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Graphical Abstract

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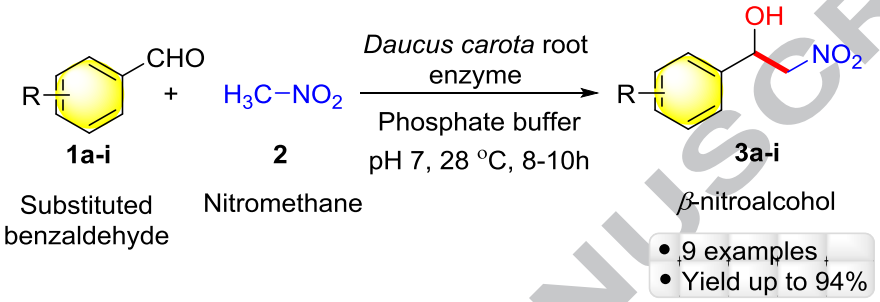

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Daucus carota root enzyme catalyzed Henry reaction: A green approach

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ABSTRACT

Enzyme from *Daucus carota* root catalyzed Henry reaction of substituted benzaldehydes and nitromethane in phosphate buffer of pH 7 at 28°C to afford *β*-nitroalcohols in excellent yields (up to 94%).

The hidden art of organic synthesis is the carbon — carbon (C — C) bond forming reactions, the fundamental tool for the synthesis of complex molecular architecture.[1-4] A number of methodologies have been reported over the years for C — C bond formation.[5-9] Among various other protocols aldol reactions are the most common and well established methods.[10] Besides this, Henry reaction of aldehydes with nitroalkanes to afford *β*-nitroalcohols which had been reported in 1895, has attracted the attention of medicinal chemists due to its applications in the synthesis of pharmaceutical agents,[11] precursors of natural products[11] and bioactive compounds having medicinal importance (Fig 1).[12]

The use of base in Henry reactions resulted in side products along with the desired *β*-nitroalcohol.[13] Although, this shortcoming has been addressed by the use of metal and organo catalysts in place of conventional bases,[14] the problem of toxicity and high cost is a deterrent. Therefore, further development was needed to provide green and efficient methodologies.

As a result of this development, enzyme promiscuity has been introduced.[15] But only a few enzymes which are both costly and substrate specific, were reported to catalyze Henry reactions.[16-17] Hence, the search for new enzymes provoked the development of enzyme catalysis which is an emerging field of modern organic synthesis.

Previously, Griengl *et. al.* reported hydroxynitrile lyases (HNLs, protease enzyme) catalyzed synthesis of *β*-hydroxynitriles and

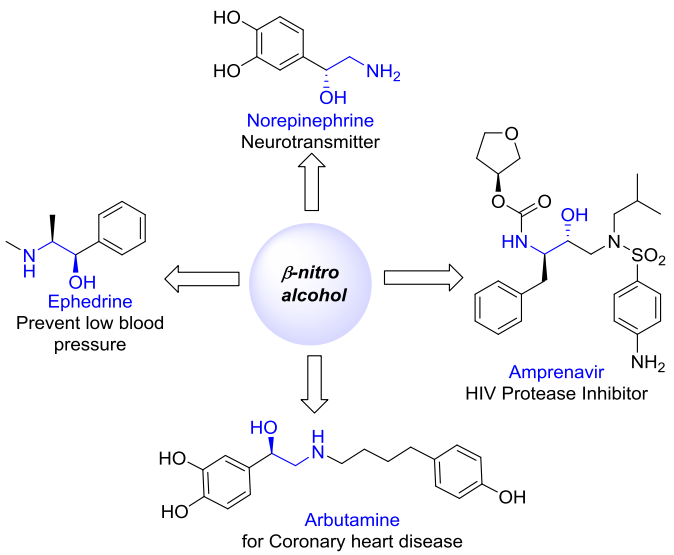

Fig1. *β*-Aminoalcohols having profound medicinal importance.

β -nitroalcohols from nitrobenzaldehydes and hydrogencyanide (HCN) or nitromethane with high substrate specificity.[15] The primary role of HNLs is to abstract the acidic proton from HCN (pK_a=9.2) and nitromethane (pK_a=10.21). There were few reports available in the literature on *Daucus carota* root mediated reduction of optically active alcohols,[18] until our recent report on its use as catalyst for the asymmetric cross aldol reaction of nitrobenzaldehyde and acetone.[19] In continuation of our prior work[19] we have deliberately used *Daucus carota* root or its enzyme in the promiscuous mixture of substituted

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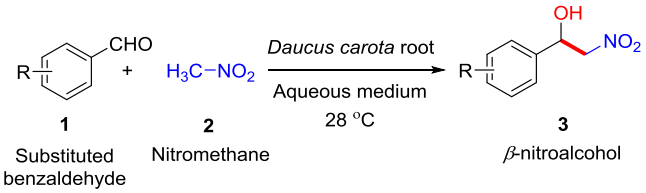
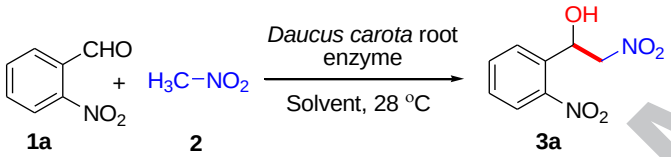
nitrobenzaldehyde and nitromethane in aqueous medium to afford *β*-nitroalcohol in excellent yields (Scheme 1).



