Catalysis Science & Technology

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Bio-catalytic asymmetric Mannich reaction of ketimines using wheat germ lipase

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Abstract:

A highly enantioselective Mannich reaction between 3-substituted-2*H*-1,4-benzoxazines and acetone catalyzed by lipase from wheat germ Type I (WGL) is described. Enantioselectivity of up to 95% ee was achieved in DMSO at 25 ^{o}C. This research provides a new and simple method for the synthesis of *β*-amino ketone derivatives and promotes the development of enzyme-catalyzed Mannich reactions.

Keywords: asymmetric Mannich reaction; wheat germ lipase; ketimine; enzyme catalysis

1. Introduction

Asymmetric Mannich reactions provide a powerful method for synthesizing *β*-amino ketone derivatives, which are useful chiral building blocks for many compounds with biological activity and pharmaceutical importance.[1] Asymmetric Mannich reactions have attracted much attention. However, efficient enantioselective access to the corresponding *β*-amino ketones via a Mannich reaction using ketimines has achieved limited success because of the relatively unreactive C=N (vs a C=O)[2] and the difficulty of controlling stereofacial differentiation due to the geometry (E or Z)

of the imine.[3] Even though some useful catalytic systems have been reported in the asymmetric addition to ketimines,[4] asymmetric additions to ketimines for synthesizing optically active *β*-amino ketone derivatives have been much less explored. To the best of our knowledge, a limited amount of previous reports have demonstrated proline-catalyzed direct Mannich addition of 3-substituted-2*H*-1,4-benzoxazines,[1d] proline-catalyzed addition of aryl trifluoromethyl ketimines,[5] proline-catalyzed addition of ketimine with aldehydes,[1a] diamine-Brønsted acid-catalyzed addition of 4-trifluoromethyldihydroquinazoline,[6] and N-(8-quinolinesulfonyl)prolinamide-catalyzed addition of 2,2,2-trifluoro-1-phenylethanimine.[7] Considering the importance of the optically active *β*-amino ketone derivatives and the applicability of the Mannich reaction, it is still desirable to explore environmentally friendly and sustainable catalysts for the asymmetric Mannich addition of ketimines to synthesize optically active *β*-amino ketone derivatives.

Enzymes as green and sustainable catalysts in organic synthesis have attracted widespread attention.[8] Because of the complex three-dimensional structure and active sites integrated therein, enzymes as catalytically active proteins possess unique functions.[9] This creates a highly specific region for specific substrates with high selectivity. However, in recent years enzyme catalytic promiscuity, in which a single active site of a given enzyme can catalyze different chemical transformations of natural or non-natural substrates,[10] has received extensive attention as more and more catalytic promiscuities of existing enzymes have been discovered. Some enzymes displayed catalytic promiscuities in catalyzing the formation of C–C and C–heteroatom bonds,[8e, 10c, 11] such as asymmetric aldol reaction,[12] asymmetric Michael addition,[13] the asymmetric synthesis of *α*-aminonitrile amides[14] as well as Mannich reaction.[10b, 15] However, promiscuity of

enzyme is still at the exploratory stage, and no general methods are available to profile enzyme catalytic promiscuity.[10b] Thus, it is important to explore enzyme promiscuous activities as much as possible.

To the best of our knowledge, the only following examples of enzyme-catalyzed Mannich reactions have been reported so far. In 2009, Yu et al. reported the first hydrolase-catalyzed Mannich reaction using lipase from *Mucor miehei* as a catalyst and an acetone/water mixture as a solvent.[15a] In 2010, the same group successfully used the lipase from *Candida rugose* in catalysis of three-component Mannich reaction[15b] In 2010, Zhang et al. reported that trypsin efficiently promoted the Mannich reaction using neat acetone as a solvent.[15c] In 2011, Gotor et al. demonstrated alcalase-catalyzed Mannich reaction between 4-nitrobenzaldehyde, acetone and aniline. Among those reactions, no enantioselectivity was obtained for the Mannich products. In 2012, our group reported the first asymmetric Mannich reaction using protease type XIV from *Streptomyces griseus* (SGP) in acetonitrile.[10b] In 2015, we found that acylase from *Aspergillus melleus* can catalyze asymmetric Mannich reaction in acetonitrile.[15e] To date, all the reported enzymatic Mannich reactions are limited in the same type of substrates (ketones, arylamines and aromatic aldehydes). Enzyme-catalyzed asymmetric Mannich addition of ketimines with acetone has not yet been reported. Thus, developing effective enzyme-catalyzed asymmetric Mannich reaction of ketimines as a more sustainable complement to chemical catalysis is still highly desirable.

