *lipase from porcine pancreas* (PPL) was used to catalyze asymmetric cross aldol reactions in ionic liquid ([BMIM][PF_6]) with deionized water for the first time in 2014 [26]. PPL exhibited high catalytic activity and good stereoselectivity in this efficient and recyclable room temperature ionic liquid in the presence of moderate water. Although the reaction yield is high, the reaction time is long.

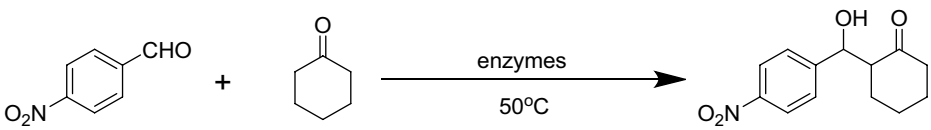
Deep eutectic solvents (DESs) are similar to ILs, with low melting point, low volatility and high thermal stability. In addition, DESs are biodegradable, non-toxic, cheap and easy to prepare. Therefore, DESs are also called "green solvent", just like ionic liquid. DESs first came to the public vision in 2001 [27]. Since then, the research on deep eutectic solvent has been developing rapidly in extraction, material synthesis, biotransformation, biocatalysis and other fields [28–31]. Gotor-Fernández and co-workers reported the lipase-catalysed aldol reaction has been performed for the first time in DESs in 2016 [32]. The aldol reaction between 4-nitrobenzaldehyde and acetone was examined in depth, with excellent compatibility being found between PPL and DESs(choline chloride/glycerol mixtures) for the formation of the aldol product in high yields.The system was compatible with a series of aromatic aldehydes and ketones including acetone, cyclopentanone and cyclohexanone. Thus, the application of DESs in other enzyme-catalyzed reactions have attracted increasing research attention.

In this study, we hope to find a more green and effective biocatalytic pathway for asymmetric aldol reaction. We selected Pepsin as a catalyst and utilized "green" solvent (water, ILs and DESs) as the reaction medium. High yields, good enantioselectivities and diastereoselectivities were also obtained in ILs and DESs in the presence of moderate water when a wide variety of substrates were simultaneously investigated.

2 Results and Discussion

It is well known that the catalytic activity of enzyme depends mainly on the type and origin of the enzyme. In this study, the aldol reaction of cyclohexanone and 4-nitrobenzaldehyde as a model reaction. Eight kinds of enzymes catalyzing aldol reaction were screened out (Table 1). It could be found that all the selected enzymes can catalyze the aldol reaction, but they all displayed poor selectivity. Among the tested enzymes, BPL showed the highest catalytic activity with a good yield of 69.8% with 8% ee(Table 1, entry

Table 1 The catalytic activities of different enzymes



Entry	Enzyme	Yield %[a]	dr(anti/syn)[a]	ee%(syn)[a]
1	Blank	< 1	–	-
2	*Bovine pancreatic lipase*(BPL)	69.8	51/49	8
3	*Lipase from Mucormiehei*(MML)	63.8	74/26	3
4	*Pepsin*	60.0	41/59	25
5	*Trypsin from porcine pancreas*	41.9	36/64	20
6	*Trypsin from bovin pancreas*	40.7	52/48	27
7	*Lipase acrylic resin from Candida antarctia*	42.6	63/37	2
8	*Subtilisin*	43.5	54/46	15
9	*Lipase from Candida rugosa*(CRL)	34.0	54/46	5

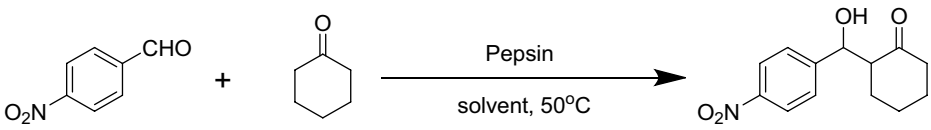
Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (2.0 mmol), and Pepsin (5 mg)at 50 °C for 24 h

[a]Yield, ee, and dr were determined by HPLC using AD-H chiral column

2), indicating that BPL had the ability to catalyze the aldol reaction, but it displayed poor selectivity under solvent-free conditions. When *Trypsin from bovin pancreas* was used as catalyst (Table 1, entry 6), the selectivity of the reaction was the highest (27%), but the yield was relatively low (40.7%). In contrast, when Pepsin was used as catalyst, the yield and selectivity of the reaction were good, which providing the aldol product in a good yield of 60.0% with 25% ee (Table 1, entry 4). To verify the specific catalytic effect of enzyme 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 24 h (Table 1, entry 1).Thus, Pepsin was chosen as the catalyst in the following experiments.

