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Enzyme-catalyzed asymmetric construction of chiral tertiary alcohols *via* aldol reaction using proteinase

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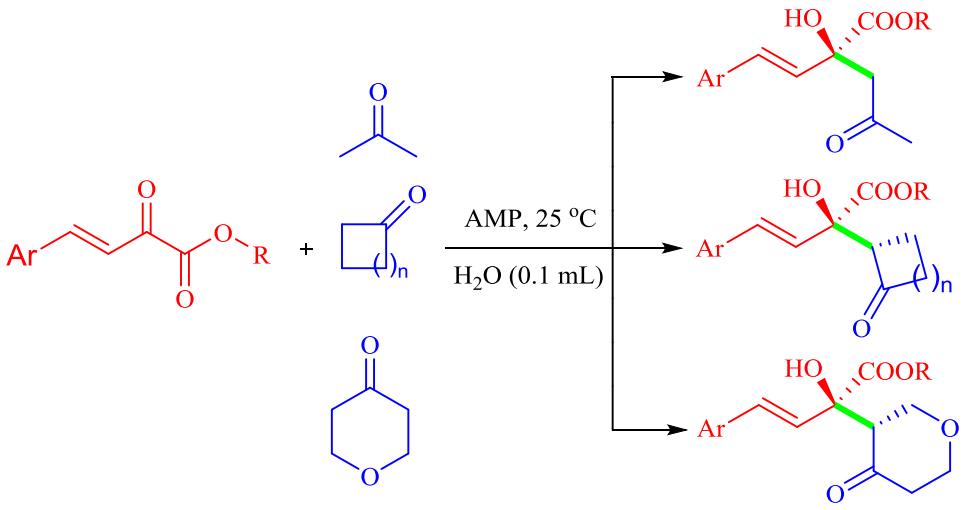
Graphical abstract



Ar = Ph, F-C_6H_4, Cl-C_6H_4, Br-C_6H_4, CH_3O-C_6H_4, CH_3-C_6H_4, pyridine
R = Me, Et, *i*-Pr, Bn
n = 1, 2, 3
AMP = proteinase from *Aspergillus melleus*

34 examples, yield up to 90%, dr up to 93:7, ee up to 70%

Chiral tertiary alcohol was obtained *via* asymmetric ketone-ketone aldol reaction using proteinase from *Aspergillus melleus* (AMP) as a sustainable biocatalyst.

Highlights

- Chiral tertiary alcohols were obtained *via* asymmetric ketone-ketone aldol reaction.
- Proteinase from *Aspergillus melleus* (AMP) was used as a sustainable biocatalyst.
- Enzymatic promiscuity was used to construct chiral tertiary alcohols.
- This work expands the application of natural enzyme in organic synthesis.

Abstract

A new enzyme-catalyzed asymmetric construction of chiral tertiary alcohols *via* asymmetric aldol reactions between β,γ-unsaturated α-keto esters and ketones was reported. Proteinase from *Aspergillus melleus* (AMP) was used as a sustainable biocatalyst. The best results can be obtained with yields of up to 90%, diastereoselectives of up to 93:7 dr, and enantioselectivities of up to 70% ee. This work not only expands the application of enzymatic promiscuity, but also provides more examples for constructing chiral tertiary alcohols.

Key words: proteinase from *Aspergillus melleus*, asymmetric aldol reaction, chiral tertiary alcohol, enzymatic promiscuity, enzyme catalysis

1. Introduction

The structural unit containing chiral tertiary alcohol is important building blocks of natural products and artificial biologically active molecules [1]. For example, Fostriecin is found possessing remarkable anti-cancer and anti-fungi properties [2]. Camptothecin (CPT) is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I (topo I). It was discovered in 1966 by Wall and Wani *et al.* in systematic screening of natural products for anticancer drugs [3]. Taxol 6 was used in the treatment of ovarian cancer, which was approved by the FDA in 1992 [4]. Efavirenz is Merck's anti-HIV drug, one of the most structurally attractive pharmaceuticals, which contains a chiral tertiary alcohol motif [5, 6]. (**Figure 1**)

Although the chiral tertiary alcohols exist widely, it is difficult to achieve these structures because of the large steric hindrance. Thus, the effective construction of chiral tertiary alcohols has attracted considerable attention from chemists and become one of the hot topics in organic synthesis. And so far, there are many encouraging enantioselective synthesis methods to construct chiral tertiary alcohols. In 1997, Evans *et al.* reported bis(oxazolinyl)pyridyl (pybox)[3] Cu(II) complexes as chiral Lewis acid catalysts to catalyze enantioselective aldol additions of enolsilanes to pyruvate esters to afford tertiary alcohols [7]. In 2005, Bolm's group used aminosulfoximines copper complexes to catalyze the same reactions [8]. Many other asymmetric synthetic methods for tertiary alcohols were also reported via Cu-catalyzed C-C bond formation such as the reactions of ketones with silyl enolates, nitromethane, acrylates, allenic esters or zinc acetylides *et al.* [9]. In 2006, Hoveyda and co-workers reported enantioselective additions of enolsilanes to α-keto esters using AgF_2 and an amino acid-based ligand that bears a pyridyl Schiff base as a chiral catalyst [10]. In 2007, Mikami and coworkers reported ketoester-ene reactions of silyl enol ethers to construct chiral tertiary alcohols by chiral dicationic palladium(II) complexes [11]. In 2007, Gong's group used an organic molecule derived from proline and 6-methyl-2-amino pyridine for the direct aldol reaction of ketones with α-keto acids, constructing chiral tertiary alcohols [12]. In 2010, Lu and co-workers reported direct asymmetric aldol reaction of acetone with α-keto esters catalyzed by primary-tertiary diamine organocatalysts [13]. In 2011, the same group disclosed an asymmetric organocatalytic MBH reaction between isatins and acrylates using β-isocupreidine as a catalyst affording chiral tertiary alcohols [14]. In 2012, Yang and co-workers reported a method of construction of chiral tertiary alcohol stereocenters via the [2,3]-Meisenheimer rearrangement [15]. In recent years, several groups reported asymmetric construction of chiral tertiary alcohols *via* aldol reaction between β,γ-unsaturated α-keto esters and cyclohexanone. So far, 9-amino(9-deoxy)epi-*Cinchona* alkaloid/acidic additive [16], chiral primary amine-imine [17], aniline/acid [18], primary amine/4-nitrophenol [19] and *trans*-siloxy-L-proline [20] have been used as the catalysts. In view of the importance

of chiral tertiary alcohols, it is still desirable to explore environmental friendly and sustainable catalysts for construction of these structures.

