

A Novel Trypsin-Catalyzed Three-Component *Mannich* Reaction

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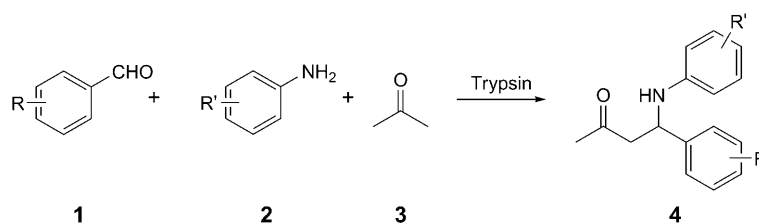
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We found that the trypsin from hog pancreas displayed high activity to promote three-component *Mannich* reaction of aromatic aldehydes, aromatic amines, and acetone with moderate-to-excellent yields. The reaction tolerates a great range of substrates and extends the application of trypsin in organic synthesis.

Introduction. – The *Mannich* reaction as a three-component reaction of an aldehyde, a primary or secondary amine, and a ketone is one of the most powerful C,C bond-forming reactions in organic synthesis [1]. It leads to β -amino carbonyl compounds, which are useful for the syntheses of N-containing compounds, such as natural products and medicinally relevant compounds [2]. The versatility and potential of these compounds to introduce both functional and structural diversity using the *Mannich* reaction have stimulated the creativity of chemists [3]. Most recently, directly catalyzed *Mannich*-type reactions have been widely investigated, utilizing different classes of catalysts, such as organometallic complexes, amino acids, and their derivatives [1b][4]. Trypsin-catalyzed *Mannich* reactions, which can be performed under mild and environment-friendly reaction conditions, have never been reported. In this article, we describe new synthetic applications of biocatalysts. We have found that some hydrolases are able to catalyze different reactions, such as *Aldol* reactions, *Michael* additions, *etc.* Here, we report on the trypsin-catalyzed three-component *Mannich* reaction of benzaldehydes **1** and anilines **2** in the presence of acetone (**3**) as the third component and solvent (*Scheme 1*).

Results and Discussion. – Acetone was used as solvent instead of the commonly used DMSO. Excellent yields were obtained in the reaction of benzaldehydes and *p*-

Scheme 1. Trypsin-Catalyzed Three-Component *Mannich* Reaction



anisidine (=4-methoxybenzenamine). A series of commercially available hydrolytic enzymes had been investigated in order to find the most suitable enzyme for the envisaged one-pot *Mannich* reaction (Table 1). We found that several enzymes displayed observable activities for this reaction, whereby trypsin from hog pancreas showed an especially significant catalytic activity. Lipase from porcine pancreas (PPL), amino lipase (AK) from *Pseudomonas fluorescens*, and α -amylase from hog pancreas also showed moderate catalytic activities, while other tested enzymes presented very low activities for this transformation. Considering these results, we performed some additional experiments to test the catalytic activity in the absence of any biocatalyst (enzyme). The reaction yielded no new product even after 96 h (Table 1, Entry 1).

Table 1. Screening the Different Enzyme Catalysts for Mannich Reaction^{a)}

Entry	Catalyst	Yield [%] ^{b)}
1	No Enzyme	3 ^{c)}
2	Trypsin from hog pancreas	83
3	α -Amylase from hog pancreas	54
4	Lipase acrylic resin from <i>Candida antarctica</i>	11
5	Lipase AY30	8
6	Lipase from porcine pancreas (PPL)	46
7	Amino lipase AK from <i>Pseudomonas fluorescens</i>	17
8	Amino lipase A from <i>Aspergillus niger</i>	20
9	Amino lipase M from <i>Mucor javanicus</i>	28
10	Cellulose (C0057)	6

^{a)} Reaction conditions: a solution of 4-nitrobenzaldehyde (0.2 mmol), *p*-anisidine (0.2 mmol), acetone (2 ml), and enzyme (20 mg) was shaken at 160 rpm at 30° for 12 h. ^{b)} Yield based on consumed *p*-anisidine. ^{c)} Reaction for 96 h.