Scheme 1. *Daucus carota* root catalyzed Henry reaction.

Initially the reaction was performed by stirring 2-nitrobenzaldehyde (**1a**, 1 mmol) and nitromethane (**2**, 1 mmol) in a suspension of freshly cut *Daucus carota* root (5.0 g) in aqueous medium for 24 hr, which resulted in the formation of *β*-nitroalcohol (**3a**) with 55% of isolated yield (Table 1, entry 2). Encouraged by this initial result we then performed the same reaction with purified enzyme (10μL) from *Daucus carota* root (see SI) and it afforded the desired *β*-nitroalcohol with 70% of isolated yield in 15 hr. Subsequently we went for optimizing the reaction protocol by changing reaction parameters (Table 1). No reaction took place in the absence of enzyme.

Table 1. Optimization of parameters for the *Daucus carota* root enzyme catalyzed Henry reaction[α]

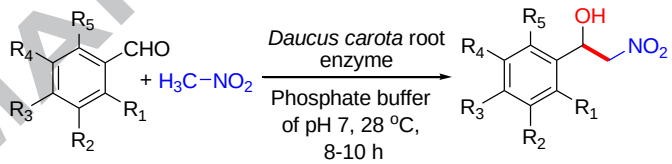


Entry	Solvent	Temperature[†] (°C)	Time[γ] (h)	Isolated yield (%)
1[#]	Water	28	24	No reaction
2[ϕ]	Water	28	24	55
3	Water	28	15	70
4	Phosphate buffer of pH 8	28	8	90
5	**Phosphate buffer of pH 7**	**28**	**8**	**93**
6	Phosphate buffer of pH 6	28	8	82
7	Phosphate buffer of pH 5	28	8	60
8	Diethylether (DEE)	28	8	No reaction
9	DEE: buffer of pH 7 (1:1)	28	12	50
10	TBME[€]	28	8	No reaction
11	TBME: buffer of pH 7 (1:1)	28	12	60
12	Acetonitrile: buffer of pH 7 (1:1)	28	24	No reaction
13	Ethanol: buffer of pH 7 (1:1)	28	24	No reaction
14	Methanol: buffer of pH 7 (1:1)	28	24	No reaction

[α] See experimental section for general reaction procedure. [#] Reaction was placed without *Daucus carota* root enzyme. [ϕ] Reaction was placed with homogenized carrot root (5g). [†] The reaction was performed at 28 °C as the enzyme was reported to perform best at that temperature.[19] [γ] Further increase of reaction time has no effect on the yield of the product. [€] *t*-Butylmethylether.

The optimized condition of the *Daucus carota* root enzyme catalyzed Henry reaction of 2-nitrobenzaldehyde and nitromethane to afford *β*-nitroalcohol in excellent yield (93%) was found to be phosphate buffer of pH 7 at 28 °C in 8 hr (Table 1, entry 5). Organic solvents did not have any positive influence in this reaction, possibly, due to denaturation of the enzyme occurred in organic solvents. A number of *β*-nitroalcohols have been synthesized under the optimized conditions and the results are summarized in table 2. All the synthesized *β*-nitroalcohols, which were racemic in nature have been characterized by ^{1}H-NMR, ^{13}C-NMR, ESI-HRMS and FT-IR spectroscopic techniques (see SI).

Table 2. Substrate scope of *Daucus carota* root enzyme catalyzed Henry reactions of substituted benzaldehyde and nitromethane.



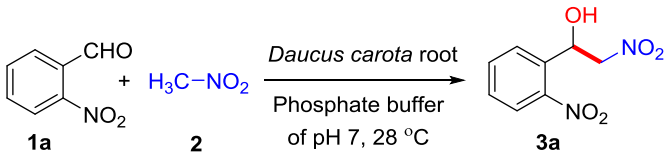
Entry	R_1	R_2	R_3	R_4	R_5	Time (h)	Product	Isolated yield (%)
1	NO_2	H	H	H	H	8	**3a**[16]	93
2	H	NO_2	H	H	H	8	**3b**[16]	94
3	H	H	NO_2	H	H	8	**3c**[16]	92
4	H	OMe	OMe	H	NO_2	10	**3d**	81
5	NO_2	H	H	Cl	H	9	**3e**[20]	90
6	H	H	OMe	H	H	10	**3f**[21]	89
7	NO_2	H	CF_3	H	H	8	**3g**	87
8	NO_2	H	NO_2	H	H	8	**3h**	91
9	CF_3	H	H	H	H	10	**3i**[21]	81

It was observed that there was almost no electronic (viz. entry 3 vs. 6) influence of aryl substituents on the yields of the products. As one would expect, the high specificity of any enzyme in catalytic activity was corroborated when nitroethane and nitropropane were used in order to broaden the scope of the reaction, failed to undergo this reaction. We also checked the reactivity of aliphatic aldehydes with nitromethane in this promiscuous reaction but disappointingly, no reaction took place. The reusability of the *Daucus carota* root enzyme was investigated in the reaction of **1a** and **2** to yield **3a** which was extracted in diethylether followed by subsequent addition of the substrates (**1a** and **2**) in the aqueous layer which contains enzyme to catalyze next batches. It was perceived that the enzyme could catalyze the Henry reaction of **1a** and **2** up to five cycles to afford **3a**, though with progressively lower isolated yields (Table 3).

