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Papain-catalyzed aldol reaction for the synthesis of trifluoromethyl carbinol derivatives

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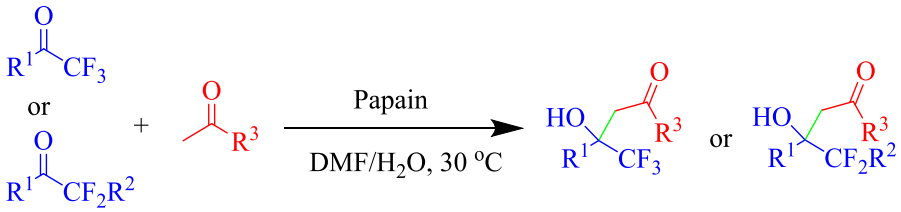
Graphical Abstract



17 examples, 42-99% yield.

Papain was used for the first time as a biocatalyst in aldolreaction between α,α,α-trifluoromethyl ketones and aliphatic ketones.

Highlights

- Papain-catalyzed aldol reaction was described.
- 17 examples of aldol products were obtained by this reaction.
- The novel reaction also expands the field of organic synthesis.
- This work promotes the development of enzyme catalytic promiscuity.

Abstract: Papain from *Carica papaya* demonstrated catalytic promiscuity was first discovered to catalyze the synthesis of trifluoromethyl carbinol derivatives via aldol reaction between α,α,α-trifluoromethyl ketones and aliphatic ketones in a mixed solvent of DMF and water. The best results of the corresponding aldol products with up to 99% yield and 30% ee were achieved.

Key words: papain; aldol reaction; trifluoromethyl carbinol derivatives; enzyme catalytic promiscuity

1. Introduction

The asymmetric aldol reaction serves as one of the most powerful carbon-carbon bond forming methods, providing access to β-hydroxycarbonyl compounds in an enantioselective fashion [1]. Ever since List [2], Barbas [3, 4], and MacMillan [5] groups reported their pioneering works on organocatalyzed enantioselective aldol reactions, a lot of efforts have been made on the organocatalysis and remarkable progress has been achieved [6-14]. However, the use of ketones as electrophilic partners, which provides access to chiral tertiary alcohols, still remains challenging due to their poor reactivity and difficulty in differentiating the two faces of the carbonyl moiety [15-17].

Organofluorine compounds are brought to the forefront as pharmaceuticals, agrochemicals, functional materials, or catalysts [18-21]. More than 20% of medicinal and agrochemical products consist of one or more fluorine atoms [22-24]. In the big family, α-trifluoromethyl tertiary alcohol compounds play an important role due to their bioactivities and stereoelectronic properties [25]. On the other hand, these compounds are important synthetic intermediates; they can be converted to α-amino acids, α-hydroxyl acids, oxiranes, and α-fluoro acids [26-31].

There is no doubt that the cross-aldol reactions of trifluoromethyl ketones with ketones could be considered as one of the most useful approaches for the construction of chiral trifluoromethyl tertiary alcohols. However, until recently, only a few cross-aldol reactions of trifluoromethyl ketones with ketones have been reported [32-43]. In 2005, Zhang and co-workers reported the first example of a proline-catalyzed asymmetric aldol addition of aryltrifluoromethyl ketones to methyl ketones, affording β-trifluoromethyl-β-hydroxyl ketones in satisfactory yields, with moderate enantioselectivity of up to 64% ee [32]. After that, other successful catalytic reaction systems with improved enantioselectivities have been disclosed by the Liu [33, 34], Yuan [35], Nakamura [36], Berkessel [37], Kokotos [38], Song [42] and Tanaka [43] groups with the use of organocatalysts.

Enzyme catalysis, as efficient and green biotransformation tools in organic synthesis, show immense advantages such as mild reaction conditions, simple

separation, good selectivity, high yields, etc [44-46]. Nowadays, a growing number of enzymes have been found to be capable to catalyze synthetic reactions which vary from their natural roles [47-49], and this phenomenon is considered as enzymatic promiscuity. In recent years, enzymatic promiscuity has been paid much attention by chemists and biochemists [50-52]. Some enzymes have exhibited their promiscuity through catalyzing the formation of C-C and C-heteroatom bonds [53, 54], such as the aldol reactions [55-57], Markovnikov additions [58], Michael additions [59], Mannich reactions [60], the asymmetric synthesis of α-aminonitrile amides [61], multi-component cascade or domino reactions, etc [62, 63]. Inspired by the pioneers' work, we wondered if it was possible to develop a novel and highly efficient catalytic system for the asymmetric aldol reactions of trifluoromethyl ketones with aliphatic ketones. After a wide screening of different hydrolases, we found that the direct asymmetric aldol reaction for the synthesis of α-trifluoromethyl tertiary alcohol derivatives could be catalyzed by papain (EC 3. 4. 22. 2), the most abundant cysteine proteinase, in the latex of the unripe fruit of *Carica papaya* [64]. Wide substrate scopes were also investigated. This finding provides a novel example of enzymatic promiscuity.

2. Results and discussion

Initial studies were undertaken using α,α,α-trifluoromethyl-phenylethanone and acetone as the model reaction. To confirm the specific catalytic effect of papain on the model reaction, some control experiments were performed (**Table 1**). These control experiments were carried out under the optimized conditions [details of the optimizations were described hereafter in the paper (from **Table 2** to **Table 5**)]. In the absence of papain, the reaction only gave the product in 5% yield (**Table 1**, entry 1). The reaction with papain provided the product in an excellent yield of 99% with 30% ee (**Table 1**, entry 2), indicating that papain preparation has a catalytic effect on the aldol reaction. Because the active site of papain consists of Cys, His and Asn residues, to further prove enzymatic activity, cysteine protease inhibitor methyl (methylsulfinyl)methyl sulfide (MMTS) [65] was used to denature papain by

