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Biocatalytic aza-Michael addition of aromatic amines to enone using α-amylase in water

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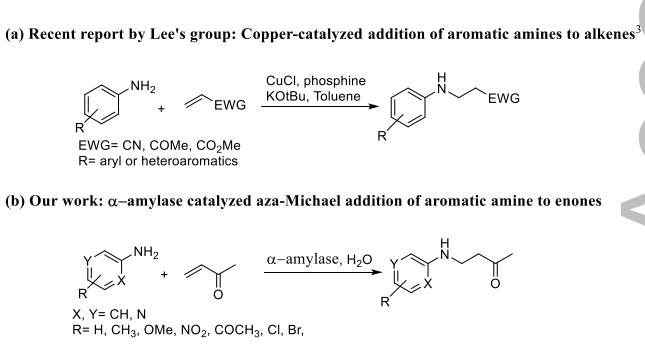
Abstract. The Michael addition of amines with enones for synthesizing ß-amino carbonyls constitutes a valuable transformation in organic chemistry. While various catalyst have been made available for catalyzing the Michael addition of aromatic amines to enones but there is no report of using α-amylase enzyme to catalyze this transformation. The α-amylase from *Aspergillus oryzae* was found to catalyze the Michael addition of various aryl (hetero) amines to methyl vinyl ketone with high catalytic efficiency (63-83% yield). A hybrid of α-amylase with copper nanoparticle (α-amylase@CuNPs) has been prepared and used to catalyze this transformation as a reusable catalyst. Further, an application of α-amylase catalyzed aza-Michael addition in the cascade reactions has been exhibited by synthesizing biologically important 3-acetyl quinoline. In addition, molecular docking and molecular dynamics (MD) simulation studies are carried out to get insight into the key interactions of the substrates with the amino acid residues near the active site and the probable reaction mechanism, which reveals Glu230 and Asn295 play a crucial role in the substrate activation process.

Keywords: Biocatalyst, α-amylase, aza-Michael addition, C-N bond formation, enone.

Introduction

The Michael addition of various nucleophiles to electron deficient alkenes is one of the valuable transformation in the area of synthetic organic chemistry.[1] In particular, aza-Michael addition which leads to the formation of a new carbon-nitrogen bond to afford ß-amino carbonyl compounds has been proved most important.[2] The ß-amino carbonyls are imperative building blocks of various biologically active compounds.[3] Furthermore, they constitute versatile intermediates for the synthesis of ß-lactams, amino alcohols, and ß-aminoacids.[4] Conventionally, the aza-Michael addition reactions are carried out for synthesizing ß-amino carbonyls under strong acidic or basic conditions.[5] There are also reports where milder Lewis acids have been used for this purpose.[6] However, the conventional methods have certain drawbacks such as requirement of high temperatures, long reaction times, poor compatibility with various substrate functional groups and generation of side products. Moreover, novel catalysts such as ß-cyclodextrin, bromo-dimethylsulfonium bromide, boric acid in water has been proved to be valuable to overcome the problems associated with conventional catalysts.[7]



Scheme 1. Aza-Michael addition of less nucleophilic aromatic amines to α, β-unsaturated olefins

Despite this progress, these methods are limited only to catalyse the aza-Michael addition of aliphatic

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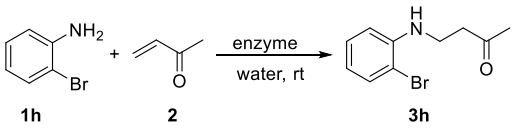
amines very efficiently. The development of a novel catalyst for the addition of less nucleophilic aromatic amines to electron deficient alkenes has seen tremendous progress in the last few years. In this context, early- and late-transition metals, ionic liquids and molecular iodine have been reported as catalysts for the Michael-addition of aromatic amines.[8] Very recently, Lee's group reported copper-catalyzed aza-Michael addition of aromatic amines to α, β-unsaturated olefins (Scheme 1a).[9] Although, this method has a few shortcomings such as use of strong bases, expensive and non-reusable ligands and generation of toxic transition-metal waste.