As a kind of green and environmental benign biocatalysts, enzymes are increasingly being used in organic synthesis. Enzymes are optimized through evolution of a specific chemical transformation with specific substrate recognition in metabolism. Despite this, many enzymes have the ability to catalyze distinctly different chemical transformation of unnatural substrates, which is called enzyme promiscuity [21-24]. Enzyme promiscuous behavior can be described as three aspects, including enzyme reaction condition promiscuity, enzyme substrate promiscuity and enzyme catalytic promiscuity. Biocatalytic promiscuity has attracted significant attention from chemists and biochemists [22, 25]. For instance, some elegant works have testified hydrolases have the ability to catalyze Michael reaction [26, 27], Mannich reaction [28, 29], Diels-Alder reaction [30], Henry reaction [31, 32], domino reaction [33, 34], and so on.

Aldol reaction is an important method in C-C bond-forming process in organic chemistry [35, 36]. Aldol reaction is generally catalyzed by the aldolase in nature; some brilliant works about aldolase-catalyzed aldol reaction have been reported [37-41]. However, some hydrolases have been found to have the aldolase activity. In 2003, Berglund and co-workers found lipase B from *Candida antarctica* (CALB) can catalyze aldol reaction between linear aldehydes [42]. Until 2008, the first asymmetric lipase-catalyzed aldol reaction was reported by the Yu group [43]. And our group has been exploring several enzyme-catalyzed ketone-aldehyde type aldol reactions, using nuclease p1, lipase and protease [44-47]. Among those investigated enzymes, proteinase from *Aspergillus melleus* (AMP) has the best catalytic effect in acetonitrile in the presence of water [47]. In order to expand the application of enzymes in organic synthesis to construct chiral tertiary alcohols, and further explore the promiscuity of enzymes, we attempted to use AMP to catalyze ketone-ketone type aldol reactions. Generally, asymmetric intermolecular aldol reactions of ketone-ketone type [48, 49] are less well developed comparing with the aldol reactions of ketone-aldehyde type and aldehyde-aldehyde type [20], and the results showed that AMP can work for this

transformation. Hence, we wish report AMP-catalyzed aldol reaction between β,γ-unsaturated α-keto esters and various ketones for the construction of chiral tertiary alcohols.

2. Experimental

2.1 *Materials*

The proteinase from *Aspergillus melleus* (AMP) [4 units/mg solid, One unit will hydrolyze casein to produce color equivalent to 1.0 micromole of tyrosine per minute at pH 7.5 at 37 ^{o}C (color per Folin Ciocalteu reagent)] was purchased from Sigma-Aldrich, Shanghai, China (P4032-25G, Lot#SLBF8373V). Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. The substrates β,γ-unsaturated α-keto esters (**1a**-**1z**) were prepared according to the literature [50].

2.2 *Analytical methods*

All reactions were monitored by thin-layer chromatography (TLC, petroleum ether/ethyl acetate as eluent) with Haiyang GF254 silica gel plates (Qingdao Haiyang chemical industry Co Ltd, Qingdao, China) using UV light, iodine and vanillic aldehyde as visualizing agents. The ^{1}H NMR and ^{13}C NMR spectra were recorded with TMS as the internal standard in $CDCl_3$ on a Bruker 600 MHz instrument at room temperature. Chemical shifts were reported in ppm from TMS with the solvent resonance as the internal standard. HRMS were recorded on a Varian 7.0 T FTICR-MS spectrometer. Melting points were taken on a WPX-4 microscopic melting point apparatus and were uncorrected (Yice instrument equipment Co Ltd, Shanghai).

2.3 *General procedure for the AMP-catalyzed asymmetric aldol reaction*

To a 10 mL round bottom flask was added with β,γ-unsaturated α-keto ester (0.3 mmol), ketone (3.0 mmol), AMP (120 U) and deionized water (0.10 mL). The

resultant mixture was stirred on a magnetic stirrer at 25 ^{o}C for the specified time and monitored by TLC. The reaction was terminated by filtering off the enzyme using filter paper, and the filter cake was washed with ethyl acetate. Then the solvent in filtrate was removed under reduced pressure. The residue was purified by silica gel flash column chromatography using 200-300 mesh silica gel at increased pressure with petroleum ether/ethyl acetate (12:1-1:1) as eluent to afford the products (Solvent ratios of the eluent mixture are listed in the Supporting Information). ^{1}H NMR and ^{13}C NMR for all the products and HRMS for unknown compounds are available in the Supporting Information.

3. Results and discussion

Initially, the aldol reaction between β,γ-unsaturated α-keto ester (**1a**) and cyclohexanone (**2**) was chosen as the model reaction. To confirm the specific catalytic effect of AMP on the model aldol reaction, some control experiments were performed (**Table 1**). When AMP was used as a catalyst in acetonitrile in the presence of water at 25 °C, the product was obtained in a yield of 34% with dr of 64/36 (**3**/**4**) and ee of 28% (for **3**) and 69% (for **4**) (**Table 1**, entry 1). In the absence of enzyme, no product was detected (**Table 1,** entry 2), which proved that AMP could catalyze the model aldol transformation. Next, non-enzyme proteins, egg albumin and bovine serum albumin, were used as catalysts, separately; low yields of 2% and 3% were obtained, respectively (**Table 1,** entries 3 and 4), which indicated that the observed results from AMP-catalyzed model reaction was not simply catalyzed by the amino acid residues on the surface of protein. Urea as a common denaturation reagent of proteins was used to pretreat AMP. The reaction with urea-pretreated AMP gave a low yield of 8% (**Table 1,** entry 5). As a control, the same amount of urea was also used to catalyze this reaction; only 1% yield was received (**Table 1,** entry 6), showing that urea alone nearly no catalytic effect on the model reaction. These results indicated that the native structure of AMP is responsible for the reaction. Since AMP is a serine protease, phenylmethanesulfonyl fluoride (PMSF) as a specific inhibitor of serine protease was

used to pretreat AMP [51]. The reaction with PMSF-pretreated AMP only gave a low yield of 5% (**Table 1**, entry 7). PMSF alone was proved no catalytic effect on the reaction (**Table 1**, entry 8). Because AMP has at least one histidine residue in its active site [52], as a modifier of the imidazole groups of histidine, diethyl cyanophosphonate (DEPC) was used to pretreat AMP [53]. DEPC-pretreated AMP nearly completely lost its catalytic activity for the model reaction; only 1% yield was detected (**Table 1**, entry 9). DEPC alone did not show any catalytic activity even after 6 days (**Table 1**, entry 10). The above control experiments suggested that the native structure of AMP is responsible for the reaction and the catalysis may take place in the active site of AMP.

Enzymes have been discovered to maintain their activities in organic solvents and applied for chemical transformations of organic compounds [54]. Thus, during the course of our initial investigation, solvents with different log *P* values were screened and the results were shown in **Table 2**. Some polar, non-polar and protic solvents were examined, and various yields and ees were obtained (**Table 2**, entries 1-10). In general, the reactions in solvents with log *P* higher than 2.00 or lower than -0.24 gave products in low ees (for **3**) (**Table 2**, entries 1, 3, 5, 6 and 8). Relatively higher ees (for **3**) were obtained when log *P* values of solvents are between 0.49 and 0.96, such as EtOAc, THF, diethyl ether and cyclohexanone (**Table 2**, entries 2, 4, 7 and 10). However, solvent log *P* did not show obvious effects on ees for **4**. Water as a solvent was also investigated; the reaction in water gave a low yield and moderate ees (**Table 2**, entry 9). Initial reaction rates in different solvents were also tested. Specifically, the substrate cyclohexanone itself as a solvent gave the best yield of 78% with 70:30 dr (**3**/**4**), 34% ee (for **3**) and 64% ee (for **4**) with the highest initial reaction rate of 2.15 (mM/h) (**Table 2,** entry 10). Considering the yield, cyclohexanone was selected as the optimal solvent. Thus, the role of cyclohexanone in this AMP-catalyzed reaction is not only one of the substrates, but a solvent.