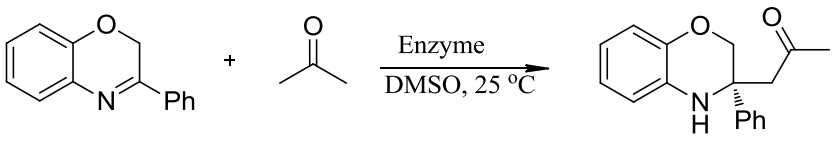
Wheat germ lipase (WGL), a lipase derived from plant, plays an important role in the growth and storage of agricultural products. In 1933, it has been preliminary purified and characterized.[16] Wheat is one of the world's most important food crops and the annual production of wheat germ is

large. Thus, the potential yield of WGL would be tremendous, and it has drawn great attention among the cereal lipase, which would be an inexpensive source of catalysts. However, as far as we know, using WGL in organic synthesis is still untapped except that our group found that WGL can catalyze asymmetric aldol reaction of tert-butyl 4-oxopiperidine-1-carboxylate with 4-nitrobenzaldehyde giving low enantioselectivity of 12% ee during our wide screening of enzymes for aldol reaction in 2012.[17] Therefore, it is significant to develop the application of WGL in organic synthesis. In this context, we wish report the WGL-catalyzed highly enantioselective Mannich reactions between 3-substituted-2*H*-1,4-benzoxazines with acetone for the synthesis of *β*-amino ketone derivatives.

2. Results and discussion

Initially, the Mannich reaction of 3-phenyl-*2H*-1,4-benzoxazine **1a**[1d, 18] and acetone was chosen as a model reaction. A series of commercially available enzymes were screened as catalysts and in those tests DMSO was used as the solvent (**Table 1**). The best yield of 22% and ee of 86% was achieved using WGL Type I as a catalyst (**Table 1**, entry 2). The model reaction with other enzymes gave lower yields and enantioselectivity (**Table 1**, entries 3-7). The enantiomeric excess was determined by HPLC analysis using a chiral column; absolute configuration was assigned by comparison with the known chiral HPLC analysis[1d]. In view of the above results, WGL Type I was identified to be the best catalyst for the further study.

Table 1. The screening of enzymes for the catalysis of the model Mannich reaction.[a]



1a **2** **3a**

Entry	Enzyme	Yield (%)[b]	ee (%)[c]
1	None	n.d.[d]	--
2	Lipase from wheat germ (WGL), Type I	22	86
3	Phosphatase acid from wheat germ, Type I	16	82
4	Nuclease p1 from *Penicillium citrinum*	6	79
5	Lipase from porcine pancreas, Type II	4	72
6	Proteinase from *Aspergillus melleus*, Type XXIII	6	70
7	Papain from *Carica pagaya*	trace	--

[a] All reactions were carried out with ketimine **1a** (0.2 mmol), acetone (4.0 mmol), enzyme (30 mg, lyophilized powder), and DMSO (1.0 mL) at 25 ^{o}C stirring for 96 h.

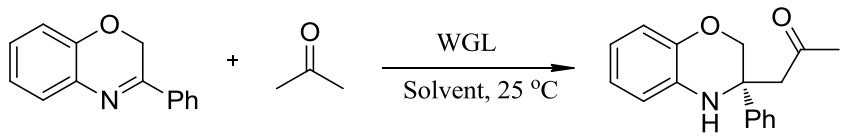
[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

[d] n.d.: no product was detected.

The nature of the reaction medium has been considered as an important parameter in enzyme-catalyzed reactions.[8c, 19] Thus, the reaction in different organic solvents was surveyed and the results were shown in **Table 2**. The data indicated that the catalytic activity and selectivity of WGL on the Mannich reaction were remarkably influenced by solvents. The reaction had the highest enantioselectivity (86% ee and 85% ee, respectively) in DMSO and DMF (**Table 2**, entries 1 and 2), but DMF had a low yield of 7%. The reaction in MeOH gave the product with a good yield of up to 88 % but with a low ee value of 39% (**Table 2**, entry 4). A 43% yield and ee value of 64% was achieved in EtOH (**Table 2**, entry 3). In the other tested solvents (CH_2Cl_2, MeCN, EtOAc, and acetone) only trace amounts of product were obtained (**Table 2**, entries 5-8). With the purpose to get high enantioselectivity in mind, and the combination of previous reports that WGL shows good stability in DMSO[20] and experimental data, DMSO was chosen as the optimum solvent for the WGL-catalyzed Mannich reaction.

