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Short communication

Knoevenagel condensation of aromatic aldehydes with active methylene compounds catalyzed by lipoprotein lipase

Yan Ding [a], Xiao Ni [b], Mengjie Gu [a], Shuang Li [a,b], He Huang [a,b,*], Yi Hu [a,b,*]

[a] College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 210009, PR China
[b] State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing Tech University, Nanjing 210009, PR China

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ABSTRACT

A screening of using different lipases to catalyze the Knoevenagel reaction was realized, and lipase lipoprotein (LPL) from *Aspergillus niger* showed the best catalytic performance. The reaction conditions including solvent, enzyme loading, and temperature were screened to improve the reaction efficiency. Various kinds of substrates were investigated, and almost all the target products were obtained in good to excellent yields (76–98%) with Z configuration exclusively. This procedure provides a novel, green and efficient method for the Knoevenagel condensation of aromatic aldehydes with active methylene compounds.

1. Introduction

Knoevenagel condensation is one of the most important carbon–carbon double-forming reactions in organic synthesis, which is reacted between carbonyl compounds and active methylene compounds and widely used in the synthesis of chemically, biologically, and pharmaceutically significant compounds. Knoevenagel reaction was classically catalyzed by organic base, Lewis acid, ionic liquids, microwave, ultrasound, etc. [1–3]. However, many of these catalytic systems suffer from disadvantages such as excess starting materials needed, not being environmentally friendly, inevitable byproducts formed, narrow substrates scope, and sometimes only moderate yields and Z/E selectivity obtained.

In recent years, enzymes as biocatalysts have attracted significant attention due to their high selectivity and mild conditions [4,5]. Biocatalytic promiscuity provides new and green tools for organic synthesis, and thus largely extends the application of enzymes [6–9]. But only limited examples of enzyme-catalyzed Knoevenagel condensation have been reported. In 2009, Yu et al. first reported that lipase from *Candida antarctica* (CAL-B) could catalyze decarboxylative Knoevenagel condensation in CH_3CN/H_2O, while a primary amine was necessarily used as an additive to form a schiff base in the course of the reaction and the active methylene compounds was limited to β-ketoesters [10]. Moreover, the mechanism of CAL-B catalyzed Knoevenagel condensation was challenged by Bornscheuer and Evitt [11]. It was reported that papain could catalyze the Knoevenagel condensation of aromatic aldehydes with 1, 3-carbonyl compounds in $DMSO/H_2O$, while long reaction time (60 °C, 120 h) and excess substrates (1.2 equivalent) was used, and for some substrates only low to moderate yields were achieved with combined Z/E products [12]. Recently, esterase BioH showed the ability to catalyze the Knoevenagel reaction in the DMF/H_2O, nevertheless long reaction time (168–200 h), large amount of excess 1,3-dicarbonyl compounds (15 equivalent) was used, and low yields (35.1–54.7%) were obtained [13]. Therefore, in the field of enzymatic Knoevenagel condensation, there are still some drawbacks needed to be overcome, such as narrow substrates scope, long reaction time, excess amount of active methylene compounds, with additives, and expensive enzymes. In continuation of our interest in the enzyme-catalyzed organic synthesis [14–16], herein, we found that several lipases displayed observable activities for Knoevenagel condensation, especially the commercially cheap available lipase lipoprotein (LPL), from *Aspergillus niger*, could efficiently catalyze the Knoevenagel condensation of aromatic aldehydes with various active methylene compounds in good to excellent yields with Z configuration exclusively.

2. Experimental

2.1. Materials

Porcine pancreas lipase (PPL, 100–400 U/mg), *Candida rugosa* lipase (CRL, 1223 U/mg) and Bovine serum albumin (BSA) were purchased from Sigma; Novozym 435 (lipase B from *Candida antarctica*, immobilized on a macroporous acrylic resin) was purchased from Novo Nordisk; Lipase PS (*Pseudomonas cepacia* lipase, ≥30 U/mg)

* Corresponding authors at: State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing Tech University, Nanjing 210009, PR China.
E-mail addresses: biotech@njtech.edu.cn (H. Huang), huyi@njtech.edu.cn (Y. Hu).