Reaction medium significantly influences the activity, stability and probably tertiary structure of the enzymes [33]. We tried to find a green medium combined with biocatalysis to achieve sustainable and environmental development standards. Thus, the influence of "green" solvents on the Pepsin-catalyzed aldol reaction was investigated in this study. The "green" solvents are water, ILs and DESs. The aldol reaction between 4-nitrobenzaldehyde and cyclohexanone was used as a model reaction. The initial experiments were performed in six pure ILs(Table 2, entries

Table 2 The effect of green solvent on the Pepsin-catalyzed asymmetric cross-aldol reaction



Entry	Solvent	Yield %[a]	dr(anti/syn)[a]	ee%(syn)[a]
1	[HOEtMIM]BF_4	36.7	48/52	84
2	[HOEtMIM]PF_6	35.5	45/55	42
3	[HOEtMIM]NO_3	14.6	62/38	74
4	[BMIM]BF_4	19.5	45/55	39
5	[BMIM]PF_6	12.0	52/48	40
6	[BMIM]NO_3	43.0	43/57	30
7	ChCl/Gly(1:2)	62.1	47/53	52
8	ChCl/EG(1:2)	32.7	53/47	35
9	deionized water	63.3	34/66	52
11	ChCl/Gly(1:2):deionized water = 5:95	60.0	41/59	41
12	ChCl/Gly(1:2):deionized water = 10:90	73.0	36/64	47
13	ChCl/Gly(1:2):deionized water = 15:85	76.2	30/70	54
14	ChCl/Gly(1:2):deionized water = 20:80	79.5	47/53	2
15	ChCl/Gly(1:2):deionized water = 25:75	82.3	36/64	52
16	ChCl/Gly(1:2):deionized water = 30:70	79.7	28/72	60
17	ChCl/Gly(1:2):deionized water = 40:60	73.4	36/64	57
18	ChCl/Gly(1:2):deionized water = 50:50	46.8	41/59	27
19	ChCl/Gly(1:2):deionized water = 80:20	17.4	38/62	41
20	[HOEtMIM]BF_4:deionized water = 5:95	64.4	36/64	60
21	[HOEtMIM]BF_4:deionized water = 10:90	79.9	33/67	52
22	[HOEtMIM]BF_4:deionized water = 15:85	82.1	29/71	70
23	[HOEtMIM]BF_4:deionized water = 20:80	87.2	35/65	54
24	[HOEtMIM]BF_4:deionized water = 25:75	88.7	33/67	72
25	[HOEtMIM]BF_4:deionized water = 30:70	91.8	39/61	63
26	[HOEtMIM]BF_4:deionized water = 40:60	64.8	40/60	35
27	[HOEtMIM]BF_4:deionized water = 50:50	57.0	46/54	21
28	[HOEtMIM]BF_4:deionized water = 80:20	57.3	41/59	42

Conditions: 4-nitrobenzaldehyde (0.1 mmol), cyclohexanone (2.0 mmol), and Pepsin (5 mg) in green solvent (0.3 mL), DES with deionized water (0.3 mL, DES/water, v/v)or in ionic liquid with deionized water (0.3 mL, ionic liquid/water, v/v)at 50 °C for 24 h

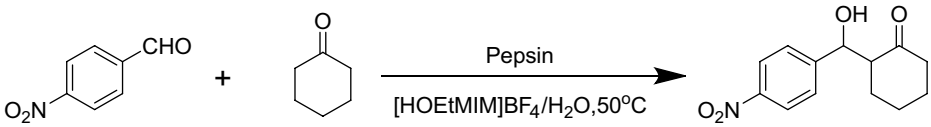
[a]Yield, ee, and dr were determined by HPLC using AD-H chiral column