Temperature has important effect on enzymatic reactions because of its effect on reaction rate, selectivity and the stability of enzymes. Thus, the effect of different temperature from 15 ^{o}C to 60 ^{o}C on the model reaction was explored (**Table 3**). With the rise in temperature, the yield of the product increased at first, reaching the highest level of 86% at 35 ^{o}C (**Table 3**, entry 5), and then decreased obviously with the continuous increase of the temperature. It is probably due to the partial denaturation of enzyme caused by higher temperature. The highest ee of 46% (for **3**) was obtained at 15 ^{o}C (**Table 3**, entry 1), and then ee values decreased remarkably with increasing the temperature. When temperature increased from 15 to 35 ^{o}C, no obvious change of ee (for **4**) was observed; further rising the temperature led to a serious decrease of ee value. In general, increasing temperature resulted in a decline of enantioselectivity, which is in accord with most reported enzyme catalyzed aldol reactions [55-59]. In terms of the yield and selectivity, 25 ^{o}C was selected as the optimal temperature.

In view of the fact that the molar ratios of substrates have great influence on reactions, the molar ratio of β,γ-unsaturated α-keto ester (**1a**) to cyclohexanone (**2**) was optimized in the AMP-catalyzed aldol reaction (**Table 4**). Both dr and ee were not obviously influenced by varying the molar ratio of substrates from 1:2 to 1:34 (**Table 4**, entries 1-8). However, the yield was significantly improved when the molar ratio of substrates increased from 1:2 to 1:10 (**Table 4**, entries 1-3). When the molar ratio is 1:2, only a low yield of 13% was obtained (**Table 4**, entry 1), but a good yield of 83% was received at the molar ratio of 1:10 (**Table 4**, entry 3). Continuously increasing the molar ratio from 1:10 to 1:34 did not lead to a remarkable change in the yield (**Table 4**, entries 3-8). Although the best yield of 87% was obtained at 1:20 (**Table 4**, entry 5), the slightly better ee was reached at 1:10 with 36% ee (for **3**) and 63% ee (for **4**) (**Table 4**, entry 3). To get better enantioselectivity and for economic consideration, 1:10 was chosen as the optimal molar ratio for the reaction.

It is known that enzymes need essentially bound water in enzymatic reactions because essential conformation of enzyme for catalytic activity is maintained directly

or indirectly by the water molecules through hydrogen bonding and other non-covalent bonds. The amount of water required to reach the maximal activity of enzyme differs in different organic solvents [60]. In this investigation, cyclohexanone was used not only as a substrate but also as a solvent, which is not miscible with water. Thus, addition of water would form a two-phase medium. To examine the effect of water addition on the reaction, the amount of water from 0 to 0.60 mL in the reaction system [**1a** (57 mg, 0.3 mmol), cyclohexanone **2** (0.31 mL, 3.0 mmol) and AMP (120 U)] was screened (**Table 5**). The reaction without water only gave a very low yield of 36% with low ee values of 11% (for **3**) and 52% (for **4**) (**Table 5**, entry 1). When increasing the amount of water from 0 to 0.10 mL, the yield increased remarkably (**Table 5**, entries 1-3). Further increasing the water led to a decrease of the yield (**Table 5**, entries 3-10). However, ee and dr remained almost unchanged for the reactions with water from 0.05 to 0.60 mL (**Table 5**, entries 2-10). The best result was obtained with the yield of 85% and 37% ee (for **3**) and 64% ee (for **4**) when 0.10 mL of water was added into the reaction system (**Table 5**, entry 3), and at this case the reaction system was still two-phase even in the presence of the substrate β,γ-unsaturated α-keto ester **1a** (57 mg, 0.3 mmol). Therefore, 0.10 mL of water was selected as the optimized water addition for the reaction.

Next, the enzyme amount was screened. When increasing the amount of AMP from 20 U to 120 U, both the yield and ee were increased (**Table 6**, entries 1-6). When the amount of AMP was increased from 120 U to 160 U, the yield and ee were almost remained unchanged (**Table 6**, entries 6-8). Therefore, 120 U of AMP was chosen as the suitable enzyme amount for the reaction.

Because the yield was low under the unoptimized conditions, to obtain as high yield as possible, the reaction time was prolonged to 144 h for the reaction (listed in **Table 1**, entry 1) based on TLC analysis. In order to compare the effects of different parameters on the reaction under the same reaction time, all the reactions in the optimization process were carried out for 144 h (**Tables 1-6**). To further investigate

the influence of time on the reaction, after optimizing a series of conditions, the time course of the reaction was explored (**Table 7**). The yield and ee increased with the reaction time extending (**Table 7**, entries 1-7). A fast increase of the yield was investigated during the first 72 h (**Table 7**, entries 1-3); after that the tendency of the yield increase slowed down (**Table 7**, entries 3-7). The best yield of 90% was reached after 168 h (**Table 7**, entry 7).

To test the generality and scope of the reaction between β,γ-unsaturated α-keto esters and cyclohexanone, different β,γ-unsaturated α-keto esters (**1**) were explored under the optimal conditions (**Table 8**). The desired products could be obtained with **1** bearing various substituents, giving yields of up to 90%, drs of up to 82/18 (**3**/**4**), and ees of up to 42% (for **3**) and 70% (for **4**) (**Table 8**). Methyl ester and ethyl ester gave better yields and ees than isopropyl ester and benzyl ester (**Table 8**, entries 1-4), probably due to the steric hindrance. β,γ-unsaturated α-keto esters (**1**) with various substituents in benzene ring such as F-, Cl-, Br-, CH_3O- and CH_3- were investigated (**Table 8**, entries 5-24). When the substituents of benzene ring were on *m*- and *p*-positions, the ethyl esters gave higher yields than the methyl esters at the same reaction time (**Table 8**, entries 5-8, 11-16, 19-24); however, when the substituents were on *o*-position, the methyl esters gave higher yields than the ethyl esters (**Table 8**, entries 9, 10, 17 and 18). For the methyl esters, the substituents of benzene ring on *o*-position gave higher yields than *m*- and *p*- positions (**Table 8**, entries 5, 7, 9, 13, 15 and 17). Moreover, β,γ-unsaturated α-keto ester (**1**) with Ar as heterocycle pyridine instead of phenyl ring could also work well in this transformation and good results of corresponding products were obtained (**Table 8**, entries 25 and 26). These compounds we synthesized can be converted to bicyclic lactones containing the unit of 2-hydroxy-γ-butyrolactone via reaction sequences of reduction and lactonization (**Scheme 1**) [20, 61], which had been used to assemble natural products and biologically active compounds [62].

