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# Enzyme-catalyzed Henry (nitroaldol) reaction

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ABSTRACT

Transglutaminase was first used to catalyze Henry reactions of aliphatic, aromatic and hetero-aromatic aldehydes with nitroalkanes. The reactions were carried out at room temperature, and the corresponding nitro alcohols were obtained in yields up to 96%.

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## 1. Introduction

The Henry (nitroaldol) reaction is a powerful and atom economical C–C bond forming reaction in synthetic organic chemistry [1–3]. The resulting β-nitro alcohols are often used as valuable synthetic intermediates in the synthesis of numerous products [4], such as 2-aminoalcohols, 2-nitroketones and nitroalkenes [5–8], which are useful for the synthesis of biologically important compounds [9,10]. In recent years, the development of new chiral catalysts for this important reaction has attracted the interest of many groups [11–18]. On the other hand, enzymes as practical catalysts have been increasingly exploited for synthetic transformations due to their simple processing requirements, high selectivity and mild reaction conditions [19,20]. However, to the best of our knowledge, there was only one group that had reported the asymmetric catalytic Henry reaction using Hydroxynitrile Lyase from *Hevea brasiliensis* (*HbHNL*) (EC 4.1.2.39) [21,22]. Therefore, the development of new enzymatic catalysts is still in great demand. Herein, we wish to report a novel discovery that the cheap and readily available transglutaminase (TGase) efficiently promotes the Henry reaction (nitroaldol) of nitroalkanes with aliphatic, aromatic and hetero-aromatic aldehydes resulting in moderate to good yields.

TGase (protein-glutamine γ-glutamyltransferase; EC 2.3.2.13) are a family of enzymes that are widely distributed in mammals [23], plants [24], fishes [25] and micro-organisms [26], and their enzymatic and physiological properties have been extensively studied [26,27]. It is well known that TGase catalyses an acyl transfer reaction between a γ-carboxyamide group of glutamine residue and ε-amino group of lysine residue or other primary amines. In this report, however, TGase was first used to catalyze Henry (nitroaldol) reaction. It shows the ability of the enzyme to catalyze synthetic reaction which varies from its natural role [28]. This is known as biocatalytic promiscuity, a new frontier which has emerged recently and largely extended the application of enzymes. Some elegant reports have addressed the importance and wide application of biocatalytic promiscuity in organic synthesis [29–46]. Herein, we report this TGase-catalyzed Henry reaction as another example of biocatalytic promiscuity.

## 2. Experimental

### 2.1. General remarks

Immobilized lipase from *Thermomyces lanuginosus* (0.25 U/mg. One unit corresponds to the amount of enzyme producing 1 μmol methyl oleate from triolein per minute at 35 °C) was purchased from Novozymes (China) Investment Co., Ltd. TGase from *Streptorerticillium griseoverticillatum* (0.06 U/mg. The activity was determined by the colorimetric hydroxamate procedure. One unit generates 1 μmol hydroxamic acid per minute at 37 °C), Pancreatin from porcin pancreas (4 U/mg. One unit of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm per minute at 40 °C and pH 7.5), Papain from fruit jam from *chaenomeles* (650 U/mg. One unit