1–6), two pure DESs (Table 2, entries 7–8) and deionized water(Table 2, entries 9). Unfortunately, Pepsin showed moderate to poor catalytic activity in all of the tested ILs. For example, Pepsin exhibited the highest selectivity (84% ee) in [HOEtMIM]BF_4, but the yield is very low, only 36.7%(Table 2, entries 1). Thus, when DESs were used as medium in the model reaction, the yields were moderate, but higher than in ionic liquids. The most likely reason for such low and moderate yields was that the high viscosity of the IL and DES systems might be limiting the mass transfer of substrates and products to and from the active site of the enzyme [34]. In principle, high viscosity could be overcome through the addition of small amounts of other solvents to the IL and DES medium. According to our previous research, water plays an important role in lipase-catalyzed aldol reaction, and the addition of water can reduce the viscosity of IL and DES. Thus, we added 20%, 50%, 60%, 70%, 75%, 80%, 85%, 90% and 95% water to the ILs and DESs respectively to investigate the optimal ILs and DES for this reaction. To our surprise, when 70% water was added to [HOEtMIM]BF_4, the yield of the reaction was increased from 36.7% to 91.8%, but the selectivity was decreased from 84 to 63%. Meanwhile, when 75% water was added to ChCl/Gly (1:2), the yield of the reaction was increased from 62.1 to 82.3%, and the selectivity(52%) was not affected. In order to prove the promotion of Pepsin-catalyzed aldol reaction by ILs and DESs, some control experiments were performed under the same conditions. In the presence of water without ILs and DESs, the yield is 63.3% with 52% ee after 24 h (Table 2, entries 9). As shown in Table 2, because in [HOEtMIM]BF_4/water (30/70), the increase of yield is relatively higher than that in ChCl/Gly(1:2)/water, we chose [HOEtMIM]BF_4/H_2O(3/7) as the reaction medium for further reaction investigations.

Then, other influencing factors were screened to obtain higher yields and stereoselectivities. The effect of the molar ratio of the substrates on the Pepsin-catalyzed aldol reaction was investigated. As shown in Table 3, when the molar ratio of 4-nitrobenzaldehyde to cyclohexanone was varied from 1:5 to 1:30, the yield was enormously improved from 60 to 90%, and the diastereoselectivity and enantioselectivity were increased. The molar ratio of 4-nitrobenzaldehyde to cyclohexanone was 1: 25, the yield (93.1%) and the enantioselectivity (63% ee) were highest. However, the yield and selectivity of the reaction decreased to some extent after increasing the mole ratio from 1:25 to 1:30. The probable reason for these observations is that the reaction reached equilibrium when the molar ratio of 4-nitrobenzaldehyde to cyclohexanone was 1:25. When the mole ratio increased from 1:25 to 1:30 (i.e. the amount of cyclohexanone increased), the by-products increased, so the yield decreased. Thus, 1:25 was selected as the optimal molar ratio for the aldol reaction.

We investigated the influence of reaction temperature on the model reaction. The model reaction was performed at four different temperatures that ranged from 30 °C to 60 °C for 24 h. As shown in Table 4, the reaction at 50 °C for 24 h offered the best result with yields up to 93.1% and the enantioselectivity (63% ee) and diastereoselectivity (46:54 dr). At the higher temperature (60 °C), both the enzyme activity and the stereoselectivity decreased. However, at the lower temperature (30 °C), the enantioselectivity (80% ee) and diastereoselectivity (28:71 dr) were highest, but the enzyme activity was poor (55.4%). The optimal conformation of

Table 3 The effect of molar ratio on the Pepsin-catalyzed asymmetric cross aldol reaction



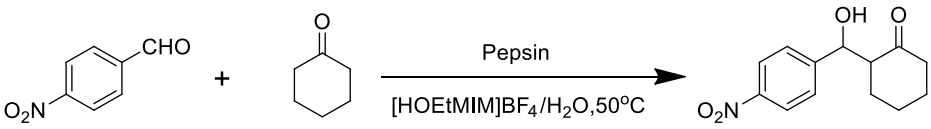
Entry	Molar ratio[a]	Yield %[b]	dr(anti/syn)[b]	ee%(syn)[b]
1	1:5	60.7	43/57	37
2	1:10	69.3	43/57	33
3	1:15	66.7	35/64	54
4	1:20	91.8	39/61	63
5	1:25	93.1	46/54	63
6	1:30	80.0	41/59	61

Conditions:4-nitrobenzaldehyde(0.1 mmol), cyclohexanone (0.5, 1, 1.5, 2.0, 2.5, 3.0 mmol), and Pepsin (5 mg) in mixed solvents(0.3 mL, [HOEtMIM]BF_4/water, 3/7) at 50 °C for 24 h

[a]Molar ratio = 4-nitrobenzaldehyde/cyclohexanone

[b]Yield, ee, and dr were determined by HPLC using AD-H chiral column

Table 4 The effect of temperature on the pepsin-catalyzed asymmetric cross aldol reaction



Entry	Temperature (°C)	Yield %[a]	dr(anti/syn)[a]	ee%(syn)[a]
1	30	55.4	28/71	80
2	40	71.7	26/74	68
3	50	93.1	46/54	63
4	60	87.6	51/49	56

Conditions:4-nitrobenzaldehyde(0.1 mmol), cyclohexanone (2.5 mmol), and Pepsin (5 mg) in mixed solvents(0.3 mL, [HOEtMIM]BF_4/water, 3/7) at 50 °C for 24 h

[a]Yield, ee, and dr were determined by HPLC using AD-H chiral column.