irreversible covalent modification, and extra MMTS was then removed by dialysis against deionized water. The reaction with MMTS pretreated papain only gave the product in a low yield of 29% with 13% ee (**Table 1**, entry 4). Meanwhile, as a comparison, papain without modification was dialyzed against deionized water and then used to catalyze the model reaction, which gave a good yield of 73% with 15% ee (**Table 1**, entry 3). The results indicated that papain still maintained most activity even undergoing the dialysis process; the chemical modification of papain by MMTS indeed caused a serious loss of activity (**Table 1**, entries 3 and 4). The above experiments suggested that cysteine is crucial for the reaction. To further confirm the catalysis of papain on the model reaction, phenylmethylsulfonyl fluoride (PMSF), an irreversible inhibitor of serine/cysteine protease which inhibits most cysteine proteases like papain, was then used to denature papain, and the reaction with PMSF-pretreated papain only gave the product in a low yield of 18% with 1% ee (**Table 1**, entry 6) which indicated that PMSF strongly inhibited enzyme activity in the aldol reaction. Furthermore, diethyl pyrocarbonate (DEPC), a known modifier of the imidazole groups of histidine [66], was used to pretreat papain. And the DEPC-pretreated papain almost completely lost its catalytic activity on the aldol reaction (**Table 1**, entry 8). In addition, because metal ions can combine with enzymes irreversibly and disrupt the three-dimensional structure of enzymes, metal ions Ag^{+} and Cu^{2+} was used to pretreat papain. It can be seen that the metal-pretreated papain almost completely lost its catalytic activity on the aldol reaction (**Table 1**, entries 10 and 12). To exclude the effect of MMTS, PMSF, DEPC, Ag^{+} and Cu^{2+} on the model reaction, blank reactions were performed with the same amounts of the above mentioned inhibitors which only gave trace amounts of the product, respectively (**Table 1**, entries 5, 7, 9, 11 and 13). It was indicated that these inhibitors alone did not have noticeable effects on the model reaction. Above control experiments suggested that the native structure of papain is responsible for the aldol reaction and the catalysis may take place in the active site of enzyme.

To better understand the relationship between natural and promiscuous activities of papain, an enzymatic assay of papain on the hydrolysis of

N-benzoyl-L-argininethylester (BAEE) as natural activity was performed. The natural activity of native and inhibitor-pretreated papain was measured. The natural activity of untreated papain was 3.20 $U \cdot mg^{-1}$, which had the best catalytic activity on the aldol reaction (**Table 1**, entry 2). After dialysis, both the natural and unnatural activities decreased slightly (**Table 1**, entry 3). However, after pretreated by MMTS, PMSF, Ag^{+} and Cu^{2+}, both the natural and unnatural activities of the papain sharply decreased (**Table 1**, entries 4, 6, 10 and 12). The results 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 papain was also responsible for its activity on the aldol reaction.

Since medium is one of the most important factors influencing the enzymatic reactions, it is necessary to investigate the effect of different solvents on the papain-catalyzed aldol reaction. Some solvents with different log *P* values were screened and the results are shown in **Table 2**. In general, the reactions in solvents with log *P* values between -1.30 and 0.28, such as DMSO, DMF, isopropanol and acetone gave products in high yields of 97-99% (**Table 2**, entries 1-4). Relatively lower yields were obtained in the solvents with log *P* values between 0.49 and 3.20, such as THF, MTBE, 1,2-diCl-ethane, butyl acetate, toluene and cyclohexane (**Table 2**, entries 6, 8-10, 12 and 13). However, exceptions were observed for the reactions with MeCN and 1,4-dioxane, in which low yields were received even though their log *P* values are -0.33 and -1.10, respectively (**Table 2**, entries 7 and 11). The model reaction under solvent-free conditions gave a yield of 81% (**Table 2**, entry 5). The results clearly indicated that the reaction yield was significantly influenced by the reaction media. Unfortunately, no satisfactory enantioselectivity was achieved in all the tested solvents. Papain showed the relatively good stereoselectivity of 27% ee in DMF with an excellent yield of 97% (**Table 2**, entry 4). Thus, DMF was chosen as the optimum solvent for the papain-catalyzed aldol reaction. The absolute configuration of the product was assigned by comparing with the known chiral HPLC analysis [38,

42].

The existence of water is crucial for the enzyme-catalyzed reactions in organic solvents, as it influences the activity, stability of the enzymes and, probably, their conformational flexibility. Thus, different water activities were screened for the papain-catalyzed aldol reaction in the mixed solvent of DMF and water (**Table 3**). Obviously, water activity had an influence on the yield of the model reaction. The lowest yield of 66% with 23% ee was gained without adding water into the reaction system (**Table 3**, entry 1). When water was added to DMF, better results were obtained. Good yields of 92-98% were achieved with water activity ranged from 0.17 to 0.42 (**Table 3**, entries 2-5). When the water activity surpassed 0.47, the reaction gave decreased yields (**Table 3**, entries 6 and 7). The best yield of 98% with 28% ee was obtained with water activity value of 0.17 (**Table 3**, entry 2). Thus, water activity value of 0.17 was selected for further studies.

After that, the effect of mole ratio of substrates on the model reaction was investigated (The data is shown in supporting information). Among the performed experiments, when the mole ratio of **1a**/**2a** was 1:10, the product was obtained in an excellent yield of 98% with the ee of 28%. Thus, the mole ratio of 1:10 (**1a**/**2a**) was identified as the optimal ratio for further studies.

Then, in order to find out a suitable enzyme loading for the reaction, the effect of papain loading on the model reaction was investigated (**Table 4**). When 32 U of papain was used, the reaction only gave a moderate yield of 61% with 29% ee (**Table 4**, entry 1). Increasing the amount of papain to 96 U led to an excellent yield of 98% with 29% ee (**Table 4**, entry 3). However, higher papain loading did not give better results; the yield and ee almost kept constant (**Table 4**, entries 3 and 4). Thus, 96 U of papain was chosen as the optimum enzyme loading for the reaction system of trifluoromethyl ketone (0.6 mmol) and acetone (6.0 mmol) in a mixed solvent of DMF (0.95 mL) and water (0.05 mL).

Since pH of the reaction system and volume of medium may also affect both stereoselectivity and activity of a reaction. So, phosphate buffer saline (pH from 4.7 to 11.1) was used to replace the water in the reaction system (buffer/DMF = 5/95, v/v) to obtain the optimum reaction conditions. Using buffer did not give obviously better results. Thus, the deionized water was still used in the mixed medium for the reaction. The volume of medium was also screened, and 1.25 mL of medium volume was chosen as the optimum condition for further studies. The data is shown in supporting information.

