Development of α-amylase catalysed organic transformations

Thesis Submitted in fulfilment of the requirement of the degree of

Doctor of Philosophy

By

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Dedicated
To MY
PARENTS
&
MY FAMILY

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Sunil Dutt

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Certificate

This is to certify that the thesis entitled **"Development of α-amylase catalysed organic transformations"** being submitted by Sunil Dutt, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala for the award of degree Doctor of Philosophy, is a record bonafide of the research work carried out by him. **Mr. Sunil Dutt** has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis. The results embodied in the thesis have not been submitted in a part or full to any other University or Institute for the award of any degree or diploma.

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Candidate's Declaration

I, hereby declare that the work presented in the thesis entitled "**Development of α-amylase catalysed organic transformations**" in fulfilment of the requirement for the award of the degree of Philosophy, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is an authentic record of my work carried out under the supervision of **Dr. Vikas Tyagi**, Assistant Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala, India. The research work embodied in the thesis has not been submitted in part or partially to any other University or Institute for the award of any degree in India or abroad.

Sunil Dutt

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LIST OF ABBREVIATIONS

AA	α-amylase
AOA	α-amylase from *Aspergillus oryaze*
BSA	α-amylase from *Bacillus Species*
PPA	α-amylase from *Porcine Pancreas*
BA	β-amylase
PPL	Lipase from *Porcine Pancreas*
MG	Myoglobin
HE	Hemin Enzyme
MV	Myoglobin Variant
LP	Lingual Lipase
CALB	Lipase from *Candida Antarctica*
CCL	Lipase from *Candida Cylindracea*
RML	Lipase from *Rhizomucor Miehei*
RAL	Lipase from Rhizopus Arrhizus
RDL	Lipase from *Rhizopus Delmar*
RJL	Lipase from Rhizopus *Javanicus*
RSL	Lipase from *Rhizopus sp.*
AMR	Aza-Michael Reaction
BAC	β-amino carbonyl compound
THF	Tetra hydro furan
DMSO	Dimethyl sulfoxide
EtOH	Ethanol
MeOH	Methanol
DMF	Dimethylformamide
NaOH	Sodium hydroxide
K_2CO_3	Potassium carbonate
$CsCO_3$	Caesium carbonate
KO^tBu	Potassium *tert*-butoxide
$(C_2H_5)_3N$	Triethylamine
$(C_2H_5)_2NH$	Diethylamine
$Pd(OAc)_2$	Palladium acetate

DBU	1,8-Diazabicyclo(5.4. 0)undec-7-ene
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladium(0)
$PdCl_2$	Palladium chloride
CuI	Copper iodide
CuO	Copper oxide
$Cu(OAC)_2$	Copper acetate
$C_6H_5NH_2$	Aniline
$C_6H_4NH_2CHO$	2-Amino benzaldehyde
$C_6H_{11}CN$	Cyclohexylisocynide
Me_3CNC	*tert*-Butyl isocyanide
$C_9H_{17}N$	1,1,3,3-*tetra* Methyl butyl isocyanide
C_6H_6NBr	2-Bromo aniline
^{1}HNMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
2DNMR	Two-dimensional nuclear magnetic resonance
COSY	^{1}H-^{1}H Correlation Spectroscopy
NOSY	Nuclear Overhauser Effect
TMS	Trimethyl silane
D_2O	Deuterium oxide
$CDCl_3$	Deuterated chloroform
C_8H_7N	Indole
GC	Gas Chromatography
HPLC	High-Performance Liquid Chromatography
CY	Conversion Yield
IY	Isolated Yield
UV	Ultra-Violet Spectroscopy
IR	Infrared Spectroscopy
GC-MS	Gas Chromatography and Mass Spectrometry
LC-MS	Liquid Chromatography with mass spectrometry

HRMS	High-resolution mass spectrometry
TLC	Thin Layer Chromatography
CC	Column Chromatography
FT-IR Fourier	Fourier Transform Infra-Red
FT-NMR	Fourier Transform-Nuclear Magnetic Resonance
XRD	X-Ray Diffraction
XPS	X-Ray Photoelectron Spectroscopy
SEM	Scanning Electron Microscopy
SAED	Selected Area Electron Diffraction
EDS	Energy Dispersive X-ray Spectroscopy
$KMnO_4$	Potassium Permanganate
PPm	Parts per million
DS	Docking Study
HA	Hybrid catalyst
IOE	Immobilization of enzym
cm^{-1}	Inverse centimetre
Mol	Mole
mmol/g	Millimole per gram
kJ mol^{-1}	Kilo joule per mole
kJ mol^{-1} K^{-1}	Kilo Joule per mole per kelvin
$JK^{-1}mol^{-1}$	Joule per Kelvin per mole
$M^{-1}s^{-1}$	Moles per litre per second
Js	Joule second
JK^{-1}	Joule per Kelvin
min^{-1}	Per minute
h^{-1}	Per hour
mL	Millilitre
µL	Microlitter
Mg	Milligram
Nm	Nanometre
Mm	Millimeter
MHz	MegaHertz

eV	Electron vol
v/v	Volume by volume
NR	No reaction
RI	Refractive Index
Eq.	Equivalent
MM	Molar Mass
EW	Equivalent Weight

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LIST OF SYMBOLS

Symbols	Description
˚A	Angstrom
A	Pre-exponential factor
C	Celsius
E_a	Activation energy
R	Gas constant
K	Rate constant
T	Temperature
ΔH‡	Enthalpy of reaction
ΔS‡	Entropy of reaction
ΔG‡	Gibb's free energy
kB	Boltzmann constant
H	Planck constant
g	Gram
◦	Degree
Λ	Wavelength
Min	Minute
h	Hour
s	Second
%	Percentage
Y	Yield
C	Conversion
S	Selectivity
d1	Relaxation delay
T1	Longest relaxation time
K	Kelvin
I	Integration

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ABSTRACT OF THE THESIS

Enzymes and whole cells are examples of biocatalysts that really can speed up and begin biochemical processes. In particular, enzymes are globular proteins formed by relatively tiny microbes that facilitate the modification of organic compounds into usable products. In addition, applying enzymes as a catalyst for unnatural reactions has been established as a valuable and environmentally sustainable method for synthetic chemistry. Besides, they provide high enantioselectivity, regioselectivity, and chemoselectivity, which could help to accelerate chemical reactions quite successfully and decrease the possibility of unwanted side reactions like racemization, decomposition, rearrangements, and isomerization. The selectivity and specificity of enzymes as a biocatalyst are also enhanced by genetic engineering to create efficient and environmentally friendly catalysts. Consequently, a critical challenge in the field of bioprocesses is the investigation of an enzyme that could facilitate a non-natural reaction. Further, enzymes have been categorized into different classes such as lyases, isomerases, ligases, oxidoreductases, transferases as well as hydrolases. Among different hydrolases, α-amylase have been used to catalyze a broad range of organic transformations. In nature, α-amylase catalyzes the hydrolysis of 1,4-glycosidic bonds in starch which leads to the formation of glucose, maltose, and dextrin. However, in the last decade, various α-amylase catalyzed non-natural organic transformations have been reported.

A crucial transformation in organic synthesis is the Michael addition of amines to enones to develop β-amino carbonyls. The Michael addition of aromatic amines to enones has been catalysed in the presence of a number of catalysts, but, no enzyme was available to catalyze this process. We first time found that α-amylase from *Aspergillus oryzae* has a superior catalytic efficiency (63-83% yield) whenever it was used to facilitate the Michael addition of various aryl (hetero) amines to methyl vinyl ketone. Additionally, in order to understand the key interactions of the substrates with the amino acid residues close to the active site as well as the most likely reaction mechanism, molecular docking, and molecular dynamics (MD) simulations were investigated. The above studies showed the significance of Glu230 and Asn295 in the substrate activation process.

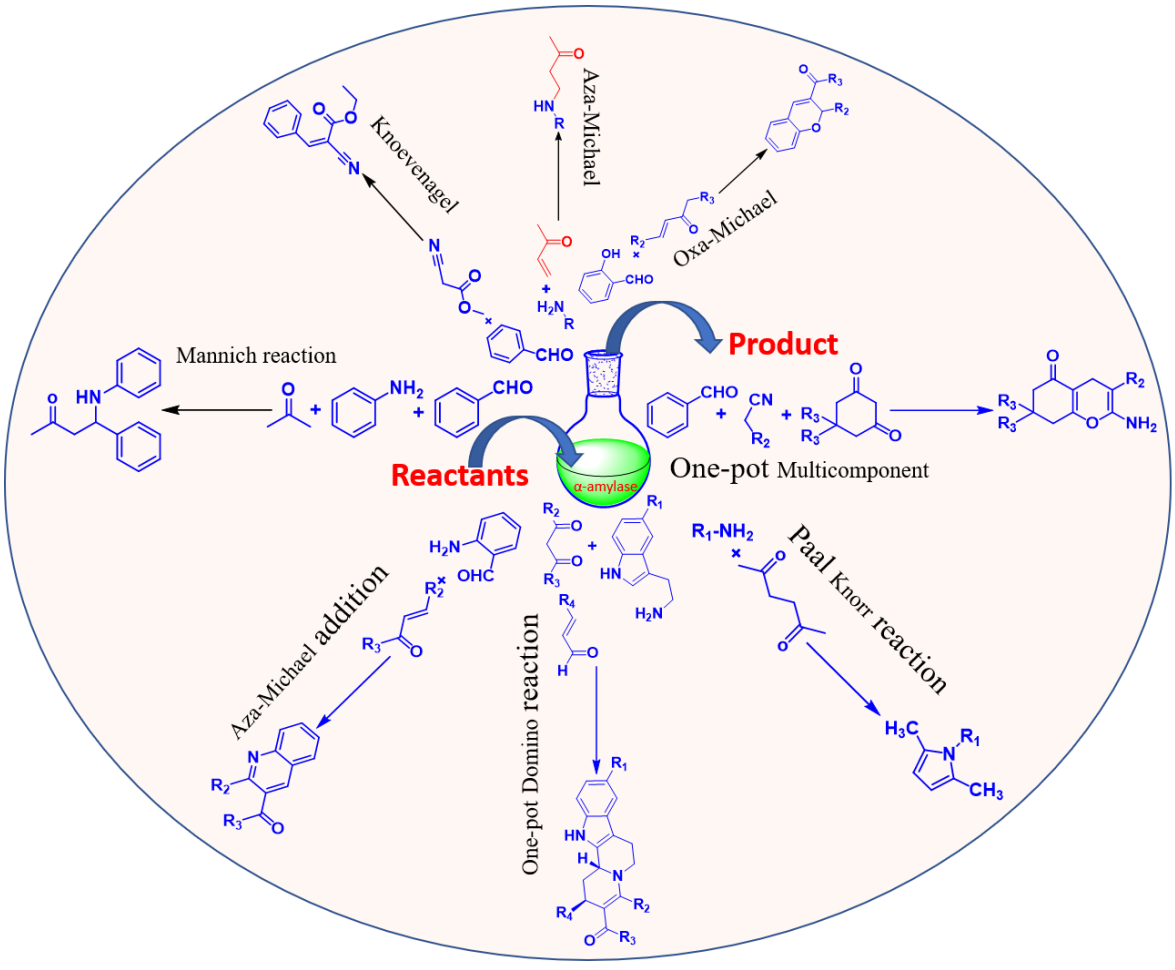
We continued to expand our research beyond and developed the derivatives of substituted quinolines. The quinoline ring system, which is widely spread in pharmaceutical drugs is essential for the growth of new drugs. The biological activities of quinoline derivatives such as antimalaria, anticancer, antiviral, antifungal, and anti-tuberculosis make them highly interesting scaffolds in medicinal chemistry. A few catalysts have previously been made accessible for the synthesis of modified quinolines. Herein, we displayed a one-pot domino

aza-Michael/Aldol/aromatization reaction catalyzed by α-amylase for the formation of modified quinolines. It was also found that the α-amylase enzyme from *Aspergillus oryzae* has high catalytic efficiency (56-86% yield) in the cascade reaction of various 2-amino benzaldehydes with, α,β-unsaturated carbonyls.

Further, we find the application of α-amylase in chemo-enzymatic synthesis. The field of using an enzyme as well as transition metals to facilitate a series of chemical reactions in a single-pot has grown significantly in recent years. In this context, we developed a one-pot synthesis and functionalization of β-aminocarbonyls using α-amylase enzyme and a Pd-based catalyst. When isocyanide has been used in the reaction, the chemo-enzymatic approach produces substituted indole derivatives with a broad variety of substitutions. Further, phenylboronic acid was used in the place of isocyanide, which provided a decent yield for the modified amino biaryls. Besides, the one-pot chemo-enzymatic method worked well when substituted 2-bromo aniline and isocyanide/phenylboronic acid were used in the reaction, and it generates the corresponding products in an isolated yield of 56–84%. All the synthesized compounds were characterized by ^{1}H NMR and ^{13}CNMR.

CHAPTER 1:
INTRODUCTION AND LITERATURE REVIEW

Graphical abstract:



CHAPTER 1:

Abstract:

Biocatalysis has emerged as a powerful technology in recent years to synthesize clinically valuable molecules. In this context, hydrolase enzymes have been used to catalyze various organic transformations. Among hydrolases, α-amylase has been recognized as a valuable biocatalyst for chemical processing in a benign reaction environment. Further, it has been widely accepted in natural transformations like hydrolysis of starch as well as in non-natural transformations due to good selectivity, broad substrate scope, and good stability in organic solvents. A number of significant organic transformations, such as the aldol condensation, Hantzsch reaction, Mannich reaction, Knoevenagel condensation, Michael addition, and the multicomponent reaction catalyzed by α-amylase have been covered in this chapter. Further, the current chapter notes that α-amylase catalyzes not only a single-step reaction but also multi-step and tandem non-natural transformations.