Table 2. The effect of solvents on the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	Solvent	Yield (%)[b]	ee (%)[c]
1	DMSO	22	86
2	DMF	7	85
3	EtOH	43	64
4	MeOH	88	39
5	CH_2Cl_2	trace	--
6	MeCN	trace	--
7	EtOAc	trace	--
8	Acetone	trace	--

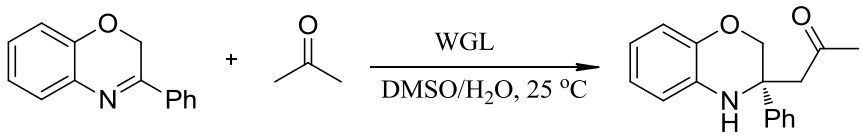
[a] The reaction conditions were as follows: ketimine **1a** (0.2 mmol), acetone (4.0 mmol), WGL (141 U, lyophilized powder), and solvent (1.0 mL) at 25 °C stirring for 96 h.

[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

In general, water has an effect on the enzyme-catalyzed reaction in organic solvents.[21] The effect of different amounts of water addition in DMSO on WGL-catalyzed model reaction was investigated (**Table 3**). Both the yield and enantioselectivity of the model Mannich reaction were reduced when water was added into the reaction system (**Table 3**, entries 2-7). When the water content exceeded 20% [$H_2O/(H_2O$ + DMSO), in vol.] the yield decreased significantly (**Table 3**, entries 5-7), probably due to the insolubility of the ketimine (**1a**) in water.[1d] The best ee value of 86% and 22% yield was obtained without adding water into the reaction system (**Table 3**, entry 1). Ultimately, DMSO (A.R.) as the optimized solvent without addition of water was selected for the further studies.

Table 3. Influence of water addition on the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	Water addition (%)	Yield (%)[b]	ee (%)[c]
1	0	22	86
2	5	14	86
3	10	11	82
4	15	11	81
5	20	3	77
6	25	3	76
7	30	1	65

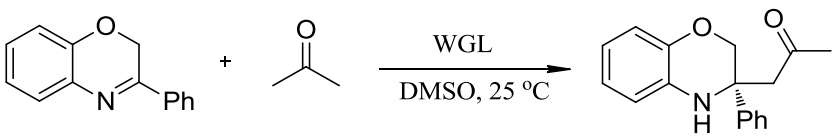
[a] The reaction conditions were as follows: ketimine **1a** (0.2 mmol), acetone (4.0 mmol), WGL (141 U, lyophilized powder), and DMSO (0.7-1.0 mL), deionized water (0-0.3 mL) at 25 °C stirring for 96 h.

[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

Among the above performed experiments, big amounts of substrate ketimine (**1a**) were left after 96 h of reaction, and the yield was very low. To further optimize the WGL-catalyzed Mannich reaction, the effect of amounts of acetone on the model reaction was investigated (**Table 4**). It could be seen that the enantioselectivity of the reaction was almost not affected by varying the amount of acetone. The best yield of 26% (**Table 4**, entry 4) was obtained when the acetone was increased to 8.0 mmol for the reaction with 0.2 mmol ketimine (**1a**). Further increasing the equivalents of acetone failed to improve the yield (**Table 4**, entries 5-12). Thus, 8.0 mmol of acetone was selected as the optimized amount for the reaction.

Table 4. The effect of amount of acetone on the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	Acetone (mmol)	Yield (%)[b]	ee (%)[c]
1	2.0	17	85

2	4.0	22	86
3	6.0	24	86
4	8.0	26	87
5	10.0	22	85
6	12.0	24	87
7	14.0	21	86
8	16.0	20	87
9	18.0	17	86
10	20.0	15	86
11	22.0	16	86
12	24.0	13	90

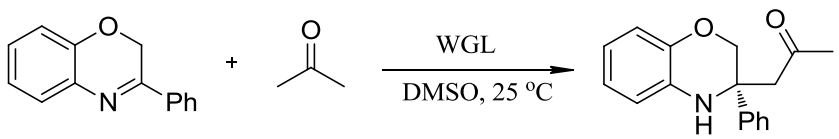
[a] The reaction conditions were as follows: ketimine **1a** (0.2 mmol), acetone (2.0-24.0 mmol), WGL (141 U, lyophilized powder), and DMSO (1.0 mL) at 25 °C stirring for 96 h.

[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

Subsequently, to find out a suitable enzyme loading for the reaction, WGL loading from 94 U to 705 U were screened for the Mannich reaction (**Table 5**). The results indicated that the enzyme loading had an obvious effect on the yield but had a miniscule effect on the enantioselectivity of the reaction. When 94 U of WGL was used, the reaction gave a low yield of only 19% (**Table 5**, entry 1). Increasing yield was observed while the enzyme loading increased from 94 U to 705 U, and the yield of the reaction was able to reach 46% (**Table 5**, entries 1-13). Taking into consideration of the yield and cost of the reaction, 423 U of WGL was chosen as the optimal amount of catalyst for the further experiments.