To further expand upon the substrate scope of ketones, various ketones including acyclic and cyclic ketones were also investigated (**Table 9**). Acetone as a representative of acyclic ketones was used to react with methyl and ethyl esters (**1**) giving low yields and ees (**Table 9**, entries 1 and 2). The cyclic ketones including four-membered ring, five-membered ring and pyranoid ring were also investigated; better results with higher yields and ees were obtained (**Table 9**, entries 3-8). In general, when reacting with these cyclic ketones, the ethyl ester gave higher yields but lower ees than the methyl ester (**Table 9** entries 3-8).

According to the literatures [51, 52], AMP is a serine protease and contains at least one histidine residue in the active site. In 2003, Berglund *et al.* reported aldol reaction catalyzed by lipase CALB, containing three amino acid residues Asp, His and Ser in active site [42]. They mutated the serine for a nonpolar residue alanine. The wild-type and the mutant enzymes were used to catalyze the aldol reaction; the results indicated that the mutant had better catalysis activity than the wild. They gave a possible explanation that in the mutant enzyme the formation of hemiacetal by serine attacking the substrate was avoided. And they proposed a mechanism, in which histidine plays a key role for the catalysis of aldol reaction. In the present work, the control experiments suggested that the native structure of AMP is responsible for the aldol reaction and the catalysis may take place in the active site of enzyme. Thus, based on the literatures and our control experiments, we attempted to propose a possible mechanism of AMP-catalyzed aldol reaction presented in **Scheme 2**. Firstly, the imidazole acts as a base to take away a proton from the ketone forming an enolate ion. Secondly, β,γ-unsaturated α-keto ester accepts the proton from imidazolium cation and combines the enolate ion forming a new carbon-carbon bond. Finally, the aldol product is formed, and released from the active site.

4. Conclusion

In conclusion, the report reveals an enzyme-catalyzed method *via* aldol reaction

between β,γ-unsaturated α-keto esters and ketones that provides a simple procedure constructing chiral tertiary alcohols. Yields of up to 90%, diastereoselectivities of up to 93:7 dr and enantioselectivities of up to 70% ee were obtained. AMP as an environment-friendly and sustainable biocatalyst displayed catalytic promiscuity in this transformation. The chiral tertiary alcohols are important building blocks of natural products and artificial biologically active molecules, but they are difficult to be constructed because of the large steric hindrance. We utilized the enzymatic promiscuity of AMP to achieve a series of chiral tertiary alcohols. Application of enzyme catalysis in organic synthesis not only develops synthetic methodology but also expands the new field of enzyme chemistry. Although enantioselectivities were not ideal in this work, it can be a building block for the future research in this field.

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References

[1] M. Shibasaki, M. Kanai, Chem. Rev. 108 (2008) 2853-2873.

[2] Y. Hayashi, H. Yamaguchi, M. Toyoshima, K. Okado, T. Toyo, M. Shoji, Chem. Eur. J. 16 (2010) 10150-10159.

[3] M.E. Wall, M.C. Wani, C.E. Cook, K.H. Palmer, J. Am. Chem. Soc. 88 (1966) 3888-3890.

[4] M. C. Wani, S.B. Horwitz, Anti-Cancer. Drug. 25 (2014), 482-487.

[5] J.W. Corbett, S.S. Ko, J.D. Rodgers, L.A. Gearhart, N.A. Magnus, L.T. Bacheler, S. Diamond, S. Jeffrey, R.M. Klabe, B.C. Cordova, S. Garber, K. Logue, G.L. Trainor, P.S. Anderson, S.K. Erickson-Viitanen, J. Med. Chem. 43 (2000) 2019-2030.

[6] O. Riant, J. Hannedouche, Org. Biomol. Chem. 5 (2007) 873-888.

[7] D.A. Evans, M.C. Kozlowski, C.S. Burgey, D.W.C. MacMillan, J. Am. Chem. Soc. 119 (1997) 7893-7894.

[8] M. Langner, P. Rémy, C. Bolm, Chem. Eur. J. 11 (2005) 6254-6265.

[9] M. Shibasaki, M. Kanai, Chem. Rev. 108 (2008) 2853-2873.

[10] L.C. Akullian, M.L. Snapper, A.H. Hoveyda, J. Am. Chem. Soc. 128 (2006) 6532-6533.

[11] K. Mikami, Y. Kawakami, K. Akiyama, K. Aikawa, J. Am. Chem. Soc. 129 (2007) 12950-12951.

[12] X.Y. Xu, Z. Tang, Y.Z. Wang, S.W. Luo, L.F. Cun, L.Z. Gong, J. Org. Chem. 72 (2007) 9905-9913.

[13] Z.Q. Jiang, Y.X. Lu, Tetrahedron Lett. 51 (2010) 1884-1886.

[14] F.R. Zhong, G.Y. Chen, Y.X. Lu, Org. Lett. 13 (2011) 82-85.

[15] H. Yang, M. Sun, S.G. Zhao, M. Zhu, Y.L. Xie, C.L. Niu, C.L. Li, J. Org. Chem. 78 (2013) 339-346.

[16] P.F. Li, S.H. Chan, A.S.C. Chan, F.Y. Kwong, Adv. Synth. Catal. 353 (2011) 1179-1184.

[17] Z.J. Mao, X. Zhu, A. Lin, W.P. Li, Y. Shi, H.B. Mao, C.J. Zhu, Y.X. Cheng. Adv. Synth. Catal. 355 (2013) 2029-2036.

[18] Y.M. Deng, L. Liu, R.G. Sarkisian, K. Wheeler, H. Wang, Z.H. Xu, Angew. Chem. Int. Ed. 52 (2013) 3663-3667.

[19] J.W. Wei, W.G. Guo, X. Zhou, Y. Liu, C. Li, Chin. J. Chem. 32 (2014) 985-990.

[20] C.W. Zheng, Y.Y. Wu, X.S. Wang, G. Zhao, Adv. Synth. Catal. 350 (2008) 2690-2694.

[21] A. Taglieber, H. Hbenreich, J. D. Carballeira, R.J.G. Mondire, M.T. Reetz. Angew. Chem. Int. Ed. 46 (2007) 8597-8600.

[22] K. Hult, P. Berglund, Trends Biotechnol. 25 (2007) 231-238.

[23] M.S. Humble, P. Berglund, Eur. J. Org. Chem. 2011, 3391-340.

[24] Z. Guan, L.Y. Li, Y.H. He, RSC Adv. 5 (2015) 16801-16814.

[25] O. Khersonsky, C. Roodveldt, D.S. Tawfik, Curr. Opin. Chem. Biol. 10 (2006) 498-508.