1.1: Introduction:

Biocatalysts are defined as natural substances like enzymes, microbes, and whole cells that accelerate or initiate chemical transformations[1-3]. Further, enzymes found in living systems, help in converting the organic molecules into valuable products without being changed themselves[4-5]. An enzyme-substrate complex is created when the substrate binds to the enzyme's active-site (E-S complex), followed by the conversion of E-S complex into an enzyme-product complex (E-P complex). As a result, a product is formed, and this enables the enzyme to be ejected without being consumed by it (Figure1.1)[6].

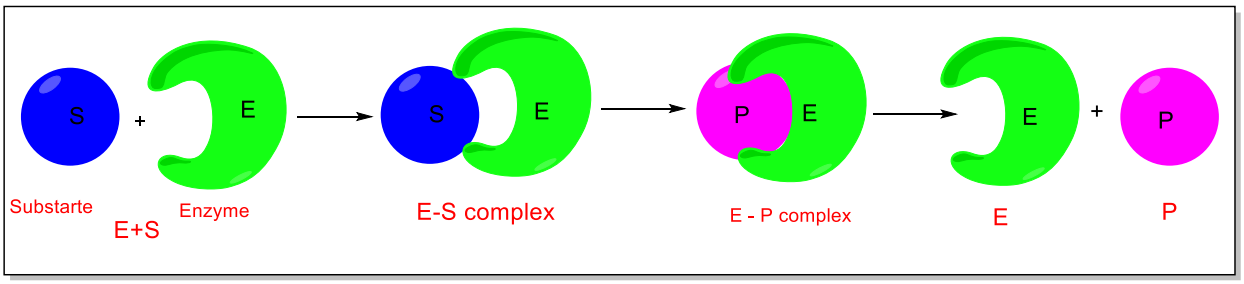


Figure 1.1: A schematic mechanism of enzyme catalysis (E=enzyme, S=substrate, P=Product).

Moreover, enzymes are made of 20 different L-amino acids that are joined together in one or more polypeptide chains. In particular, the amino acid residues present in enzymes are composed of

various polar as well as non-polar groups that provide specific chemical features that help the enzymes to fold and form unique shapes which play a key role in their activity and selectivity during a chemical catalysis[7-9]. Further, enzymes have been classified into seven categories based on the reaction they catalysed in the nature:[10]

1. Oxidoreductase (EC 1): *These enzymes increase the rate of redox reactions and in this process, electrons are gained and ripped off by reactants.*

2. Transferases (EC 2): *It involves the transfer of an atom or group from one molecule to another.*

3. Hydrolases (EC 3): *These enzymes catalyse the hydrolysis reaction and break each molecule into two different molecules by removing the hydroxyl group from one of the products and the hydrogen atom from another product.*

4. Lyases (EC 4): *These enzymes are very similar to the hydrolase enzyme, but they do not remove the water molecule. It facilitates the addition of a group across a double bond and also removes any two groups to form a double bond.*

5. Isomerases (EC 5): *It helps in speeding up the isomerization reaction, in which all the original atoms remain in their positions but are rearranged to form an isomer of the reactant.*

6. Ligases (EC 6): *These enzymes help in the joining of two molecules together. These enzymes use the energy broken down by ATP.*

7. Translocases (EC 7): *These enzymes facilitate the passage of ions or molecules through membranes.*

Notwithstanding, enzymes catalyse chemical reactions with high enantioselectivity, regioselectivity, and chemoselectivity, reducing the likelihood of side reactions such as racemization, decomposition, rearrangements, and isomerization.[11-18] Further, the concept of green chemistry is fundamentally linked to enzyme catalysis, as enzymes can be obtained from renewable sources, and are capable to catalyse various chemical reactions under the sustainable reaction conditions.[19-22] In addition, genetic engineering improves the selectivity and specificity of the enzymes and makes them an efficient eco-friendly catalysts.[23-24] Due to these advantages, the use of enzyme to catalyse the organic transformations those were known for particular enzyme in nature (primary function), have gained exponentially in the last decades.[25-28] However, in the

past few years, enzyme have been explored for their secondary functions in synthetic organic chemistry which is also known as promiscuity of enzyme.[29-30] For example, lipase enzyme catalyse the reaction of a broad range of carboxylic acids and alcohols, which is a primary function of lipase. Although, it also catalyses C–C bond formation reactions, which is known as promiscuity of lipase. Thus, finding a novel promiscuous functions for an enzyme is a longstanding challenge in the field of biocatalysis. On the other hand, hydrolase is a class of enzymes that use water molecules to break a chemical bond, which generally results in dividing a bigger molecule into smaller molecules.[31] Among different hydrolases, α-amylase catalyse the hydrolysis of α-1,4-glycosidic linkages in starch (a primary function for α-amylase enzyme) which leads to the formation of glucose, maltose, and dextrin (Figure 1.2).[32-34] 11In this chapter, we have covered the research papers published during the last decade reporting the promiscuity of α-amylase enzyme for a number of organic transformation.

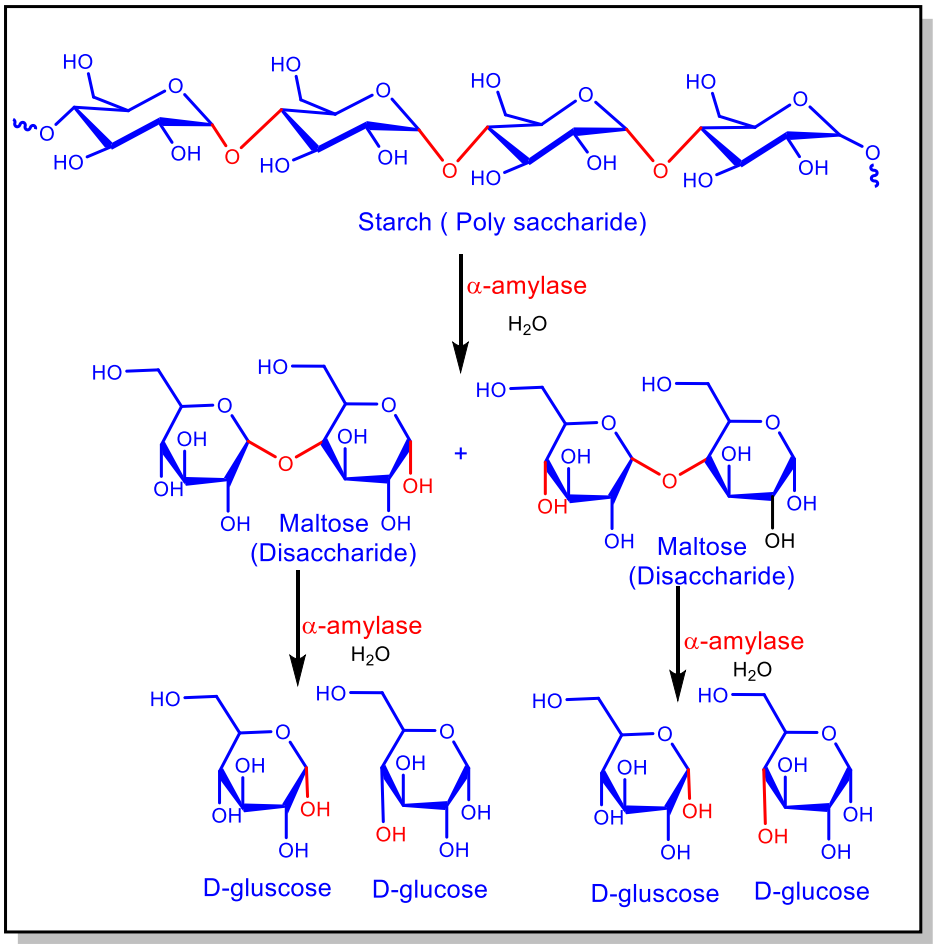


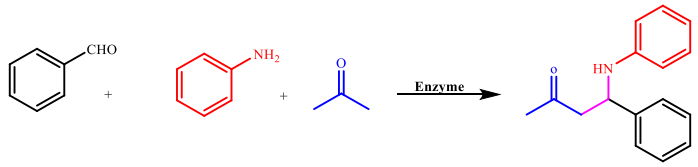
Figure 1.2: α-Amylase catalyzed hydrolysis of α-1, 4-linkages converting starch to glucose

1.2: Literature review:

1.2.1: Enzyme catalyzed one-pot Mannich reaction:

In 2010, P. F. Zhang and co-workers reported an unprecedented one-pot Mannich reaction of aniline (**1**), benzaldehyde (**2**), and acetone (**3**) as a reactant as well as a solvent to construct the β-aminoketones (**4**) (Scheme 1.1). [35] First, they tested the influence of different biocatalysts on the model reaction and obtained moderate to significant catalytic activities only in the case of α-amylase and trypsin enzyme (Table 1.1, entry 1-2), while other enzymes showed very low catalytic activities. So, they selected the trypsin enzyme which provided the best conversion for further optimization of the reaction conditions. To improve the yield of trypsin catalyzed reaction first they screened different solvents and obtained higher conversion (93%) in the mixture of acetone and ethanol (ratio=2:0.5 v/v). A very low yield of 2-5% was noticed when the reaction was performed in the solvents like dichloromethane, toluene, and carbon tetrachloride. The other influencing factors such as temperature, enzyme concentration, and reaction time were also tested. The results showed that the highest yields (up to 93%) were determined when 20 mg trypsin in 2 ml acetone was used at 37°C for 48 h. To verify the feasibility of this protocol, the substitution of various groups such as electron-withdrawing groups on benzaldehyde were tested. In this context, 4-nitrobenzaldehyde provided the highest yield (93%), while p-anisaldehyde gave a lower yield i.e 45% (Scheme 1.1). Further, the reaction also provided the desired products in good to excellent yields in the case of halide substituents.

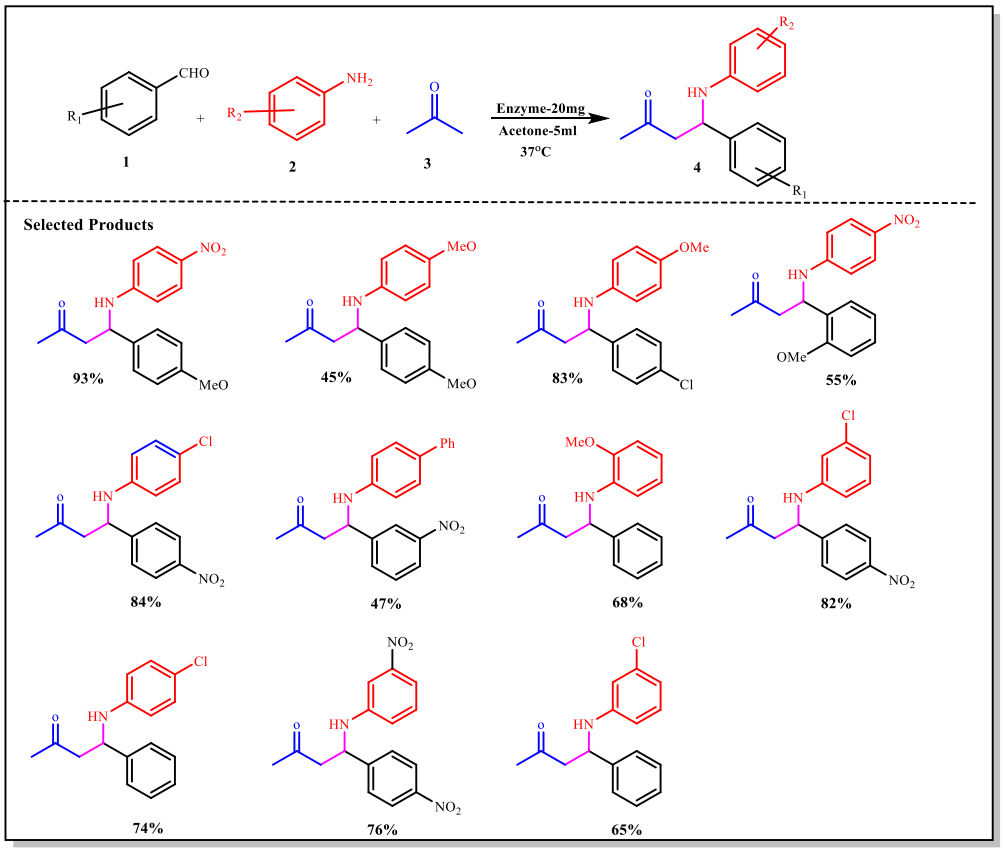
Table 1.1: Enzyme catalyzed Mannich reaction[a]



Entry	enzyme	%Yield[b]
1	Trypsin (*Hog pancreas*)	83%
2	α-Amylase (*Hog pancreas*)	54%
3	Lipase AR (*Candida antarctica*)	11%
4	Lipase AY30	8%
5	Lipase (*Porcine pancreas*)	46%
6	Lipase AK (*Pseudomonas fluorescens*)	17%
7	Lipase A (*Aspergillus niger*)	20%

8	Lipase M (*Mucor javanicus*)	28%
9	Cellulose	6%

[a]**Reaction conditions:** 4-nitrobenzaldehyde (0.2 mmol), p-anisidine (0.2 mmol), acetone (2 ml), and enzyme (20 mg) were stirred at 160 rpm at 30°C for 12 h. [b]Yield dependent on p-anisidine consumption.



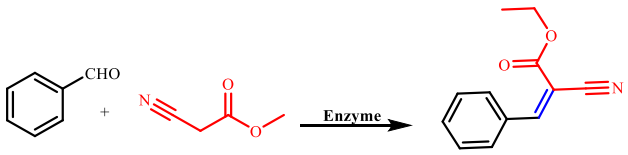
[a]**Reaction conditions:** A solution of the corresponding aldehyde (1 mmol), amine (1 mmol), acetone (5 ml), and trypsin (20 mg) were stirred at 160 rpm at 37°C for 24-48 h.