Table 5. Effect of enzyme loading on the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	Enzyme loading (U)	Yield (%)[b]	ee (%)[c]
1	94	19	87
2	118	24	86
3	141	26	86
4	165	28	86

5	188	31	87
6	235	34	86
7	282	35	87
8	329	36	87
9	376	39	87
10	423	40	87
11	470	41	87
12	564	44	87
13	705	46	87

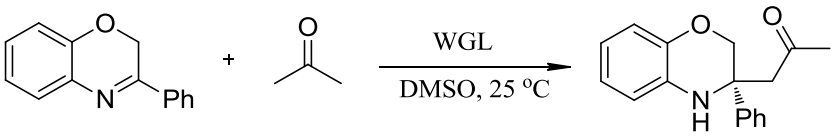
[a] The reaction conditions were as follows: ketimine **1a** (0.2 mmol), acetone (8.0 mmol), WGL (94-705 U, lyophilized powder), and DMSO (1.0 mL) at 25 ^{o}C stirring for 96 h.

[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

To further optimize the reaction conditions, the effect of solvent volume (DMSO, 0.50-3.00 mL) on the reaction was investigated (**Table 6**). The enantioselectivity of the product almost kept a constant value of approximately 86% ee during the whole phase of the reaction. When the volume of DMSO from 1.00 mL to 1.50 mL was used, slightly better yields were obtained (**Table 6**, entries 3-5). Based on these experiments, 1.00 mL of DMSO was chosen as the optimal solvent volume.

Table 6. Effect of DMSO volume on the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	DMSO (mL)	Yield (%)[b]	ee (%)[c]
1	0.50	38	86
2	0.75	39	85
3	1.00	40	87
4	1.25	39	86
5	1.50	40	87
6	2.00	38	85
7	2.50	36	86
8	3.00	35	85

[a] The reaction conditions were as follows: ketimine **1a** (0.2 mmol), acetone (8.0 mmol), WGL (423 U, lyophilized

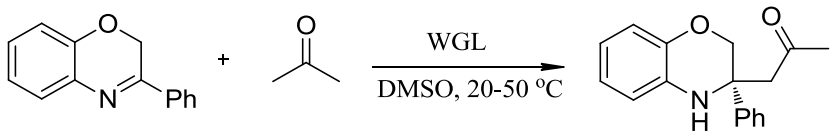
powder), and DMSO (0.50-3.00 mL) at 25 °C stirring for 96 h.

[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

Temperature is another important factor for the enzyme-catalyzed reactions because of its significant effects on enzyme stability and the rate of reactions.[22] The effect of temperature on the WGL-catalyzed Mannich reaction was investigated at different temperatures ranging from 20 °C to 50 °C (**Table 7**). The reaction exhibited the best enantioselectivity of 87% ee at 25 °C, which gave a yield of 40% (**Table 7**, entry 2). Although the yield of the reaction could be slightly increased by raising the temperature, the decreased of ee values were observed (**Table 7**, entries 3-5). Moreover, higher temperature (50 °C) brought an obvious decline in enantioselectivity and yield was also reduced (**Table 7**, entry 6). Thus, to obtain the best enantioselectivity, 25 °C was chosen as the optimal temperature.

Table 7. Effect of temperature on the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	Temperature (°C)	Yield (%)[b]	ee (%)[c]
1	20	26	86
2	25	40	87
3	30	43	82
4	35	46	80
5	40	47	78
6	50	43	55

[a] The reaction conditions were as follows: ketimine **1a** (0.2 mmol), acetone (8.0 mmol), WGL (423 U, lyophilized powder), and DMSO (1.0 mL) at 20-50 °C stirring for 96 h.

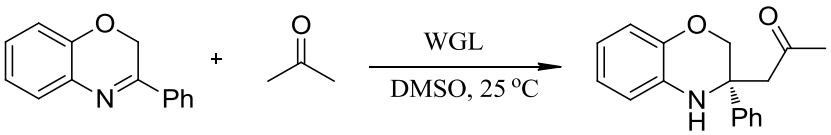
[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

Time course of the WGL-catalyzed Mannich reaction was investigated (**Table 8**). Extending

the reaction time led to an increase of the yield (**Table 8**, entries 1-9), and the best yield of 51% was obtained after 144 h (**Table 8**, entry 9). Further prolonging the reaction time did not lead to any increase of the yield (**Table 8**, entry 10). Meanwhile, the ee value remained nearly constant during the reaction.