The reaction medium has been recognized to be one of the most important factors influencing the enzymatic reaction. We screened the reaction in various organic solvents (Table 2). We found that acetone as reactant and solvent, and acetone in EtOH were the most efficient media to promote the *Mannich* reaction, and the *Mannich* products were obtained in similar yields of 89 and 86%, respectively. Other solvents, such as CH₂Cl₂, CCl₄, and toluene did not promote the product formation (Table 2, Entries 2–4), and other polar solvents (Table 2, Entries 6–9) led to moderate yields.

In previous studies of enzymatic promiscuity, H₂O has been considered as a very important factor in enzymatic activity leading to an acceleration of the enzyme-catalyzed reaction [5]. Therefore, we performed some experiments to optimize the percentage of H₂O in H₂O/acetone binary mixtures for this reaction. The effect of H₂O concentration on the considered trypsin-catalyzed *Mannich* reaction is illustrated in the Figure. Apparently, 25% of H₂O added to the system was optimal for the trypsin-catalyzed reaction. To our surprise, the yield of the reaction with 25% H₂O in acetone is even a little smaller than that in pure acetone. In other words, for optimal reaction conditions, H₂O is not necessary in this trypsin-catalyzed three-component *Mannich* reaction.

Table 2. Optimization of the Mannich Reaction in Various Solvents^{a)}

Entry	Solvent	Time [h]	Yield [%]
1	Hexane	24	56
2	CCl ₄	24	2
3	Toluene	24	3
4	CH ₂ Cl ₂	24	5
5	EtOH	24	86
6	THF	24	46
7	MeCN	24	53
8	DMF	24	35
9	DMSO	24	48
10	Acetone	12	80
11	Acetone	24	89

^{a)} Reaction conditions: a solution of aldehyde (0.2 mmol), amine (0.2 mmol), acetone (2 ml), solvent (3 ml), and trypsin (20 mg) was shaken at 160 rpm at 37° for 24 h.

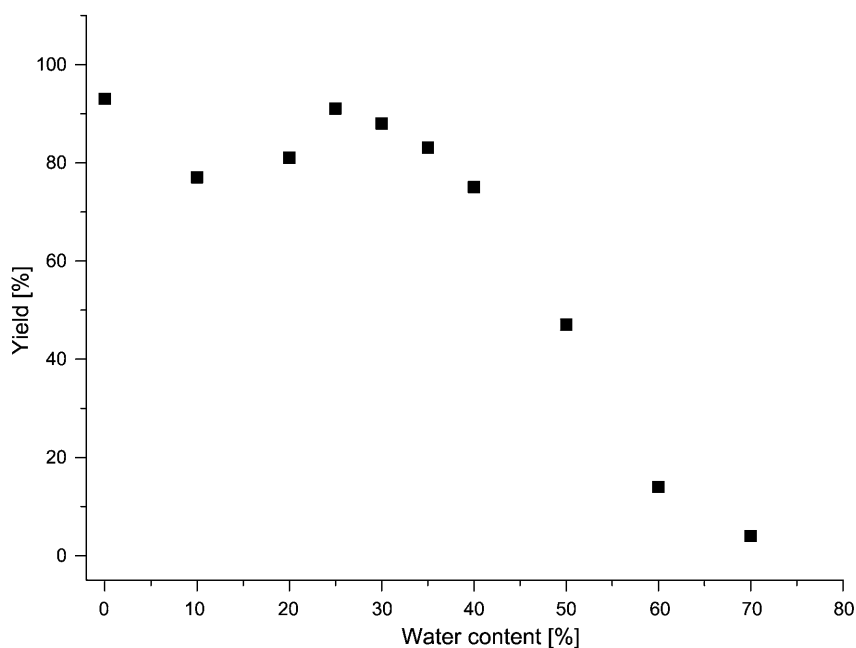


Figure. Influence of H₂O on the trypsin-catalyzed Mannich reaction. Conditions: 4-nitrobenzaldehyde (0.2 mmol), *p*-anisidine (0.2 mmol), acetone (2 ml), and enzyme (20 mg) was stirred at 160 rpm at 37° for 48 h; deionized H₂O from 0 to 70% (H₂O/(H₂O + acetone) (v/v)). Yield of Mannich product was determined by HPLC.