Temperature is a crucial factor affecting stability of enzymes, as well as stereoselectivity and reaction rate of enzymatic reactions. Hence, it was essential to investigate the influence of temperature on the papain-catalyzed aldol reaction. As shown in **Table 5**, rising the temperature from 10 °C to 30 °C led to an increase of both yield and ee (**Table 5**, entries 1-5). Further increasing the temperature to 50 °C, the yield and ee changed to be steady (**Table 5**, entries 6-8). The model reaction exhibited the best yield of 99% with 30% ee at 30 °C (**Table 5**, entry 5). The blank reaction at 30 °C was also conducted, which only gave a low yield of 5% (**Table 5**, entry 9). Thus, 30 °C was selected as the optimal temperature.

With these optimized conditions in hand, we next evaluated the applicability scope of catalytic promiscuity embodied by papain. Some other substrates including various trifluoroacetophenones and ketones were investigated to expand upon the papain-catalyzed aldol reaction. The results are summarized in **Table 6**. To our delight, the reactions between acetone and various aryltrifluoromethyl ketones with either electron-rich or electron-deficient substituents gave products in excellent yields of 90-97% (**Table 6**, entries 1-6). However, when the phenyl ring was replaced by a perfluorophenyl moiety, the yield dropped significantly. Perfluoroacetophenone reacted with acetone gave an inferior yield of 42% (**Table 6**, entry 7). In the case of

perfluoroalkylphenyl ketones, the high yields of 95% and 96% were obtained (**Table 6**, entries 8 and 9). Even the CF_3 group was replaced by the less electron-withdrawing CF_2Cl group an excellent yield of 99% was still received (**Table 6**, entry 10). The substitutional-trifluoroacetone containing heteroatom reacting with acetone also resulted in an excellent yield of 92% (**Table 6**, entry 11). Besides acetone, some other aliphatic ketones were also used to react with trifluoroacetophenone. When 2-hydroxyacetone was utilized, a mixture of two diastereomers was obtained in almost equal amounts with the moderate yield of 70% (**Table 6**, entry 12). The reactions with 2-butanone and 2-pentanone gave acceptable yields of 72% and 75%, respectively (**Table 6**, entries 13 and 14). Methoxyacetone could also react with trifluoroacetophenone giving a yield of 56% (**Table 6**, entry 15). Moreover, this papain-catalyzed aldol reaction could be expanded to alkyltrifluoromethyl ketones. For instance, ethyl 4,4,4-trifluoroacetoacetate and ethyl trifluoropyruvate reacted with acetone giving corresponding products in moderate yields of 58% and 67%, respectively (**Table 6**, entries 16 and 17). Unfortunately, the enantioselectivities were not satisfactory; the ee values observed were no more than 30% (data are not shown in **Table 6**).

Furthermore, the kinetic parameters (Michaelis constant K_M, catalytic constant k_{cat} and k_{cat}/K_M) of the papain-catalyzed aldol reaction for each of the substrates in the model reaction were determined (**Table 7**). It can be seen that the K_M for each substrate was quite high, showing that the affinity of papain to non-native substrates was very poor. But the K_M of acetone (**2a**) was lower than trifluoromethyl ketone (**1a**), indicating that the affinity of papain with acetone is better than trifluoromethyl ketone. Based on these results, we speculate that the papain-catalyzed aldol reaction of acetone and trifluoromethyl ketone possibly takes place through the intermediate enolate ion, generated from acetone mediated by enzyme (as shown in the possible mechanism we proposed in **Scheme 1**). Meanwhile, both the k_{cat} and k_{cat}/K_M were very low, indicating that the reaction of papain for the promiscuous substrates was

very slow.

It is known that the active site residues Cys-25, His-159 and Asn-175 (papain numbering) are involved in the papain catalytic process for hydrolysis. Cysteine and histidine form the catalytic dyad in the active site of cysteine proteases. They are thought to be present as a thiolate-imidazolium ion pair Cys 25-S^-/His 159-Im^+H in the mature enzyme [67-69], which is stabilized by Asn-175 via keeping the imidazole ring of His-159 in a favorable orientation [70]. A three-dimensional X-ray study at a resolution of 2.8 Å has revealed that the single polypeptide chain of 212 residues is folded into two distinct parts which are divided by a cleft. The active site cysteine residue (Cys-25) is part of the L1 α-helix at the surface of the left domain, while the histidine (His-159) is in a β sheet at the surface of the right domain of the enzyme [71, 72]. Our control experiments suggested that the native structure of papain is responsible for the aldol reaction and the catalysis may take place in the active site of enzyme. In 2003, Berglund *et al.* reported an aldol reaction catalyzed by Lipase B *Candida antarctica* (CALB), in which the control reactions were run with covalently inhibited wild-type CALB using the inhibitor methyl p-nitrophenyl n-hexylphos-phonate. The inhibited enzyme did not show any lipase activity, and the specific activity for the aldol reaction was as that of albumin. Then they mutated the serine for a nonpolar residue alanine. The wild-type and the mutant enzymes were used to catalyze the aldol reaction between aldehydes and ketones, 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 [73], and serine does not directly participate in the catalytic process. Based on the mechanism of the lipase CALB catalyzed aldol reaction proposed by Berglund *et al.* and our previous work on the direct asymmetric aldol reaction of aldehydes with ketones catalyzed by chymopapain, a papain-like cysteine protease [74], we hypothesized a mechanism for

the papain-catalyzed aldol reaction (**Scheme 1**). Firstly, the ketone is deprotonated by His residue forming an enolate anion. Secondly, another substrate trifluoromethyl ketone combines with the enolate anion and accepts a proton from imidazolium cation leading to the aldol adduct. Eventually, the product is released from the active site.