Table 8. Time course of the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	Time (h)	Yield (%)[b]	ee (%)[c]
1	12	9	87
2	24	15	87
3	36	19	86
4	48	28	86
5	60	30	87
6	72	33	87
7	96	40	87
8	120	49	86
9	144	51	86
10	168	48	86

[a] The reaction conditions were as follows: ketimine **1a** (0.2 mmol), acetone (8.0 mmol), WGL (423 U, lyophilized powder), and DMSO (1.0 mL) at 25 °C stirring for 12-168 h.

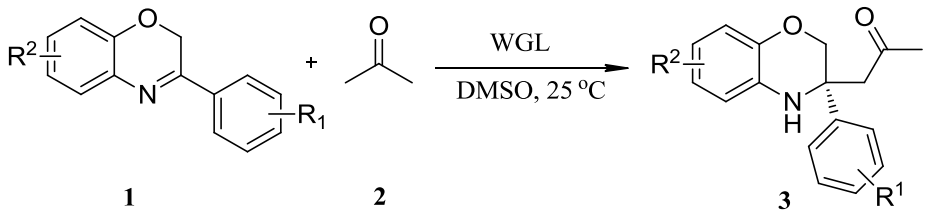
[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

With the optimized conditions in hand, several substrates were tested to expand upon this novel WGL-catalyzed asymmetric Mannich reaction (**Table 9**). The reactions with ketimines containing a neutral or electron-donating group as R^1 or R^2 (**Table 9**, entries 1-3, 11 and 12) gave better yields than those containing an electron-withdrawing group (**Table 9**, entries 5-7 and 13-16). The best yield of 54% was obtained with the ketimine (R^1 = 4-CH_3, R^2 = H) (**Table 9**, entry 2). Notably, the highest enantioselectivity of 95% ee was observed for the reaction with

4-phenyl-substituted ketimine (**Table 9**, entry 3). The reaction with ketimine containing a naphthyl or 3,4-dichlorostyryl group was also successfully carried out to afford the corresponding products (**Table 9**, entries 4 and 10). In general, the desired products were obtained with good enantioselectivities but in low yields. In all cases, high amounts of starting materials remained unreactive, and small amounts of unidentified by-products were observed in some reactions. When other aliphatic and aromatic ketones such as 2-butanone, cyclohexanone, cyclopentanone, 3-pentanone, 1,3-cyclohexanedione, acetophenone, 4'-chloroacetophenone, and 4'-methylacetophenone were employed instead of acetone in this Mannich reaction with **1a**, no desired products were received; starting materials remained unreactive.

Table 9. Scope of the WGL-catalyzed asymmetric Mannich reaction.[a]



1 **2** **3**

Entry	R^1	R^2	Product	Time (h)	Yield (%)[b]	ee (%)[c]
1	H	H	**3a**	120	49	87
2	4-CH_3	H	**3b**	96	54	83
3	4-Ph	H	**3c**	72	31	95
4	2-naphthy	H	**3d**	108	20	85
5	4-F	H	**3e**	120	21	87
6	4-Cl	H	**3f**	120	17	86
7	4-NO_2	H	**3g**	120	21	89
8	3-Cl	H	**3h**	120	18	85
9	3-Br	H	**3i**	120	20	82
10	3,4-2Cl	H	**3j**	108	21	86
11	H	4-CH_3	**3k**	96	37	87
12	H	3-CH_3	**3l**	96	27	83
13	H	4-Cl	**3m**	96	17	89
14	H	3-Cl	**3n**	96	15	86
15	H	4-F	**3o**	96	19	84
16	H	4-NO_2	**3p**	96	16	73

[a] The reaction conditions were as follows: a mixture of ketimine **1** (0.2 mmol), acetone (8.0 mmol) and WGL (423

U, lyophilized powder) in DMSO (1.0 mL) at 25 °C.

[b] Yield of the isolated product after chromatography on silica gel.