Scheme 1.1: Enzyme catalyzed one-pot Mannich reaction to synthesized β-aminoketones

1.2.2: Enzyme-catalysed Knoevenagel condensation reaction:

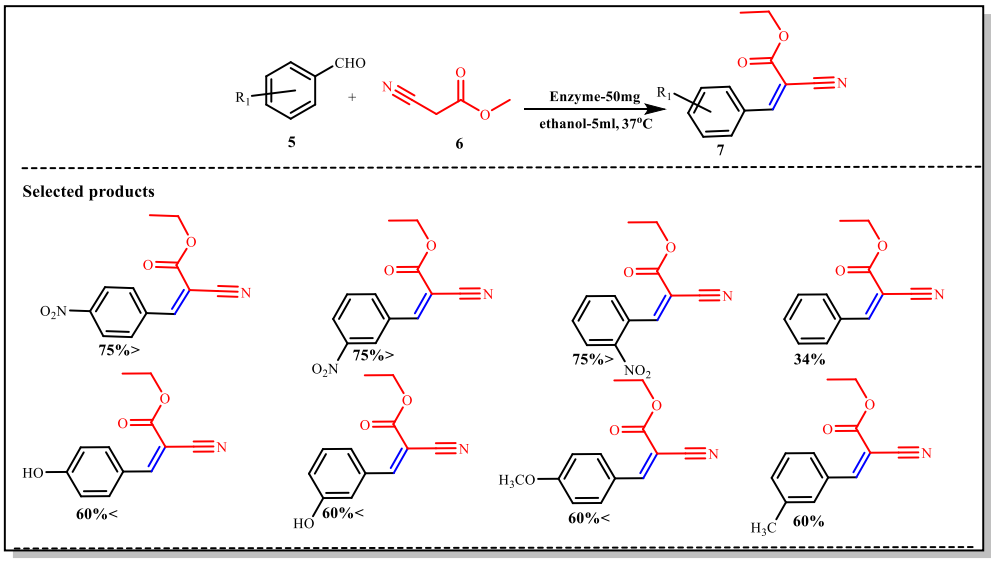
X. Z. Chen's group 2010 performed the enzymatic tandem Knoevenagel condensation and esterification reaction using benzaldehyde (**5**) and methyl cyanoacetate (**6**) and constructed a range of methyl-2-cyno-3-phenyl acrylate derivatives (**7**) (Scheme 1.2).[36] They started their investigation by screening different biocatalysts including lipases obtained from different sources along with α-amylase as well as BSA protein (Bovine serum albumin). They found that lipase from *porcine pancreas* (PPL) was superior biocatalyst. Also, α-amylase from *hog pancreas* showed moderate to high catalytic activity i.e. 74% yield, (Table 1.2, entry 1, 8), while other catalyst such as lipase

Table 1.2: Lipase catalyzed tandem Knoevenagel condensation and esterification reaction[a]



Entry	Enzyme	%Yield[b]
1	Lipase (*Porcine pancreas*)	85%
2	Lipase A (*Aspergillus niger*)	35%
3	Lipase M (*Mucor javanicus*)	40%
4	LipaseAK(*Pseudomonas fluorescens*)	4%
5	Lipase AY30 (*Candida rugosa*)	2%
6	Lipase (Recombinant,*Candida antarctica*)	5%
7	BSA	3%
8	α-Amylase (*Hog pancreas*)	74%
9	Lipase (d*enatured, Porcine pancreas*)	3%
10	Control	3%

[a]**Reaction condition:** Enzyme (50 mg), benzaldehyde (1 mmol), methyl cyanoacetate (1 mmol), and DMSO (5 ml), at 37°C were stirred at 150 rpm for 12h. [b]Yield of product.



[a]**Reaction condition:** Enzyme (50 mg), benzaldehyde (1 mmol), methyl cyanoacetate (1 mmol), and DMSO (5 ml) at 40°C were stirred at 150 rpm for 12h.

Scheme 1.2: Tandem Knoevenagel condensation and esterification reaction catalyzed by enzyme in ethanol

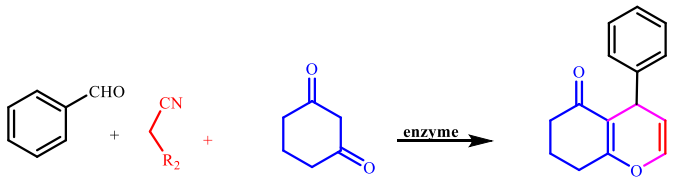
AY30, BSA, and the control reaction (in the absence of enzyme) showed the low catalytic activities (Table 1.2, entries 5,7 &10). These observations rule out the possibility that the enzymes tertiary structure only acts as polymeric support and instead indicates that it plays a crucial role in the catalysis.

To examine the generality of this protocol, the substrate scope was done. In this context, NO_2-substitution at different positions of the aldehyde gave the highest yield i.e. 75%, however, moderate to poor yield was observed in the case of -OH, OCH_3, and -Cl on the aldehyde (Scheme 1.2).

1.2.3. Enzyme-catalysed synthesis of tetrahydrochromenes

In 2011, P. F. Zhang's group reported an enzyme-catalyzed one-pot reaction to construct the tetrahydrochromene derivatives in a mixture of ethanol and water (Scheme 1.3). [37] They started this study by using benzaldehyde (**8**), malononitrile (**9**), and cyclohexane-1,3-dione (**10**) in the presence of PPL as a biocatalyst in a mixture of ethanol and water as a solvent. Further, a number of biocatalyst were screened (Table 1.3), but, the α-amylase from the *Porcine pancreas* and lipase from the *Porcine pancreas* (PPL) showed the highest catalytic efficiency with a conversion yield of 76% to 86% respectively (Table 1.3, entries 4&8). Further, a number of experiments were performed to confirm the catalytic role of PPL in this transformation and in this context reaction was set-up with BSA and denatured PPL, as a result no product was obtained (Table 1.3, entries 9-10).These studies suggested that the active-site of PPL enzyme playing a significant role in this catalysis. Interestingly, the reaction turned out solvent selective, i.e. it did not work when dichloromethane, toluene, n-hexane, and water were used as solvents. Next, to prove the generality of the reaction, they used various aromatic aldehydes, malonitil, and 1,3-diketone as starting materials (Scheme 1.3).

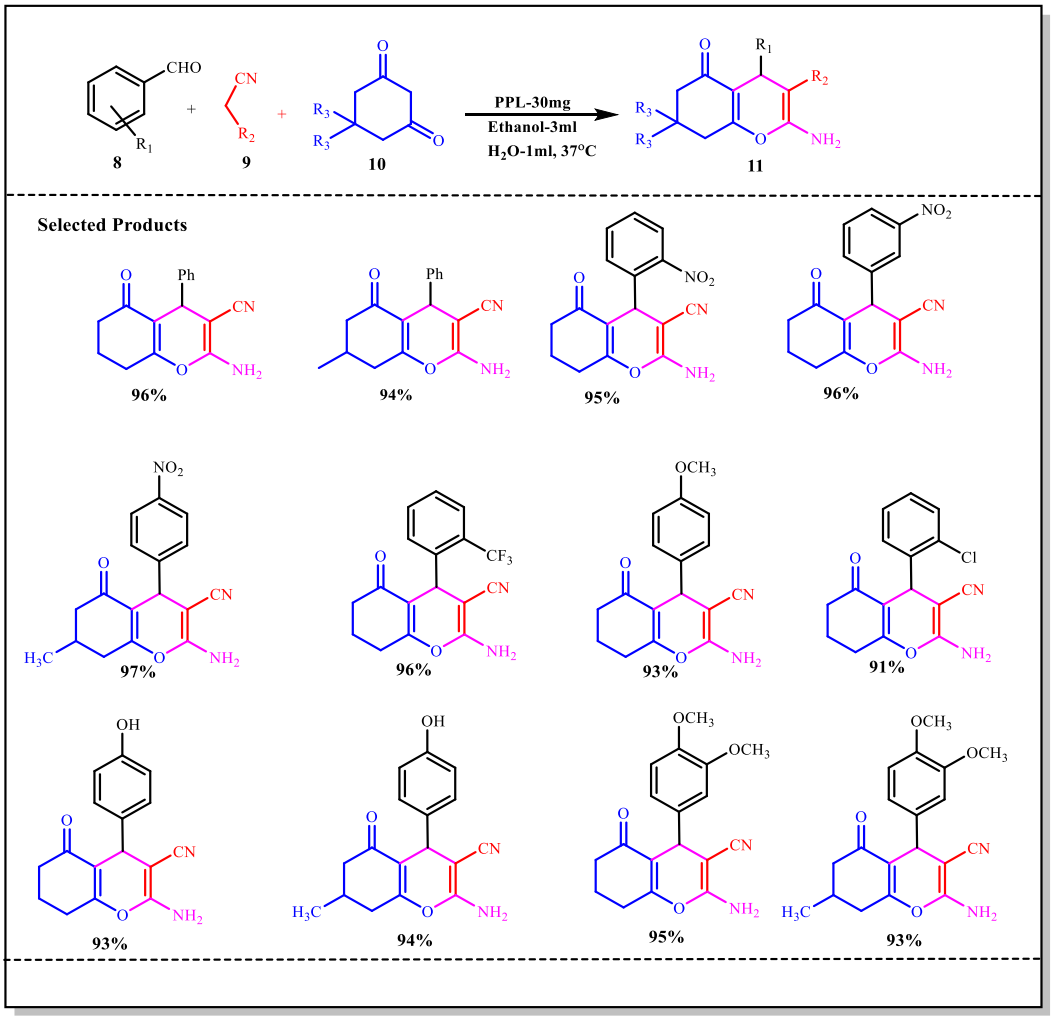
Table 1.3: Screening of different enzymes for the multi-component reaction[a]



Entry	enzyme	%Yield[b]
1	Trypsin (*Porcine pancreas*)	60%
2	Pepsin (*Hog stomach*)	52%

3	α-Amylase (*Aspergillus oryzae*)	5%
4	α-Amylase (*Hog pancreas*)	76%
5	α-Amylase (*Bacillus subtilis*)	9%
6	Amano lipase M (*Mucor javanicus*)	18%
7	Amano lipase A (*Aspergillus niger*)	24%
8	Lipase (*Porcine pancreas*)	86%
9	Lipase (*Porcine pancreas*)	Trace
10	BSA	Trace

[a]**Reaction conditions:** Benzaldehyde (1 mmol), malononitrile (1 mmol), cyclohexane-1,3-dione (1 mmol), enzyme (30 mg), and EtOH (4 mL) were stirred at 160 rpm at 35 °C for 1 h.



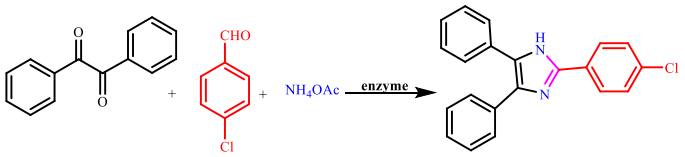
[a]**Reaction conditions:** Aldehydes (1 mmol), malononitrile (1 mmol), 1,3-dicarbonyls (1 mmol), PPL (30 mg), ethanol (4 mL), and water (1 mL) were stirred at 160 rpm at 35 °C. [b]Isolated yield

Scheme 1.3: One-pot multistep reaction catalyzed by PPL enzyme to synthesize the tetrahydrochromene compound

1.2.4. Enzymatic synthesis of 2,4,5-trisubstituted imidazoles

Later, Zhang's group developed a simple and efficient method to construct 2,4,5-trisubstituted imidazole (**15**) from benzil (**12**), aldehyde (**13**), and ammonium acetate (**14**) using Lipase AT 30 under mild reaction conditions (Scheme 1.4). [38] To find the most efficient biocatalyst, the catalytic efficiency of different enzymes for the model reaction was screened. They found the maximum conversion yield (65% to 71%), when α-amylase from *Aspergillus oryza* and Lipase AT30 were used, while other biocatalysts illustrated lower activities (Table 1.4, entry 1-4). The effect of different solvents such as ethanol, methanol, water, acetonitrile, ethyl acetate, and isopropanol was studied, but ethanol was chosen as the best solvent which gave the highest yield i.e. 75%. Also, it was observed that when the temperature was increased from 25°C-40°C, the highest yield of 72% was obtained. Next, they set-up the reaction with various aromatic and heterocyclic aldehydes to test the substrate scope. The experimental results showed that all aromatic aldehydes with different functional groups (electron-donating and electron-withdrawing) worked well and provided the imidazole compounds with moderate to excellent yields i. e. 65 to 87 % (Scheme 1.4).

