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

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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.

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

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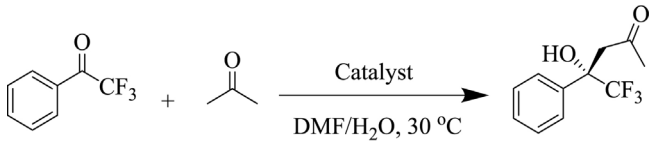
Table 1
Control experiments[a].



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

Entry	Catalyst	Yield (%)[b]	Ee (%)[c]	Natural activity (U·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	–	–

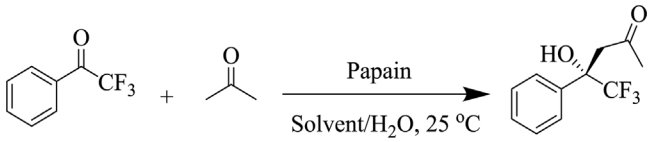
[a] Unless otherwise noted, reaction conditions: a mixture of trifluoromethyl ketone (0.6 mmol), acetone (6.0 mmol), papain (96 U), DMF (1.19 mL), deionized water (0.06 mL) at 30 °C for 72 h.
[b] Yield refers to the isolated yield.
[c] Ee was determined by HPLC analysis using a chiral column (OD-H).
[d] Definition of enzyme activity (U 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 (0.43 M) (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 °C 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.

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-trifluoromethylphenylethanone 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

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).