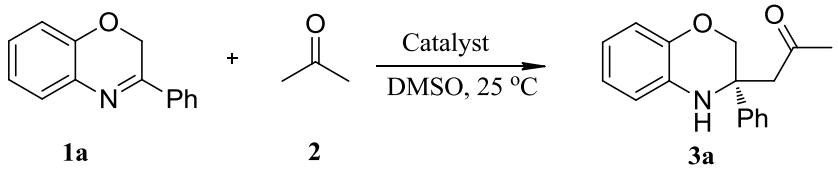
[c] Determined by HPLC analysis using a chiral column [1d] (for details, please see the Supporting Information).[1d]

Finally, to confirm the specific catalytic effect of WGL on the Mannich reaction and to further understand this reaction, some control experiments were performed (**Table 10**). In the absence of WGL, no reaction was detected (**Table 10**, entry 1). The model reaction with WGL gave the product with a yield of 49% and 87% ee under the optimized reaction conditions (**Table 10**, entry 2), which indicated WGL preparation had a catalytic effect on the Mannich reaction. In order to further prove that the three-dimensional protein structure of WGL was important for the model reaction, several types of inhibitors and denaturants were tested. Guanidine hydrochloride (GuHCl), a compound that can break the three-dimensional structure of protein [23] was used to pretreat WGL. The reaction with GuHCl-pretreated WGL only gave a low yield of 14% with 53% ee (**Table 10**, entry 3). GuHCl alone was also proved to not catalyze the reaction (**Table 10**, entry 4). According to the work reported by E. A. Motina et al.,[24] the catalytic activity of WGL decreased after incubated with phenylmethylsulfonyl fluoride (PMSF), diethyl cyanophosphonate (DEPC), dicyclohexylcarbodiimide (DCC), or ethylenediaminetetraacetic acid (EDTA). PMSF, a specific inhibitor of serine of enzymes,[25] was used to pretreat WGL. Both the yield and enantioselectivity of the model reaction with PMSF-pretreated WGL reduced (**Table 10**, entry 5). DEPC, a known modifier of the imidazole groups of histidine, [26] was used to pretreat WGL and the catalytic activity and enantioselectivity of WGL were decreased (**Table 10**, entry 7). An almost complete inhibition of the catalytic activity of WGL in the Mannich reaction was observed by using DCC, a well-known effective modifier of carboxylic groups of aspartic or glutamic acids,[27] which only gave a low yield of 8% (**Table 10**, entry 9). EDTA, a chelating agent for the

majority of divalent metal ions,[28] caused a yield decrease in the WGL-catalyzed Mannich reaction to only 13% yield (**Table 10**, entry 11). The above mentioned inhibitors alone were verified no effect on the model reaction (**Table 10**, entries 6, 8, 10 and 12). These results further confirmed that WGL indeed catalyzed the Mannich reaction, and once the enzyme denatured, its catalytic ability in the Mannich reaction decreased. The above data suggests that divalent metal ions existing in enzyme play a key role in the catalytic event. The imidazole groups of histidine, the hydroxyl groups of serine, and carboxylic groups of aspartic or glutamic acids may be involved in the catalytic activity.

To explore the relationship between natural and promiscuous activities of WGL, an enzymatic assay of WGL on the hydrolysis of triacetin as natural activity was performed. Native enzyme showed an activity of 6.3 units per mg protein (**Table10**, entry 2). Since the inhibitor-pretreated WGL partially lost its catalytic ability for the Mannich reaction, as a comparison we checked its natural activity on hydrolyzing triacetin. The results indicated that the inhibitor-pretreated WGL also lost its natural activity in certain degrees (**Table10**, entries 3, 5, 7, 9 and 11). These data demonstrated that both the natural and promiscuous activities of the enzyme were inhibited by the above mentioned inhibitors. Based on the above experiments, it can be inferred that the natural active center of WGL was responsible for its activity in the Mannich reaction.

Table 10. Control experiments for the WGL-catalyzed asymmetric Mannich reaction.[a]



1a **2** **3a**

Entry	Catalyst	Yield (%)[b]	ee (%)[c]	Natural activity (U mg^{-1} protein)
1	None	n.d.[d]	--	--
2	WGL	49	87	6.3

3	WGL pretreated with GuHCl[e]	14	53	2.1
4	GuHCl[f]	n.d.	--	--
5	WGL pretreated with PMSF[g]	21	55	3.6
6	PMSF[h]	n.d.	--	--
7	WGL pretreated with DEPC[i]	19	74	3.9
8	DEPC[j]	n.d.	--	--
9	WGL pretreated with DCC[k]	8	75	1.7
10	DCC[l]	n.d.	--	--
11	WGL pretreated with EDTA[m]	13	87	2.9
12	EDTA[n]	n.d.	--	--

[a] Unless otherwise specified, all reactions were carried out with ketimine **1a** (0.2 mmol), acetone (8.0 mmol), WGL (423 U, lyophilized powder), and DMSO (1.0 mL) at 25 ^{o}C stirring for 120 h.

[b] Yield of the isolated product after chromatography on silica gel.

[c] Determined by HPLC analysis using a chiral column (AD-H).[1d]

[d] n.d.: no product was detected.