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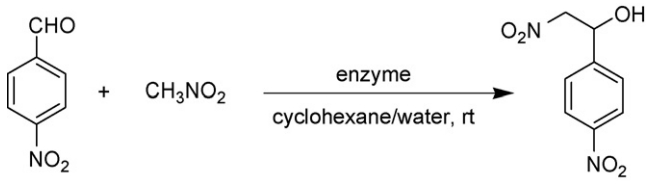
of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm/min at 37 °C and pH 7.0), Lysozyme from hen egg white (20,000 U/mg. The activity determination was according to the procedure described by Shugar [47]. One unit of activity was defined as the amount of enzyme that lowers 0.001 absorbance at 450 nm/min), Chymosin from fruit jam from *chaenomeles* (20 U/mg. The activity determination was according to the procedure described by Lowry et al. [48]. One unit of milk-clotting activity was defined as the amount of enzyme required to clot 1 ml of milk in 40 min at 35 °C), Nuclease from *Penicillium citrinum* (5 U/mg. The activity determination was according to the procedure described by Eaves and Jeffries [49]. One unit of activity was defined as the amount of the enzyme which liberates the digestion product not precipitated by the ammonium molybdate-perchloric acid reagent and gives an extinction change of 1 at 260 nm), Bromelain from pineapple peduncle (500 U/mg. One unit of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm/min at 37 °C and pH 7.0), and Cellulase from *Trichoderma* (10 U/mg. One unit of activity was defined as the amount of enzyme which released 1.0 mg of glucose from cellulose in 1 h at 40 °C and pH 4.8) were purchased from Guangxi Nanning Pangbo Biological Engineering Co. Ltd. (Nanning, China). Alkaline proteinase from *Bacillus licheniformis* No 2709 (200 U/mg. One unit of activity is the amount of enzyme that liberates 1.0 μequiv. of tyrosine from casein per minute at 40 °C and pH 10.5), Acidic proteinase from *Aspergillus usamii* No 537 (50 U/mg. One unit of activity is the amount of enzyme that liberates 1.0 μequiv. of tyrosine from casein per minute at 40 °C and pH 3.0), Neutral proteinase from *Bacillus subtilis* A.S.1.398 (130 U/mg. One unit of activity is the amount of enzyme that liberates 1.0 μequiv. of tyrosine from casein per minute at 30 °C and pH 7.5), and Trypsin from porcin pancreas (4 U/mg. One unit of activity was defined as the amount of the enzyme to produce TCA-soluble hydrolysis products from casein, which gives an absorbance value equivalent to 1 μg of tyrosine at 275 nm/min at 40 °C and pH 8.0) were purchased from Wuxi Xuemei Enzyme Co. Ltd. (WuXi, China). Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. All reactions were monitored by thin-layer chromatography (TLC) with Haiyang GF254 silica gel plates. Flash column chromatography was carried out using 100–200 mesh silica gel at increased pressure.

### 2.2. Representative procedure for enzyme-catalyzed Henry reactions (products **2a–s**, **3a–d**, **4a–d**)

For **2a–s**: a 25 ml round-bottomed flask was charged with the enzyme (200 mg), deionized water (3 ml) and $CH_2Cl_2$ (5 ml), to which the aldehyde (200 mg) and nitromethane (2 g, 32 mmol) were introduced. The resulting solution was stirred for the specified amount of time at rt (20–30 °C).

For **3a–d** and **4a–d**: a 25 ml round-bottomed flask was charged with the enzyme (200 mg), deionized water (3 ml) and $CH_2Cl_2$ (5 ml), to which the aldehyde (1 mmol) and nitroalkane (25 mmol) were introduced. The resulting solution was stirred for the specified amount of time at 30 °C.

The reaction was terminated by filtering the enzyme. $CH_2Cl_2$ was used to wash the filter paper to assure that products obtained were all dissolved in the filtrate. The filtrate was extracted three times with 20 ml of $CH_2Cl_2$. The combined extracts were dried over anhydrous $Na_2SO_4$ and the solvent was then removed under reduced pressure. The crude products were purified by column chromatography with petroleum ether/ethyl acetate as eluent.




**Scheme 1.** Enzyme-catalyzed Henry reaction of 4-nitrobenzaldehyde and nitromethane.

## 3. Results and discussion

Initial studies were undertaken using 4-nitrobenzaldehyde and nitromethane as a model reaction. We chose a cyclohexane/water 10:1 (v/v) system as the reaction medium. The reaction was performed at room temperature (20–30 °C) (Scheme 1). In order to select the appropriate enzymes, by using the model system several commercially available enzymes were screened (Table 1).

As shown in Table 1, the best result (93% yield) was achieved by using TGase as catalyst after 32 h (Table 1, entry 1). Immobilized lipase and Pancreatin also showed moderate catalytic activities (Table 1, entries 2 and 3), while some tested enzymes including Papain, Lysozyme, Chymosin, Alkaline proteinase, and Nuclease presented low activities in this reaction (Table 1, entries 4–8). In addition, in the presence of Acidic proteinase, Neutral proteinase, Trypsin, Bromelain and Cellulase, respectively, only trace product was observed on TLC (Table 1, entries 9–13).

