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Henry reaction catalyzed by Lipase A from *Aspergillus niger*

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The Henry reaction of aromatic aldehydes and nitroalkanes could be performed by catalyst of Lipase A from *Aspergillus niger* in organic/water medium, and the corresponding Henry products were obtained in yields up to 94%.

Keywords: Lipase; Henry reaction; β-Nitroalkanols; enzyme catalysis; promiscuity

Introduction

Carbon–carbon bond forming reaction is one of the most basic reactions in organic chemistry, and the Henry (nitroadldol) reaction is an important C–C bond-formation reaction (*1–5*). The resulting optically active β-Nitroalcohols can be synthetized between a nitroalkane and a carbonyl compound (*6–10*). β-Nitroalcohols have been applied to the synthesis of many key intermediates to access biologically active compounds, such as natural products, insecticides, fungicides, and antibiotics (*11–17*). Since Henry reaction was reported in 1895 (*18*), various catalysts have been employed to perform the Henry reaction, including rare earth metal complexes, chiral metal catalysts, organocatalysts, and so on (*5, 19–23*). For example, some Henry reactions catalyzed by strong bases have been successful reported in good yields, but a great many of useless side products were produced in such processes (*6*, *24*). Therefore, it is necessary to develop green and efficient catalysts for Henry reaction.

Biocatalysis is an efficient green tool for modern organic synthesis because of its high selectivity, mild reaction conditions, low energy requirements, and few byproducts (*25–29*). Traditionally, the remarkable specificity of enzyme catalysts has been highlighted largely, and the 'darker' side of enzyme cross-reactivity was ignored. However, in the past decade, some extra activities were discovered for nonnatural substrate or alternative chemical transformation, which was called catalytic promiscuity (*30–33*). As a new frontier in biocatalysis, biocatalytic promiscuity provides new tools for organic synthesis and thus expands largely the application of enzymes. But only a few enzyme classes have been reported to catalyze Henry reactions (*34–37*).

Herein, as a part of our continuing interest in green chemistry and biocatalysis, we wish to report a hydrolase-catalyzed Henry reaction using Lipase A from *Aspergillus niger* as a catalyst. A series of aromatic aldehydes and nitroalkanes could participate in the reaction with moderate to good yields.

Results and discussion

Initially, the reaction of 4-nitrobenzaldehyde and nitromethane was carried out in *n*-propanol/water [2:1 (v/v)] at 30°C to screen the catalytic specificity of different enzymes. As shown in Table 1, eight enzymes were researched in this reaction, and the best yield (93%) was achieved using ANL as a catalyst (Table 1, entry 1); RNL, MJL, PFL, and papain also showed high catalytic activities (Table 1, entries 2–5), while other enzymes were low efficient (Table 1, entries 6–8). In order to confirm that the catalytic activity of ANL for Henry reaction did not arise from unspecific amino acids, nonenzyme protein BSA and denatured ANL were also used as catalysts. The results indicated that BSA and denatured ANL only gave product in low yields (Table 1, entries 9–10). Furthermore, the model reaction was carried out in the absence of any biocatalysts, and only trace product was detected after 24 h (Table 1, entry 11). Therefore, we chose ANL as catalyst for Henry reaction.

The solvent is also important in the enzymatic reactions due to its effects on the enzyme activation, stability and solubility. So some conventional solvents were screened for the better yield. As shown in Table 2, in the mixture of water and organic solvents, we found that *n*-propanol gave the best yield of 93% (Table 2, entry 1), while in the other tested solvents, such as THF, acetonitrile, and cyclohexane, the

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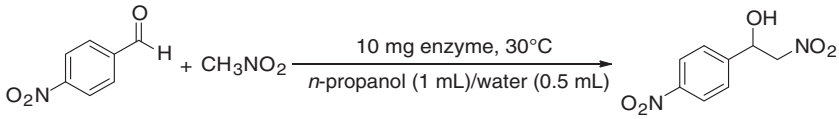
Table 1. The Henry reaction catalyzed by different enzymes.[a]