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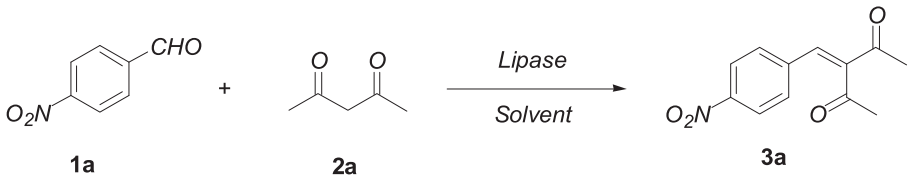


Scheme 1. Lipase-catalyzed Knoevenagel condensation of 4-nitrobenzaldehyde and acetylacetone.

were kindly donated by Amano Pharmaceuticals; Lipase lipoprotein from *Aspergillus niger* (LPL, 223 U/mg), was purchased from Ningxia Sunson group corporation. Other reagents were commercially available and were used without further purification.

2.2. General methods

Melting points were determined on WRS-1B digital melting point apparatus and were not corrected. The NMR spectra were measured on a Bruker Advance 2B 400 MHz instrument with $CDCl_3$ or $(CD_3)_2SO$ as the solvent and TMS as internal standard. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. Routine monitoring of reaction was performed by TLC using precoated Haiyang GF254 silica gel TLC plates. All the column chromatography separations were done by using silica gel (100–200 mesh) at increased pressure.

2.3. General procedure for lipase-catalyzed Knoevenagel reaction

A mixture of aromatic aldehyde (2 mmol), lipase (200 mg) and active methylene compound (2 mmol) in DMSO (5 mL, dried by anhydrous Na_2SO_4) was stirred at 35 °C. The reaction was monitored by thin layer chromatography (eluent: ethyl acetate/petroleum ether = 1:1, v/v) and terminated by filtering off the catalyst. The filtrate was diluted with ethyl acetate (10 mL) and washed with water (5 mL × 2). The aqueous phase was back-extracted with ethyl acetate (10 mL × 2). Combined organic phase was washed with water and dried over anhydrous Na_2SO_4, then concentrated to give the crude product which was further purified by flash column chromatography (eluent: ethyl acetate/petroleum ether = 1:8–1:2, v/v).

Table 1
Activities of different lipases for Knoevenagel condensation of 4-nitrobenzaldehyde and acetylacetone[a].

Entry	Catalyst	Yield (%)[b]
1	No enzyme	–
2	PPL	32
3	PSL	38
4	CRL	50
5	Novozym 435	46
6	LPL	55
7	BSA	25
8[c]	Denatured LPL	12

[a] Reaction conditions: lipase (200 mg), 4-nitrobenzaldehyde (2 mmol), acetylacetone (2 mmol), DMSO (4 mL), water (1 mL), 35 °C, 60 h.
[b] Isolated yield.
[c] Denatured LPL was obtained by treating at 100 °C for 24 h.

3. Results and discussion

3.1. Catalytic activities of different lipases for Knoevenagel condensation

In initial research, the reaction of 4-nitrobenzaldehyde **1a** and the less active methylene compounds acetylacetone **2a** were used as the model reaction (Scheme 1). Five lipases from different sources were screened to catalyze this Knoevenagel condensation and the results were shown in Table 1. Lipase lipoprotein (LPL) (Table 1, entry 6) was identified to be the most efficient catalyst for this Knoevenagel condensation, and no detectable products were obtained (Table 1, entry 1) in the absence of enzyme. BSA and denatured LPL (Table 1, entries 8–9) also showed the ability to catalyze the reaction, while their catalytic activities were less efficient. These results proved that the specific tertiary structure of lipase was important to this reaction [17].