On the other hand, the use of enzymes for catalysing the valuable organic transformations has exponentially grown in the current times.[10] Further, a number of enzymes either in the free form or in immobilized form have been reported for catalysing the aza-Michael addition reaction of amines with electron deficient alkenes.[11] In this context, various lipase enzymes have been used as a catalyst.[12] Lin's group have been reported alkaline protease or hydrolase enzymes to catalysed the Michael addition of imidazole with acrylates.[11b, 11c] Very recently, Chen *et al.* used lipase enzyme to catalysed the aza-Michael addition of amines to acrylates in supercritical carbon dioxide.[13] Moreover, the α-amylase enzymes are known for catalyzing the hydrolysis of α-glucosidic linkages of polysaccharides such as starch or glycogen in nature.[14] Over the years, α-amylase have been used to catalyse various organic reactions.[15] Recently, Guan and co-workers have reported a one-pot synthesis of nitrocyclopropane using α-amylase enzyme as a catalyst.[16] Additionally, Yu et al. developed α-amylase catalyse synthesis of highly substituted indoloquinolizines using tryptamines, β-ketoesters and α, β-unsaturated aldehydes.[17] But, to the best of our knowledge there is no previous report of using α-amylase as a catalyst for the aza-Michael addition reaction of amines to enones. Herein, we report α-amylase from *Aspergillus oryzae* [E.C. 3.2.1.1] as an efficient biocatalyst for catalysing the aza-Michael addition of less nucleophilic aryl (hetero) amine derivatives to enone (methyl vinyl ketone).

Results and Discussion

We started our investigations by selecting 2-bromoaniline and methyl vinyl ketone as the model substrates and screened different enzymes to catalyze the aza-Michael addition reaction of model substrates (Table 1). To our surprise only α-amylase gave the desired product (3h) in 28% conversion yield (entry 4, Table 1). Further, this reaction gave 45% conversion when performed at 40°C using α-amylase as catalyst in water as a solvent (entry 5, Table 1). After having an enzyme in hand for this addition reaction, we moved to screen various reaction conditions such as solvents, temperature and amount of reagents and catalyst to improve the conversion of this transformation. First, we evaluated the effectiveness of different organic solvents such as DMSO, THF and hexane in place of water (Table 2). Among the various solvents tested for this addition reaction water remains the best choice. Also, we checked the different percentage of DMSO in water as a solvent just to make both starting material completely soluble during reaction (entry 5-7, Table 2). We obtained maximum conversion when we used 10% DMSO-water mixture (1:9 v/v) as a solvent (entry 6, Table 2).

Table 1. Screening of different enzymes for Michael addition of 2-bromoaniline to methyl vinyl ketone[a]

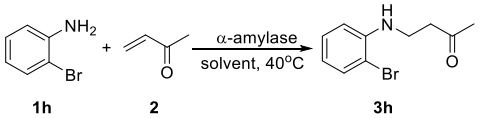


1h 2 3h

entry	enzymes	[b]conversion%
1	*Rhizomucor miehei lipase* (RML)	NR
2	*Pseudomonas stutzeri lipase* (PSL)	NR
3	*Candida antarctica lipase B* (CALB)	trace
4	α-amylase from *A. oryzae*	28 %
5	α-amylase from *A. oryzae*	[c]45 %
6	blank	[d]NR

[a)]Reaction conditions: 1.0 equiv. of 2-bromoaniline (0.58 mmol) and 1.2 equiv. of methyl vinyl ketone (0.69 mmol), 1 mL of enzyme (1 mg/mL in H_2O) and 1 mL of H_2O were added to a glass tube having a teflon cap and stirred at room temperature for overnight. [b)]based on HPLC, [c)]temperature 40°C, [d)]Blank: conversion under same conditions without enzyme.