We also performed some control experiments to demonstrate the specific catalytic effect of the TGase. Just as we expected, the Henry reaction of 4-nitrobenzaldehyde and nitromethane in the absence of enzyme showed low yield adduct (10%) even after 5 days (Table 1, entry 14). Moreover, the reaction catalyzed by non-enzyme protein bovine serum albumin (B.S.A.) gave product only

**Table 1**
The catalytic activities of different enzymes in Henry reaction between 4-nitrobenzaldehyde and nitromethane.[a].

| Entry | Catalyst | Time (h) | Yield (%)[b] |
|---|---|---|---|
| 1 | TGase from *Streptorerticillium griseoverticillatum* | 32 | 93 |
| 2 | Immobilized lipase from *Thermomyces lanuginosus* | 48 | 64 |
| 3 | Pancreatin from porcin pancreas | 48 | 41 |
| 4 | Papain from fruit jam from *chaenomeles* | 48 | 15 |
| 5 | Lysozyme from hen egg white | 48 | 13 |
| 6 | Chymosin from fruit jam from *chaenomeles* | 48 | 13 |
| 7 | Alkaline proteinase from *Bacillus licheniformis* No 2709 | 48 | 12 |
| 8 | Nuclease from *Penicillium citrinum* | 48 | 11 |
| 9 | Acidic proteinase from *Aspergillus usamii* No 537 | 48 | Trace |
| 10 | Neutral proteinase from *Bacillus subtilis* A.S.1.398 | 48 | Trace |
| 11 | Trypsin from porcin pancreas | 48 | Trace |
| 12 | Bromelain from pineapple peduncle | 48 | Trace |
| 13 | Cellulase from *Trichoderma* | 48 | Trace |
| 14 | No enzyme | 120 | 10 |
| 15 | Bovine serum albumin (B.S.A.) | 48 | 16 |
| 16 | TGase denatured with EDTA [c] | 120 | 0 |
| 17 | TGase inhibited with NBS[d] | 48 | 12 |

[a] All reactions were carried out using 4-nitrobenzaldehyde (200 mg, 1.3 mmol), nitromethane (2 g, 32 mmol), deionized water (0.5 ml), cyclohexane (5 ml) and enzyme (200 mg) at rt (20–30 °C).
[b] Yield of the isolated product after chromatography on silica gel.
[c] Pre-treated with EDTA at 100 °C for 24 h.
[d] Pre-treated with NBS at 25 °C for 24 h.

**Table 2**
Henry reaction of 4-nitrobenzaldehyde and nitromethane catalyzed by TGase in different solvents.[a].

| Entry | Solvent | Time (h) | Yield (%)[b] |
|---|---|---|---|
| 1 | DMSO | 59 | 73 |
| 2 | DMF | 59 | 85 |
| 3 | THF | 59 | 80 |
| 4 | TBME | 59 | 90 |
| 5 | $CH_2Cl_2$ | 59 | 90 |
| 6 | Cyclohexane | 59 | 93 |
| 7 | $H_2O$ | 59 | 32 |
| 8 | Solvent-free | 59 | 92 |

[a] All reactions were carried out using 4-nitrobenzaldehyde (200 mg, 1.3 mmol), TGase (200 mg), nitromethane (2 g, 32 mmol), deionized water (0.5 ml) and organic solvent (5 ml) at rt (20–30 °C).
[b] Yield of the isolated product after chromatography on silica gel.

in 16% yield (Table 1, entry 15), which excluded the possibility of protein catalysis. When the reactants were incubated with EDTA-denatured TGase (pre-treated with EDTA at 100 °C for 24 h), no product was detected even after 5 days (Table 1, entry 16), suggesting that the promiscuity of TGase was metal-dependent, and the tertiary structure of the enzyme might also be necessary. In addition, a strong inhibition of the catalytic activity of TGase in Henry reaction was observed by using NBS (N-Bromosuccinimide) (pre-treated with NBS at 25 °C for 24 h). The reaction catalyzed by NBS-inhibited TGase gave product only in 12% yield after 48 h (Table 1, entry 17). These results implied that the reaction must take place in a specific fashion on the catalytic site of TGase. Therefore, we chose TGase as catalyst for Henry reaction in our study. TGase was from *S. griseoverticillatum* (0.06 U/mg. The activity was determined by the colorimetric hydroxamate procedure. One unit generates 1 μmol hydroxamic acid per minute at 37 °C).