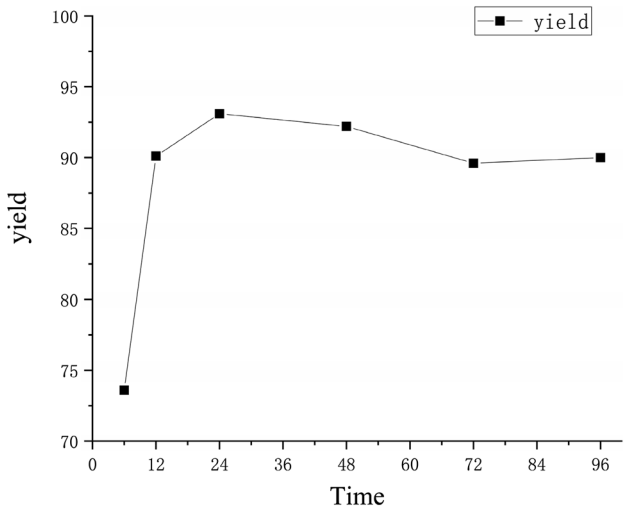


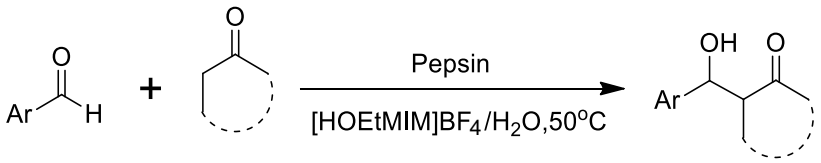
Fig. 1 The effect of reaction time on the Pepsin-catalyzed asymmetric cross aldol reaction[a,b]. ([a]Conditions:4-nitrobenzaldehyde(0.1 mmol), cyclohexanone (2.5 mmol), and Pepsin (5 mg) in in mixed solvents(0.3 mL, [HOEtMIM]BF_4/water, 3/7) at 50 °C for 24 h. [b]Yield was determined by HPLC using AD-H chiral column.)

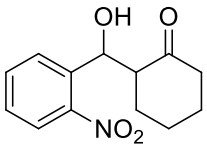
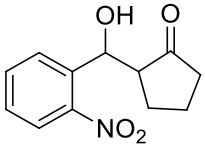
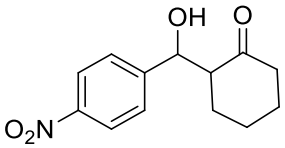
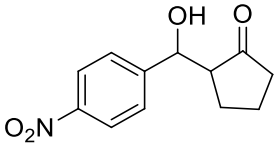
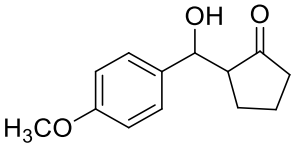
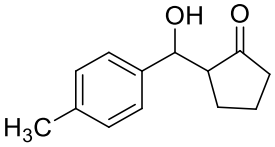
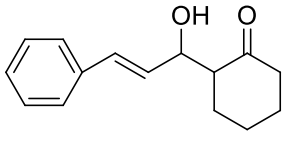
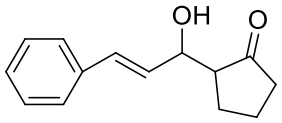
enzyme is most likely to occur at the optimum temperature, while the most unfavorable conformation is at the higher temperature. To obtain the best yield, diastereoselectivity and enantioselectivity, the reaction temperature of 50 °C was chosen for the Pepsin-catalyzed aldol reaction.

Then, the influence of reation time on the model reaction was examined. As shown Fig. 1., the yield was increasing with the extension of reaction time from 6 to 24 h. When reaction time was 24 h, the yield was highest (93.1%). Then, the reaction time continues to be extended to 48 h, 72 h and 96 h, the yield did not inreased. The probable reason for these observations is also that the reaction reached equilibrium when the reaction time was 24 h. Therefore, no obvious improvements were observed when the reaction time was prolonged. So, 24 h was selected as the optimal reaction time for the aldol reaction.