Table 2. Screening of solvents for α-amylase-catalyzed addition of 2-bromoaniline to methyl vinyl ketone[a]



1h 2 3h

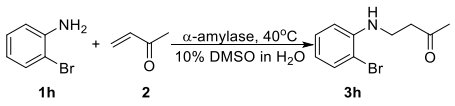
entry	solvents	[b]conversion
1	H_2O	45%
2	DMSO	42%
3	THF	31%

4	Hexanes	15%
5	5% DMSO-water mixture	51%
6	10% DMSO-water mixture	65%
7	20% DMSO-water mixture	64%

[a)]Reaction conditions: 1.0 equiv. of 2-bromoaniline (0.58 mmol) and 1.2 equiv. of methyl vinyl ketone (0.69 mmol), 1 mL of α-amylase (1 mg/mL in H_2O) and 1 mL of solvent were added to a glass tube having a teflon cap and stirred at 40°C for overnight, [b)]based on HPLC.

Further, we tested the effect of different molar ratios of 2-bromoanilne and methyl vinyl ketone on the conversion of this addition reaction (Table 3) and found that 1:1.2 molar ratio of 2-bromo aniline and methyl vinyl ketone remained the best choice to attain the maximum conversion (entry 2, Table 3). We also increased the temperature of this reaction upto 80°C but found that 40°C was the optimum temperature for getting the highest conversion.

Table 3. Optimization of the substrates molar ratio and reaction temperature[a]

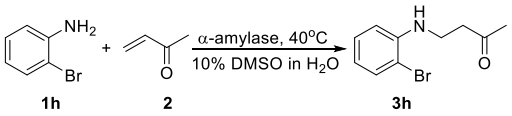


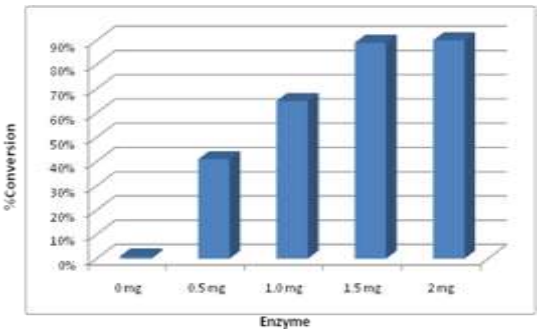
entry	ratio of reagents (1a:2a)	temperature	[b]conversion
1	1:1	40°C	54%
2	**1:1.2**	**40°C**	**65%**
3	1:1.5	40°C	64%
4	1:0.5	40°C	34%
5	1:1.2	50°C	63%
6	1:1.2	60°C	59%
7	1:1.2	80°C	43%

[a)]Reaction conditions: 2-bromoaniline (0.58 mmol) and methyl vinyl ketone in 200 µL of DMSO, 1 mL of α-amylase (1 mg/mL in H_2O) and 0.8 mL of H_2O were added to a glass tube having a teflon cap and stirred for overnight, [b)]based on HPLC.

In the final phase of optimization, we increased the concentration of enzyme to improve the yield of this transformation (Figure 1). To our delight, high yield (89%) could be obtained even at low catalyst loading (1.5 mg/mL); this undoubtedly proved the efficiency of α-amylase as a catalyst for this addition reaction.

Figure 1. Optimization of the concentration of α-amylase[a]





[a)]Reaction conditions: 1.0 equiv. of 2-bromo aniline (0.58 mmol) and 1.2 equiv. of methyl vinyl ketone (0.69 mmol) in 200 µL of DMSO, 1 mL of α-amylase in H_2O (having 0.5 1.0, 1.5 and 2.0 mg/mL conc. respectively) and 0.8 mL of H_2O were added to a glass tube having a teflon cap and stirred at 40°C for overnight, [b)]based on HPLC, %error = ±4.7%.

After having the optimized conditions for the aza-Michael addition of 2-bromo aniline (1 equiv.) and methyl vinyl ketone (1.2 equiv.) using α-amylase (1.5 mg/mL) as catalyst in 2 mL of 10% DMSO-water mixture as the solvent at 40°C for overnight, we explored the scope of different substitution on aryl(hetero) amines under the optimized reaction conditions (Table 4). The reaction of aniline with methyl vinyl ketone produced the Michael addition product (3a) in 74% yield (entry 1, Table 4). Then, we tested the effect of electron-donating groups such as -Me and -OMe at the arene ring which allowed the reaction to afford the products in good yields (entry 2-3, Table 4). On the other hand, the presence of electron-withdrawing group such as $-NO_2$, $-COCH_3$ at the arene ring of aniline has significant effect on the reactivity of the reaction and afforded the desired products in slightly lower yield (entry 4-6, Table 4). A much lower yield was obtained when $–NO_2$ group was at ortho-position (entry 4, Table 4). Moreover, the