Entry	Catalyst	Yield (%)[b]
1	Lipase A from *Aspergillus niger* (ANL)	93
2	Lipase from *Rhizopus niveus* (RNL)	90
3	Lipase M from *Mucor javanicus* (MJL)	87
4	Lipase from *Pseudomonas fluorescens* (PFL)	82
5	Papain from papaya latex	78
6	Lipase from *Candida rugosa* (CRL)	51
7	Lipase PS, from *Burkholderia cepacia* (BCL)	24
8	Pepsin from porcine gastric mucosa	21
9	Bovine serum albumin (BSA)	16
10	Denatured ANL[c]	13
11	No enzyme	Trace

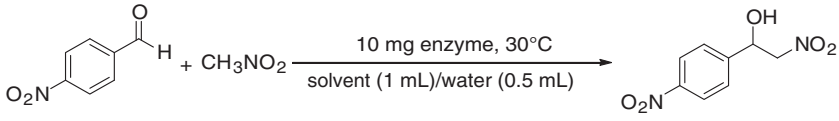
[a]The reaction was conducted using 4-nitrobenzaldehyde (0.5 mmol), nitromethane (10 mmol), and enzyme (10 mg) in *n*-propanol (1 mL) and deionized water (0.5 mL) at 30°C for 24 h.
[b]Yield of the isolated product after chromatography on silica gel.
[c]Pretreated with EDTA at 100°C for 12 h.

corresponding product was obtained in good yields (Table 2, entries 2–4), and the yield of 70% was given in DMSO and DMF (Table 2, entries 5–6). However, the reaction gave low yield in solvent-free conditions even after 48 h (Table 2, entry 8). Based on the above results, we chose *n*-propanol as the optimum solvent for the next study.

The water content plays an important role in enzymatic reactions. To enhance the catalytic activities of the enzyme, we analyzed the influence of water concentration. As shown in Figure 1, the reaction rate could be accelerated by increasing the concentration of water, and the highest yield of 93% was obtained when 0.5 mL water was used (in 1 mL *n*-propanol). However, once the water content exceeded 0.5 mL, the yield reduced slightly. Probably, excessive water could lead to a serious decrease of the solubility of substrates. The results also indicated that water was obviously essential in this enzyme-catalyzed Henry reaction. Thus, the optimum water content for the reaction is 0.5 mL in 1.0 mL organic solvent.

To further optimize reaction conditions, the effects of enzyme loading (Figure 2), mole ratio (Figure 3), and temperature (Table 3) on the ANL-catalyzed

Table 2. The Henry reaction under different solvents.[a]



Entry	Solvent	Time/h	Yield (%)[b]
1	*n*-propanol	24	93
2	THF	24	84
3	Acetonitrile	24	83
4	Cyclohexane	24	76
5	DMSO	24	70
6	DMF	24	70
7	H_2O	24	59
8	Solvent-free	48	12

[a]The reaction was conducted using 4-nitrobenzaldehyde (0.5 mmol), nitromethane (10 mmol), and ANL (10 mg) in organic solvents (1 mL) and deionized water (0.5 mL) at 30°C.
[b]Yield of the isolated product after chromatography on silica gel.

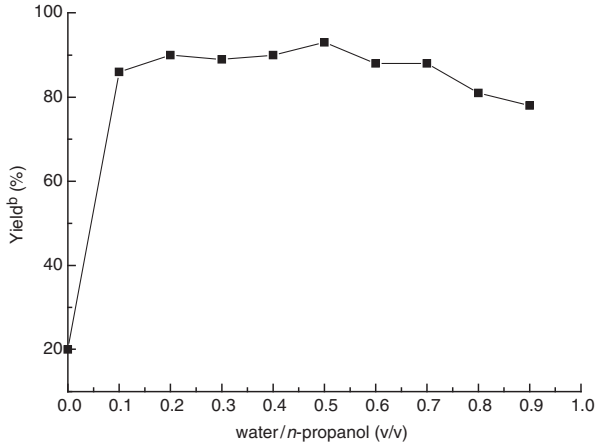


Figure 1. The influence of water concentration on the lipase-catalyzed Henry reaction.[a]
[a]Conditions: 4-nitrobenzaldehyde (0.5 mmol), nitromethane (10 mmol), ANL (10 mg) in *n*-propanol (1 mL) and water (0–0.9 mL) at 30°C for 24 h.
[b]Yield of the isolated product after chromatography on silica gel.