3.2. Effect of solvents on the Knoevenagel condensation catalyzed by LPL

Based on the concept that organic media is one of the most important factors influencing the enzyme catalytic performance [18–21], seven common used organic solvents were investigated (Table 2). The results indicated that DMSO was the best reaction medium for the model reaction, while no clear correlation between solvent polarity and the enzyme activity was observed, as is consistent with the literature [12]. The role of water content in the reaction medium is crucial, as it dramatically influences both the activity and stability of the enzymes and, probably, their conformational flexibility [22,23]. Several results also indicated that water was essential in the enzyme catalyzed

Table 2
Effect of organic solvent on the Knoevenagel condensation of 4-nitrobenzaldehyde and acetylacetone[a].

Entry	Solvent	Yield (%)[b]
1	CH_2Cl_2	1
2	Toluene	2
3	MTBE	11
4	CH_3CN	28
5	Ethanol	29
6	THF	40
7	DMSO	55
8[c]	DMSO	46
9[d]	DMSO	77

[a] Reaction conditions: enzyme (200 mg), 4-nitrobenzaldehyde (2 mmol), acetylacetone (2 mmol), solvents (4 mL), water (1 mL), 35 °C, 60 h.
[b] Isolated yield.
[c] DMSO (4.75 mL), water (0.25 mL).
[d] DMSO (5 mL), no water was added.

Table 3
Effects of enzyme amount, temperature and reaction time on the Knoevenagel condensation of 4-nitrobenzaldehyde and acetylacetone[a].

Entry	Enzyme amount (mg)	Temperature (°C)	Time (h)	Yield (%)[b]
1	200	35	24	45
2	200	35	36	63
3	200	35	48	76
4	200	35	60	77
5	25	35	48	53
6	50	35	48	70
7	75	35	48	81
8	100	35	48	81
9	75	25	48	50
10	75	45	48	70
11	75	55	48	54

[a] Reaction conditions: 4-nitrobenzaldehyde (2 mmol), acetylacetone (2 mmol), DMSO (5 mL).
[b] Isolated yield.

Scheme 2. Lipase-catalyzed Knoevenagel condensation of various aromatic aldehydes and active methylene compounds.

reactions of carbon–carbon bond formation [24,25]. Interestingly, we found that the yield could be raised up to 77% from 46% without the addition of water, as is greatly different from previous reported enzyme catalyzed Knoevenagel reaction [10,12,13,26].

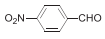
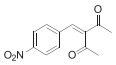
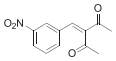
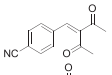
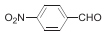
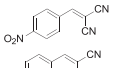
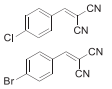
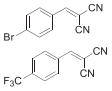
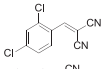
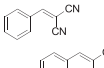
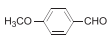
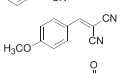
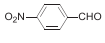
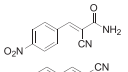
3.3. Effects of enzyme amount, temperature and reaction time on the Knoevenagel condensation catalyzed by LPL

In order to improve the reaction yields, other influencing factors such as reaction time, enzyme loading and reaction temperature were further investigated (Table 3). The results showed that the yield could be improved up to 81% when LPL loading was decreased to 75 mg and reacted at 35 °C for 48 h in DMSO. Interestingly, when the enzyme amount was up to 200 mg, the yield decreased slightly. We supposed that large amounts of enzyme employed may result in protein agglomeration and possible diffusion problem. Similar results that excess enzyme presented in the reaction system decreased the efficiency in other enzymatic reactions have also been reported [27–29].

3.4. LPL-catalyzed Knoevenagel condensation of various aromatic aldehydes and active methylene compounds

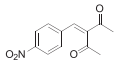
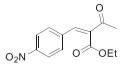
Based on the above results, the Knoevenagel condensation reactions of various different aromatic aldehydes and active methylene compounds were conducted in order to investigate the generality for the scope of this new catalysis method (Scheme 2). The results were summarized in Table 4. All the products were characterized by NMR and melting points that were consistent with literature data. It was found

Table 4
Knoevenagel reaction of aromatic aldehydes with active methylene compounds[a].