The selection of organic solvents is also crucial in the enzyme-catalyzed reactions, due to their effects on the enzyme stability and the solubility of substrates. Thus, a preliminary solvent screen was performed (Table 2). We found that the reaction using tert-butyl methyl ether (TBME), dichloromethane or cyclohexane gave the anticipant product in excellent yield (Table 2, entries 4–6), while moderate yield was obtained with DMSO, DMF or THF (Table 2, entries 1–3). It seems likely that less polar solvents favor the reaction. In addition, the reaction in deionized water gave the product in low yield (Table 2, entry 7). It may be attributed to the poor solubility of substrates in water. Interestingly, even in solvent-free conditions, the high yield was obtained (Table 2, entry 8). Based on the results of solvent screen, in view of the good ability to resolve the wide scope of substrates, we chose dichloromethane as the optimum solvent for the Henry reaction.

For the enzymes require a specific amount of water bound to them to maintain activities, it is important to confirm the optimal water content in reaction system. Therefore, we analyzed the effects of the amount of added $H_2O$ on the reaction rate (Fig. 1).

From Fig. 1, it was found that the rate of the enzymatic reaction can be accelerated by increasing the concentration of water used, and reaches the highest rate at 0.6 water content (water/dichloromethane, v/v). However, once the water content surpasses 0.6, the yield of Henry product decreased evidently, probably due to the insolubility of the substrates. Thus, the optimum water content for the reaction was 0.6 (water/dichloromethane, v/v). The results indicated that water is obviously essential in the enzyme-catalyzed Henry reaction.

To optimize experimental conditions, we then examined the effect of nitromethane stoichiometry on the reaction. From Table 3, the best result was achieved by using 25 or 30 equiv. of nitromethane. Thus, we chose 25 equiv. of nitromethane as the optimum molar equivalent for the Henry reaction.

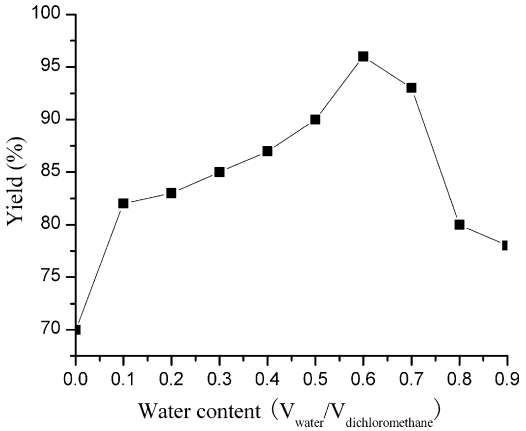



**Fig. 1.** Influence of water content in organic solvent on the yield of the TGase-catalyzed Henry reaction. *Conditions*: TGase (200 mg), nitromethane (2 g, 32 mmol), 4-nitrobenzaldehyde (200 mg, 1.3 mmol) and dichloromethane (5 ml) was stirred at rt (20–30 °C) for 48 h. Deionized water was added from 0 to 0.9 [water/dichloromethane, v/v].

We next investigated the effect of TGase loading on the reaction. As shown in Table 4, evident difference in the chemical yields (68% and 89%) was observed between TGase loading of 50 mg and 100 mg. However, once the quantity of TGase surpasses 100 mg, the yield of product kept unchanged as 90%. The results showed that 100 mg of TGase was the optimum quantity for the Henry reaction between 4-nitrobenzaldehyde and nitromethane. However, in consideration of reaction rates of some less active substrates, we chose 200 mg of TGase as the optimum quantity for the Henry reaction.

We further studied the effect of temperature on the reaction. From Table 5, there were no significant differences in yields between room temperature (20–30 °C), 30 °C and 35 °C for the model reaction of 4-nitrobenzaldehyde and nitromethane. In view

**Table 3**
Effect of molar equivalents of nitromethane on the yield of Henry reaction.[a].

| Entry | Nitromethane (mmol) | 4-Nitrobenzaldehyde (mmol) | Time (h) | Yield (%)[b] |
|---|---|---|---|---|
| 1 | 1 | 1 | 48 | 73 |
| 2 | 2 | 1 | 48 | 74 |
| 3 | 3 | 1 | 48 | 74 |
| 4 | 4 | 1 | 48 | 74 |
| 5 | 5 | 1 | 48 | 76 |
| 6 | 10 | 1 | 48 | 84 |
| 7 | 15 | 1 | 48 | 89 |
| 8 | 20 | 1 | 48 | 90 |
| 9 | 25 | 1 | 48 | 93 |
| 10 | 30 | 1 | 48 | 93 |

[a] All reactions were carried out using 4-nitrobenzaldehyde (151 mg, 1 mmol), TGase (200 mg), deionized water (3 ml), $CH_2Cl_2$ (5 ml) and specified amount of nitromethane at rt (20–30 °C).
[b] Yield of the isolated product after chromatography on silica gel.