Table 1.4: Effect of enzyme on the model reaction to synthesize the 2,4,5-trisubstituted imidazole derivatives[a]

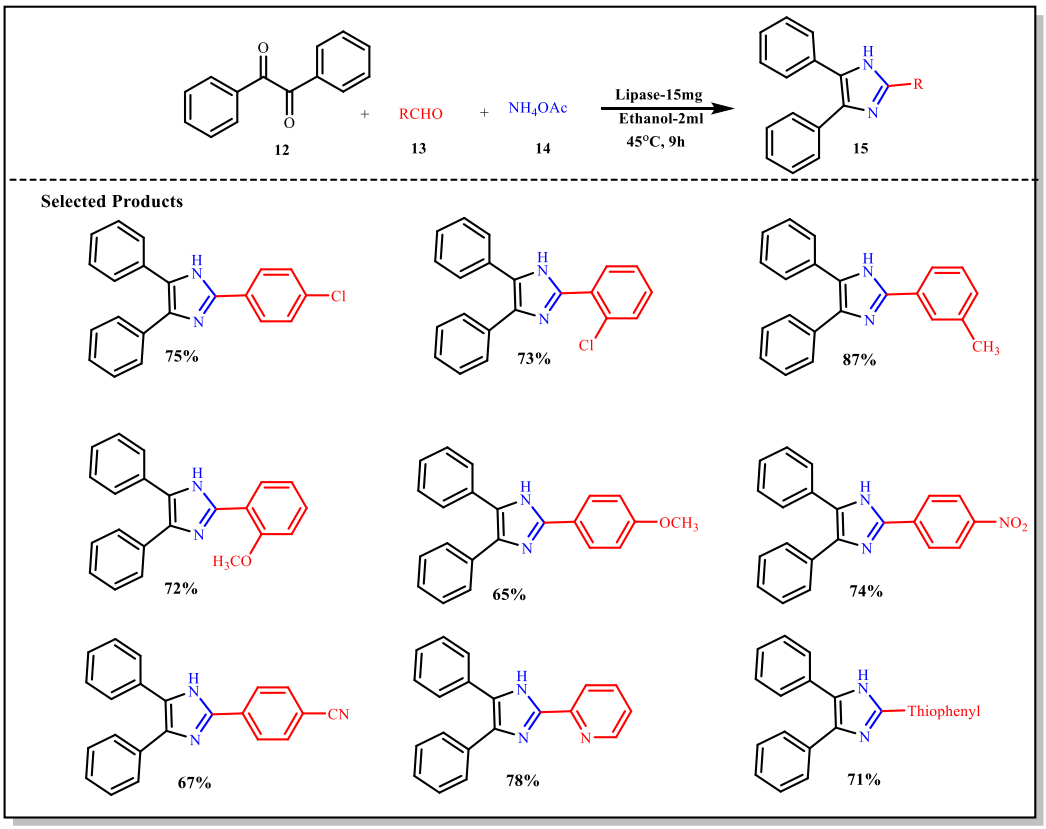


Entry	enzyme	%Yield[b]
1	Lipase AT30	71%
2	Trypsin (*Porcine pancreas*)	56%
3	Diastase	60%
4	α-Amylase (*Aspergillus oryzae*)	65%
5	α-Amylase (*Hog pancreas*)	66%
6	Albumin (*Bovine*)	45%

[a]**Reaction conditions:** Benzil (1 mmol), aldehyde (l mmol), and ammonium acetate (l mmol), solvent (2 mL), enzyme (15 mg), at 45°C, were shaken at 160 rpm for 9h. [b]Yields were determined by GC.

Further, they performed control experiment with BSA and got 45% conversion yield and hypothesized that BSA can bind with organic molecules through reversible non-covalent complexation in its hydrophobic pockets, creating an environment favorable for the reaction.

However, it requires further validation by set-up the reaction with denatured enzyme or in the presence of enzyme inhibitors.



[a]**Reaction conditions:** Benzil (1 mmol), aldehyde (l mmol), and ammonium acetate (l mmol), ethanol (2 mL), Lipase (15 mg), at 45°C, were stirred at 160 rpm for 9 h. [b]Yields were determined by GC (Gas Chromatography).

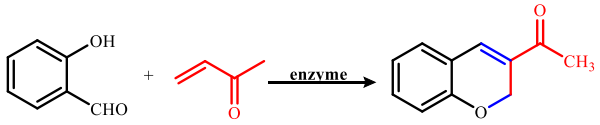
Scheme 1.4: One-pot synthesis of 2,4,5-trisubstituted imidazoles using enzyme

1.2.5. Enzymatic synthesis of 3-substituted 2H-chromenes

In 2013, Yu's group demonstrated the one-pot oxa-Michael addition/aldol condensation reaction of salicylaldehyde (**16**) with methyl vinyl ketone (**17**) to synthesize 3-substituted 2H-chromene derivatives (**18**) using α-amylase from *B.subtilis* (Scheme 1.5). [39] This transformation was optimized by screening different enzymes along with different solvents and reaction temperatures. They found α-amylase in DMSO at 50°C as the best combination to get 3-substituted 2H-chromenes in 36% yield (Table 1.5, entry 1). While, other enzymes like PPL, AMA, MJL, BPL, OAA, and HPA showed no catalytic efficiency (Table 1.5, entry 2-6). To show the precise catalytic impact of α-amylase from *B.subtilis*, some well-controlled experiments were carried out. In this context, no product was found when reactions were run without any biocatalyst or with denatured α-amylase from *B.subtilis, and* BSA (Table 1.5, entries 7-9). All of the findings indicated that the

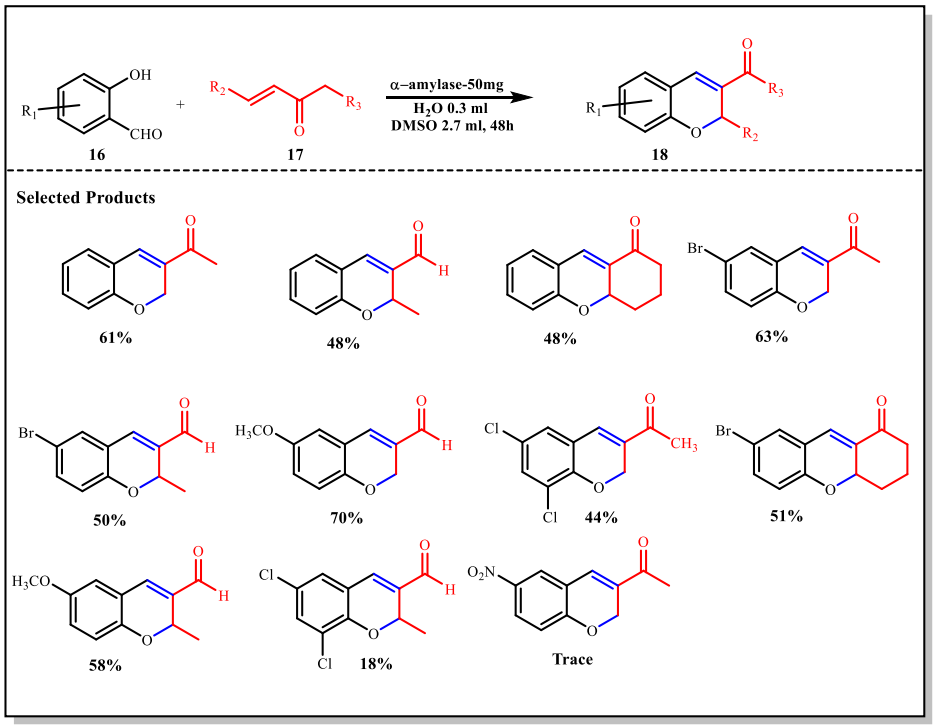
domino reaction must be catalysed by the unique active-site and spatial conformation of α-amylase from *B. subtilis*. Next, the effect of the molar ratio was examined and it was found that varying the molar ratio of vinyl ketone and salicylaldehyde from 1:1 to 1:10 increased the product yield from 7% to 45%. Furthermore, the influence of temperature was examined, however, the yield increased slightly between 50°-80°C, and the highest yield of 58% was achieved at 80° C after 48 h. A panel of 2H-chromene derivatives was constructed in good to excellent yields using α-amylase from *B. subtilis* as a catalyst in DMSO (Scheme 1.5).

Table 1.5: α-Amylase from *B. subtilis* catalyzed domino oxa-Michael/aldol condensation reaction[a]



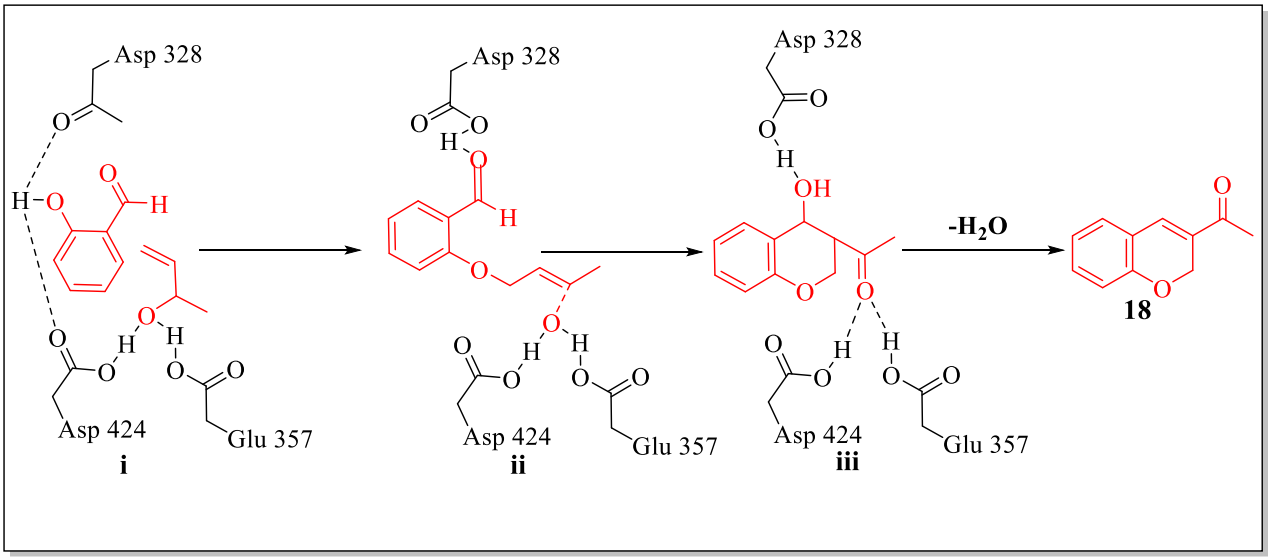
Entry	Solvent	%Yield[b]
1	α-Amylase (*Bacillus subtilis*)	36%
2	Lipase (*Porcine pancreas*)	Trace
3	Lipase M (*Mucor javanicus*)	Trace
4	Acylase I (*Aspergillus melleus*)	Trace
5	α-Amylase (*Hog pancreas*)	Trace
6	α-Amylase (*Aspergillus oryzae*)	Trace
7	BSA	NR
8	-	NR
9	denatured α-Amylase (*Bacillus subtilis*)	NR

[a]**Reaction conditions:** Salicylaldehyde (0.2 mmol), methyl vinyl ketone (0.5 mmol), enzyme (10 mg) were stirred at 200 rpm at 50°C for 48 h. [b]Conversion yields are determined by HPLC (High-Performance Liquid Chromatography), NR=no reaction.



[a]**Reaction Conditions:** α-amylase (*B. subtilis*) (50 mg), salicylaldehyde (1mmol), α,β-unsaturated ketones (5 mmol), 300 µL H_2O, 2700 µL DMSO, 200 rpm, 50°C, 48 h. [b]Isolated yield of product.

Scheme 1.5: Domino oxa-Michael addition/aldol condensation reaction catalyzed by α-amylase



Scheme 1.6: Plausible reaction mechanism for domino oxa-Michael addition/aldol condensation reaction

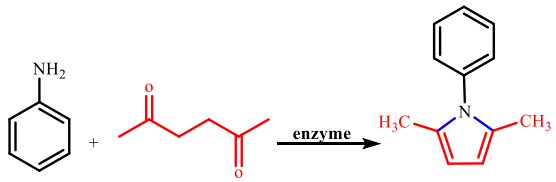
The proposed reaction mechanism for domino aldol condensation reaction promoted by α-amylase is shown in (Scheme 1.6). The amino acids (Asp328, Asp424, Glu 357) of the amylase first

stimulated the salicylaldehyde. The active salicylaldehyde was then targeted to methyl vinyl ketone, resulting in a new C-O bond through oxa-Michael addition. Following that, an intramolecular aldol reaction occurred, resulting in the development of a carbon-carbon bond. Subsequently, the resultant adduct was dehydrated, and the desired product was produced.

1.2.6. α-Amylase catalyzed Paal-Knorr reaction

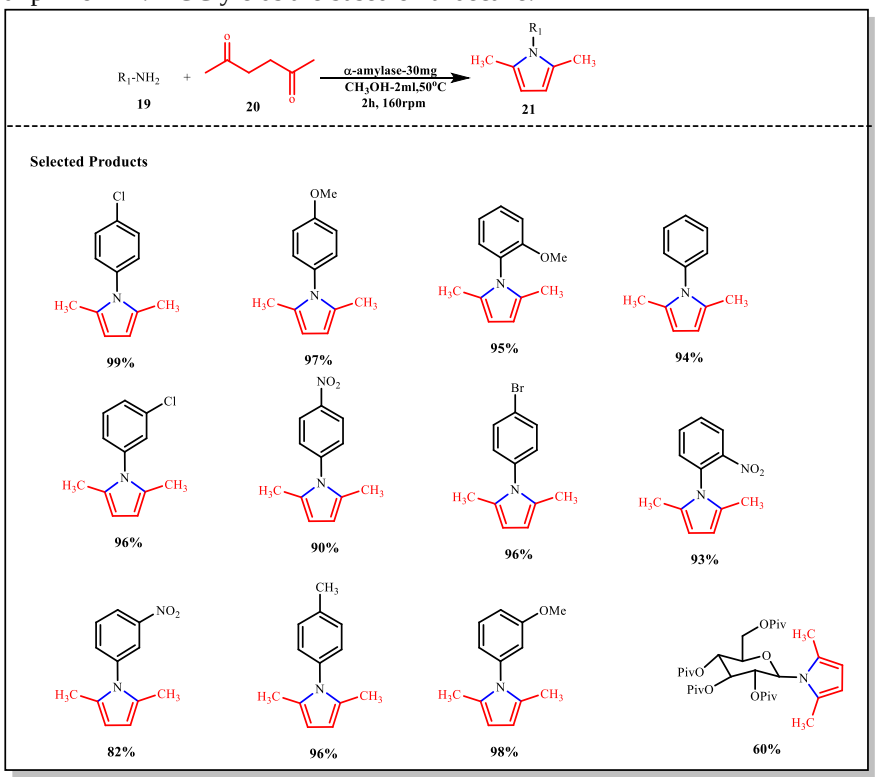
Later, Zhang et. al. reported the Paal-Knorr reaction of aniline (**19**) with 2,5-hexanedione (**20**) to synthesize the substituted pyrroles (**21**) using α-amylase from the *Porcine pancreas* (Scheme 1.7).[40] To obtain the optimized reaction conditions, different hydrolase enzymes were screened and found that α-amylase from *Hog pancreas* was superior and gave the highest yield of product i.e 94% (Table 1.6, entry 1). In addition, the influence of solvents such as methanol, ethanol, dichloromethane, tetrahydrofuran, water, and acrylonitrile was tested, showing that the reaction proceeded smoothly in methanol. To confirm the catalytic role of α-amylase, two control experiments were performed. First, the enzyme was treated with an aqueous urea solution at 100 °C and refluxed for 24 h to totally inactivate it and dried, then it was used as a catalyst in the model reaction. Second, the control reaction was performed in the absence of α-amylase. As a result, very poor yields i.e. 17 and 18% only was obtained, which suggested that the α-amylase has a catalytic role in this transformation. Furthermore, the influence of factors such as temperature and enzyme concentration on the reaction was investigated and it was observed that the highest yield was achieved when the temperature was increased from 35°C to 50°C. A series of pyrrole derivatives were synthesized under mild reaction conditions and the products were formed in good to excellent yields (60-99%) (Scheme 1.7). A plausible reaction mechanism for the construction of *N*-substituted pyrrole derivatives through the Paal-Knorr reaction was presented in Scheme 1.8. Initially, the amylase stimulates one carbonyl of 2,5-hexadione (**i**), as well as the amino group, operating as a nucleophile which reacts with the electron-rich carbonyl to give intermediate (**ii**) and accomplish dehydration via a hydrogen bond, that is hit mostly by the intramolecular amino group to construct (**iii**) and accomplish water loss from (**iv**) through use of hydrogen bond to produce the product (**21**).