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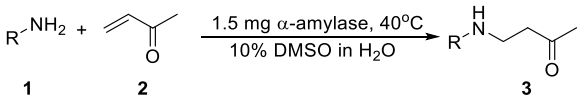
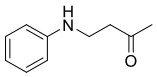
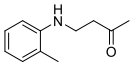
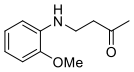
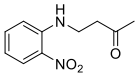
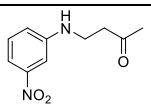
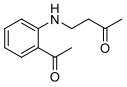
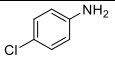
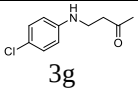
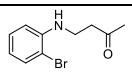
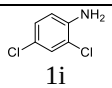
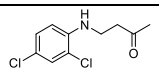
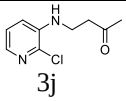
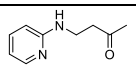
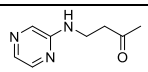
presence of halides such as -Cl, -Br and -F on different positions of aryl amines slightly increased the yield of the reaction (entry 7-9, Table 4). Gratifyingly, the nitrogen containing heteroaromatic amines also reacted well and gave the corresponding products in very good yield (entry 10-12, Table 4).

Table 4. Scope of substrates for the α-amylase catalyzed Michael addition reaction[a]



1 2 3

entry	amines	products	[b]yield
1	 1a	 3a	74%
2	 1b	 3b	81%
3	 1c	 3c	77%
4	 1d	 3d	53%
5	 1e	 3e	69%
6	 1f	 3f	63%
7	 1g	 3g	83%
8	 1h	 3h	82%
9	 1i	 3i	71%
10	 1j	 3j	81%
11	 1k	 3k	75%
12	 1l	 3l	69%

[a)]Reaction conditions:1.0 equiv. of substituted aromatic amines and 1.2 equiv. of enones in 200 µL of DMSO, 1.5 mL of α-amylase (1 mg/mL in H_2O) and 0.3 mL of H_2O were added to a glass tube having a teflon cap and stirred at 40°C for overnight, [b)]isolated yields.

In the second phase of our endeavor, we tried to isolate the α-amylase after the completion of the first catalytic cycle for reusing it in further catalytic cycles, but we were unsuccessful to isolate the α-amylase from the reaction. Recently, there are various reports for synthesizing a hybrid of enzyme with metal nanoparticles which increase the operational stability to use it in organic transformation as reusable catalyst.[18] Very recently Bäckvall's group reported a Pd(0)-CALB biohybrid catalyst and used it in a cascade reaction.[19] Encouraged by previous reports, we developed histidine protected biogenic copper nanoparticles (CuNPs) and stabilized them on α-amylase enzyme. The synthesized enzyme-nanocluster is termed as "α-amylase@CuNPs".[20] The characterization of the novel α-amylase@CuNPs was done using various techniques such as UV-visible, DLS, FT-IR, TEM and EDS (The details of synthetic procedure and characterization is given in supporting information).

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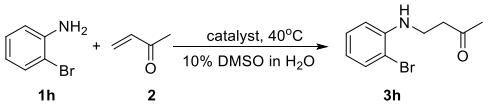
Next, we tested the hybrid catalyst α-amylase@CuNPs for catalyzing the aza-Michael addition reaction of 2-bromoaniline with methyl vinyl ketone (Table 5). To our delight, we obtained very similar results when comparing the catalytic efficiency of this hybrid with the free enzyme (entry 1-2, Table 5). Subsequently, we tested α-amylase@CuNPs for reusability and the results are shown in Figure 2. These results indicated that hybrid catalyst can be use many times with high catalytic efficiency due to improved operational stability under the optimized reaction conditions.