**Table 4**
Effect of TGase loading on the yield of Henry reaction.[a].

| Entry | TGase (mg) | Time (h) | Yield (%)[b] |
|---|---|---|---|
| 1 | 50 | 48 | 68 |
| 2 | 100 | 48 | 89 |
| 3 | 200 | 48 | 90 |
| 4 | 300 | 48 | 90 |
| 5 | 400 | 48 | 90 |
| 6 | 500 | 48 | 89 |

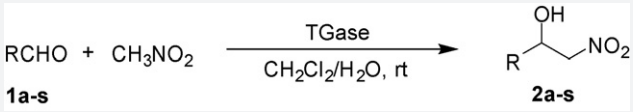
[a] All reactions were carried out using 4-nitrobenzaldehyde (151 mg, 1 mmol), nitromethane (1.5 g, 25 mmol), deionized water (3 ml), $CH_2Cl_2$ (5 ml) and specified amount of TGase at rt (20–30 °C).
[b] Yield of the isolated product after chromatography on silica gel.

**Table 5**
Effect of temperature on the yield of Henry reaction.[a].

| Entry | Temp. (°C) | Time (h) | Yield (%)[b] |
|---|---|---|---|
| 1 | 30 | 48 | 92 |
| 2 | 35 | 48 | 92 |
| 3 | rt[c] | 48 | 90 |

[a] All reactions were carried out using 4-nitrobenzaldehyde (151 mg, 1 mmol), TGase (200 mg), nitromethane (1.5 g, 25 mmol), deionized water (3 ml) and $CH_2Cl_2$ (5 ml) at specified temperature.
[b] Yield of the isolated product after chromatography on silica gel.
[c] 20–30 °C.

**Table 6**
TGase-catalyzed Henry reaction of aldehydes with nitromethane.[a].



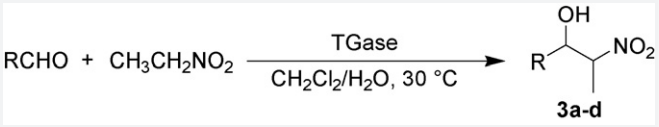

| Entry | R | Time (h) | Yield of **2** (%)[b] |
|---|---|---|---|
| 1 | Ph(**1a**) | 96 | 58 |
| 2 | 2-$MeOC_6H_4$(**1b**) | 120 | 51 |
| 3 | 3-$MeOC_6H_4$(**1c**) | 120 | 59 |
| 4 | 4-$MeOC_6H_4$(**1d**) | 120 | 50 |
| 5 | 4-$CH_3C_6H_4$(**1e**) | 120 | 60 |
| 6 | 2-$NO_2C_6H_4$(**1f**) | 120 | 86 |
| 7 | 3-$NO_2C_6H_4$(**1g**) | 120 | 62 |
| 8 | 4-$NO_2C_6H_4$(**1h**) | 48 | 96 |
| 9 | 4-$FC_6H_4$(**1i**) | 120 | 68 |
| 10 | 4-$BrC_6H_4$(**1j**) | 120 | 68 |
| 11 | 2-$ClC_6H_4$(**1k**) | 120 | 77 |
| 12 | 3-$ClC_6H_4$(**1l**) | 120 | 64 |
| 13 | 4-$ClC_6H_4$(**1m**) | 120 | 64 |
| 14 | 4-$CNC_6H_4$(**1n**) | 120 | 79 |
| 15 | 2-Thienyl(**1o**) | 120 | 21 |
| 16 | 2-Furyl(**1p**) | 120 | 12 |
| 17 | Ethyl(**1q**) | 48 | 61 |
| 18 | n-Propyl(**1r**) | 48 | 70 |
| 19 | Isobutyl(**1s**) | 48 | 71 |

[a] All reactions were carried out using aldehyde **1** (200 mg), TGase (200 mg), nitromethane (2 g, 32 mmol), deionized water (3 ml) and $CH_2Cl_2$ (5 ml) at rt (20–30 °C).
[b] Yield of the isolated product after chromatography on silica gel.

of the boiling point of $CH_2Cl_2$, we chose 30 °C as the optimum temperature for the Henry reaction.