Table 1.6: Paal-Knorr reaction of aniline with 2,5-hexadiketone catalyzed by α-amylase and synthesized the N-substituted pyrrole derivatives[a].



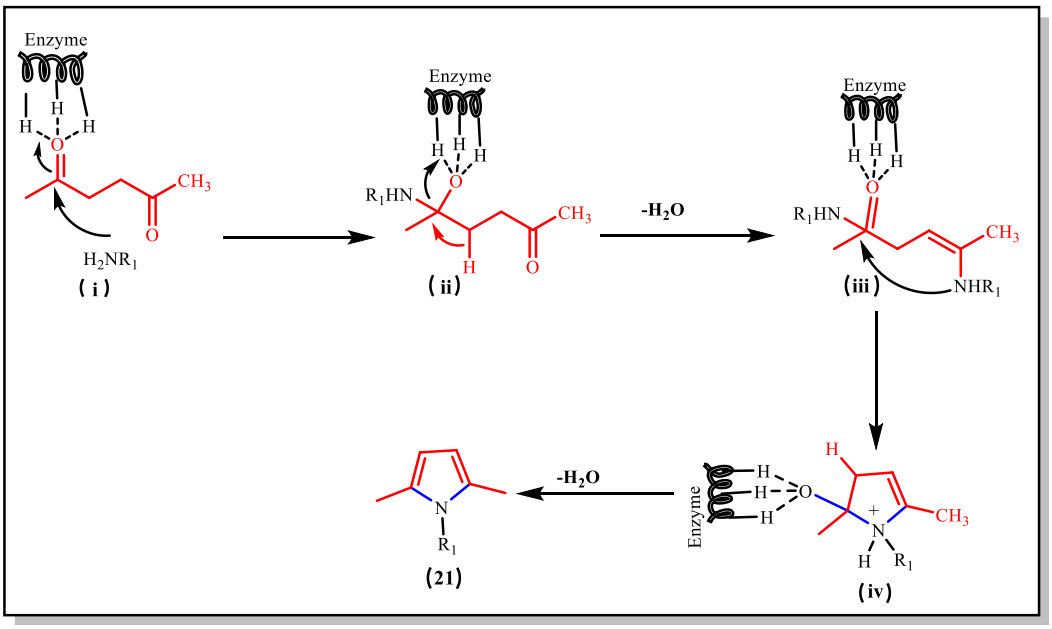
Entry	Enzyme	%Yield[b]
1	α-Amylase (*Hog pancreas*)	94%
2	Trypsin (*Porcine pancreas*)	79%
3	Diastase	75%
4	α-Amylase (*Aspergillus oryzae*)	65%
5	Albumin (*Bovine*)	46%
6	Lipase AT30	53%
7	Lipase M (*Mucor javanicus*)	47%
8	Blank	18%

[a]**Reaction conditions:** 2,5-hexanedione (l.5mmol), aniline (l.5mmol), methanol (2mL), enzyme (25mg), at 50°C, were stirred at 160 rpm for 2 h. [b]GC yields are based on tridecane.



[a]**Reaction conditions:** 2,5-hexanedione (l.0mmol), amine (l.0mmol), methanol (2mL), α-Amylase from *hog pancreas* (30mg), at 50°C, were shaken at 160 rpm for 2 h. [b]Isolated yield.

Scheme 1.7: α-Amylase catalyzed the Paal-Knorr reaction to synthesize the substituted pyrrole derivatives

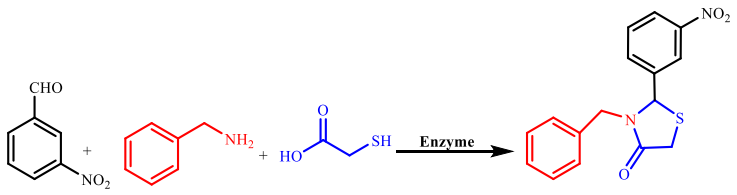


Scheme 1.8: The proposed reaction mechanism for the construction of substituted pyrrole compounds

1.2.7. One-pot synthesis of thiazole derivatives

In the same year, Zhang's group developed the one-pot multicomponent reaction of 3-nitrobenzaldehyde, benzylamine with mercaptoacetic acid and constructed the substituted 4-thiazolidinone derivatives (Scheme 1.9). [41] In order to optimize the reaction conditions different enzymes were studied and it was found that α-amylase from *Aspergillus oryzae* and trypsin from *Porcine pancreas* (PPT) allowed the reaction to proceed smoothly and provided the highest yields (73 & 82%) (Table 1.7, entries 3&5). While, other biocatalysts like , AMJ, AHP showed lower catalytic activity and attained modest yields in the range of 63–64% (Table 1.7, entries 6-8). Further, a limited amount of product was obtained, when reaction was performed with inactivated enzyme or in the absence of catalyst (blank control). However, they obtained 60% yield when BSA was employed as a catalyst in this reaction. These results suggested that trypsin from *Porcine Pancreas* (PPT) only improves the conversion of this transformation . Next, various solvents were employed in the model reaction and dichloromethane was found to be the best solvent. In order to check the catalytic productivity of the biocatalyst, the influence of other factors such as enzyme amount, temperature, and reaction time were examined and it was noticed that when temperature increased from 25° to 40ºC the reaction yield got improved. To prove the generality of this

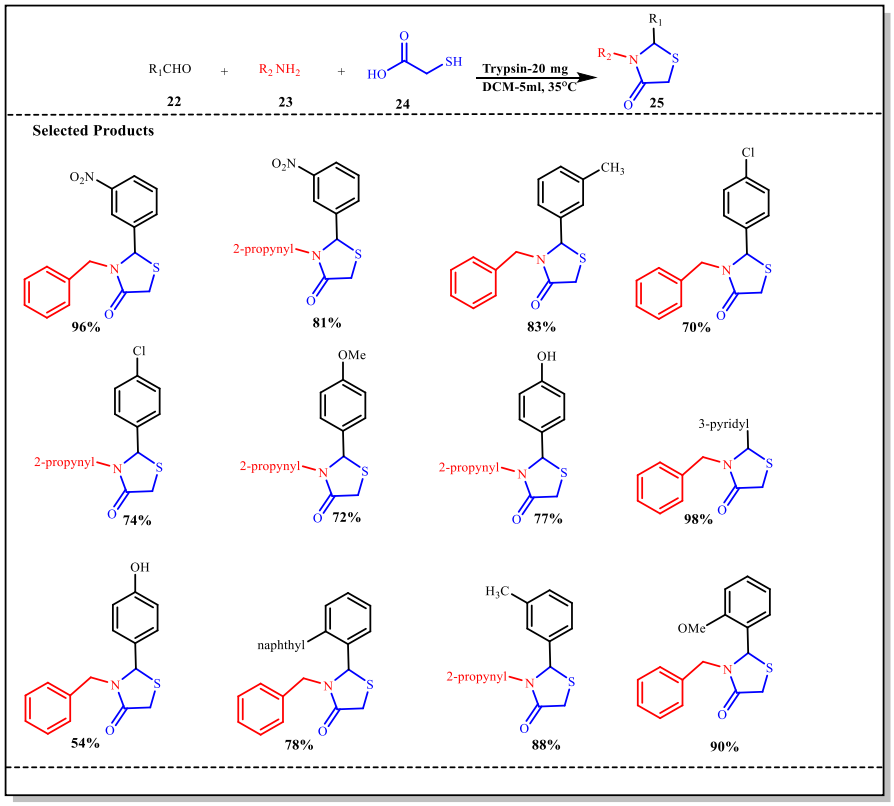
Table 1.7. Trypsin catalysed the one-pot synthesis of 4-thiazolidinone[a]



Entry	enzyme	%Yield[b]
1	Trypsin (*Porcine pancreas*)	96%
2	Lipase AY30	85%
3	Lipase (*Porcine pancreas*)	82%
4	Diastase (*Aspergillus oryzae*)	75%
5	α-Amylase (*Aspergillus oryzae*)	73%
6	α -Amylase (*Hog pancreas*)	64%
7	Amano lipase M (*Mucor javanicu*)	63%
8	BSA	60%

[a]**Reaction conditions:** Benzylamine (1 mmol), 3-nitrobenzaldehyde (1 mmol), and mercaptoacetic acid (1 mmol), enzyme (20 mg), dichloromethane (5 mL), were shaken at 160 rpm at 35°C for 4 h. [b] Tridecane is used as an internal standard to calculate GC yields.

protocol, various amines and aldehydes were screened. The results showed that all aldehydes and amines reacted well and the 4-thiazolidinone derivatives were determined in good to excellent yields (54-98%) (Scheme 1.9). It proves that biocatalysts have a wide range of tolerance to substituted aldehydes and amines.



[a]**Reaction conditions:** Amines (1 mmol), aldehyde (1 mmol), and mercaptoacetic acid (1 mmol), trypsin (20 mg), dichloromethane (5 mL), were stirred at 160 rpm at 35°C. [b]Tridecane is used as an internal standard to calculate GC yields.

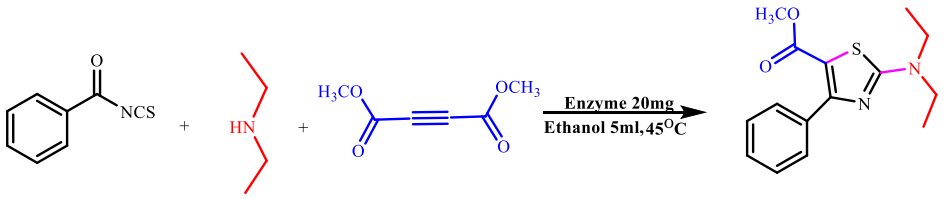
Scheme 1.9 : One-pot construction of 4-thiazolidinones using a biocatalyst

1.2.8. Enzymatic synthesis of thiazole derivatives

In their next study, Zhang's group reported a novel one-pot multistep chemoenzymatic synthesis of thiazole derivatives (**29**) using benzoyl isothiocyanate (**26**), secondary amines (**27**), and dialkyl acetylene dicarboxylates (**28**) (Scheme 1.10). [42] During this study, they examined different enzymes such as trypsin from *Porcine pancreas* (PPT), α-amylase from *Porcine pancreas*, Diastase, α-amylase from *Aspergillus oryzae*, lipase AT30, Amano-lipase M from *Mucor javanicus* and bovine serum albumin along with solvents, temperature and reaction time. It was found that trypsin from *Porcine pancreatic* has better catalytic efficiency in ethanol at 45°C and provided the highest yield of 90 % (Table 1.8, entry, 1). Additionally, the reaction was set-up with the bovine serum albumin (BSA) and obtained the product in 50% yield, which demonstrated that non-enzyme proteins may also catalyse to this reaction, but the yield was significantly lower than