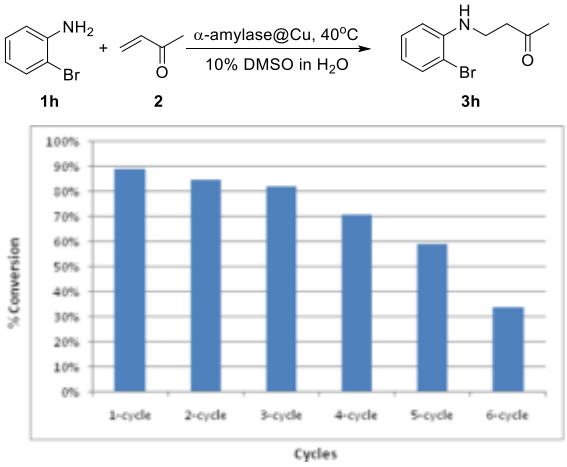
Table 5. Comparison of the catalytic activities between α-amylase and α-amylase@CuNPs[a]



entry	catalyst	[b]conversion
1	α-amylase	89%
2	**amylase@CuNPs**	**91%**
3	$CuSO_4$	15%
4	CuNPs	trace
5	L-Histidine	no reaction

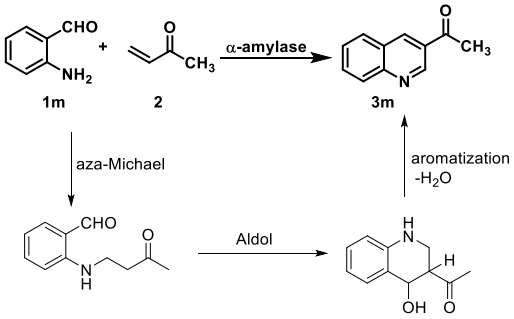
[a)]Reaction conditions: 1.0 equiv. of 2-bromoaniline (0.58 mmol) and 1.2 equiv. of methyl vinyl ketone (0.69 mmol) in 200 µL of DMSO, 1.5 mL of catalyst (1 mg/mL in H_2O) and 0.3 mL of H_2O were added to a glass tube having a teflon cap and stirred at 40°C for overnight, [b)]based on HPLC.

Figure 2. Reusability test of amylase@Cu for the Michael addition of 2-bromo aniline with vinyl ketone[a]



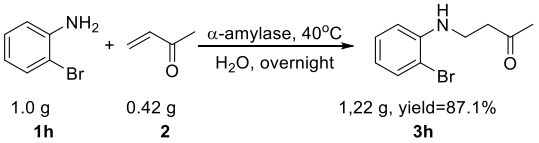
[a)]Reaction conditions: 1.0 equiv. of 2-bromoaniline (0.58 mmol) and 1.2 equiv. of methyl vinyl ketone (0.69 mmol) in 200 µL of DMSO, 1.5 mL of α-amylase@CuNPs (1 mg/mL in H_2O) and 0.3 mL of H_2O were added to a glass tube having a teflon cap and stirred at 40°C for overnight, [b)]based on HPLC, %error = ±6.2%

In addition, the tandem Michael addition/Aldol condensation reaction to synthesize various biologically important heterocyclic compounds has gained significant attention over the years.[21] To exhibit the application of α-amylase in the cascade reaction consisting aza-Michael addition/Aldol condensation, we started a reaction of 2-amino benzaldehyde (1m) and methyl vinyl ketone (2) at 40°C. Gratifyingly, we obtained the desired product 3-acetyl quinolone (3m) in 26% yield with ~93% purity (Scheme 2, Figure S8). However, the conversion of this reaction was lower and require additional investigation to improve the yield.



Scheme 2: Synthesis of 3-acetyl quinoline via α-amylase catalyzed aza-Michael addition/Aldol condensation/aromatization cascade reaction.

Table 6: Scale-up synthesis of ß-amino carbonyl (3h) and calculation of green chemistry metrics[a]



entry	metrics	results
1	isolated yield	87.1%
2	selectivity	100%
3	E-factor	0.16
4	PMI	1.16
5	atom-economy	100%
6	reaction mass efficiency	86.1%

[a)]Reaction conditions: 2-bromoaniline (1.0 g, 5.81 mmol, 1 equiv.) and methyl vinyl ketone (0.426 g, 5.81 mmol, 1.0 equiv.) in 2.0 mL of DMSO, 15 mL of α-amylase (1 mg/mL in H_2O) and 3 mL of H_2O were added and stirred the resulting mixture at 40°C for overnight.

