Lipase-catalysed decarboxylative aldol reaction and decarboxylative Knoevenagel reaction†

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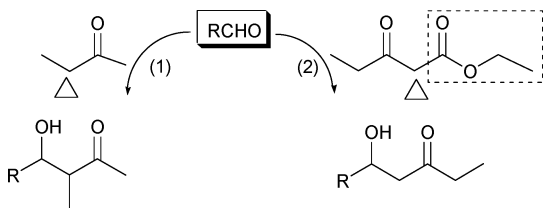
Received 21st July 2009, Accepted 1st September 2009
First published as an Advance Article on the web 14th September 2009
DOI: 10.1039/b914653a

Acrylic resin immobilized *Candida antarctica* lipase B (CAL-B) is able to catalyse decarboxylative aldol reaction and decarboxylative Knoevenagel reaction with good to excellent yields.

Biocatalysis is an efficient and green tool for modern organic synthesis due to its high selectivity and mild conditions.[1] Recently, a new frontier in biocatalysis is catalytic promiscuity, which is mainly exploring the catalytic activities of enzymes on the unnatural substrates or alternative reactions.[2] Biocatalytic promiscuity provides new tools for organic synthesis, and thus expands largely the application of enzymes. To the best of our knowledge, some promiscuous biocatalytic basic reactions such as aldol[3a–b] and Mannich condensations[3c] and Michael[3d–e] and Markovnikov additions[3f] have been reported. However, more complex and useful reaction systems are relatively scarce. Hilvert and coworkers reported that oxaloacetate went through decarboxylation and then an aldol reaction with aldehydes in the presence of macrophomate synthase.[4] Ohta *et al.* demonstrated a decarboxylase catalysed intramolecular aldol reaction after a decarboxylation process first.[5] Nevertheless, limited industrial outputs of these enzymes restrict their large-scale application. Utilizing the promiscuity of widely-used hydrolases to accomplish some novel processes becomes interesting and challenging.

As one of the important carbon–carbon bond formation reactions in organic synthesis, the decarboxylative aldol reaction provides a good protocol for regioselective aldol reactions (Scheme 1). Though lots of efforts have been contributed, the most common approaches require strong bases[6] or metal catalysts.[7] Thus, the application of enzymes will effectively circumvent the harsh reaction conditions.



Scheme 1 (1) General aldol reaction. (2) Decarboxylative aldol reaction, a good protocol for regioselective aldol reaction.

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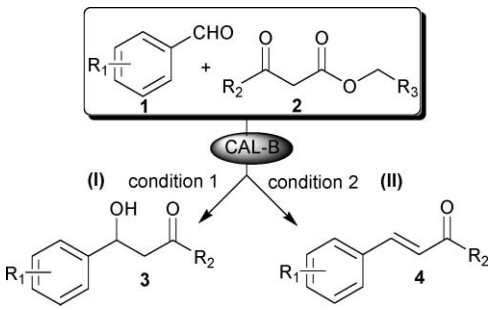
† Electronic supplementary information (ESI) available: Experiment details, characterization and spectra. See DOI: 10.1039/b914653a

In continuation of our work with enzymatic synthetic methodologies, we report herein two environmentally-benign decarboxylative addition protocols. CAL-B was found to be able to catalyse decarboxylative aldol reactions and decarboxylative Knoevenagel reactions. Good substrate scopes were obtained, as well as yields (Scheme 2).