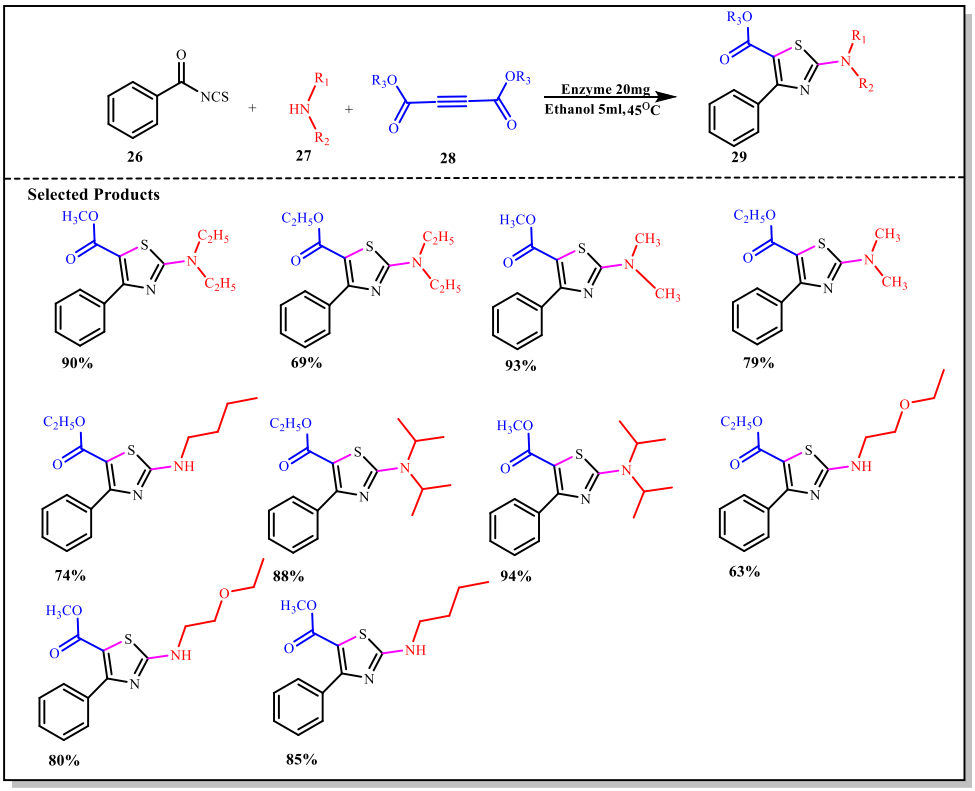
obtained with *Porcine pancreas* (PPT) (Table 1.8, entry 8). To further demonstrate PPT's catalytic capability, experiments were run without any enzyme and with denatured PPT as a result obtained the corresponding product in trace amount only. These findings suggested that the reaction proceeded in a specific way on *Porcine pancreas*'s catalytic site. Thus, in this chemoenzymatic one-pot synthesis of thiazole derivatives, porcine pancreas trypsin serves as a major catalytic component. The facile workup, high isolated yield, and mild reaction conditions make it a valuable tool for expanding the scope of chemoenzymatic synthesis (Scheme 1.10). The proposed reaction mechanism for this transformation was presented in (Scheme 1.11). The synthesis of N-benzoyl thiourea intermediate (**ii**) produced by the introduction of secondary amines to benzoyl isothiocyanate produces a product (**iii**). Trypsin stimulates the carbonyl of compound (**iii**) and converts it to precursors (**iv**) and (**v**), from which the final product (**29**) is produced in high yields with no by-products.

Table 1.8: One-pot chemo-enzymatic synthesis of thiazole derivatives[a]



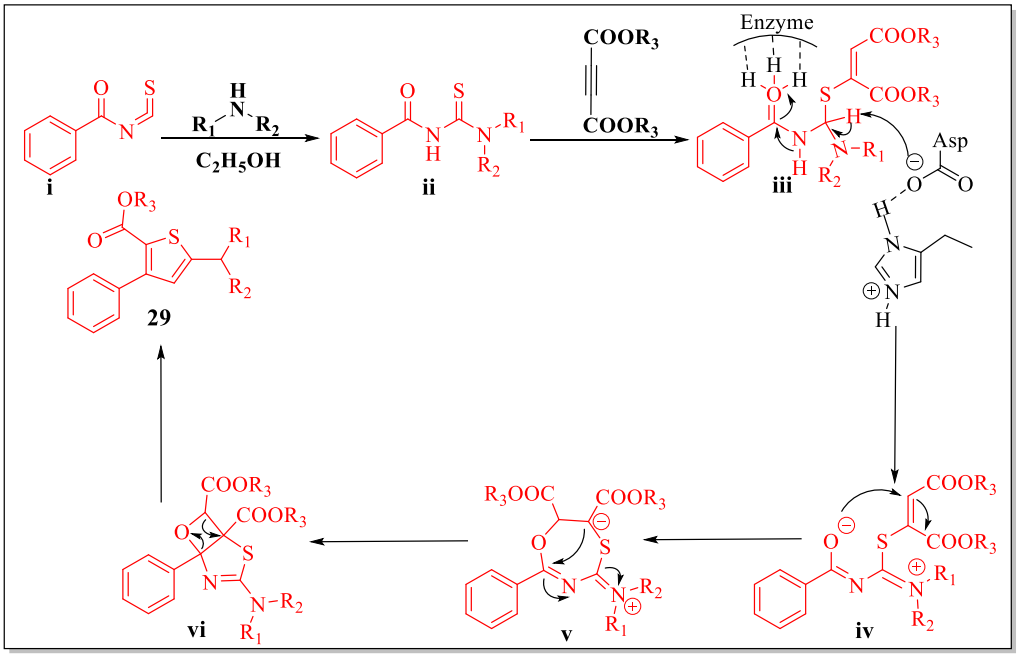
Entry	enzyme	enzyme dosage (mg)	Time (h)	%Yield[b]
1	Trypsin (*Porcine pancreas*)	20	7	90%
2	α-Amylase (*Hog pancreas*)	20	7	60%
3	Diastase (*Aspergillus oryzae*)	20	7	67%
4	α-Amylase (*Aspergillus oryzae*)	20	7	53%
5	Lipase AT30	20	7	61%
6	Lipase M (*Mucor javanicus*)	20	7	40%
7	Lipase (*Porcine pancreas*)	20	7	63%
8	BSA	20	7	50%
9	Blank	20	7	Trace

[a]**Reaction condition:** Diethylamine (1 mmol), benzoyl isothiocyanate (1 mmol), and dimethyl but-2-ynedioate (1 mmol), enzyme (20 mg), ethanol (5 ml), were stirred at 160 rpm at 45°C. GC yields based on internal standard



[a]**Reaction conditions:** Secondary amines (1 mmol), benzoyl isothiocyanate (1 mmol), and dimethyl but-2-ynedioate (1 mmol), Trypsin from porcine pancreas (20 mg), ethanol (5 mL), were stirred at 160 rpm at 45°C.

Scheme 1.10: Enzymatic synthesis of thiazole derivatives via one-pot multicomponent reaction

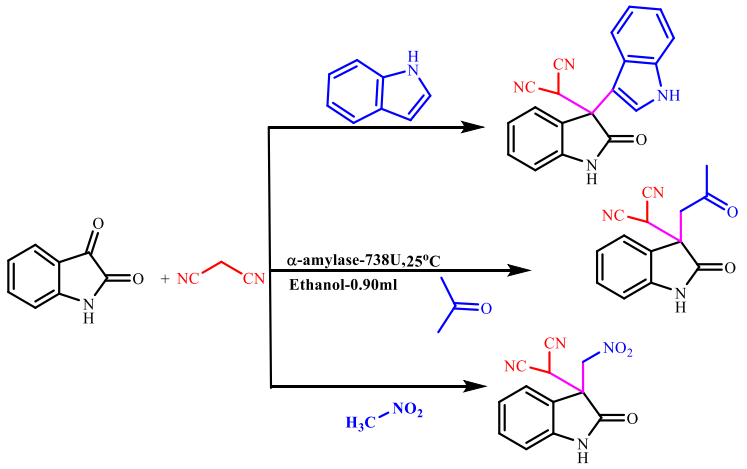


Scheme 1.11: Purposed mechanism for one-pot multicomponent reaction to synthesized the thiazole derivatives

1.2.9. α-Amylase catalysed one-pot Knoevenagel reaction/Michael/cyclization reaction

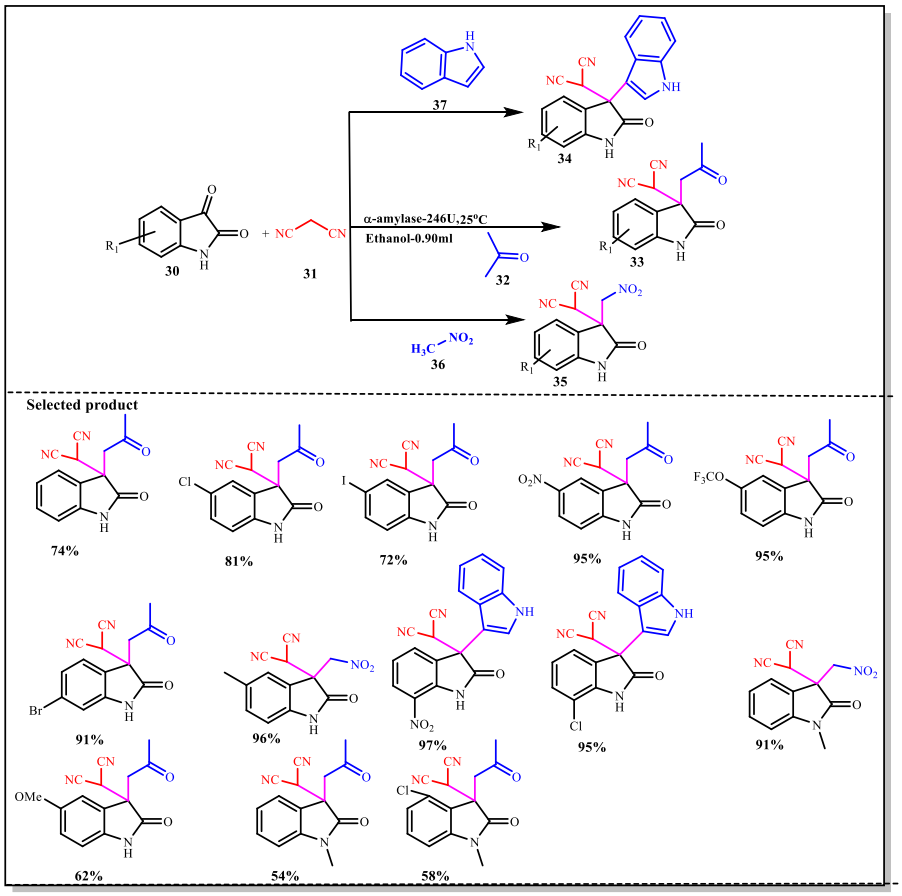
In 2015, Guan et al. described a one-pot multicomponent reaction of isatin (**30**), malononitrile (**31**), and active methylene compounds (**32**) to construct 3,3-disubstituted oxindoles and spirooxindolepyrans (**33**) via Knoevenagel or cyclization reaction using α-amylase (Scheme 1.12). [43] Initially, different solvent systems were screened in order to create a suitable medium for this reaction. In particular, the enzyme-catalyzed reaction provided affordable yields of 43–55% in polar solvents compared to non-polar solvents. It has been observed that when α-amylase is pre-treated with urea and miglitol, a very low yield of 21% to 33% is achieved which confirms the role of α-amylase enzyme in this reaction (Table 1.9, entries 2&5). To check the feasibility of this protocol, they replaced different groups of isatin along with malononitrile and acetone and provided high yields of desired products ranging from 43% to 95% (Scheme 1.12). Even when acetone was replaced with nitromethane (**34**) and indole (**35**), it still promoted the reaction and gave a good yield. The plausible reaction mechanism for the construction of spiro oxindole pyrans was illustrated through the reaction of isatin, malononitrile, and acetone (Scheme 1.13). Initially, the Asp197 amino residue of α-amylase behaved as a base to extract acid hydrogens from acetone, and Glu233 tends to add a hydrogens to the acetone carbonyl to construct the enol. The enol then interacts with the spontaneously founded Knoevenagel adduct through a Michael addition with the help of Asp197 and Glu233 to obtain the 3,3-disubstituted oxindole.

Table 1.9: Synthesize 3,3-disubstituted oxindoles and spiro-oxindole derivatives using α-Amylase[a]



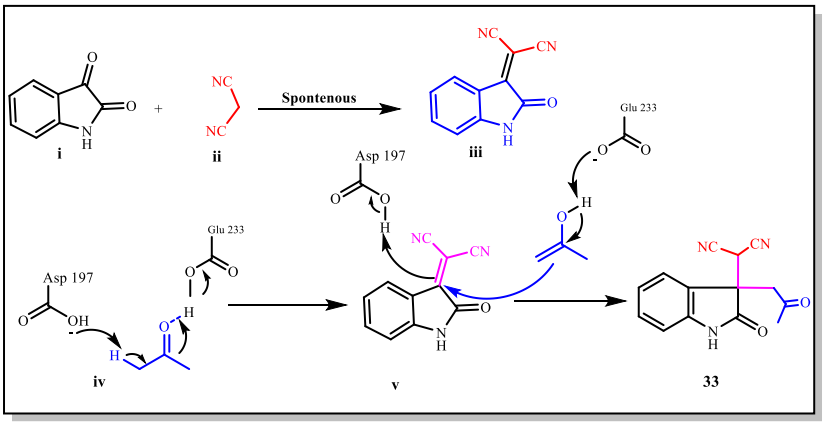
Entry	Catalyst	Time (h)	%Yield[b]
1	α-Amylase (*Hog pancreas*)	2	55%
2	α-Amylase (pretreated with urea)	3	21%
3	α-Amylase (pretreated with $CuSO_4$)	3	Trace
4	α-Amylase (pretreated with $AgNO_3$)	3	NR
5	α-Amylase (pretreated with miglitol)	3	33%
6	Urea	3	[d]NR
7	$AgNO_3$	3	[d]NR

[a]Reaction conditions: Isatin (0.25 mmol), malononitrile (0.25 mmol), acetone (2.5 mmol), α-amylase (738 U), ethanol (0.90 mL), and deionized water (0.10 mL) at 25 C. [b]Isolated yield of product. [c]Unit definition (U mg^{-1}). [d]NR = No reaction



[a]**Reaction conditions:** Isatin (0.25 mmol), malononitrile (0.25 mmol), acetone or indole (0.38mmol) or nitometane (7.5 mmol), α-amylase (246 U), ethanol (0.90 mL), and deionized water (0.10 mL) were stirred at 25°C. [b]Isolated yield of product.