Other influencing factors such as temperature, concentration of catalyst, and reaction time have also been investigated. The results showed that the desired products were obtained in yields up to 93% when 20 mg of trypsin in 2 ml of acetone at 37°/48 h were used. Encouraged by this remarkable result and in order to expand the scope of

this new catalyst, we have subjected various aromatic aldehydes, aromatic amines, and acetone to the three-component *Mannich* reaction (Table 3). Trypsin turned out to be an excellent catalyst for all *Mannich* reactions. Excellent yields of β -amino ketones were obtained from benzaldehydes with electron-acceptor substituents, specifically 4-nitrobenzaldehyde. *p*-Anisaldehyde with MeO as electron-donor substituent gave a lower yield. Fortunately, aromatic amines with MeO as electron-donating group afforded the desired products in good yields.

Table 3. *One-Pot, Three-Component Mannich Reaction*^{a)}

$$\text{R}^1\text{CHO} + \text{R}^2\text{NH}_2 + \text{CH}_3\text{COCH}_3 \xrightarrow{\text{Trypsin}} \text{CH}_3\text{C(=O)CH}_2\text{CH(R}^1\text{)NHR}^2$$

4a – 4k

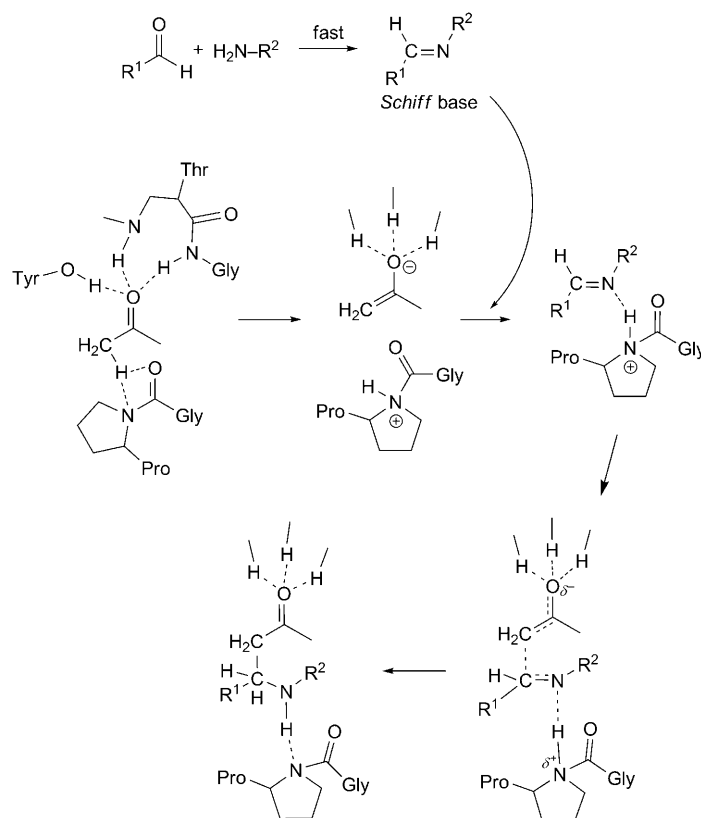
Entry	Product	R ¹	R ²	Yield [%] ^{b)}
1	4a	4-NO ₂ -C ₆ H ₄	4-MeO-C ₆ H ₄	93
2	4b	4-MeO-C ₆ H ₄	4-MeO-C ₆ H ₄	45
3	4c	4-Cl-C ₆ H ₄	4-MeO-C ₆ H ₄	83
4	4d	4-NO ₂ -C ₆ H ₄	2-MeO-C ₆ H ₄	55
5	4e	4-NO ₂ -C ₆ H ₄	4-Cl-C ₆ H ₄	84
6	4f	4-NO ₂ -C ₆ H ₄	3-Cl-C ₆ H ₄	82
7	4g	4-NO ₂ -C ₆ H ₄	3-NO ₂ -C ₆ H ₄	76
8	4h	Ph	2-MeO-C ₆ H ₄	68
9	4i	Ph	4-Cl-C ₆ H ₄	74
10	4j	Ph	3-Cl-C ₆ H ₄	65
11	4k	Ph	3-NO ₂ -C ₆ H ₄	47

^{a)} Reaction conditions: a solution of the corresponding aldehyde (1 mmol), and amine (1 mmol), acetone (5 ml), and trypsin (20 mg) was shaken at 160 rpm at 37° for 24–48 h. ^{b)} Yield of isolated product.