[e] The mixture of WGL (423 U), deionized water (1 mL) and GuHCl (6.0 mmol) was stirred at 30 ^{o}C for 8 h and then water was removed by lyophilization before use.

[f] GuHCl (6.0 mmol) was used instead of WGL (423 U).

[g] The mixture of WGL (423 U), THF (1 mL) and PMSF (0.6 mmol) was stirred at 30 ^{o}C for 2 h and then THF was removed under reduced pressure before use.

[h] PMSF (0.6 mmol) was used instead of WGL (423 U).

[i] The mixture of WGL (423 U), phosphate buffer solution (NaH_2PO_4-Na_2HPO_4, pH 8.04) (1 mL) and DEPC (0.3 mmol) was stirred at 37 ^{o}C for 2 h and then water was removed by lyophilization before use.

[j] DEPC (0.3 mmol) was used instead of WGL (423 U).

[k] The mixture of WGL (423 U), deionized water (1 mL) and DCC (1.0 mmol) was stirred at 30 ^{o}C for 2 h and then water was removed by lyophilization before use.

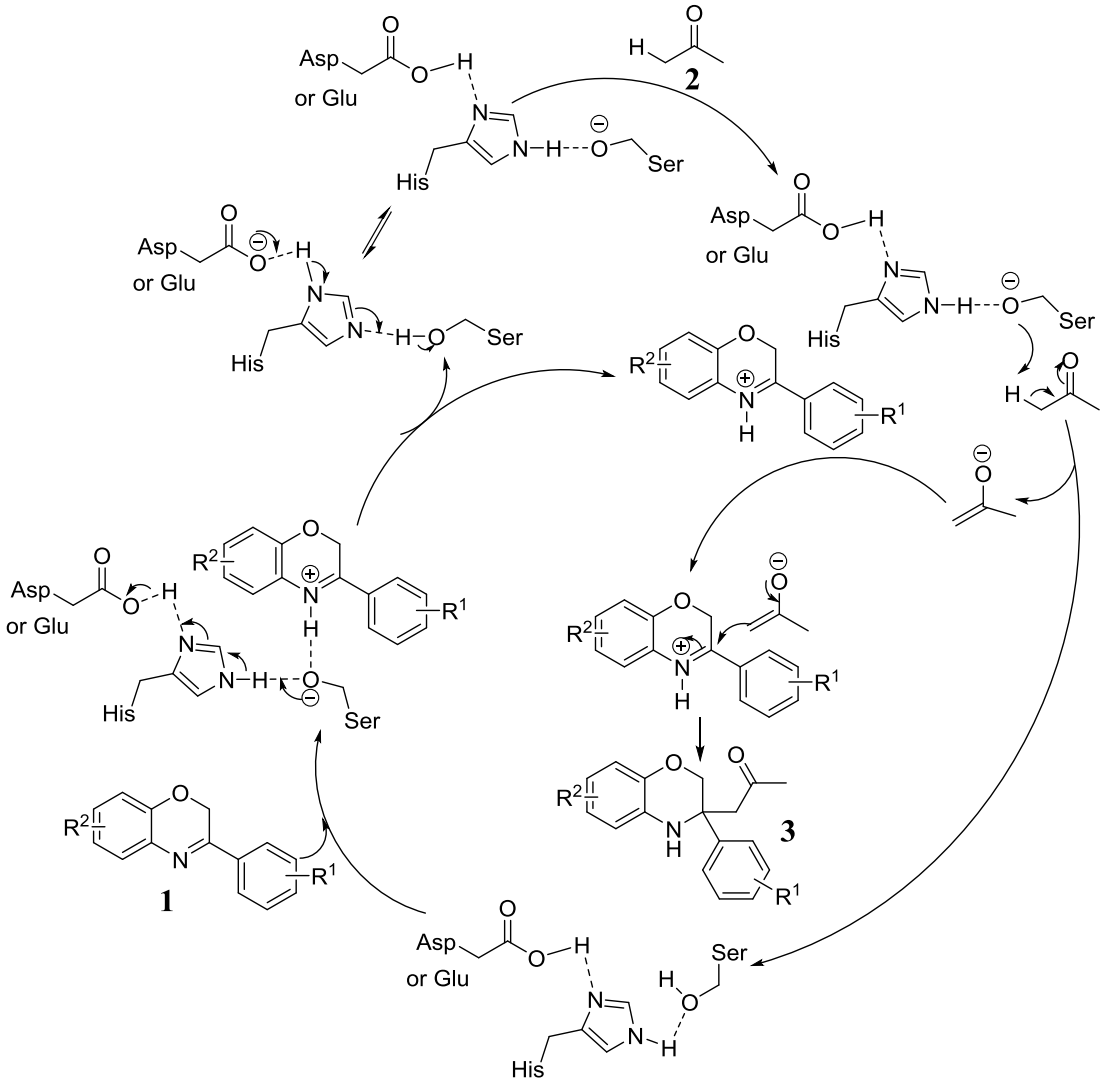
[l] DCC (1.0 mmol) was used instead of WGL (423 U).