[26] K.L. Xu, Z. Guan, Y.H. He, J. Mol. Catal., B: Enzym 71 (2011) 108-112.

[27] M. Svedendahl, K. Hult, P. Berglund, J. Am. Chem. Soc. 127 (2005) 17988-17989.

[28] Y. Xue, L.P. Li, Y.H. He, Z. Guan, Sci. Rep. 2 (2012) 761, DOI: 10.1038/srep00761.

[29] K. Li, T. He, C. Li, X.W. Feng, N. Wang, X.Q. Yu, Green Chem. 11 (2009) 777-779.

[30] Y.H. He, W. Hu, Z. Guan, J. Org. Chem. 77 (2012) 200-207.

[31] R.C. Tang, Z. Guan, Y.H. He, W. Zhu, J. Mol. Catal., B: Enzym 63 (2010) 62-67.

[32] N. Gao, Y.L. Chen, Y.H. He, Z. Guan, RSC Adv. 3 (2013) 16850-16856.

[33] C.H. Wang, Z. Guan, Y.H. He. Green Chem. 13 (2011) 2048-2054.

[34] T. He, Q.Q. Zeng, D.C. Yang, Y.H. He, Z. Guan, RSC Adv. 5 (2015) 37843-37852.

[35] T.D. Machajewski, C.H. Wong, Angew. Chem. Int. Ed. 39 (2000) 1352-1374.

[36] R. Mestres, Green Chem. 6 (2004) 583-603.

[37] O. Eyrisch, W.D. Fessner, Angew. Chem., Int. Ed. Engl. 34 (1995) 1639-1641.

[38] P. Clapés, W.D. Fessner, G.A. Sprenger, A.K. Samland, Curr. Opin. Chem. Biol. 14 (2010) 154-167.

[39] W.D. Fessner, V. Helaine, Curr. Opin. Biotechnol. 12 (2001) 574-586.

[40] T.D. Machajewski, C.H. Wong, Angew. Chem. Int. Ed. 39 (2000) 1353-1374.

[41] A. Heine, G. DeSantis, J.G. Luz, M. Mitchell, C.H. Wong, I.A. Wilson, Science, 294 (2001) 369-374.

[42] C. Branneby, P. Carlqvist, A. Magnusson, K. Hult, T. Brinck, P. Berglund, J. Am. Chem. Soc. 125 (2003) 874-875.

[43] C. Li, X.W. Feng, N. Wang, Y.J. Zhou, X.Q. Yu, Green Chem. 10 (2008) 616-618.

[44] H.H. Li, Y.H. He, Y. Yuan, Z. Guan, Green Chem. 13 (2011) 185-189.

[45] Z, Guan, J.P. Fu, Y.H. He, Tetrahedron Lett. 53 (2012) 4959-4961.

[46] Y.H. He, H.H. Li, Y.L. Chen, Y. Xue, Y. Yuan, Z. Guan, Adv. Synth. Catal. 354

(2012) 712-719.

[47] Y. Yi, Z. Guan, Y.H. He, Sci. Chin. Chem. 56 (2013) 939-944.

[48] O. Tokuda, T. Kano, W.G. Gao, T. Ikemoto, K. Maruoka. Org. Lett. 7 (2005) 5103-5105.

[49] S. Konda, Q.S. Guo, M. Abe, H.C. Huang, H. Arman, J.C.G. Zhao, J. Org. Chem. 80 (2015) 806-815.

[50] L. Gremaud, A. Alexakis, Angew. Chem. Int. Ed. 51 (2012) 794-797.

[51] M. Luisetti, P.D. Piccioni, K. Dyne, M. Donnini, A. Bulgheroni, L. Pasturenzi, A.M. Donnetta, V. Peona, Int. J. Tiss. Reac. XIII 4 (1991) 187-192.

[52] Shinichi Kobayashi, Teijiro Tazawa, Masanori Sasaki, Setsuo Kiryu, Mamoru Sugiura. Chem. Pharm. Bull. 32 (1984) 3111-3117.

[53] G. Gesecken, Z. Physiol. Chem. 359 (1978) 1086-1087.

[54] K. Griebenow, A.M. Klibanov, J. Am. Chem. Soc. 47(1996) 11695-11700.

[55] L.Y. Li, D.C. Yang, Z. Guan, Y.H. He, Tetrahedron 71 (2015) 1659-1667.

[56] Y.L. Chen, W. Li, Y. Liu, Z. Guan, Y.H. He, J. Mol. Catal., B: Enzym 87 (2013) 83-87.

[57] Z. Guan, J.P. Fu, Y.H. He, Tetrahedron Lett 53 (2012) 4959–4961.

[58] H.H. Li, Y.H. He, Y. Yuan, Z. Guan, Green Chem. 13 (2011) 185-189.

[59] B.H. Xie, W. Li, Y. Liu, H.H. Li, Z. Guan, Y.H. He, Tetrahedron 68 (2012) 3160-3164.

[60] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 8017-8021.

[61] X.Y. Xu, Z. Tang, Y.Z. Wang, S.W. Luo, L.F. Cun, L.Z. Gong, J. Org. Chem. 72 (2007) 9905-9913.

[62] A.G.H. Wee, B. Liu, L. Zhang, J. Org. Chem. 57 (1992) 4404-4414.