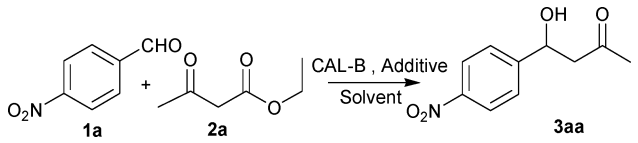
Scheme 2 (I) CAL-B catalysed decarboxylative aldol reaction. (II) CAL-B catalysed decarboxylative Knoevengel reaction

Initially, we focused on demonstrating the specific catalytic effect of the CAL-B in the decarboxylative aldol reaction by performing some control experiments. The reaction of 4-nitrobenzaldehyde (**1a**) with ethyl acetoacetate (**2a**) in the absence of CAL-B led to no product being detected, even after two weeks. When the reactants were incubated with denatured CAL-B or bovine serum albumin (B.S.A.), it was equal to the background reaction, suggesting that the tertiary structure of the enzyme was necessary and the effect of the polymeric support was excluded (for details, see ESI).†

For optimization of the decarboxylative aldol reaction conditions, the reaction of **1a** and **2a** was chosen as a model. The results are summarized in Table 1. To our delight, a very interesting result was obtained using an amine as additive. The best results were obtained in up to 96% yield with 10 mol% 1,4,7,10-tetraazacyclododecane (cyclen) added in acetonitrile after 20 h. No product was detected only using cylen in the absence of CAL-B, it showed that cyclen may cooperate with CAL-B to promote the reaction. Though CAL-B showed high activity in this decarboxylative aldol reaction, the product **3aa** exhibited little enantioselectivity, which was similar to other promiscuous CAL-B catalyzed reactions.[3a,3d]

Subsequently, some substituted aromatic aldehydes and β-ketoesters were tested under the optimized conditions, the results are shown in Table 2. Though only aldehydes with strong electron-withdrawing group can take part in the reaction (Table 2, entry 1–3), β-ketoesters have an extensive scope (Table 2, entry 4–11), and good to excellent yields were obtained.

Table 1 Reaction conditions optimization for decarboxylative aldol reaction[a]



Entry	Solvent	Additive	t (h)	Yield (%)[b]
1	$CHCl_3$	—	72	25
2	Toluene	—	72	28
3	*t*-BuOMe	—	72	53
4	THF	—	72	74
5	CH_3CN	—	72	83
6	CH_3CN	Et_3N	30	87
7	CH_3CN	Cyclen[d]	20	96 (0)[c]

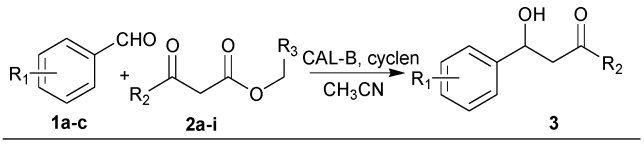
[a] Reaction conditions: **1a** (0.1 mmol), **2b** (0.12 mmol), CAL-B 10 mg, additive 10% mol, solvent 0.5 mL, 30 °C. [b] Isolated yields. [c] The value given in parenthesis refers to yield without CAL-B. [d] Cyclen = 1,4,7,10-tetraazacyclododecane.

Substrate **2a** and **2b** indicated that group R_3 had no obvious effect on the reaction (Table 2, entries 1 and 4). They had the same product, and both of them obtained excellent yields. When the methylene of *β*-ketoesters was substituted by ethyl, the reaction didn't take place (Table 2, entry 8), whereas high yield was observed for substrate **2g** due to reduction of the steric hindrance (Table 2, entry 9), indicating that the reaction system was sensitive to steric effects.

Interestingly, when a primary amine such as aniline, *p*-toluidine and benzylamine was added to the decarboxylative aldol reaction, *α*, *β*-unsaturated ketones were obtained as the final products due to the occurrence of the decarboxylative Knoevenagel reaction. After some optimization, we found a good result using water as cosolvent (Table S1, ESI).† The best results were obtained in 90% yield with 5% (v/v) water added in acetonitrile. We next investigated a series of substituted aromatic aldehydes and *β*-ketoesters, and this time a wide range of aromatic aldehydes were able to take part in the reaction (Table 3, entry 1–9). A Schiff base was formed firstly as a result of adding aniline. As the reactivity of the Schiff base is higher than aldehyde, so good substrate scope was obtained. The yields of aldehydes with electron-withdrawing groups were higher than those with electron-donating groups. Also, no product was observed for **2f** due to steric effects. In contrast, diethyl malonate and ethyl cyanoacetate were also tested, which are often used in Knoevenagel reactions, but no corresponding products were formed. It suggested that the structure of the *β*-ketoester was necessary for CAL-B catalysed decarboxylative Knoevenagel reactions. As an effective immobilized hydrolase, CAL-B is frequently applied in the pharmaceutical industry. It was easy to recycle from the reaction system and makes full use of its catalytic function. When CAL-B was reused for five times in the decarboxylative Knoevenagel reaction, the yield of product only decreased slightly (Fig S1).†