In order to assess the scalability of α-amylase-catalyzed aza-Michael addition, the reaction of 2-bromoaniline (1.0 g) and methyl vinyl ketone (0.426 g, 1.0 equiv.) to synthesize the aza-Michael product (3h) using α-amylase (15 ml, 1mg/ml in H_2O) was carried out (Table 6). The successful isolation of 1.22 g of (3h) in 95% purity from this reaction proved the synthetic utility of α-amylase in the synthesis of ß-amino carbonyl compounds in gram scales. Furthermore, we calculated the green chemistry metrics for this reaction such as E-factor, PMI, and atom-economy and reaction mass efficiency to display the high greenness of this protocol and compiled the results in Table 6.

Active site structure and proposed mechanism

A modeling of the interaction of the substrates with the key amino acids of the α-amylase at the active site was carried out to investigate the role play by the enzyme in catalyzing the aza-Michael addition of aromatic amines to enones. There have been several experimental and computational reports on the binding site of this particular strain. [22] These studies revealed that a catalytic triad of Glu230, Asp297 and Asp206 constitutes the active site. Hence we started with the crystal structure of the α-amylase from *Aspergillus oryzae* (PDB id : 6TAA)[22a] for molecular modeling. To explore the initial orientation of substrates inside the binding site, first we have docked the substrates, aniline and 2-butenone (methyl vinyl ketone), in the active site by using AutoDock Vina software.[23] Then we have carried out 50 ns Molecular Dynamics (MD) simulation to get the most preferable orientation of the substrates inside the binding site using GPU version64 of the AMBER 16 package.[24] The details of the molecular docking and MD simulations are given in supporting information. The orientation of the substrate in the active site in one of the representative snapshots from MD simulation and the key interactions of the substrates with nearby amino acids are shown in Figure 3. We have observed that the nucleophile, aniline, forms a strong hydrogen bond (1.85 Å) with the Glu230 and thus making it a stronger nucleophile. On the other hand, the carbonyl group of 2-butenone forms a strong hydrogen bond with Asn295 (1.92 Å) and hence becoming a better electron acceptor in protein environment. Thus, based on the substrate positioning in the active site we have identified that the Glu230 and Asn295 play crucial roles in activating the substrates for aza-Michael addition reaction. In order to validate our claim that the strong H-bonding with the Glu230 increases the nucleophilicity of the aniline, we have carried out a Natural bond orbital (NBO)[25] charge analysis on the model system comprised of aniline molecule H-bonded to a glutamic acid residue using Density Functional Theory (DFT). The structure of the model is shown in Figure 4. We have observed a development of a negative charge density (-0.055) on the H-bonded aniline moiety suggesting an increase in electron density on the aniline due to the H-bond formation with glutamic acid residue. Hence, we can conclude that H-bonding with Glu230 makes the aniline a better nucleophile as compared to free aniline. A similar trend is observed for H-bonded 2-bromoaniline moiety for which even higher negative charge density (-0.062) is formed on the 2-bromo aniline moiety (Figure S9).

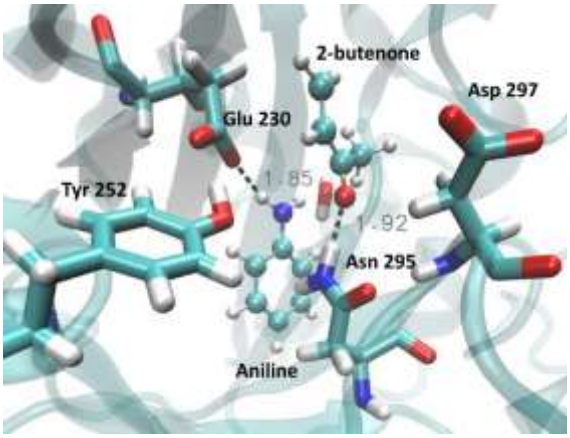


Figure 3: Overview of substrates (aniline and 2-butenone) accommodated in the active site of α-amylase. The representative snapshot was taken from molecular dynamic (MD) simulation, revealing substrates (aniline and 2-butenone) in the binding pocket of α-amylase and the surrounding residues.