Based on the mechanism of lipase-catalyzed [6] organic reaction which has been widely accepted, we assume that a similar mechanism is involved in the trypsin-catalyzed *Mannich* reaction (Scheme 2). The *in situ* generated *Schiff* base complexes with the trypsin. At the same time, the enolate anion is stabilized by the oxyanion hole. Then, the enolate anion attacks the *Schiff* base complexed with trypsin and a new C–C bond is formed. H-atom transfer to the O-atom from acetone takes place in a concerted process. Finally, the *Mannich* adduct is released from the oxyanion hole. We will study this proposed mechanism further in detail.

In conclusion, we have found out that several enzymes, especially trypsin, have remarkable catalytic activity to promote three-component *Mannich* reaction. This reaction can be carried out under mild conditions both in organic and in aqueous solvents, and provides β -amino carbonyl compounds in high yields. The trypsin-catalyzed *Mannich* reaction covers a great range of substrates and further extends the application of enzymes in the organic synthesis.

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Scheme 2. *Proposed Mechanism of the Trypsin-Catalyzed Mannich Reaction*


Experimental Part

General. Commercial reagents were used without further purification, unless otherwise indicated. All solvents were distilled prior to use. Reactions were performed in oven-dried glassware. Flash chromatography (FC): silica gel 60 (SiO_2 , 230–400 mesh; *Fluka*). Anal. TLC: *Merck* precoated TLC (silica gel 60 *F254*) plates. C_{18} column was used in the HPLC experiments with MeOH/ H_2O 70:30 (v/v), 0.8 ml/min; UV detection at 254 nm. M.p.: *X4-Data* microscopic melting-point apparatus; uncorrected. IR Spectra: *Nicolet 380* FT-IR spectrophotometer using KBr discs. 1H - and ^{13}C -NMR spectra: *Bruker Avance 400* spectrometer in $CDCl_3$ using TMS as internal standard. ESI-MS: *Bruker Esquire 3000 plus* spectrometer.

General Procedure. 4-[(4-Methoxyphenyl)amino]-4-(4-nitrophenyl)butan-2-one (**4a**). A soln. of 4-nitrobenzaldehyde (1 mmol), *p*-anisidine (1 mmol), acetone (5 ml), and trypsin (30 mg) was shaken at 160 rpm at 37° for 24 h. The residue was then filtered off, and the solvent was evaporated. A single product was obtained by FC (petroleum ether (PE)/AcOEt 4:1 (v/v)). 1H -NMR: 8.19–8.17 (*m*, 2 arom. H); 7.57–7.55 (*m*, 2 arom. H); 6.71–6.67 (*m*, 2 arom. H); 6.48–6.46 (*m*, 2 arom. H); 4.86 (*t*, $J=6.4$, CH_2CH); 3.70 (*s*, MeO); 2.96 (*d*, $J=9.6$, CH_2); 2.15 (*s*, Me). ESI-MS: 314 ($[M+Na]^+$).

4-(4-Methoxyphenyl)-4-[(4-methoxyphenyl)amino]butan-2-one (**4b**). 1H -NMR: 7.26–7.24 (*m*, 2 arom. H); 6.84–6.82 (*m*, 2 arom. H); 6.89–6.87 (*m*, 2 arom. H); 6.51–6.49 (*m*, 2 arom. H); 4.70 (*t*, $J=6.4$, CH_2CH); 3.76 (*s*, MeO); 3.68 (*s*, MeO); 2.87 (*d*, $J=6.4$, CH_2); 2.07 (*s*, Me). ESI-MS: 299 ($[M+Na]^+$).

4-[(4-Chlorophenyl)-4-[(4-methoxyphenyl)amino]butan-2-one (**4c**). $^1\text{H-NMR}$: 7.13–7.11 (*m*, 2 arom. H); 6.62–6.58 (*m*, 4 arom. H); 6.44–6.42 (*m*, 2 arom. H); 4.70 (*q*, $J = 5.2$, CH_2CH); 2.70 (*m*, $J = 6.4$, CH_2); 2.05 (*s*, Me). ESI-MS: 303 ($[M + \text{Na}]^+$).