To realize the reaction mechanism, we first performed the experiment using acetone instead of ethyl acetoacetate, no product was detected. It proved indirectly that decarboxylation was not occurring before addition; then a tentative reaction mechanism was proposed. CAL-B catalysed aldol condensation

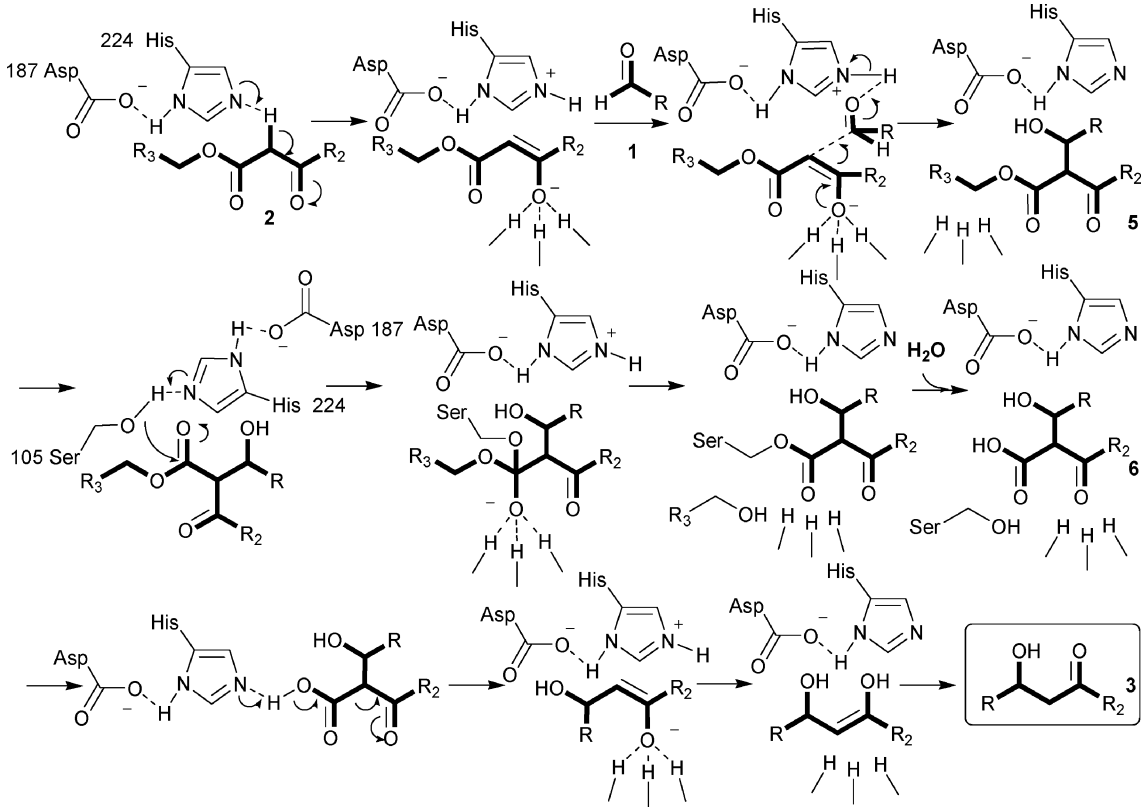
Table 2 Decarboxylative aldol reaction of aromatic aldehydes and *β*-ketoesters[a]