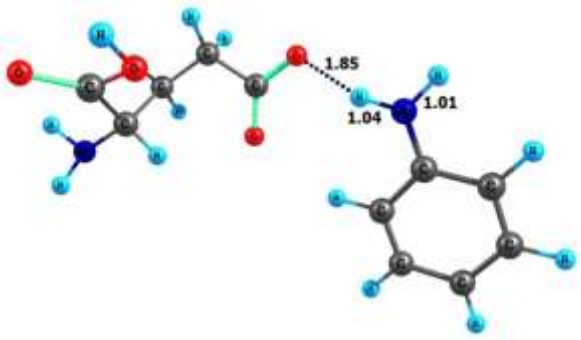
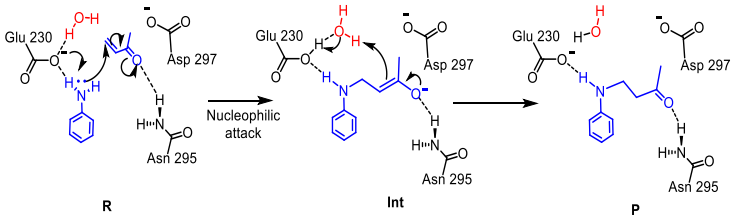


Figure 4: The optimized structure of model system comprised of aniline H-bonded to a glutamic acid. Relevent bond distances are given in Å.

On the basis of the active site structure, we have proposed a probable mechanism in which Glu230 acts as an acid/base residue. The proposed mechanism is shown in Scheme 3. In the first step of the reaction, there will be a nucleophilic attack by the lone pair of nitrogen of aniline to the C=C of the 2-butenone. This nucleophilic attack step will be facilitated by Glu230, which acts as a base, by accepting the proton from –NH_2 group of aniline. The intermediate (Int) generated in this step will have higher electron density on the O-atom of the carbonyl group of 2-butenone, which is stabilized by the strong H-bonding with the Asn295. In the subsequent step the product (P) will be formed by rearrangement of electrons and transfer of proton from Glu230. The transfer of proton can be assisted by water molecule which is used as solvent as shown in Scheme 3. The verification of the proposed mechanism using Quantum Mechanical/ Molecular Mechanical (QM/MM) approach would give us more insights in terms of energetics but that would be a study on its own and will be done in future.



Scheme 3: Proposed mechanism for aza-Michael addition of aniline to 2-butenone using α-amylase.

To get more evidence about the role of Glu230 in the catalysis of aza-Michael addition, we have started a control experiment in the presence of starch which has been known as a natural substrate of α-amylase and Glu230 plays a role in the hydrolysis of this.[26] The results showed that the addition of starch decreased the yield of the reaction from 88% to 50.9% (Scheme S1 & Figure S7) due to the competition reaction between starch and aza-Michael addition substrates, this indicates that Glu230 plays a role in the catalysis of aza-Michael addition reaction of aromatic amines with enones. However, further investigation such as mutagenesis of active site are required to confirm the role of Glu230 in the catalysis.

Conclusion

In summary, we have developed the first example of biocatalytic aza-Michael addition of less nucleophilic aromatic amines to enone using α-amylase as a catalyst. This strategy could be applied to a number of substituted anilines and heteroaromatic amines in combination with methyl vinyl ketone. A hybrid of α-amylase with copper nanoparticles was synthesized and used as a reusable catalyst in many catalytic cycles with high efficiency. The scalability of this biocatalytic transformation was exhibited by synthesizing ß-amino carbonyl derivative (3h) in gram scale and the green chemistry metrics for this reaction such as E-factor, PMI, atom-economy and reaction mass efficiency were calculated which displayed the high greenness of this protocol. Moreover, the α-amylase catalyzed aza-Michael addition was further employed in a cascade reaction to synthesize biologically important 3-acetyl quinoline. In addition, an analysis of binding of substrates at the active site using molecular docking and molecular dynamics studies revealed that Glu230 acts as an acid/base catalyst and Asn295 activates the enols through strong hydrogen bonding. Finally, this work expands the application of α-amylase to catalyze the valuable transformations in the organic chemistry.