Henry reaction were investigated successively. The enzyme loading exhibited an unobvious influence on the yield of the Henry reaction, but the effects of mole ratio and temperature were obvious. Consequently, we chose enzyme loading of 10 mg, mole ratio 26:1, and 30°C as the best conditions.

In a similar fashion, the reactions of nitromethane with a variety of benzaldehyde were investigated under the optimized reaction condition. We found that the reaction is general and applicable to benzaldehyde containing nitro, chlorine, hydroxy, and

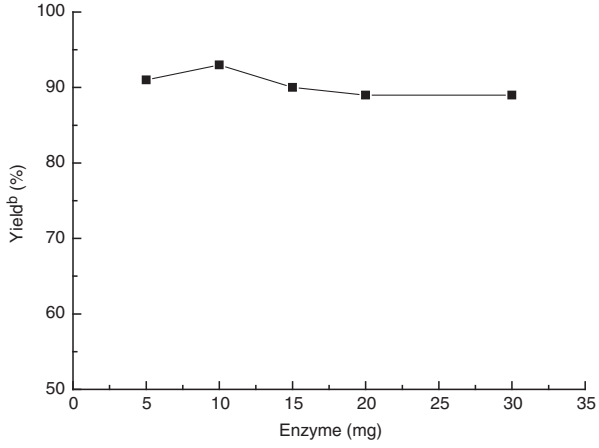


Figure 2. The influence of enzyme loading on the yield of Henry reaction.[a]
[a]Conditions: 4-nitrobenzaldehyde (0.5 mmol), nitromethane (10 mmol) and specified amount of ANL in *n*-propanol (1 mL) and water (0.5 mL) at 30°C for 24 h.
[b]Yield of the isolated product after chromatography on silica gel.

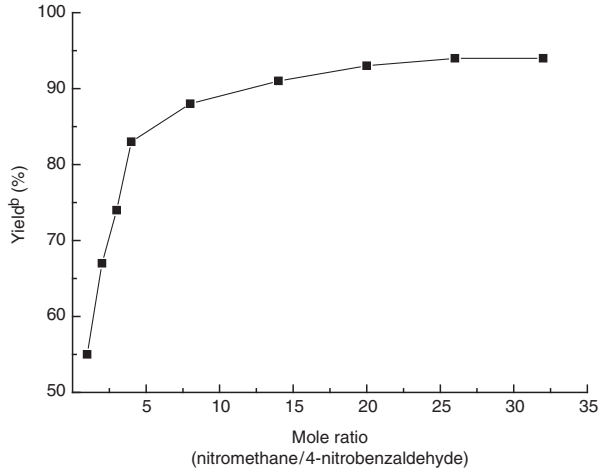


Figure 3. The influence of mole ratio on the yield of Henry reaction.[a]
[a]Conditions: 4-nitrobenzaldehyde (0.5 mmol), specified amount of nitroalkane and ANL (10 mg) in *n*-propanol (1 mL) and water (0.5 mL) at 30°C for 24 h.
[b]Yield of the isolated product after chromatography on silica gel.

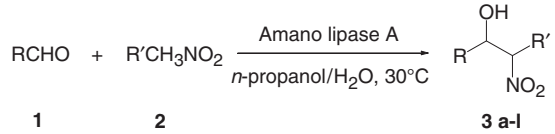
methyl (Table 4, entries 1–9). Aromatic aldehydes bearing a strong electron-withdrawing group (such as nitro) provided β-nitro alcohols with better yields ranging from 89% to 94% (Table 4, entries 2–4), when aromatic aldehydes containing an electron-donating group or a weak electron-withdrawing group, such as, hydroxyl, methyl, and chlorine, obtained middle to good yields (Table 4, entries 7–9). This is because electron-withdrawing groups could enhance the electrophilicity of carbonyl carbon in aldehyde which facilitates the reaction, while electron-donating groups render it less electrophilic (*34*). At the same time, in order to explore the generality of the method developed for Henry reaction, we also conducted experiments in nitroethane and nitropropane, and better results were obtained (Table 4, entries 10–12). Unfortunately, ANL only

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

Entry	Temperature (°C)	Yield (%)[b]
1	20	90
2	30	94
3	40	92
4	50	71
5	60	71

[a]The reaction was conducted using 4-nitrobenzaldehyde (0.5 mmol), nitromethane (13 mmol), and ANL (10 mg) in *n*-propanol (1 mL) and deionized water (0.5 mL) at specified temperature for 24 h.
[b]Yield of the isolated product after chromatography on silica gel.