3. Conclusion

In summary, we developed a novel synthetic methodology for the synthesis of functionalized trifluoromethyl carbinol derivatives via enzyme-catalyzed aldol reaction of trifluoromethyl ketones and aliphatic ketones in DMF/H_2O system under mild reaction conditions. Papain was used as an economically feasible and environmentally friendly biocatalyst. Yields of up to 99% and ee of up to 30% were achieved from readily available starting materials without using any additives. Although the enantioselectivities were not satisfied, to the best of our knowledge, this is the first example of enzyme-catalyzed aldol reaction between trifluoromethyl ketones and aliphatic ketones. The use of ketones as electrophilic partners to provide access to chiral tertiary alcohols is challenging due to their poor reactivity and difficulty in differentiating the two faces of the carbonyl moiety. This work expands the application of enzyme in organic synthesis, and may be a useful building block for future study on papain catalytic promiscuity. Using the abundant papain as a catalyst could be developed as a potentially valuable method which can be a complement to the traditional chemical catalysis.

4. Experimental section

4.1 General procedure for the papain-catalyzed aldol reaction

To a 10 mL round bottom flask was added with trifluoromethyl ketone (0.6 mmol), ketone (6.0 mmol), DMF (1.19 mL), deionized water (0.06 mL) and papain (96 U). The flask was covered with a rubber stopper. The resultant mixture was stirred on a magnetic stirrer for the specified time at 30 °C, and monitored by TLC [petroleum ether/ethyl acetate (V_{PE}/V_{EA} = 3:1)]. The reactions were terminated by filtering the enzyme. The ethyl acetate was employed to wash the residue on the filter paper to

assure that products obtained were all dissolved in the filtrate. The filtrate was washed with water for three times, and then dried over anhydrous Na_2SO_4. The organic solvent was then removed under reduced pressure. The crude products were purified by silica gel column chromatography with petroleum ether/ethyl acetate as eluent (V_{PE}/V_{EA} = 7:1 to 10:1).

4.2 Materials

Papain was purchased from Sigma-Aldrich, Shanghai, China. Papain from *Carica papaya* (76220-25G, Lot# BCBD3116V, 3.6 units/mg solid), unit definition: one unit corresponds to the amount of enzyme which hydrolyzes 1 μmol N-benzoyl-L-argininethylester (BAEE) per minute at pH 6.2 and 25 °C. Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification.

4.3 Analytical Methods

Reactions were monitored by thin-layer chromatography (TLC) with Haiyang GF254 silica gel plates (Qingdao Haiyang chemical industry Co Ltd, Qingdao, China) using UV light and vanillic aldehyde as visualizing agents. Flash column chromatography was performed using 200-300 mesh silica gel at increased pressure. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker-AM 600 (600 MHz) (Bruker BioSpin AG Ltd., Beijing, China). Chemical shifts were reported in ppm from TMS with the solvent resonance as the internal standard. Data were reported as follows: chemical shifts (δ) in ppm, coupling constants (*J*) in Hz, and solvent ($CDCl_3$). High-resolution mass spectra were obtained by using ESI ionization sources (Varian 7.0 T FTICR-MS). Melting points were taken on a WPX-4 apparatus and were uncorrected (Yice instrument equipment Co Ltd, Shanghai). The enantiomeric excesses (ee) of aldol products were determined by chiral HPLC analysis performed using Chiralpak AD-H, AS-H, Chiralcel OJ-H, OD-H columns (Daicel Chiral Technologies CO., LTD.; Shanghai, China). Saturated vapor pressure was determined by a DPCY-2C apparatus (company of NJUWH, Nanjing, China). Absolute

configurations of the products were determined by comparing with the known chiral HPLC analysis. All the aldol products are known compounds except **3c** and **3o** (for details about references, ^{1}H NMR and ^{13}C NMR of **3a-q**, HRMS of **3c** and **3o,** and HPLC charts of **3a**, please see the supporting information)

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References

[1] Review: B. M. Trost, C. S. Brindle, Chem. Soc. Rev. 39 (2010) 1600-1632.

[2] W. Notz, B. List, J. Am. Chem. Soc.122 (2000) 7386-7387.

[3] K. Sakthivel, W. Notz, T. Bui, C. F. Barbas, J. Am. Chem. Soc. 123 (2001) 5260-5267.

[4] A. Cordova, W. Notz, C. F. Barbas, J. Org. Chem.67 (2002) 301-303.

[5] A. B. Northrup, D. W. C. MacMillan, J. Am. Chem. Soc. 124 (2002) 6798-6799.

[6] R. Mahrwald, Modern Methods in Stereoselective Aldol Reactions; Wiley-VCH: Weinheim. 2013.

[7] B. M. Trost, C. S. Brindle, C. S. Chem. Soc. Rev. 39 (2010) 1600-1632.

[8] H. Torii, M. Nakadai, K. Ishihara, S. Saito, H. Yamamoto, Angew. Chem. Int. Ed. 43 (2004) 1983-1986.

[9] J. Franzen, M. Marigo, D. Fielenbach, T. C. Wabnitz, A. Kjaersgaard, K. A. Jorgensen, J. Am. Chem. Soc. 127 (2005) 18296-18304.

[10] K. Liu, H.-F. Cui, J. Nie, K.-Y. Dong, X.-J. Li, J.-A. Ma, Org. Lett. 9 (2007) 923-925.

[11] Y. Hayashi, T. Itoh, S. Aratake, H. Ishikawa, Angew. Chem. Int. Ed. 47 (2008) 2082-2084.

[12] K. Nakayama, K. Maruoka, J. Am. Chem. Soc. 130 (2008) 17666-17667.

[13] X. Ma, C.-S. Da, L. Yi, Y.-N. Jia, Q.-P. Guo, L.-P. Che, F.-C. Wu, J.-R.Wang, W.-P. Li, Tetrahedron: Asymmetry. 20 (2009) 1419-1424.

[14] J.-H. Lin, C.-P. Zhang, J.-C. Xiao, Green Chem. 11 (2009) 1750-1753.

[15] G. Guillena, C. Najera, D.-J. Ramon, Tetrahedron: Asymmetry. 18 (2007) 2249-2293.

[16] S. Adachi, T. Herada, Eur. J. Org. Chem. (2009) 3661-3671.

[17] W.-B. Chen, X.-L. Du, L.-F. Cun, X.-M. Zhang, W.-C. Yuan, Tetrahedron. 66 (2010) 1441-1446.