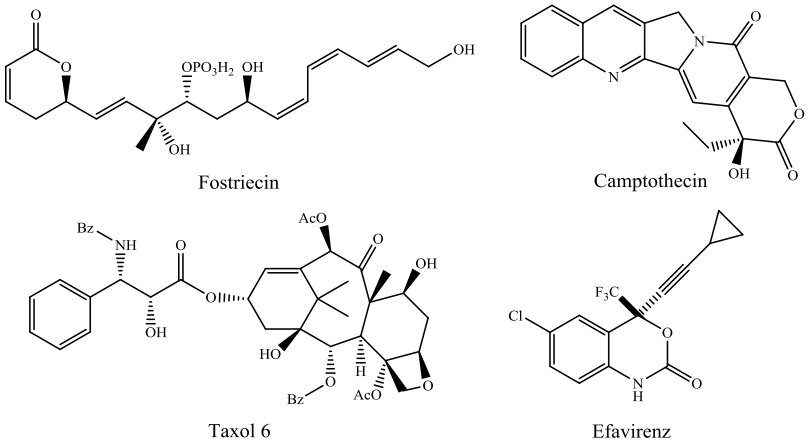
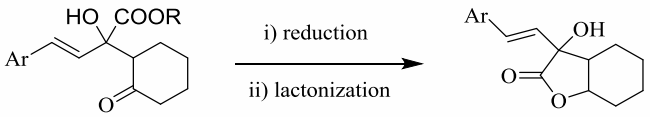
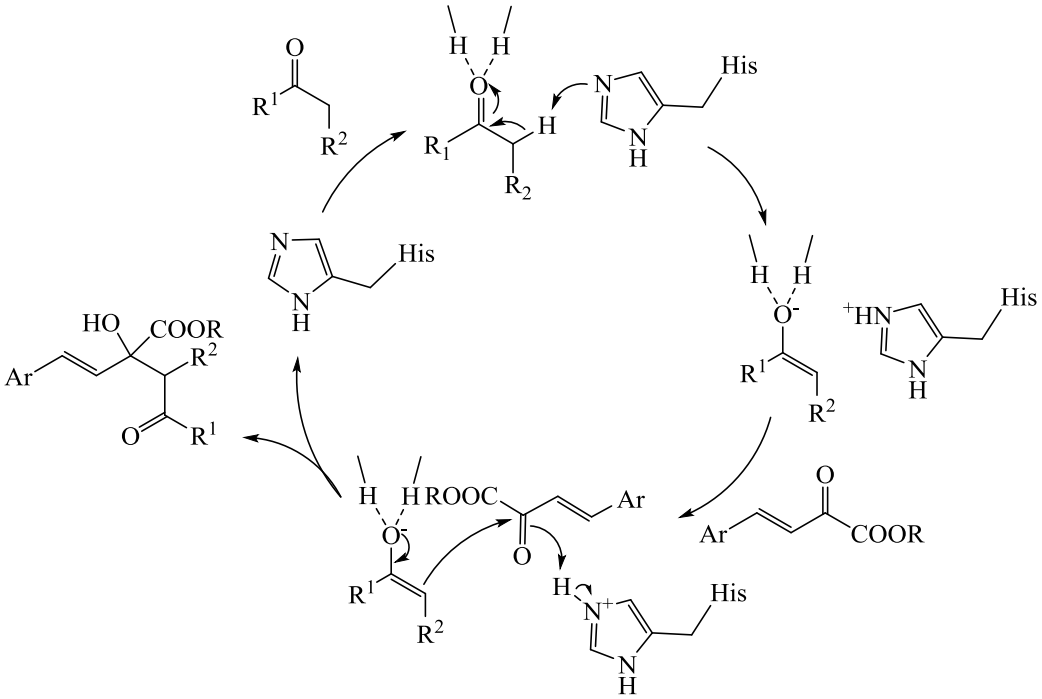


Figure 1 Some examples of compounds (natural and artificial) carrying chiral tertiary alcohol.

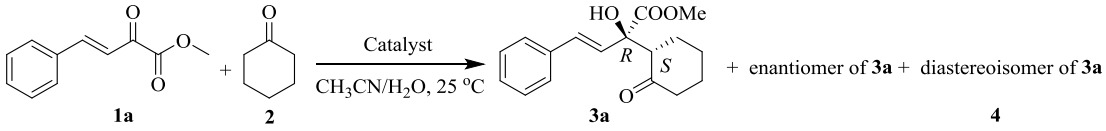


Scheme 1. Transformation of the products to bicyclic lactones.



Scheme 2 Proposed mechanism for the AMP-catalyzed aldol reaction of β,γ-unsaturated α-keto esters and ketones.

Table 1. Control experiments.[a]



Entry	Catalyst	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[b]	ee (for **3**) (%)[c]	ee (for **4**) (%)[d]
1	AMP (120 U)	34	64/36	28	69
2	No enzyme	n.d.[k]	--	--	--
3	Egg albumin (30 mg)	2	83/17	0	6
4	Bovine serium albumin (30 mg)	3	82/18	0	5
5	AMP (pretreated with 2.5 M urea)[e]	8	67/33	13	61
6	Urea[f]	1	78/22	0	0
7	AMP (pretreated with PMSF)[g]	5	63/37	0	8
8	PMSF[h]	n.d.[k]	--	--	--
9	AMP (pretreated with DEPC)[i]	1	63/37	14	59
10	DEPC[j]	n.d.[k]	--	--	--

[a] Unless otherwise noted, the reaction was conducted using **1a** (0.3 mmol), **2** (0.16 mL, 5 equiv), catalyst, CH_3CN (0.90 mL), and deionized water (0.10 mL), and the mixture was stirred at 25 °C for 144 h.

[b] Yield and dr were determined by chiral HPLC analysis (Chiralpak AS-H column).

[c] Enantiomeric excess of **3a** determined by chiral HPLC analysis (Chiralpak AS-H column).

[d] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis (Chiralpak AS-H column).

[e] AMP (120 U) in urea solution (2.5 M) [urea (150 mg) in 1 mL H_2O] was stirred at 25 °C for 24 h and then water was removed by lyophilization before use.

[f] Urea (150 mg) was used instead of AMP.

[g] AMP (120 U) and PMSF (50 mg) in THF (2 mL) was stirred at 25 °C for 24 h, then THF was removed under reduced pressure before use.

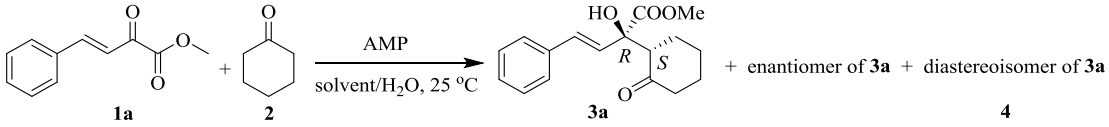
[h] PMSF (50 mg) was used instead of AMP.

[i] AMP (120 U), phosphate buffer solution (NaH_2PO_4-Na_2HPO_4, pH 8.02) (1 mL) and DEPC (0.3 mmol) was stirred at 37 °C for 2 h and then water was removed by lyophilization before use.

[j] DEPC (0.3 mmol) was used instead of AMP.

[k] n.d.: No product was detected.

Table 2. The screening of solvents.[a]



Entry	Solvent	Log *P*	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[b]	ee (for **3**) (%)[c]	ee (for **4**) (%)[d]	Initial reaction rate (mM/h)[e]
1	Toluene	2.50	14	76/24	4	27	0.03
2	EtOAc	0.73	16	75/25	32	54	0.09
3	$CHCl_3$	2.00	17	67/33	7	54	0.08
4	THF	0.49	25	72/28	46	65	0.78
5	EtOH	-0.24	31	77/23	21	41	0.94
6	CH_3CN	-0.33	34	64/36	28	69	0.26
7	Diethyl ether	0.85	43	76/24	37	52	0.54
8	*n*-Hexane	3.50	45	77/23	29	44	0.58
9	H_2O	--	25	75/25	36	53	0.28
10	Cyclohexanone	0.96	78	70/30	34	64	2.15

[a] Reaction conditions: **1a** (0.3 mmol), **2** (0.16 mL, 5 equiv), AMP (120 U), solvent (0.9 mL) and deionized water (0.1 mL) at 25 °C for 144 h.

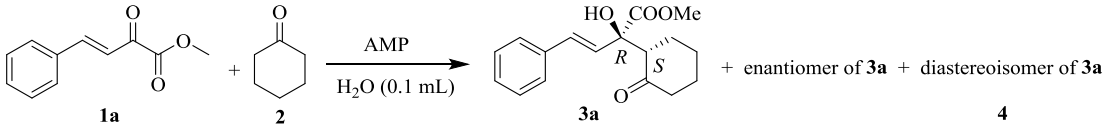
[b] Yield and dr were determined by chiral HPLC analysis (Chiralpak AS-H column).