Table 4. Investigation of the reactant scope of the ANL-catalyzed Henry reaction.[a]



Entry	1, Aldehyde	2, Nitroalkane	3, Product	Time (h)	Yield (%)[b]
1	benzaldehyde	CH_3NO_2	3a	120	24
2	*o*-nitrobenzaldehyde	CH_3NO_2	3b	80	90
3	*m*-nitrobenzaldehyde	CH_3NO_2	3c	120	89
4	*p*-nitrobenzaldehyde	CH_3NO_2	3h	24	94
5	*o*-chlorobenzaldehyde	CH_3NO_2	3d	120	58
6	*p*-chlorobenzaldehyde	CH_3NO_2	3j	120	71
7	*o*-hydroxybenzaldehyde	CH_3NO_2	3e	120	77
8	*p*-hydroxybenzaldehyde	CH_3NO_2	3f	120	69
9	*p*-methyltolualdehyde	CH_3NO_2	3g	120	52
10	*p*-nitrobenzaldehyde	$CH_3CH_2NO_2$	3i	120	76
11	*p*-chlorobenzaldehyde	$CH_3CH_2NO_2$	3k	120	88
12	*p*-chlorobenzaldehyde	$CH_3CH_2CH_2NO_2$	3l	120	45

[a]The reaction was conducted using aldehyde (0.5 mmol), nitroalkanes (13 mmol), ANL (10 mg) in *n*-propanol (1 mL) and deionized water (0.5 mL) at 30°C.
[b]Yield of the isolated product after chromatography on silica gel.

displayed negligible stereoselectivities for most of the substrates.

Experimental section

Materials

Lipase from *Rhizopus niveus* (≥1.5 u/mg, Sigma-Aldrich Co. LLC), lipase A from *Aspergillus niger* (≥ 1.2 u/mg, Amano Enzyme Inc), lipase M from *Mucor javanicus* (≥1.0 u/g, Amano Enzyme Inc), lipase PS from *Burkholderia cepacia* (≥30,000 u/g, Amano Enzyme Inc), lipase from *Pseudomonas fluorescens* (≥20,000 u/mg, Amano Enzyme Inc), lipase from *Candida rugosa* (≥2 u/mg, Sigma-Aldrich Co. LLC), pepsin from porcine gastric mucosa (≥250 u/mg, Sigma-Aldrich Co. LLC), and papain from papaya latex (1.5–10 u/mg, Sigma-Aldrich Co. LLC), Bovine serum albumin (Aladdin Co., Ltd.) Other reagents were obtained from commercial suppliers and were used without further purification unless otherwise noted.

Analytical methods

All reactions were monitored by thin-layer chromatography (TLC) with Haiyang GF254 silica gel plates. Flash column chromatography was carried out using 200–300 mesh silica gel at increased pressure. The ^1H-NMR spectra were recorded with TMS as internal standard on a Bruker AMX-400MHz spectrometer. All the products are known compounds.

Typical procedure for lipase-catalyzed Henry reaction

A 10 mL test tube was charged with the aldehyde (0.5 mmol), nitroalkane (13 mmol), and ANL (10 mg) in *n*-propanol (1 mL) and deionized water (0.5 mL), then shaken at 200 rpm at 30°C. After completion of the reaction, enzyme was filtered off to stop the reaction, and the solvent was then removed under reduced pressure. The crude residue was purified by flash column chromatography on silica gel, using ethyl acetate-petroleum ether as a mobile phase.

Conclusion

In summary, the ANL-catalyzed Henry reaction of aromatic aldehydes and nitroalkanes was reported, which provides a simple efficient method for the synthesis of β-nitroalcohols. The influences of reaction conditions, including solvent, water content, enzyme loading, mole ratio, and temperature, were investigated. The present method has many obvious advantages compared to those reported in the literature, including the mild and green reaction conditions, simplicity, high-efficiency, and good yield.

Acknowledgments

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