[18] R. Filler, Y. Kobayashi, L. N. Yagupolskii, Eds. Compounds in Medicinal and Biochemical Applications; Elsevier: Amsterdam. 1993.

[19] T. Hiyama, Ed. : Chemistry and Applications; Springer: New York. 2000.

[20] P. Kirsch, Modern Fluoroorganic Chemistry: Synthesis, Reactivity, Application; Wiley-VCH: Weinheim. 2004.

[21] S. Purser, P.-R. Moore, S. Swallow, V. Gouverneur, Chem. Soc. Rev. 37 (2008) 320- 330.

[22] K.-S. Ritter,Chem. Eng. News. 90 (2012) 10-17.

[23] P. Zhang, C. Wolf, J. Org. Chem. 77 (2012) 8840-8844.

[24] K. Muller, C. Faeh, F. Diederich, Science. 317 (2007) 1881-1886.

[25] J. Nie, H.-C. Guo, D. Cahard, J.-A. Ma, Chem. Rev. 111 (2011) 455-529.

[26] E. J. Corey, J. O. Link, J. Am. Chem. Soc. 114 (1992) 1906-1908.

[27] A. P. Khrimian, J. E. Oliver, R. M. Waters, S. Panicker, J. M. Nicholson, J. A. Klun, Tetrahedron: Asymmetry. 7 (1996) 37- 40.

[28] T. Fujisawa, T. Ito, K. Fujimoto, M. Shimizu, H. Wynberg, E. G. J. Staring, Tetrahedron Lett. 38 (1997) 1593-1596.

[29] Z. Wang, S. Campagna, K. Yang, G. Xu, M. E. Pierce, J. M. Fortunak, P. N. Confalone, J. Org. Chem. 65 (2000) 1889-1891.

[30] B. Jiang, Y.-G. Si, Adv. Synth. Catal. 346 (2004) 669-674.

[31] R. Bejot, S. Tisserand, L. M. Reddy, D. K. Barma, R. Baati, J. R. Falck, C. Mioskowski, Angew. Chem. Int. Ed. 44 (2005) 2008-2011.

[32] L.-H. Qiu, Z.-X. Shen, C.-Q. Shi, Y.-H. Liu, Y.-W. Zhang, Chin. J. Chem. 23

(2005) 584-588.

[33] X.-J. Wang, Y. Zhao, J. T. Liu, Org. Lett. 9 (2007) 1343-1345.

[34] X.-J. Wang, Y. Zhao, J.-T. Liu, Synthesis. 24 (2008) 3967-3973.

[35] D.-H. Zhang, C.-Y. Yuan, Tetrahedron. 64 (2008) 2480-2488.

[36] N. Hara, R. Tamura, Y. Funahashi, S. Nakamura, Org. Lett. 13 (2011) 1662-1665.

[37] N. Duangdee, W. Harnying, G. Rulli, J. M. Neudorfl, H. Groger, A. Berkessel, J. Am. Chem. Soc. 134 (2012) 11196-11205.

[38] C. G. Kokotos, J. Org. Chem. 77 (2012) 1131-1135.

[39] Y. Zheng, H.-Y. Xiong, J. Nie, M.-Q. Hua, J.-A. Ma, Chem. Commun. 48 (2012) 4308-4310.

[40] J. Lin, T.-R. Kang, Q.-Z. Liu, L. He, Tetrahedron: Asymmetry. 25 (2014) 949-955.

[41] W. Yang, Y.-M. Cui, W. Zhou, L. Li, K.-F. Yang, Z.-J. Zheng, Y.-X. Lu, L.-W. Xu, Synlett. 25 (2014) 1461-1465.

[42] H. Zong, H.-Y. Huang, G. L. Bian, L. Song. J. Org. Chem. 79 (2014) 11768-11773.

[43] D. X. Zhang , F. Tanaka. Adv. Synth. Catal. 357 (2015) 3458-3462.

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

[45] S. Akai, K. Tanimoto , Y. Kita, Angew. Chem. Int. Ed. 43 (2004) 1407-1410.

[46] S.-J. Chai, Y.-F. Lai, J.-C. Xu, H. Zheng, Q. Zhu , P.-F. Zhang, Adv. Synth. Catal. 353 (2011) 580-582.

[47] O. P. Ward, A. Singh, Curr. Opin. Biotechnol. 11 (2000) 520-526.

[48] Babtie, A.; Tokuriki, N.; Hollfelder, F. Curr. Opin. Chem. Biol. 14 (2010) 200.

[49] M. S. Humble, P. Berglund, Eur. J. Org. Chem. (2011) 3391-3401.

[50] A. M. Klibanov, Nature. 409 (2001) 241-246.

[51] Q. Wu, B.-K. Liu , X.-F. Lin, Curr. Org. Chem. 14 (2010) 1966-1988.

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

[53] E. Busto, V. Gotor-Fernandez , V. Gotor, Chem. Soc. Rev. 39 (2010) 4504-4523.

[54] I. Nobeli, A. D. Favia , J. M. Thornton, Nat. Biotechnol. 27 (2009) 157-167.

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

616-618.

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

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

[58] W.-B. Wu, J.-M. Xu, Q. Wu, D.-S. Lv , X.-F. Lin, Adv. Synth. Catal. 348 (2006) 487-492.

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

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

[61] P. Vongvilai, M. Linder, M. Sakulsombat, M. S. Humble, P. Berglund, T. Brinck, O. Ramström, Angew. Chem. Int. Ed. 50 (2011) 6592-6595.

[62]Y.-H. He, T. He, J.-T. Guo, R. Li,Y. Xiang, Z. Guan, Catal. Sci. Technol. 6 (2016) 2239-2248.

[63] L.-P. Li, X. Cai, Y. Xiang,Y. Zhang, J. Song, D.-C. Yang, Z. Guan , Y.-H. He, Green Chem. 17 (2015) 3148-3156.

[64] S. Zucker, D. J. Buttle, M. J. H. Nicklin, A. J. Barrett, Biochim. Biophys. Acta. 828 (1985) 196-204.

[65] M. St-Vincent, M. Dickman, J. Chem. Educ. 81 (2004) 1048-1050.

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

[67]A. C. Storer, R. Menard, Methods in Enzymology. 244 (1994) 486-500.