Earlier, it was reported that the lysine residue of HNLs acted as base to abstract a methylene proton from nitromethane to favor

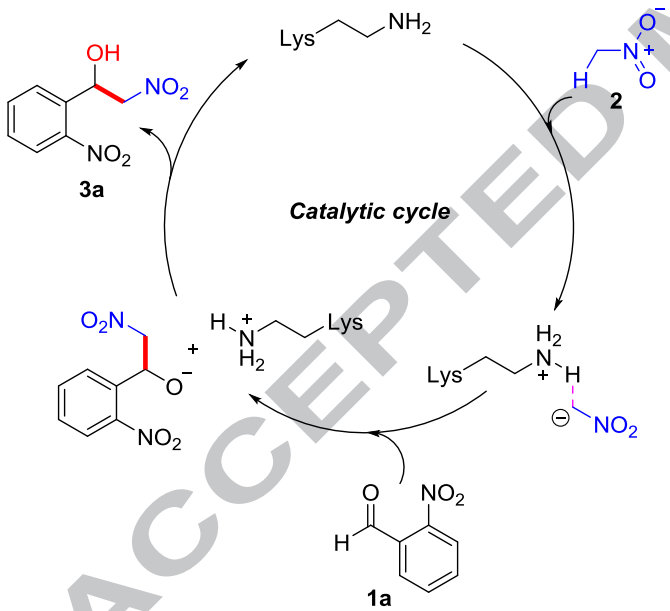
the reaction with nitrobenzaldehyde.[15b] Recently, we disclosed that the *Daucus carota* root enzyme also contains Lysine residue which was proposed to favor the asymmetric cross aldol reaction of nitrobenzaldehyde and acetone.[19] Therefore, we could visualize that the catalytic site of the enzyme[19] which may contain lysine residue could activate the methylene proton of nitromethane (**2**) to favor Henry reaction with 2-nitrobenzaldehyde (**1a**), that resulted in the formation of *β*-nitroalcohol (**3a**) (Scheme 2). The non-stereospecificity of the products (**3a-i)** could be due to lack of binding of the substrate (**2**) with the amino acid residues present in the active sites of enzyme.

Table 3. Investigation of recyclability of *Daucus carota* root enzyme in Henry reaction[δ]



No of cycle	Time (h)	% of yield
1	8	93
2	8	72
3	8	50
4	8	35
5	8	20

[δ]See experimental section for reaction procedure.



Scheme 2. Plausible mechanism of *Daucus carota* root enzyme catalyzed Henry reaction of 2-nitrobenzaldehyde (**1a**) and nitromethane (**2**).

In conclusion we have disclosed yet another catalytic activity of *Daucus carota* root enzyme towards Henry reaction in aqueous medium. The impact of the reaction conditions including solvent, pH of the reaction medium has been explored and extent of the reaction investigated. The substrate specificity of the enzyme may be exploited in the synthesis of *β*-nitroalcohols which are important intermediates of many pharmaceuticals and natural products. The present methodology has some genuine and competitive advantages over the reported ones, including simple and mild reaction condition, high efficiency and excellent isolated yield of the products.

Experimental Procedure:

An aliquot (10 μL, 0.7 μg) of enzyme solution of concentration 0.07 mg/mL was taken in 1 mL of phosphate buffer of pH 7. Then 2-nitrobenzaldehyde (**1a**; 100 mg, 0.66 mmol) and nitromethane (**2**, 35 μL, 0.66 mmol) were added to the reaction mixture and it was stirred at 28 °C for 8 hr. The reaction was monitored by TLC on silica gel G using 15% ethyl acetate in petroleum ether as mobile phase. The reaction mass was then diluted with 50 mL of diethylether. The organic layer was separated and passed through anhydrous Na_2SO_4. The solvent was removed in rotary evaporator to obtain a yellow sticky mass (152 mg). The product was purified by column chromatography using 60–120 mesh silica gel and eluting with 10–12% of ethyl acetate in petroleum ether. A sticky mass obtained which was washed with diethyl ether to get a yellow solid **3a** (130 mg, yield 93%).

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Supplementary Data:

Supplementary data (spectroscopic data of compound **3a–i**, copies of ^{1}H and ^{13}C NMR spectra) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tetlet.xxxx.xx.xxx. These data include MOL files and InChiKeys of the important compounds described in this article.

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Highlights:

- *Daucus carota* root enzyme catalyzed Henry reaction has been reported.
- The products were obtained in excellent yields (up to 94%).
- This enzymatic C— C bond formation reaction is highly substrate specific.
- A plausible reaction mechanism has been highlighted in this report.