With the optimized conditions in hand, some other aldehydes were used to expand upon this TGase-catalyzed Henry reaction to show the generality and scope of this new enzymatic promiscuity. The results are summarized in Table 6. It can be seen that a wide range of aromatic, hetero-aromatic and aliphatic aldehydes can effectively participate in the reaction. In general, aromatic aldehydes bearing an electron-withdrawing substituent furnished β-nitro alcohols with yields well above 62% (Table 6, entries 6–14). Especially, 2-nitrobenzaldehyde and 4-nitrobenzaldehyde

**Table 7**
TGase-catalyzed Henry reaction of aldehydes with nitroethane.[a].



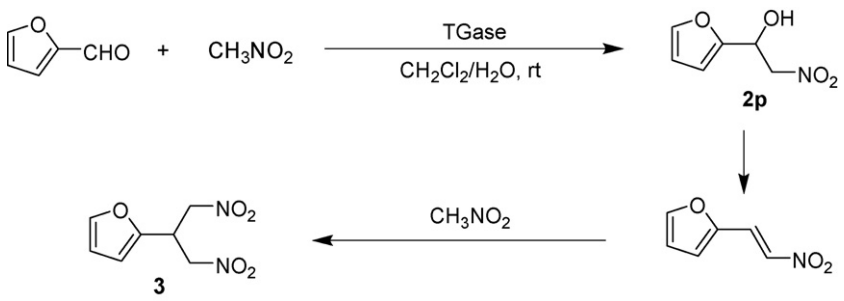

| Entry | R | Time (h) | Yield (%)[b] | Anti:syn[c] |
|---|---|---|---|---|
| 1 | 4-$MeOC_6H_4$(**1d**) | 144 | 40 | 1:2.3 |
| 2 | 4-$CH_3C_6H_4$(**1e**) | 144 | 46 | 1:1.3 |
| 3 | 4-$NO_2C_6H_4$(**1h**) | 72 | 90 | 1:1.3 |
| 4 | Isobutyl(**1s**) | 72 | 77 | 1:1 |

[a] All reactions were carried out using aldehyde (1 mmol), TGase (200 mg), nitroethane (1.5 g, 25 mmol), deionized water (3 ml) and $CH_2Cl_2$ (5 ml) at 30 °C.
[b] Yield of the isolated product after chromatography on silica gel.
[c] Determined by HPLC on chiral stationary phase (AD-H) and $^1H$ NMR.

gave corresponding product in 86% and 96% yield respectively (Table 6, entries 6 and 8). In contrast, aromatic aldehydes containing an electron-donating group gave the products in relatively low yields (Table 6, entries 2–5). This is because electron-withdrawing groups enhance the electrophilicity of carbonyl carbons in aldehydes which facilitates the reaction, while electron-donating groups render it less electrophilic. Moreover, it is clear that the reactions using aliphatic aldehydes were faster and provided the corresponding adducts with satisfied yields (Table 6, entries 17–19). This is probably due to the relatively small steric hinderance of alkyls.

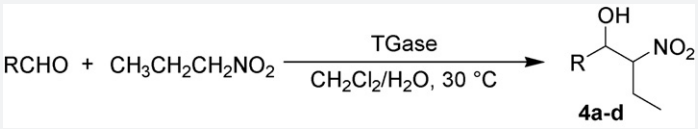
In the cases of hetero-aromatic aldehydes **1o** and **1p**, the reaction was obviously affected by the heteroatom of the hetero-aromatic ring, and both **1o** and **1p** gave anticipant product β-nitroalcohols in low yields (Table 6, entries 15 and 16). Interestingly, the tandem Henry/Michael reaction was observed with 2-furaldehyde (**1p**), and the unexpected 1,3-dinitro compound **3** was obtained in 36% yield while expected β-nitroalcohol **2p** was only received in 12% yield (Scheme 2).