[68] H.H. Otto, T. Schirmeister, Chem. Rev. 97 (1997) 133-171.

[69]L. Polgar. Handbook of proteolytic Enzymes, 2nd ed, London: Elsevier. (2004) 1072-1079.

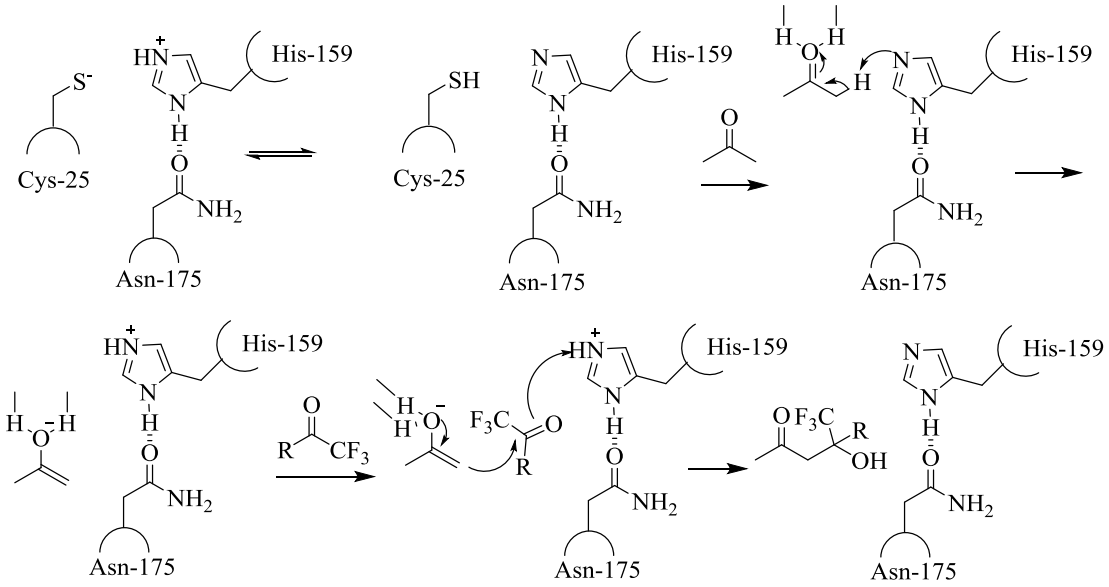
[70] M.J. Harrison, N.A. Burton, I.H. Hillier, J. Am. Chem. Soc. 119 (1997) 12285-12291.

[71] J. Drenth, J. N. Jaansonius, R. Koekoek, H. M. Swen , B.G. Wolthers, Nature. 218 (1968) 929-932.

[72] I. G. Kamphuis, K. H. Kalk, M. B. A. Swarte, J. Drenth, J. Mol. Biol. 17 (1984) 233-256.

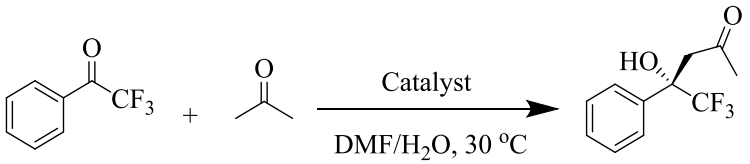
[73] C. Branneby, P. Carlqvist, A. Magnusson, K. Hult, T. Brinck, P. Berglund, J. Am. Chem. Soc. 125 (2003) 874-875.

[74] Y.-H. He, H.-H. Li, Y.-L. Chen, Y. Xue, Y. Yuan, Z. Guan, Adv. Synth. Catal. 354 (2012) 712-719.



Scheme 1 Possible mechanism for the papain-catalyzed aldol reaction.

Table 1. Control experiments[a].



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

Entry	Catalyst	Yield (%)[b]	Ee (%)[c]	Natural activity ($U \cdot mg^{-1}$)[d]
1	No enzyme	5	0	--
2	Papain	99	30	3.20
3	Papain[e]	73	15	2.30
4	Papain (pretreated with 3.68 M MMTS)[f]	29	13	0.47
5	MMTS (0.9 mL)	Trace	--	--
6	Papain (pretreated with 0.43 M PMSF)[g]	18	1	0.28
7	PMSF (75 mg)	Trace	--	--
8	Papain (pretreated with 0.3 M DEPC)[h]	Trace	--	--
9	DEPC (43.3 μL)	Trace	--	--
10	Papain (pretreated with 0.25 M Ag^{+})[i]	Trace	--	0.19
11	$AgNO_3$ (42.5 mg)	Trace	--	--
12	Papain (pretreated with 0.25 M Cu^{2+})[j]	Trace	--	0.19
13	$CuSO_4$ (39.9 mg)	Trace	--	--

[a] Unless otherwise noted, reaction conditions: a mixture of trifluoromethyl ketone (0.6 mmol), acetone (6.0 mmol), papain (96 U), DMF (1.19 mL), deionized water (0.06 mL) at 30 °C for 72 h..

[b] Yield refers to the isolated yield.

[c] Ee was determined by HPLC analysis using a chiral column (OD-H).

[d] Definition of enzyme activity ($U \cdot mg^{-1}$): one unit corresponds to the amount of enzyme which hydrolyzes 1 μmol N-benzoyl-L-argininethylester (BAEE) per minute at pH 6.2 and 25 °C.

[e] Papain was dialyzed against deionized water and then water was removed by lyophilization.

[f] Papain (96 U) in MMTS solution (3.68 M) (0.9 mL MMTS in 1.5 mL MeCN) was stirred at rt for 1 h, and then the 1 mL pH 5.0 acetic buffer was added. After stirred at rt for 12 h the mixture was dialyzed against deionized water, and water was removed

by lyophilization.

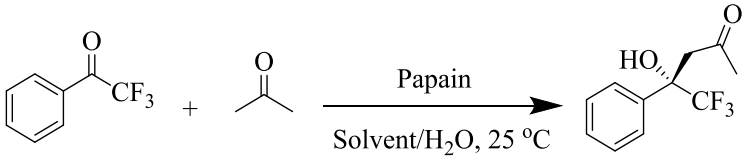
[g] Papain (96 U) in PMSF solution (0.43M) (75 mg PMSF in 1.0 mL dried THF) was stirred at rt for 12 h, and then organic solvent was removed under reduced pressure.