[m] The mixture of WGL (423 U), deionized water (1 mL) and DETA (0.5 mmol) was stirred at 30 ^{o}C for 2 h and then water was removed by lyophilization before use.

[n] EDTA (0.5 mmol) was used instead of WGL (423 U).

The imidazole groups of histidine, the hydroxyl groups of serine, carboxylic groups of aspartic or glutamic acids play an important role in the natural catalytic function of WGL.[24] Based on the above control and comparison experiments, it can be inferred that the His, Ser, Asp or Glu residues located in the active centre are also crucial for this enzymatic Mannich reaction. According to the literature,[29] trypsin-like serine protease triad forms the catalytic centre of some triacylglycerol lipases. So, we attempted to propose a mechanism for the WGL-catalyzed Mannich reaction (**Scheme 1**). Firstly, the ketimines **1** are activated by Ser from the Asp (or Glu) -His-Ser

catalytic triad in the active centre of WGL and the protonated ketamines are obtained. Secondly, a proton is transferred from the acetone to the Ser in the Asp (or Glu) -His-Ser catalytic triad and the enolate ion is formed. Thirdly, the protonated ketamines are attacked by the enolate ion through a Mannich process. Then, the products **3** are generated.



Scheme 1. Proposed mechanism for the WGL-catalyzed Mannich reaction.

Conclusions

In summary, WGL was used for the first time as a safe, economical, and eco-friendly biocatalyst in asymmetric Mannich reaction for the synthesis of *β*-amino ketone derivatives. The

influence of several factors including water content, solvent, amount of substrate, enzyme loading, solvent volume, and temperature effects were investigated. The desired products were obtained in excellent enantioselectivities (up to 95% ee) with a wide range of substrates. To confirm the catalytic promiscuity of WGL, several control experiments were conducted. Results indicated that the imidazole groups of histidines, the hydroxyl groups of serines, carboxylic groups of aspartic or glutamic acids, and divalent metal ions may have an effect on the catalytic activity of WGL in the Mannich reaction. By comparing the natural and unnatural activity, it can be speculated that the natural active center of WGL was also responsible for its activity in the Mannich reaction. As a novel case of the enzyme catalytic promiscuity, this work not only expands the application of WGL in organic synthesis, but also provides useful insights into enzyme promiscuity. Although the yields are not good enough at present, it may be a building block for the future research to develop a potentially valuable method using the abundant WGL as a catalyst which can be a sustainable complement to the traditional chemical catalysis.

3. Experimental Section

General procedure for the WGL-catalyzed Mannich reaction

To the mixture of ketimine **1** (0.2 mmol) and WGL (423 U) in DMSO (1.0 mL) was added acetone (8.0 mmol). This reaction mixture was stirred at 25 ^{o}C for the specified reaction time and monitored by TLC. The reaction was terminated by filtering out the enzyme (with 40 mm Buchner funnel and qualitative filter paper), and the filter cake was washed with ethyl acetate (3×10 mL). The filtrate was washed three times with water. The organic phase was dried over anhydrous Na_2SO_4, and the solvents were removed under reduced pressure. The crude products were purified

by column chromatography on a silica gel (petroleum ether/EtOAc: 20/1~10/1) and gave the desired Mannich adducts. The enantiomeric excess was determined by HPLC. Racemic Mannich adducts were obtained via the Mannich reactions catalyzed by tetrahydropyrrole in DMSO.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 21276211 and No. 21472152).

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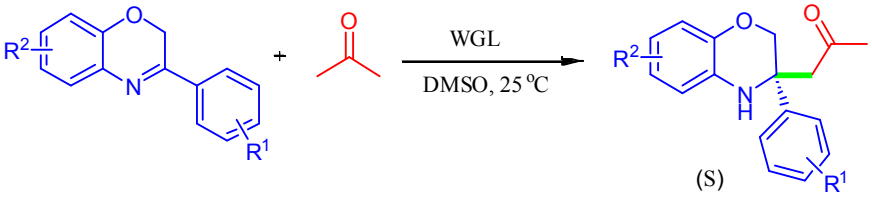

16 examples, yields: 15-54%, ee up to 95%.

Wheat germ lipase (WGL) was used for the first time as a biocatalyst in asymmetric Mannich reaction of ketimines.