Scheme 1.12: One-pot multicomponent reaction catalyzed by an α-amylase enzyme



Scheme 1.13: Plausible mechanism for the assembly of 3,3-disubstituted oxindole

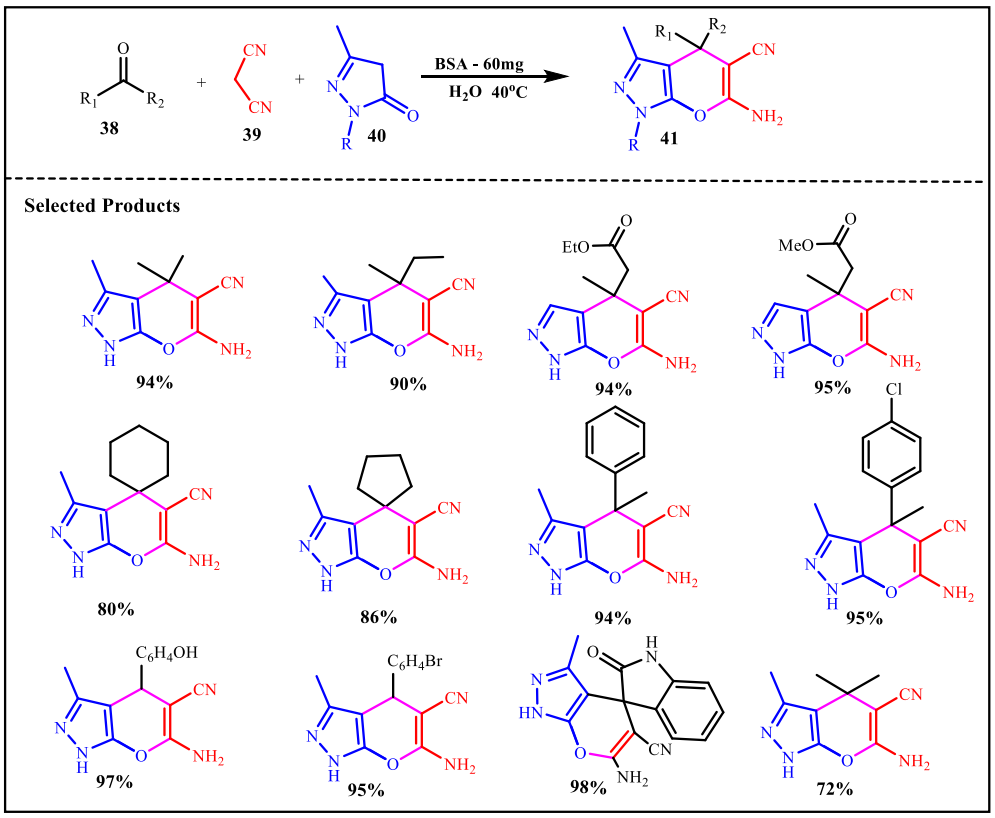
1.2.10. Synthesis of dihydropyrano [2,3-c]pyrazole derivatives

In 2016, Chaudhari and co-workers presented the highly efficient and environmentally friendly method for synthesizing dihydropyrano[2,3-c]pyrazoles and spiro[indoline-3,4-pyrano[2,3-c]pyrazole] derivatives (**41**) from acetone (aldehyde, isatin) (**38**), malononitrile (**39**), and pyrazolone (**40**) (Scheme 1.14). Next, eight commercially available biocatalysts were screened to test their activity for the synthesis of dihydropyrano[2,3-c]pyrazole derivatives. [44] When lipase from *Candida rugosa*, α-amylase, trypsin from *porcine pancreatic*, and papain were used in the model reaction, very poor to moderate yields were observed, however, in the lacking of a catalyst, only 20% yield was found. The yield significantly enhanced to 94% when BSA was added, most likely providing catalysis due to the wide variety of amino acids found on its surface. (Table 1.10, entry 7). The effect of different solvents with different polarities was also investigated and found that the combination of water and ethanol provided best yield. Afterwards, different amounts of enzyme were used to optimize the enzyme concentration and 60 mg of BSA was sufficient to obtain the highest conversion (Scheme 1.14).

Table 1.10: One-pot synthesis of dihydropyrano[2,3-c]pyrazoles[a]

Entry	enzyme	%Yield[b]
1	Lipase (*Candida rugosa*)	13%
2	Lipase (*Porcine pancreas*)	35%
3	Trypsin (*Bovine*)	57%
4	Trypsin (*Porcine Pancreas*)	30%
5	Papain	40%
6	Diastase α-Amylase	25%
7	BSA	94%

[a]**Reaction conditions:** Acetone (2 mmol), malononitrile (2 mmol), pyrazolone (2 mmol), enzyme (60 mg) and H_2O-EtOH (7:3, 10 ml) at 40°C for 1 h, [b]Isolated yield.



[a]**Reaction conditions:** Ketone (2 mmol), malononitrile (2 mmol), pyrazolone (2 mmol), BSA (60 mg) and H_2O-EtOH (7:3, 10 mL) at 40°C. [b]Isolated yield.

Scheme 1.14: One-pot synthesis of dihydropyrano[2,3-c]pyrazole derivatives

1.2.11. α-amylase catalysed Michael addition reaction

In 2016, Guan and co-workers demonstrated the one-pot synthesis of nitrocyclopropane (**44, 45**) using the α-amylase enzyme as a biocatalyst through a Michael addition-initiated ring-closure sequence reaction of bromonitromethane (**43**) with enone (**42**) under mild reaction conditions (Scheme 1.15). During this work, they examined various α-amylases, lipases, and protease enzymes, but found that α-amylase from the *Porcine pancreas* provided the desired product with a maximum yield of 53% (Table 1.11, entry 1). Next, different solvents were screened in this ring-closure reaction, but methanol turned out to be the best solvent and the reaction provided an 84% yield with enantioselectivity up to 17 %. In order to investigate the specific catalytic effect of -amylase on the model reaction, multiple control experiments were performed to confirm the role of catalyst. The blank experiment showed only a very small amount of product even after 120 h. Next, the metal ions Cu2+ and Ag+ were utilised as denaturation agents, and the reaction with the metal ion pre-treated enzyme was set-up which produced the product in trace amounts only. In addition, to prove the feasibility of this reaction, they tested a series of cyclic enones and obtained the corresponding products in moderate to decent yields (55-93%) [45] (Scheme 1.15). A plausible reaction mechanism for the α-amylase-catalyzed Michael-initiated ring-closure sequence transformation was demonstrated in (Scheme 1.16). Initially, the α-amylase's Asp197 amino residue abstracts a portion of bromotromethane and manifestations an imine type intermediate (**i**) with the help of Glu233. Furthermore, an intermolecular Michael addition occurs by (**i**) attacking the cyclohexanone to produce intermediates (**ii**). Subsequently, the ring-closure reaction undergoes to provide the desired product (**45**).

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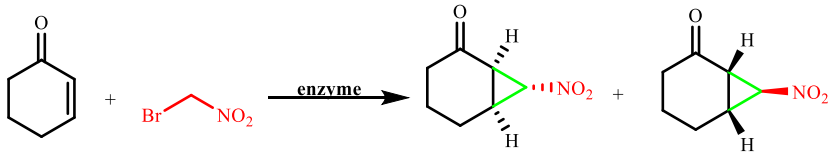
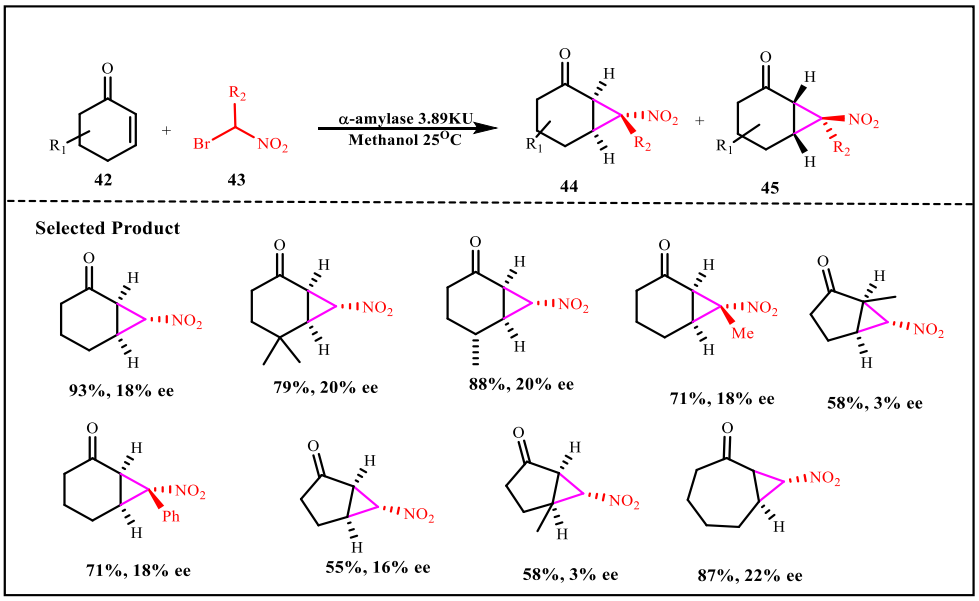
Table 1.11: α-mylase catalyzed one-pot Michael addition-initiated ring-closure reaction



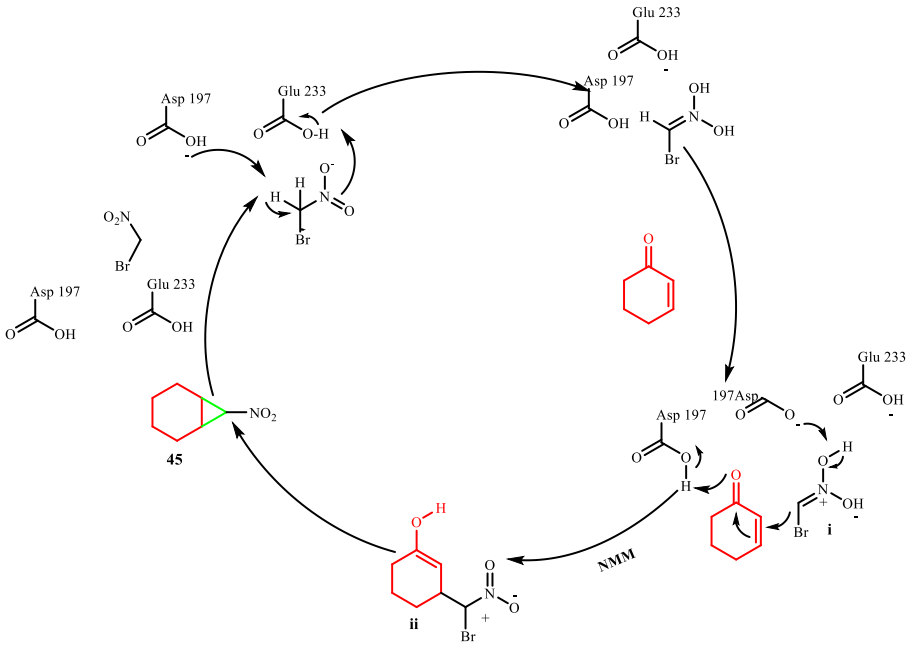
entry	enzyme	Yield%
1	α -Amylase (*Hog pancreas*)	53%
2	Amyl glucosidase (*Aspergillus niger*)	30%
3	α-Amylase (*Aspergillus oryzae*)	9%
4	Protease (*Aspergillus saitoi*)	17%
5	Protease (*Rhizopus sp.*)	15%
6	Protease (*Streptomyces griseus*)	15%
7	Lipase (Pseudomonas cepacian)	13%

[a]**Reaction conditions:** Enone (0.30 mmol), bromonitroalkane(0.25 mmol), enzyme and NMM(0.25 mmol) in MeCN (0.9 mL) and deionized water (0.1 mL) at 25°C for 120 h. [b]Isolated yield. [c]ee was determined by HPLC with Chiral Pak AD-H column.



[a]**Reaction conditions:** Enone (0.50 mmol), bromonitroalkane (0.25 mmol), α-amylase (3.89 kU) and NMM (0.25 mmol) in MeOH (0.9 mL) and deionized water (0.1 mL) at 25 °C. [b]Isolated yield. [c]ee was determined by HPLC with Chiral Pak AD-H column.