[h] To papain (96 U) in phosphate buffer solution (NaH_2PO_4-Na_2HPO_4, pH 8.04) (1 mL) was added DEPC (0.3 mmol). The mixture was stirred at 37 ^oC for 2 h, and then water was removed by lyophilization.

[i] Papain (96 U) in Cu^{2+} solution (0.25 M) (39.9 mg $CuSO_4$ in 1.0 mL deionized water) was stirred at rt for 12 h, and then water was removed by lyophilization.

[j] Papain (96 U) in Ag^+ solution (0.25 M) (42.5 mg $AgNO_3$ in 1.0 mL deionized water) was stirred at rt for 12 h, and then water was removed by lyophilization.

Table 2. Effect of solvents on the papain-catalyzed aldol reaction [a].



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

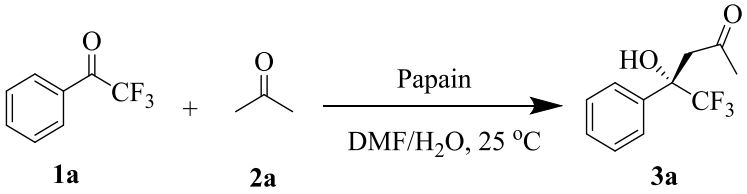
Entry	Solvent	Log *P*	Yield (%)[b]	Ee (% [c]
1	DMSO	-1.30	99	24
2	Isopropanol	0.28	99	24
3	Acetone	-0.23	99	23
4	DMF	-1.00	97	27
5	Solvent-free	--	81	25
6	Cyclohexane	3.20	67	28
7	MeCN	-0.33	60	27
8	MTBE	0.90	56	20
9	THF	0.49	42	23
10	1,2-DiCl-ethane	1.50	41	17
11	1,4-Dioxane	-1.10	40	19
12	Toluene	2.50	36	16
13	Butyl acetate	1.70	31	17

[a] The reactions were conducted using trifluoromethyl ketone (0.6 mmol), acetone (6.0 mmol), papain (160 U), solvent (0.9 mL), deionized water (0.1 mL) at 25 °C for 72 h.

[b] Yield refers to the isolated yield.

[c] Ee was determined by HPLC analysis using a chiral column (OD-H).

Table 3. Effect of water activity on the papain-catalyzed aldol reaction [a].



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

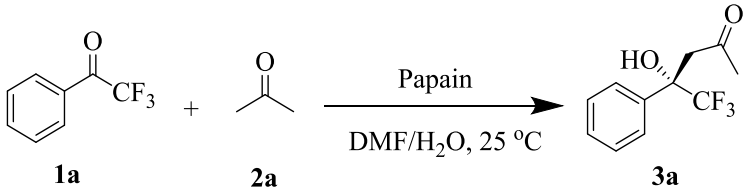
Entry	Water / DMF (v/v)	Water activity	Yield (%)[b]	Ee (%)[c]
1	0/1.00	--	66	23
2	0.05/0.95	0.17	98	28
3	0.10/0.90	0.27	97	27
4	0.15/0.85	0.34	95	25
5	0.20/0.80	0.42	92	26
6	0.25/0.75	0.47	84	23
7	0.30/0.70	0.54	76	23

[a] The reaction conditions were as follows: trifluoromethyl ketone (0.6 mmol), acetone (6.0 mmol), papain (160 U), DMF (1.00-0.70 mL), deionized water (0-0.30 mL) at 25 °C for 72 h.

[b] Yield refers to the isolated yield.

[c] Ee was determined by HPLC analysis using a chiral column (OD-H).

Table 4. Effect of enzyme loading on the papain-catalyzed aldol reaction [a].



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

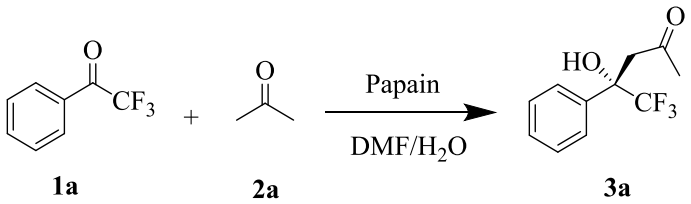
Entry	Papain loading (U)	Yield (%)[b]	Ee (%)[c]
1	32	61	29
2	64	86	30
3	96	98	29
4	128	98	29

[a] The reaction conditions were as follows: trifluoromethyl ketone (0.6 mmol), acetone (6.0 mmol), papain (32-128 U), DMF (0.95 mL), deionized water (0.05 mL) at 25 °C for 72 h.

[b] Yield refers to the isolated yield.

[c] Ee was determined by HPLC analysis using a chiral column (OD-H).

Table 5. Effect of temperature on the papain-catalyzed aldol reaction [a].



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

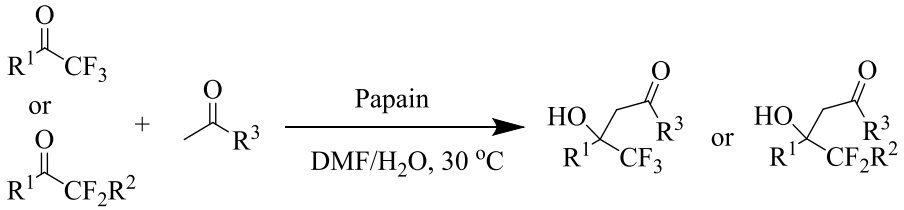
Entry	T (°C)	Yield (%)[b]	Ee (%)[c]
1	10	25	3
2	15	54	17
3	20	78	23
4	25	99	29
5	30	99	30
6	35	98	29
7	40	97	30
8	50	96	29
9	30 (no enzyme)	5	--

[a] The reactions conditions were as follows: trifluoromethyl ketone (0.6 mmol), acetone (6.0 mmol), papain (96 U), DMF(1.19 mL) , deionized water (0.06 mL) at 10-50 °C for 72 h.