4-[(2-Methoxyphenyl)amino]-4-(4-nitrophenyl)butan-2-one (**4d**). $^1\text{H-NMR}$: 8.16–8.13 (*m*, 2 arom. H); 7.55–7.53 (*m*, 2 arom. H); 6.77–6.27 (*m*, 4 arom. H); 4.96 (*t*, $J = 6.4$, CH_2CH); 3.87 (*s*, MeO); 2.98 (*m*, $J = 9.6$, CH_2); 2.14 (*s*, Me). ESI-MS: 314 ($[M + \text{Na}]^+$).

4-[(4-Chlorophenyl)amino]-4-(4-nitrophenyl)butan-2-one (**4e**). $^1\text{H-NMR}$: 8.18–8.16 (*m*, 2 arom. H); 7.55–7.53 (*m*, 2 arom. H); 7.04–7.02 (*m*, 2 arom. H); 6.42–6.40 (*m*, 2 arom. H); 4.88 (*t*, $J = 6.0$, $\text{H}-\text{C}-\text{Ph}$); 4.67 (*s*, NH); 2.98 (*d*, $J = 5.6$, CH_2); 2.15 (*s*, Me). ESI-MS: 318 ($[M + \text{Na}]^+$).

4-[(3-Chlorophenyl)amino]-4-(4-nitrophenyl)butan-2-one (**4f**). $^1\text{H-NMR}$: 8.20–8.18 (*m*, 2 arom. H); 7.56–7.54 (*m*, 2 arom. H); 7.02–6.98 (*m*, 1 arom. H); 6.67–6.36 (*m*, 3 arom. H); 4.90 (*t*, $J = 6.4$, CH_2CH); 4.75 (*s*, NH); 2.99 (*d*, $J = 6.4$, CH_2); 2.15 (*s*, Me). ESI-MS: 318 ($[M + \text{Na}]^+$).

4-(4-Nitrophenyl)-4-[(3-nitrophenyl)amino]butan-2-one (**4g**). $^1\text{H-NMR}$: 8.18–8.16 (*m*, 2 arom. H); 7.57–7.55 (*m*, 2 arom. H); 7.50–6.76 (*m*, 4 arom. H); 5.18 (*s*, NH); 4.98 (*t*, $J = 6.4$, CH_2CH); 3.03 (*m*, $J = 6.4$, CH_2); 2.16 (*s*, Me). ESI-MS: 329 ($[M + \text{Na}]^+$).

4-[(2-Methoxyphenyl)amino]-4-phenylbutan-2-one (**4h**). $^1\text{H-NMR}$: 7.38–7.21 (*m*, 5 arom. H); 6.77–6.42 (*m*, 4 arom. H); 4.88 (*t*, $J = 6.4$, CH_2CH); 4.80 (*s*, NH); 3.07 (*s*, MeO); 2.96 (*m*, $J = 9.2$, CH_2); 2.12 (*s*, Me). ESI-MS: 269 ($[M + \text{Na}]^+$).

4-[(4-Chlorophenyl)amino]-4-phenylbutan-2-one (**4i**). $^1\text{H-NMR}$: 7.34–7.27 (*m*, 5 arom. H); 7.04–7.02 (*m*, 2 arom. H); 6.47–6.46 (*m*, 2 arom. H); 4.79 (*t*, $J = 6.4$, CH_2CH); 4.57 (*s*, NH); 2.92 (*d*, $J = 6.0$, CH_2); 2.11 (*s*, Me). ESI-MS: 273 ($[M + \text{Na}]^+$).

4-[(3-Chlorophenyl)amino]-4-phenylbutan-2-one (**4j**). $^1\text{H-NMR}$: 7.34–7.24 (*m*, 5 arom. H); 7.01–6.40 (*m*, 4 arom. H); 4.81 (*t*, $J = 6.4$, CH_2CH); 4.61 (*s*, NH); 2.92 (*d*, $J = 6.4$, CH_2); 2.09 (*s*, Me). ESI-MS: 273 ($[M + \text{Na}]^+$).

4-[(3-Nitrophenyl)amino]-4-phenylbutan-2-one (**4k**). $^1\text{H-NMR}$: 7.40–7.33 (*m*, 5 arom. H); 7.28–7.16 (*m*, 3 arom. H); 6.82–6.80 (*m*, 1 arom. H); 4.87 (*t*, $J = 6.0$, CH_2CH); 2.96 (*d*, $J = 6.4$, CH_2); 2.11 (*s*, Me). ESI-MS: 284 ($[M + \text{Na}]^+$).

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