The reaction was then extended to nitroethane and nitropropane. As shown in Tables 7 and 8, the electronic effects of the substituent in the aromatic aldehydes affected the reactions in the same way as using nitromethane. As expected, 4-nitrobenzaldehyde, an aldehyde bearing an electron-withdrawing group, gave high yields of 90% with nitroethane (Table 7, entry 3), and 94% with nitropropane (Table 8, entry 3). The aldehyde with electron-donating group gave moderate to modest yields (Tables 7 and 8, entries 1 and 2). Moreover, the aliphatic aldehydes also gave satisfied yields (Tables 7 and 8, entry 4). In addition, anti/syn selectivity was observed in the reactions of nitroethane and nitropropane with aromatic aldehydes, and the syn products were obtained chiefly (Tables 7 and 8, entries 1–3). However, when 3-methyl-butanal (**1s**) was used, the adducts showed no anti/syn selectivity (Tables 7 and 8, entry 4). Finally, it can be found that the



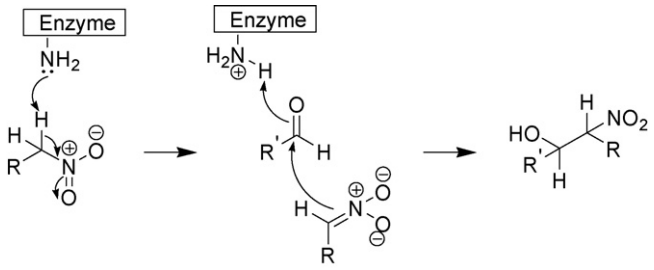

**Scheme 2.** Tandem Henry/Michael reaction between 2-furaldehyde and nitromethane.

**Table 8**
TGase-catalyzed Henry reaction of aldehydes with nitropropane.[a].




| Entry | R | Time (h) | Yield (%)[b] | Anti:syn[c] |
|---|---|---|---|---|
| 1 | 4-$MeOC_6H_4$(**1d**) | 168 | 22 | 1:2.9 |
| 2 | 4-$CH_3C_6H_4$(**1e**) | 168 | 35 | 1:1.7 |
| 3 | 4-$NO_2C_6H_4$(**1h**) | 72 | 94 | 1:3.8 |
| 4 | Isobutyl(**1s**) | 72 | 53 | 1:1 |

[a] All reactions were carried out using aldehyde (1 mmol), TGase (200 mg), nitropropane (2.2 g, 25 mmol), deionized water (3 ml) and $CH_2Cl_2$ (5 ml) at 30 °C.
[b] Yield of the isolated product after chromatography on silica gel.
[c] Determined by HPLC on chiral stationary phase (AD-H) and $^1H$ NMR.




**Scheme 3.** Proposed mechanism of enzymatic Henry reaction.

length of carbon chain in the nitroalkane also obviously affected the enzymatic Henry reaction. Generally, longer reaction times were required, and lower yields were obtained with nitroethane and nitropropane in comparison to nitromethane, due to steric hinderance.

Based on the above results, we proposed the possible reaction mechanism of TGase-catalyzed Henry reaction, depicted in Scheme 3. From the experiments catalyzed by denatured enzyme, inhibited enzyme and non-enzyme protein as well as blank experiment (Table 1), it is reasonable to think that the reaction takes place in a specific fashion on the catalytic site of TGase. Firstly, an amine group on the reaction site assists the deprotonation of nitroalkane to give nitronate. Secondly, nucleophilic attack of nitronate onto carbonyl carbon in aldehyde forms C–C bond. Meanwhile, the resulted aminium on enzyme provides a proton to carbonyl oxygen to give β-nitro alcohol.

Finally, although various conditions were tested to improve the enantioselectivity, no clear progress has been made at this time. Further studies on enzymatic asymmetric Henry reactions are still in progress in our lab.

## 4. Conclusion

In conclusion, we describe here the first TGase-catalyzed Henry reaction. The cheap and readily available TGase efficiently catalyzed the Henry reaction of nitroalkanes with aliphatic, aromatic and hetero-aromatic aldehydes. The influence of reaction conditions including solvents, water content, stoichiometry of nitroalkane, loading of catalyst and temperature was also investigated. This TGase-catalyzed Henry reaction provides a novel case of catalytic promiscuity and might be a useful synthetic method for application.

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