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 Pepsin-catalyzed aldol reaction. The results are summarized in Table 5. The highest yield of 93.1%, the best diastereoselectivity of 5:95 dr and the best enantioselectivity of 96% ee were achieved. Generally, benzaldehydes with strong electron-withdrawing substituents were better acceptors, producing better yields (Table 5, entries 1–4). The reactions of aromatic aldehydes with strong electron-withdrawing substituents provided products in the highest yield of 93.1%. However, for aromatic aldehydes with electron-donating substituents or no substituents, such as 4-methyoxybenzaldehyde and benzaldehyde, the yields were very low with only a trace of product obtained (Table 5, entries 5–6). Furthermore, we examined the effect of two ketones. Because the volume of cyclopentanone is smaller than that of cyclohexanone, cyclopentanone is easier to attack aromatic aldehydes. Then, the aldol reaction of cyclopentanone with aldehyde afforded the products in better yields than that of cyclohexanone, except for p-nitrobenzaldehyde. This may be because for p-nitrobenzaldehyde, the electronic effect plays a leading role. Moreover, the substituent positions 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. This may be because the steric hindrance of substituents on benzaldehyde had a great influence on the yield and stereoselectivity of the reaction. All of these results may illustrate that Pepsin in [HOEtMIM]BF_4/H_2O exhibited outstanding activity and stereoselectivity.

Table 5 Substrate scope of the pepsin-catalysed asymmetric cross aldol reaction



Entry	Product	Time(h)	Yield %[a]	dr(anti/syn)[a]	ee%(syn)[a]
1		48	21.5	52/48	62
2		48	79.5	54/46	66
3		24	93.1	46/54	63
4		24	57.5	31/69	60
5		96	Trace	-	-
6		96	Trace	-	-
9		48	49.2	5/95	65
10		48	58.8	49/51	96

Reaction conditions: aromatic aldehyde (0.1 mmol), cycloketone (2.5 mmol) and Pepsin (5 mg) in mixed solvents(0.3 mL, [HOEtMIM]BF_4/water, 3/7) at 50 °C

[a]Yield, ee, and dr were determined by HPLC using AD-H chiral column.

3 Conclusions

To sum up, in the present work, the Pepsin-catalyzed cross-aldol reaction between aromatic aldehydes and cyclic ketones was promoted by ILs and DESs. It was more meaningful to find that ILs/water and DESs/water system had decisive influence on the activity and stereoselectivity of Pepsin. Excitingly, ILs and DESs exhibited excellent recyclability and recoverability, as the recovered ILs and DESs were recycled for next reaction.The reactions could be performed smoothly between a wide range of aromatic aldehydes and cyclic ketones in [HOEtMIM]BF_4/water(3/7), and good to excellent yields as well as better selectivity were obtained. As a green and efficient synthetic method, it not only expands the application fields of enzymatic promiscuity, but might be a potential synthetic method for industrial application.

4 Experimental

4.1 Materials and Analytical Methods

All enzymes were purchased from Sigma-Aldrich Co. LLC (US), BPL was purchased from Aladdin Co., Ltd. (Shanghai, China). 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), 1-butyl-3-methylimidazolium nitrate ([BMIM][NO_3]), 1-hydroxyethyl-3-methylimidazolium tetrafluoroborate ([HOEtMIM][BF_4]), 1-hydroxyethyl-3-methylimidazolium hexafluorophosphate ([HOEtMIM][PF_6]), 1-hydroxyethyl-3-methyl-

imidazolium nitrate ([HOEtMIM][NO_3]) were purchased from ShangHai Cheng Jie Chemical Co. LTD. (China). Choline chloride (ChCl) was purchased from Aladdin Co., Ltd. (Shanghai, China). Glycerol(Gly) and ehylene glycol (EG) were purchased from Tianjin Damao Chemical Reagent Factory. 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).

4.2 General Procedure for DESs Preparation

In this study, DESs were prepared according to the procedures reported in the literature [32]. Choline chloride (50.0 mmol, 6.98 g) and glycerol (100.0 mmol, 7.30 mL) or ethylene glycol (100.0 mmol, 5.56 mL) were mixed and stirred at 80 °C until a clear solution was obtained. The prepared DESs were cooled and used for the enzyme-catalyzed the aldol reaction without any purification.

4.3 General Procedure for Aldol Reaction

Aldehyde (0.1 mmol), Pepsin (5 mg), cyclic ketone (2.5 mmol), [HOEtMIM]BF_4 (0.09 mL) and deionized water (0.21 mL) were added to an Erlenmeyer flask at 250 rpm at 50 °C. After completion of the reaction, the product was directly analyzed by HPLC in comparison with a standard sample. 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.