[c] Enantiomeric excess of **3a** determined by chiral HPLC analysis (Chiralpak AS-H column).

[d] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis (Chiralpak AS-H column).

[e] The initial reaction rate refers to the increase of product concentration (mM/h), which was detected by HPLC analysis (Chiralpak AS-H column).

Table 3. Effect of temperature on the reaction.[a]



Entry	Temperature (°C)	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[b]	ee (for **3**) (%)[c]	ee (for **4**) (%)[d]
1	15	46	70/30	46	63
2	20	60	71/29	38	58
3	25	80	73/27	33	64
4	30	85	68/32	26	65
5	35	86	68/32	16	59
6	40	80	68/32	10	43
7	50	70	72/28	10	28
8	60	66	77/23	5	7

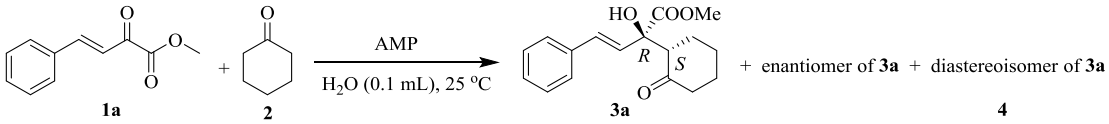
[a] Reaction conditions: **1a** (0.3 mmol), **2** (1.06 mL, 34 equiv), AMP (120 U) and deionized water (0.10 mL) at specified temperature for 144 h.

[b] Yield and dr were determined by chiral HPLC analysis (Chiralpak AS-H column).

[c] Enantiomeric excess of **3a** determined by chiral HPLC analysis (Chiralpak AS-H column).

[d] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis (Chiralpak AS-H column).

Table 4. The screening for molar ratio of substrates.[a]



Entry	Molar ratio of **1a**:**2**	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[b]	ee (for **3**) (%)[c]	ee (for **4**) (%)[d]
1	1:2	13	76/24	34	56
2	1:5	61	76/24	37	58
3	1:10	83	74/26	36	63
4	1:15	86	74/26	33	65
5	1:20	87	73/27	32	64
6	1:25	82	74/26	32	62
7	1:30	83	73/27	31	62
8	1:34	80	73/27	30	60

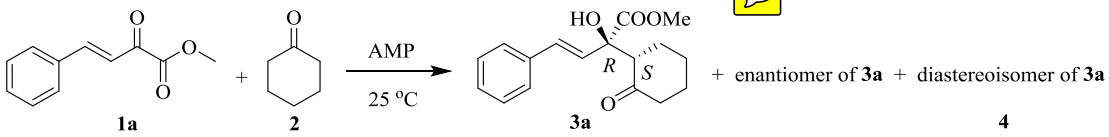
[a] Reaction conditions: **1a** (0.3 mmol), **2** (0.062-1.06 mL, 0.6-10.2 mmol), AMP (120 U) and deionized water (0.10 mL) at 25 °C for 144 h.

[b] Yield and dr were determined by chiral HPLC analysis (Chiralpak AS-H column).

[c] Enantiomeric excess of **3a** determined by chiral HPLC analysis (Chiralpak AS-H column).

[d] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis (Chiralpak AS-H column).

Table 5. Effect of amount of water on the reaction.[a]



Entry	Amount of water (mL)	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[b]	ee (for **3**) (%)[c]	ee (for **4**) (%)[d]
1	0	36	71/29	11	52
2	0.05	60	75/25	34	63
3	0.10	85	75/25	37	64
4	0.15	81	74/26	36	64
5	0.20	71	74/26	36	64
6	0.25	68	74/26	36	65
7	0.30	63	75/25	36	65
8	0.40	58	75/25	37	68
9	0.50	54	75/25	36	66
10	0.60	47	75/25	36	66

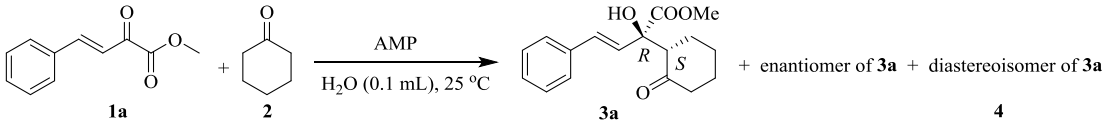
[a] Reaction conditions: **1a** (0.3 mmol), **2** (0.31 mL, 3.0 mmol), AMP (120 U) and deionized water (0-0.60 mL) at 25 °C for 144 h.

[b] Yield and dr were determined by chiral HPLC analysis (Chiralpak AS-H column).

[c] Enantiomeric excess of **3a** determined by chiral HPLC analysis (Chiralpak AS-H column).

[d] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis (Chiralpak AS-H column).

Table 6. The screening of the amount of AMP.[a]



1a 2 3a 4

Entry	The amount of AMP (U)	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[b]	ee (for **3**) (%)[c]	ee (for **4**) (%)[d]
1	20	39	75/25	23	47
2	40	48	75/25	23	44
3	60	62	75/25	23	45
4	80	70	73/27	37	64
5	100	81	74/26	38	63
6	120	85	73/27	38	63
7	140	86	75/25	38	64
8	160	87	73/27	38	63

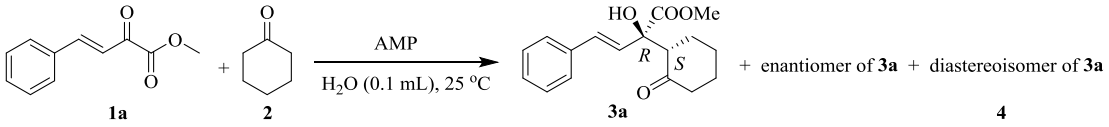
[a] Reaction conditions: **1a** (0.3 mmol), **2** (0.31 mL, 3.0 mmol), AMP (20-160 U) and deionized water (0.10 mL) at 25 °C for 144 h.

[b] Yield and dr were determined by chiral HPLC analysis (Chiralpak AS-H column).

[c] Enantiomeric excess of **3a** determined by chiral HPLC analysis (Chiralpak AS-H column).

[d] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis (Chiralpak AS-H column).

Table 7. The time course of the reaction.[a]



1a 2 3a 4

Entry	Time (h)	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[b]	ee (for **3**) (%)[c]	ee (for **4**) (%)[d]
1	24	40	73/27	24	53
2	48	65	73/27	28	57
3	72	75	73/27	29	56
4	96	79	73/27	35	59
5	120	82	74/26	37	63
6	144	87	74/26	38	63
7	168	90	73/27	38	64

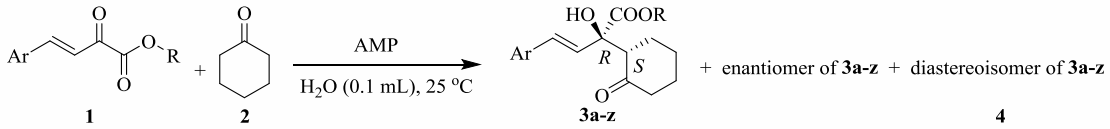
[a] Reaction conditions: **1a** (0.3 mmol), **2** (0.31 mL, 3.0 mmol), AMP (120 U) and deionized water (0.10 mL) at 25 °C.