Experimental Section

General information: All reagents and solvents were purchased from commercial sources and used without purification. The sterile distilled water was used for the preparation of all aqueous solutions. The α-amylase from *Aspergillus oryzae* (powder, ~30 U/mg) and other enzymes were bought from Sigma-Aldrich. The NMR spectra were recorded on a Jeol-400 MHz or Bruker-400 MHz spectrometer in deuterated solvents with TMS as internal reference (chemical shifts δ in ppm, coupling constant *J* in Hz.). Multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), multiplet (m), and broad singlet (brs). The reaction progress was monitored by thin layer chromatography (TLC) on pre-coated silica gel plates.

General Procedure for the α-amylase catalyzed aza-Michael Addition: To a 10 mL glass tube equipped with a megnetic stirrer bar added 1.5 mL of α-amylase (1 mg/mL in H_2O) and 0.3 mL of H_2O. Afterward, added aromatic amines (100 mg, 1 equiv.) and methyl vinyl ketone (1.2 equiv.) after dissolving in 200 µL of DMSO and stirred the reaction mixture at 40 °C for overnight. After completion of the reaction as indicated by TLC, the resulting mixture was filtered through a small pad of celite and washed the celite two times with ethyl acetate. Further, the organic layer from filterate was extracted using ethyl acetate and dried over sodium sulfate. The volatiles were evaporated under reduced pressure and the residue was purified by column

chromatography on silica gel (eluent: hexane/ EtOAc) affording the corresponding products 3a-3l in very good yields.

Procedure for the α-amylase@CuNPs catalyzed aza-Michael addition: To a 10 mL glass tube equipped with a megnetic stirrer bar added 1.5 mL of hybrid catalyst α-amylase@CuNPs (1.0 mg/ml in H_2O) and 0.3 mL of H_2O at room temperature. After that, added 2-bromoaniline (0.58 mmol, 1 equiv.) and methyl vinyl ketones (0.69 mmol, 1.2 equiv.) after dissolving in 200 µL of DMSO and stirred the reaction mixture at 40°C for overnight. After completion of the reaction as indicated by TLC, the resulting mixtures was transferred to the 10 mL tube and centrifuge for 10 min. @ 10,000 RPM, the hybrid catalyst precipitate as a pellet which was reused for further catalytic cycle and the supernatant was used to extract crude product using ethyl acetate affording the corresponding product (3h) in 91 % conversion yield. To run the reusability experiment, dissolved the pellets obtained from previous cycles into 1.8 mL of H_2O and then followed the same prcodure.

Procedure for the α-amylase catalyzed cascade reaction to synthesize 3-acetyl quinoline (3m): To a 10 mL glass tube equipped with a megnetic stirrer bar added 1.5 mL of α-amylase (1.0 mg/mL in H_2O) and 0.3 mL of H_2O. Subsequently, added 2-aminobenzaldehyde (100 mg, 1 equiv.) and methyl vinyl ketone (1.2 equiv.) after dissolving in 200 µL of DMSO and stirred the reaction mixture at 40 °C and monitored the reaction using TLC. After 72 h, the resulting mixture was filtered through a small pad of celite and washed the celite two times with ethyl acetate. Next, the organic layor from filterate was extracted using ethyl acetate and dried over sodium sulfate. The volatiles were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (eluent: hexane/ EtOAc) affording the corresponding products 3q in 26 % isolated yield.

Author Contributions

SD and VT designed and executed all the reactions, characterization using NMR and Mass spectra and prepared the manuscript. DC and VG synthesized and characterized α-amylase@CuNPs, NG did TEM study of α-amylase@CuNPs. DM executed computational study to get information about the mechanism.

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FULL PAPER

Biocatalytic aza-Michael addition of aromatic amines to enone using α-amylase in water

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