4.4 General Procedure for Recovering and Recycling IL and DES

IL and DES were recovered according to the procedures reported in the literature [32, 35]. The reaction on the reusability of IL and DES was conducted under the optimal reaction conditions. After the reaction had been completed, enzyme and the product were filtered, and IL or DES was washed with diethyl ether for many times in order to extract the residual substrates. After 12 h in the vacuum drying oven, the recycled IL or DES was reused in the next reaction under the same conditions.

4.5 2-(hydroxy(2-nitrophenyl)methyl) cyclohexanone

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95(d, J = 8.1 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.73 (t, J = 7.5 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 5.92 (s, 0.32H), 5.42(d, J = 7.1 Hz, 0.67H), 4.09(s, 0.87H), 2.84–2.70 (m, 1H), 2.40–2.28 (m, 2H), 2.07 (s, 1H), 1.86–1.54 (m, 5H).

Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:

2-propanol), 25 °C, 254 nm, 0.5 mL/min; major enantiomer tr = 14.7 min, minor enantiomer tr = 15.9 min.

4.6 2-(Hydroxy(2-nitrophenyl)methyl) cyclopentanone

^{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, 0.34H), 5.45 (d, J = 7.0 Hz, 0.65H), 4.19 (s, 0.89H), 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, 3H).

Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane: 2-propanol), 25 °C, 254 nm, 0.5 mL/min; major enantiomer tr = 13.4 min, minor enantiomer tr = 14.3 min.

4.7 2-(Hydroxy(4-nitrophenyl)methyl) cyclohexanone

^{1}H NMR (400 MHz, $CDCCl_3$): δ 8.21 (d, J = 8.1 Hz, 2H), 7.50 (d, J = 7.4 Hz, 2H), 5.49 (s, 0.45H), 4.90 (d, J = 8.4 Hz, 0.45H), 4.08(s, 0.55H), 3.17(s, 0.47H), 2.64–2.56 (m, J = 13.8 Hz, 1H), 2.54–2.45 (m, 1H), 2.44–2.38 (m, J = 19.3 Hz, 1H), 2.15 − 2.10 (d, J = 12.8 Hz, 1H), 1.83 (d, J = 12.7 Hz, 1H), 1.73 − 1.45 (m, 4H). 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; major enantiomer tr = 25.4 min, minor enantiomer tr = 18.9 min.

4.8 2-(Hydroxy(4-nitrophenyl)methyl) cyclopentanone

^{1}H NMR (400 MHz, CD_3COCD_3): δ 8.22 (d, J = 7.6 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H), 5.43 (s, 0.43H), 4.85 (d, J = 9.2 Hz, 0.50H), 4.77(s, 0.62H), 2.57(s, 0.44H), 2.53–2.09 (m, 3H), 2.00(s,1H), 1.90 -1.44 (m, 3H).

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 = 23.2 min, minor enantiomer tr = 18.6 min.

4.9 (E)-2-(1-hydroxy-3-phenylallyl)cyclohexanone

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60–7.30 (m, 5H), 6.73 (d, J = 16.0 Hz, 1H), 6.27 (d, J = 11.8 Hz, 1H), 4.33 (m, J = 8.6 Hz, 1H), 3.66 (m, J = 9.8 Hz, 1H), 3.53 (m, J = 7.0 Hz, 1H), 2.03 (d, J = 8.4 Hz, 2H), 1.95–1.49 (m, 6H).

Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (90:10 hexane:propanol), 25 °C, 254 nm, 1.0 mL/min; major enantiomer tr = 5.5 min, minor enantiomer tr = 7.7 min.

4.10 (E)-2-(1-hydroxy-3-phenylallyl) cyclopentanone

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–7.29 (m, 5H), 6.64 (d, J = 15.5 Hz, 1H), 6.31–6.17 (m, 1H), 4.36–4.32 (m, 1H), 3.69–3.65 (m, 1H), 3.54 (m, J = 7.0 Hz, 1H), 2.51–2.22 (m, 3H), 2.21–1.79 (m, 3H). 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; major enantiomer tr = 12.0 min, minor enantiomer tr = 9.9 min.

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Compliance with Ethical Standards

Conflict of interest All authors of this paper are aware of the submission and agree to its publication and declare no conflicts of interest.

Research Involving Human and Animal Participants This study does not cover human participants and/or animal studies.

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