[b] Yield and dr were determined by chiral HPLC analysis (Chiralpak AS-H column).

[c] Enantiomeric excess of **3a** determined by chiral HPLC analysis (Chiralpak AS-H column).

[d] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis (Chiralpak AS-H column).

Table 8. Substrate scope of β,γ-unsaturated α-keto esters (1).[a]



Entry	Ar	R	Major product	Time (h)	Yield (**3**+**4**) (%)[b]	dr (**3**/**4**)[c]	ee (for **3**) (%)[d]	ee (for **4**) (%)[e]
1	Ph	Me	**3a**	168	90	73/27	38	64
2	Ph	Et	**3b**	127	79	81/19	17	42
3	Ph	*i*-Pr	**3c**	112	31	82/18	6	38
4	Ph	Bn	**3d**	120	32	78/22	12	50
5	4-FC_6H_4	Me	**3e**	114	39	69/31	19	61
6	4-FC_6H_4	Et	**3f**	114	50	78/22	26	50
7	3-FC_6H_4,	Me	**3g**	114	42	74/26	31	62
8	3-FC_6H_4	Et	**3h**	114	59	74/26	12	54
9	2-FC_6H_4	Me	**3i**	96	64	69/31	22	70
10	2-FC_6H_4	Et	**3j**	96	50	73/27	23	64
11	3-ClC_6H_4	Me	**3k**	113	59	69/31	34	64
12	3-ClC_6H_4	Et	**3l**	113	61	74/26	30	52
13	4-BrC_6H_4	Me	**3m**	114	34	71/29	28	62
14	4-BrC_6H_4	Et	**3n**	114	49	73/27	16	52
15	3-BrC_6H_4	Me	**3o**	113	40	69/31	36	65
16	3-BrC_6H_4	Et	**3p**	113	56	67/33	18	52
17	2-BrC_6H_4	Me	**3q**	114	55	69/31	26	70
18	2-BrC_6H_4	Et	**3r**	114	37	74/26	15	54
19	4-$CH_3OC_6H_4$	Me	**3s**	113	20	70/30	29	60
20	4-$CH_3OC_6H_4$	Et	**3t**	113	34	72/28	13	60
21	3-$CH_3OC_6H_4$	Me	**3u**	113	53	70/30	37	67

22	3-$CH_3OC_6H_4$	Et	**3v**	113	66	70/30	30	57
23	4-$CH_3C_6H_4$	Me	**3w**	119	47	73/27	29	59
24	4-$CH_3C_6H_4$	Et	**3x**	119	52	82/18	21	33
25	3-Pyridyl	Me	**3y**	127	78	69/31	42	65
26	3-Pyridyl	Et	**3z**	127	86	68/32	37	53

[a] Reaction conditions: **1** (0.3 mmol), **2** (0.31 mL, 3.0 mmol), AMP (120 U), and H_2O (0.10 mL) at 25 °C.

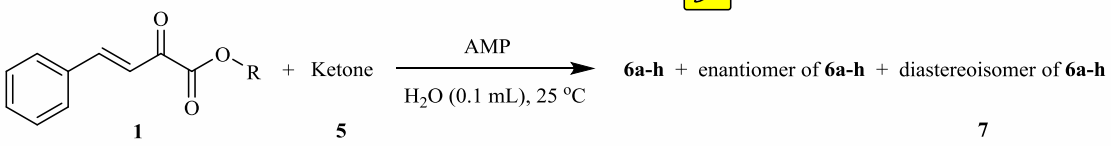
[b] Yield of the isolated product after chromatography on silica gel.

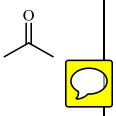
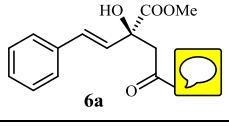
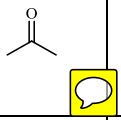
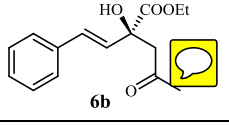
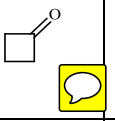
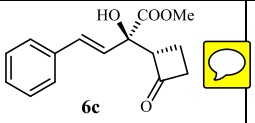
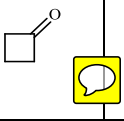
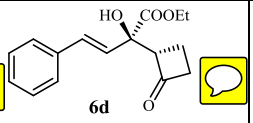
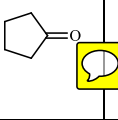
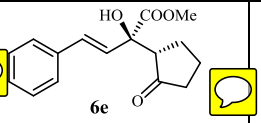
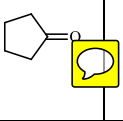
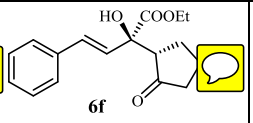
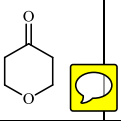
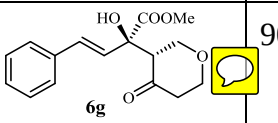
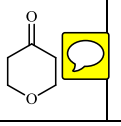
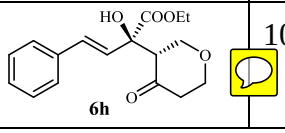
[c] The dr was determined by chiral HPLC analysis.

[d] Enantiomeric excess of **3a-z** determined by chiral HPLC analysis (for details, please see the Supporting Information).

[e] Absolute configuration of **4** was not ascertained and ee was determined by chiral HPLC analysis.

Table 9. Substrate scope of ketones (5).[a]



Entry	R	5	Major product	Time (h)	Yield (**6**+**7**) (%)[b]	dr (**6**/**7**)[c]	ee (for **6**) (%)[d]	ee (for **7**) (%)[e]
1	Me		 **6a**	94	37	--	10	--
2	Et		 **6b**	117	29	--	7	--
3	Me		 **6c**	94	41	58/42	21	10
4	Et		 **6d**	104	54	54/46	17	13
5	Me		 **6e**	91	48	75/25	12	28
6	Et		 **6f**	117	68	77/23	4	22
7	Me		 **6g**	90	67	79/21	18	48
8	Et		 **6h**	101	73	93/7	2	14

[a] Reaction conditions: **1** (0.3 mmol), **5** (3.0 mmol), AMP (120 U) and deionized water (0.10 mL) at 25 °C.

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

[c] The dr was determined by chiral HPLC analysis.

[d] Enantiomeric excess of **6a-h** determined by chiral HPLC analysis (for details, please see the Supporting Information).

[e] Absolute configuration of **7** was not ascertained and ee was determined by chiral HPLC analysis.