Entry	Aromatic aldehydes	Active methylene compounds	Product	Time (h)	Yield (%)[b]
1				48	81/68 [c]/45[d]
2				48	79
3				48	80
4				48	76
5				72	44
6				12	95
7				12	94
8				12	92
9				12	85
10				12	81
11				24	87
12				24	98
13				24	83
14				48	94
15				72	98
16				48	74
17				48	76
18				48	69

[a] Reaction conditions: enzyme (75 mg), active methylene compounds (2 mmol), acetylacetone (2 mmol), DMSO (5 mL), 35 °C.
[b] Isolated yield.
[c] Second recycling of LPL.
[d] Third recycling of LPL.

Table 5
Comparison of our works with previously described literatures.

Product	This work		Literatures		
	Time (h)	Yield (%)	Time (h)	Yield (%)	Ref.
	48	80	48	64[a]	[32]
			168	55[b]	[13]
	48	74	120	30[c]	[12]
			72	40[d]	[33]

[a] Yield for reaction catalyzed by 20 mol% L-lysine in water at ambient temperature, aldehyde (1 mmol), acetylacetone (1 mmol).
[b] Yield for reaction catalyzed by 15 mg *E. coli* BioH esterase in water/(water + DMF), v/v = 20% at 37 °C, aldehyde (0.5 mmol), acetylacetone (7.5 mmol).
[c] Yield for reaction catalyzed by 150 mg papain from the latex of carica papaya in water/(water + DMSO), v/v = 25% at 60 °C, aldehyde (1 mmol), acetoacetic ester (1 mmol).
[d] Yield for reaction catalyzed by 70 mg protease from *Bacillus licheniformis* in water/(water + DMSO), v/v = 5% at 45 °C, aldehyde (0.5 mmol), acetoacetic ester (0.6 mmol).

that a wide range of aromatic aldehydes could effectively participate in LPL catalyzed Knoevenagel reaction to give the corresponding products. For acetylacetone, the less active methylene compound, the substitution with electron-withdrawing group is necessary to obtain good yield. For malononitrile, the more active methylene compound, no matter substitution with electron-withdrawing or electron-donating group, excellent yields (81–98%) were obtained (Table 4, entries 6–12). For other active methylene compounds such as acetoacetic ester, cyanoacetic ester and cyanoacetamide, the reaction could also proceed smoothly and good to excellent yields were obtained (Table 4, entries 13–16). Moreover, it is noteworthy that satisfactory results were also observed for heterocycle methylene compounds (Table 4, entry 18). The condensation product, 5-arylidenethiazolidine-2, 4-dione constitutes an important class of chemically, biologically and pharmaceutically significant compounds [30]. Besides, only Z-isomers were obtained under our reaction conditions by comparing the NMR spectra of products with literatures [31]. Finally, the recycling of enzyme was investigated (Table 4, entry 1). Disappointedly, the yield dropped evidently after third reaction cycles. The decrease in yield upon repeated use could be probably ascribable to the gradual denaturation of the enzyme in the strong polar organic phase [20]. Actually, after third recycling, we measured the hydrolytic activity of LPL decreased obviously from original 223 U/mg to 5 U/mg.

In order to compare our procedure with the previous reported methods, some representative literatures data were summarized in Table 5. Obviously, our method exhibits pronounced rate accelerations and higher yields are obtained.

4. Conclusions

In conclusion, we have developed an enzymatic method for Knoevenagel condensation between aromatic aldehydes and a wide range of active methylene compounds. LPL, with a mild, cheap, commercially available and environmentally benign quality, could catalyze the Knoevenagel condensation effectively with good to excellent yields and Z selectivity in short reaction times without the need of additive and excess substrates. This case of biocatalyst not only expanded the application of lipases to new chemical transformations, but also could be developed into a potentially valuable method for organic synthesis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.catcom.2015.02.007.

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