Entry	1	2	3	Yield (%)[b]
1	**1a** *p*-NO_2	**2a**	**3aa**	96
2	**1b** *o*-NO_2	**2a**	**3ba**	81[c]
3	**1c** *m*-NO_2	**2a**	**3ca**	87[c]
4	**1a**	**2b**	**3ab**	97
5	**1a**	**2c**	**3ac**	95
6	**1a**	**2d**	**3ad**	94
7	**1a**	**2e**	**3ae**	91
8	**1a**	**2f**	—	N.R.
9	**1a**	**2g**	**3ag**	95
10	**1a**	**2h**	**3ah**	87[c]
11	**1a**	**2i**	**3ai**	85[c]

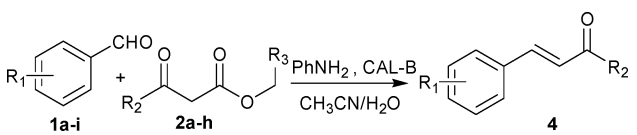
[a] Reaction conditions: **1** (0.1 mmol), **2** (0.12 mmol), cyclen (0.01 mmol), CAL-B 10 mg, CH_3CN 0.5 mL, 30 °C, 20 h. [b] Isolated yields. [c] 30 h. N.R. means no reaction.

between **1** and **2**, then a sequential process of hydrolysis and decarboxylation was carried out (Scheme 3). Firstly, **5** was generated through an aldol condensation after the enol form of substrate **2** was stabilized by the Asp-His dyad and oxyanion hole in the active site. Then the Asp-His-Ser triad catalysed the hydrolysis of **5** to get acid **6**. Subsequently, carbon dioxide was released from **6** after an electron transfer process under the influence of the Asp-His dyad and oxyanion hole. Then the final product **3** was formed. For decarboxylative Knoevenagel reactions, a Schiff base was formed firstly, then



Scheme 3 Proposed mechanism of decarboxylative aldol reaction

Table 3 Decarboxylative Knoevenagel of aromatic aldehydes and *β*-ketoesters[a]



Entry	1	2	4	t (h)	Yield (%)[b]
1	**1a** *p*-NO_2	**2a**	**4aa**	12	90
2	**1b** *o*-NO_2	**2a**	**4ba**	24	85
3	**1c** *m*-NO_2	**2a**	**4ca**	24	88
4	**1d** *p*-CN	**2a**	**4da**	24	89
5	**1e** H	**2a**	**4ea**	24	62
6	**1f** *p*-CH_3	**2a**	**4fa**	24	81
7	**1g** *p*-CH_3O	**2a**	**4ga**	24	72
8	**1h** *o*-CH_3O	**2a**	**4ha**	24	58
9	**1i** *m*-CH_3O	**2a**	**4ia**	24	56
10	**1a**	**2b**	**4ab**	12	91
11	**1a**	**2c**	**4ac**	24	88
12	**1a**	**2d**	**4ad**	24	86
13	**1a**	**2e**	**4ae**	24	82
14	**1a**	**2f**	—	24	N.R.
15	**1a**	**2g**	**4ag**	12	85
16	**1a**	**2h**	**4ah**	24	84

[a] Reaction conditions: **1** (0.1 mmol), **2** (0.12 mmol), $PhNH_2$ (0.05 mmol), CAL-B 10 mg, CH_3CN 475 μL, H_2O 25 μL, 30 °C. [b] Isolated yields. N.R. means no reaction.

a similar sequential process of addition, elimination, hydrolysis and decarboxylation was taken place to get the final products.

In conclusion, here we report an unprecedented CAL-B catalysed decarboxylative aldol reaction and decarboxylative Knoevenagel reaction. It provides a novel case of biocatalytic promiscuity and might be a potential synthetic method for organic chemistry.

Acknowledgements

This work was financially supported by the National Science Foundation of China (Nos. 20725206, 20732004 and 20702034) and the Specialized Research Fund for the Doctoral Program of Higher Education. We also thank the Sichuan University Analytical and Testing Centre for NMR analysis.

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