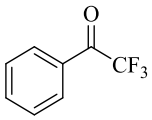
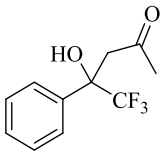
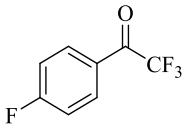
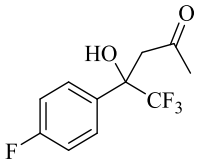
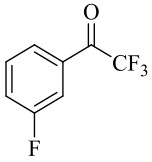
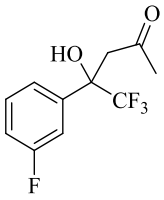
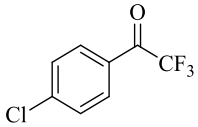
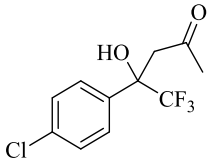
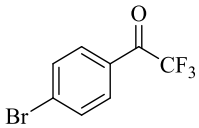
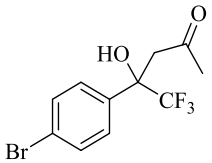
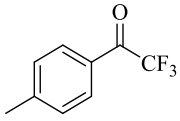
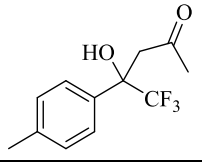
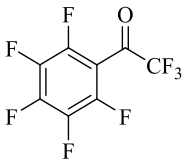
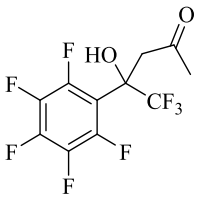
[b] Yield refers to the isolated yield.

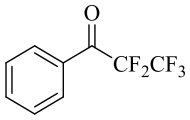
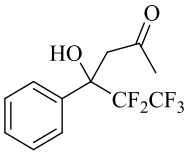
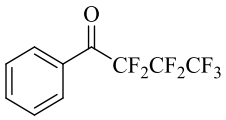
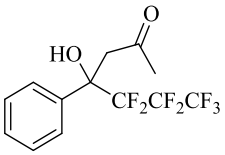
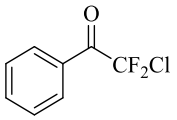
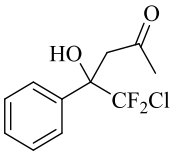
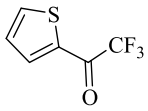
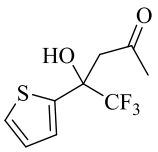
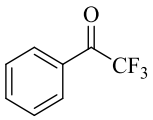
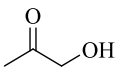
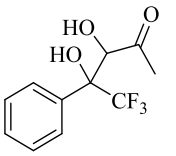
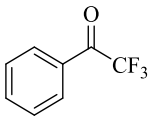
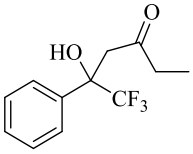
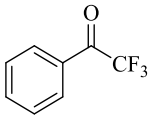
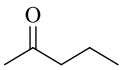
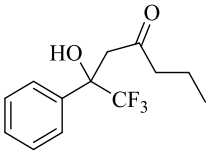
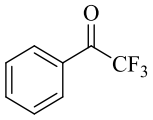
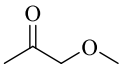
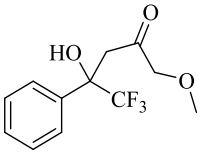
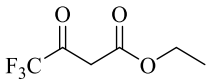
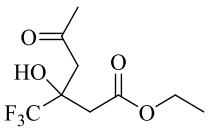
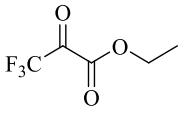
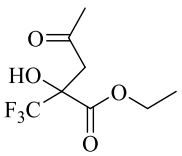
[c] Ee was determined by HPLC analysis using a chiral column (OD-H).

Table 6. Substrate scope of the papain-catalyzed aldol reaction [a].



1 **2** **3**

Entry	**1**	**2**	No.	Product	Time (h)	Yield (%)[b]
1			**3a**		48	97
2			**3b**		48	90
3			**3c**		48	95
4			**3d**		48	96
5			**3e**		48	94
6			**3f**		48	97
7[c]			**3g**		72	42

8			**3h**		48	95
9			**3i**		48	96
10			**3j**		48	99
11			**3k**		48	92
12[d]			**3l**		72	70
13[c]			**3m**		72	72
14[c]			**3n**		72	75
15[c]			**3o**		72	56
16[c]			**3p**		72	58
17[c]			**3q**		72	67

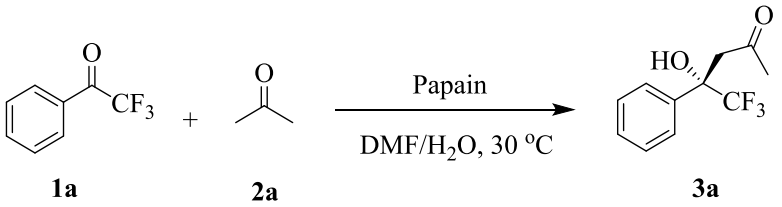
[a] Unless otherwise noted, reaction conditions: a mixture of **1** (0.6 mmol), **2** (6.0 mmol), papain (96 U), DMF (1.19 mL), deionized water (0.06 mL) at 30 °C.

[b] Yield refers to the isolated yield.

[c] Papain loading of 192 U.

[d] Papain loading of 192 U, dr (52:48) was determined by ^{1}H NMR and HPLC (chiral column AS-H).

Table 7. Kinetic parameters for the papain-catalyzed aldol reaction [a].



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

Entry	Substrate	K_M (M)	k_{cat} (h^{-1})	k_{cat}/K_M (M^{-1} h^{-1})
1[b]	Trifluoromethyl ketone (**1a**)	0.102	98.5	965.7
2[c]	Acetone (**2a**)	0.051	65.0	1274.5

[a] The reactions were carried out in DMF (0.24 mL) and deionized water (0.01 mL) at 30 °C for 1 h; the kinetic parameters were obtained at the enzyme concentration of 1.04 mM. The experiments were based on HPLC determination of the product.

[b] The concentration of **1a** varied from 0.10 M to 0.58 M, with **2a** (4.80 M).

[c] The concentration of **2a** varied from 0.12 M to 0.72 M, with **1a** (0.48 M).