Scheme 1.15: One-pot synthesis of nitrocyclopropane using α-amylase enzyme

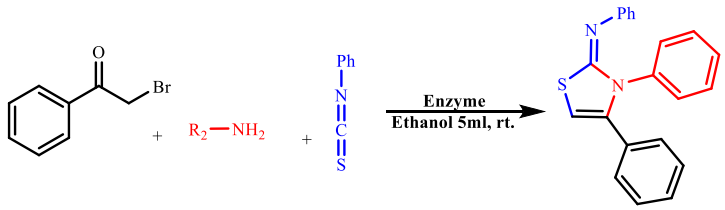


Scheme 1.16: Proposed mechanism of α-amylase catalysed ring closure sequence

1.2.12: Enzymatic synthesis of substituted thiazole-imine derivatives

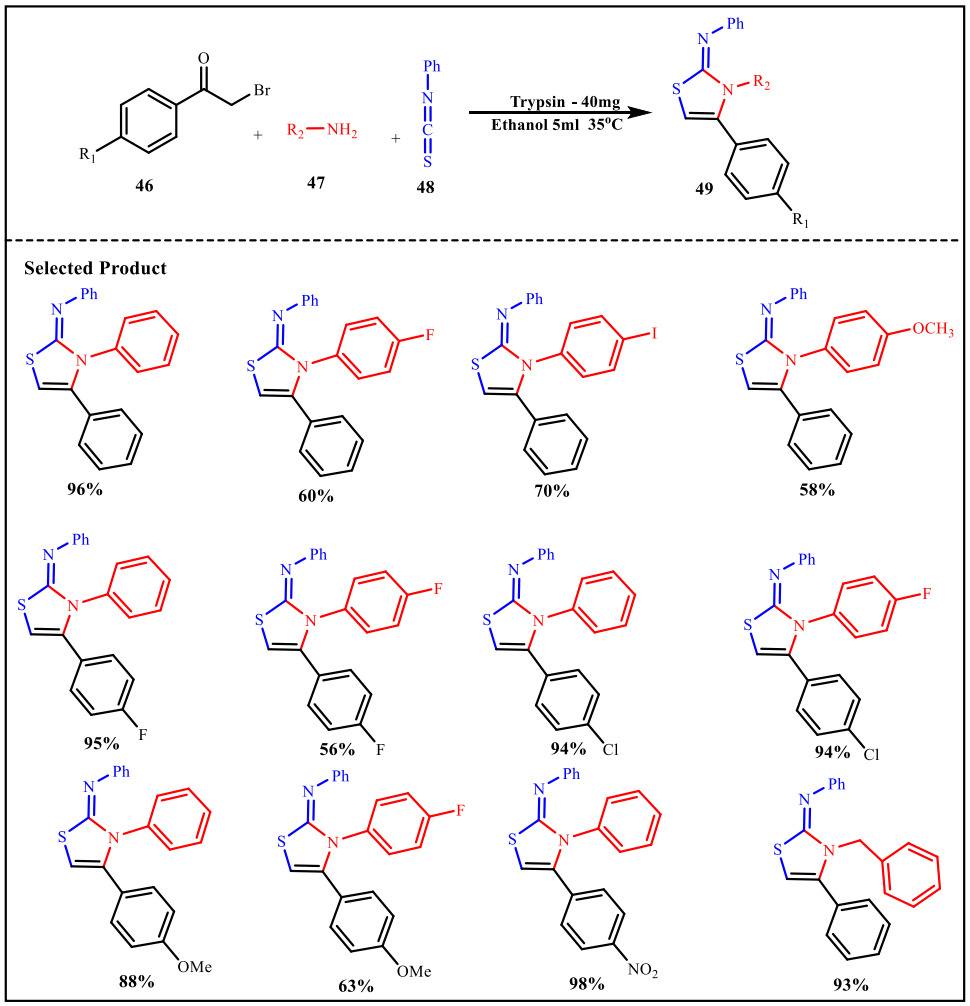
Li and his group reported the one-pot synthesis of substituted thiazole-imines (**49**) from aromatic bromoketones (**46**), primary amines (**47**), and phenyl isothiocyanate (**48**) (Scheme 1.16). Initially, various commercially available enzymes were screened such as , hemoglobin, lipase, lysozyme, α-amylase, and trypsin. In this context, when this reaction was carried out with α-amylase, moderate catalytic activity was observed and a yield of 53% was obtained (Table 1.12, entry 6).[46] Surprisingly, trypsin from the *Porcine pancreas* yielded the highest yield of the product i.e. 87% (Table 1.12, entry 10). Futher, when this reaction was tested with BSA only got 27% conversion yield. To find the appropriate solvent for this reaction, eight protic and aprotic solvents were chosen. Only three protic solvents, such as EtOH, aqueous EtOH, and MeOH, showed high catalytic efficiency for this reaction, whereas ethyl acetate, chloroform, DMSO, ethylene glycol, and toluene showed very poor to moderate catalytic activity. The effect of enzyme concentration, temperature, and reaction time on the model reaction was also investigated, and it was discovered that a 40 mg enzyme concentration at 35°C in ethanol was the best-optimized reaction condition. Further, the 3CRs' scope, generality, and effectiveness were also assessed. Under optimized conditions, a sequence of bromoacetophenone supplemented arylamine or benzylamine, and phenyl isothiocyanate was used in the equimolar amounts of Trypsin. In all circumstances, the reactions proceeded efficiently and successfully, affording the estimated thiazole-2-imine derivatives in good to excellent yields ranging from 55 to 98% (Scheme 1.17). The transformation's probable mechanism is described in (Scheme 1.18). The asymmetrical thiourea (**iii**) was most likely made from the primary amine (**i**) bonded in Trypsin's S1 pocket and phenyl isothiocyanate (**ii**). Followed by nucleophilic alkylation of bromoketones leads to the formation of intermediate (**iv**) which was then converted into intermediate (**v**). The carbonyl group of intermediate (**v**) had been stimulated by the oxyanion tunnel of Trypsin and cyclized to obtain intermediate (**vi**), whose positive charge had been fascinated and stabilized by Asp-189 positioned inside the S1 pocket. After deprotonation and dehydration, intermediate (**vi**) could have been transformed into the final product (**49**) by trypsin's triad (His-57, Asp-102, and Ser-195).

Table 1.12: Trypsin-catalyzed multicomponent one-pot reaction in different solvents[a]



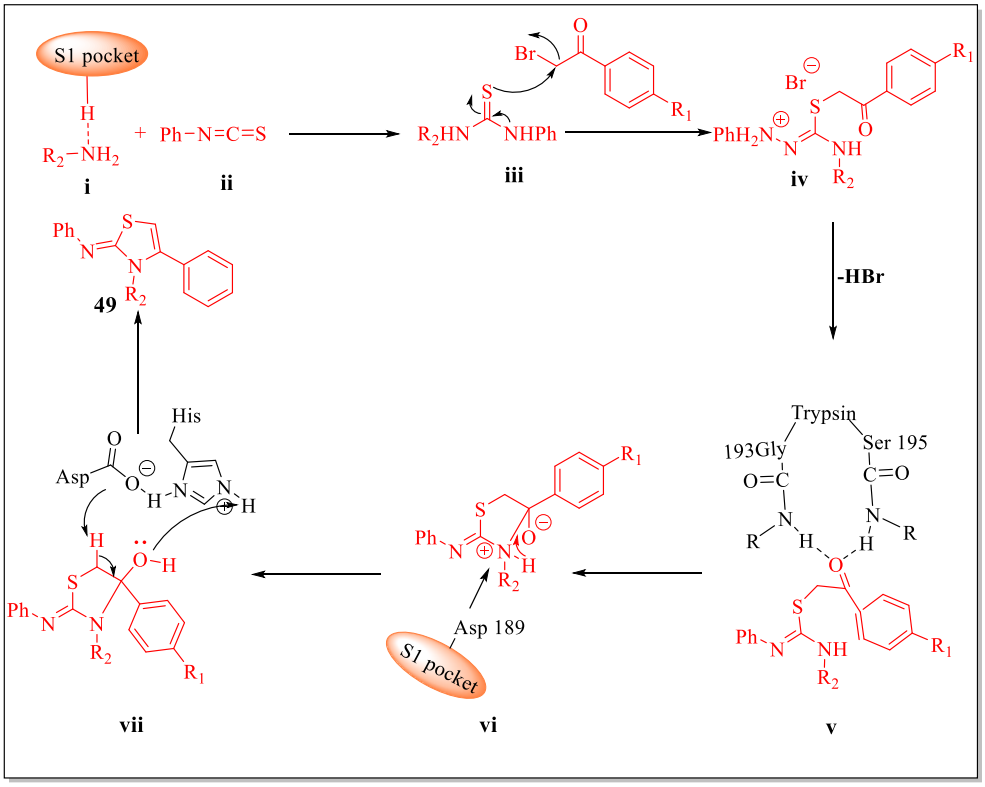
Entry	Enzyme	Enzyme amount(mg)	Time (min.)	%Yield[b]
1	Egg white albumin	50	90	20%
2	Hemoglobin (*Bovine erythrocytes*)	50	90	24%
3	Albumin Bovine V (*Bovine serum*)	50	90	27%
4	Lipase (*Porcine pancreas*)	50	90	30%
5	Lysozyme (*Chinken egg*)	50	90	36%
6	α-Amylase (*Bacillus submerged fermentation*)	50	90	53%
7	Trypsin (*Porcine pancreas*)	10	30	58%
8	Trypsin (*Porcine pancreas*)	20	30	63%
9	Trypsin (*Porcine pancreas*)	30	30	74%
10	Trypsin (*Porcine pancreas*)	40	30	87%
11	Trypsin (*Porcine pancreas*)	50	30	81%
12	Trypsin (*Porcine pancreas*)	60	30	81%

[a]**Reaction condition:** α-bromophenylacetone (1 mmol), aniline (1 mmol), and phenyl isothiocyanate (1 mmol), various enzyme in EtOH (5 ml) at rt. [b]Isoalted yield.



[a]**Reaction condition:** α-bromophenylacetone (1 mmol), primary amine (1 mmol), and phenyl isothiocyanate (1 mmol), catalyst (40 mg) in 75% ethanol (v/v, 5 ml) at 35°C. [b]Isoalted yield.

Scheme 1.17: Enzyme-catalysed one-pot reaction to synthesized substituted thiazole-imine derivatives



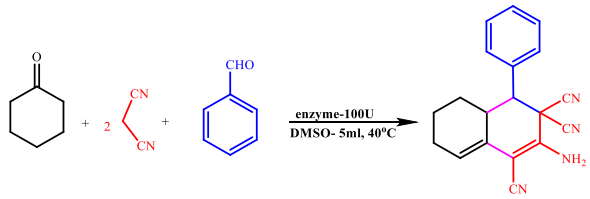
Scheme 1.18: Plausible mechanism for the construction of thiazole-2-imines

1.2.13. Synthesis of ortho-amino carbonitriles using biocatalyst

In 2018, Chaudhari *et. al.* demonstrated a new strategy for eco-friendly synthesis of *ortho*-amino carbonitrile (**53**) through a multicomponent reaction in DMSO at 40°C. First, the influence of different commercial enzymes such as PPL, CRL, ANL, AMA, and α-amylase was tested on the model reaction of malononitrile (**51**), cyclohexanone (**50**), and benzaldehyde (**52**). Although, PPL showed high catalytic activity, and an isolated yield of 54% was achieved (Table 1.13, entry 1).[47] Further solvent, enzyme concentration, and reaction conditions were optimized. The effect of different solvents such as acetonitrile, chloroform, DMSO, DMF, ethanol, *t*-butanol, THF, and water on the model reaction was studied but obtained the highest isolated yield of 54% in DMSO due to the high solubility of the substrates. The addition of electron-accepting and electron-releasing groups on the aldehyde resulted in the highest isolated yields, ranging from 80% to 98%, extending the reaction's feasibility (Scheme 1.19). Further, PMS (phenylmethylsulfonylfluoride), an inhibitor, was used to pre-treat PPL, and used the pre-treated enzyme in the reaction as a result there was no reaction. Also, there was no reaction when urea treated denatured PPL was employed

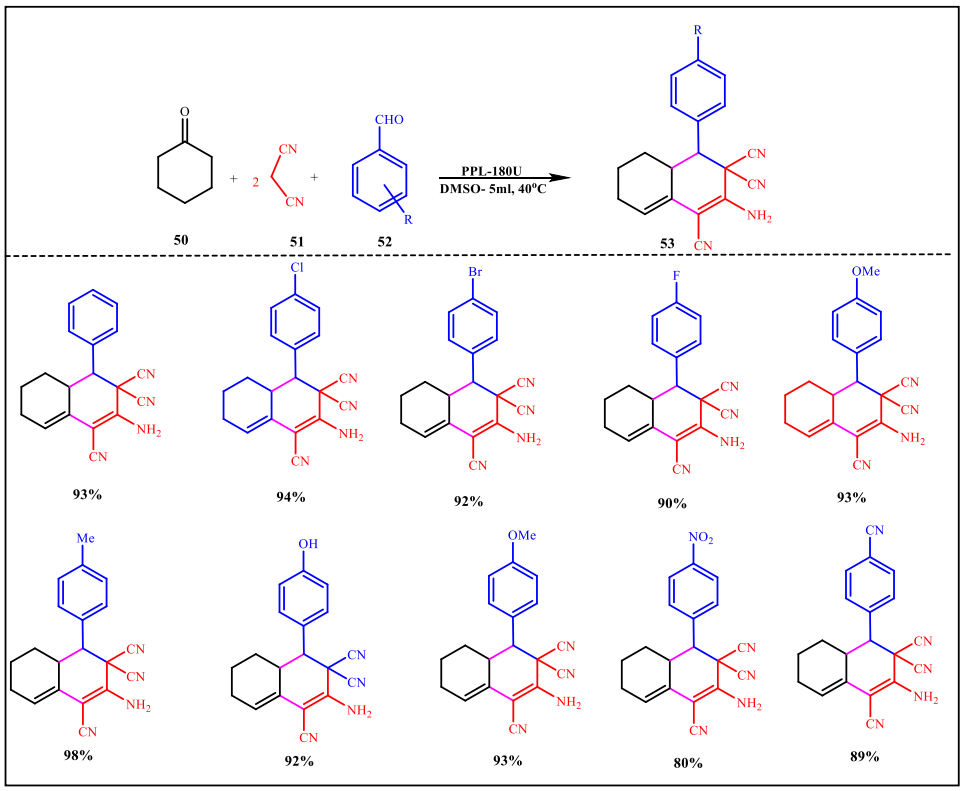
in the reaction. Thus, they came to the conclusion that the enzyme's active site is required for this process.

Table1.13: Lipase-catalyzed synthesis of the compound *ortho*-aminocarbonitrile[a]



Entry	enzyme	%Yield[b]
1	Lipase (*Porcine pancreas*)	54%
2	Lipase (*Candida rugosa*)	40%
3	Lipase (*Aspergillus niger*)	34%
4	Acylase (*Aspergillus mellus*)	48%
5	Trypsin (*Porcine Pancreas*)	Trace
6	Diastase α-Amylase	15%
7	Blank	NR

[a]**Reactionconditions:** Cyclohexanone (2 mmol), malononitrile (4 mmol), benzaldehyde (2 mmol), enzyme (100 U), in solvent DMSO (5 ml) at 40°C for 12 h. [b]Isolated yield.



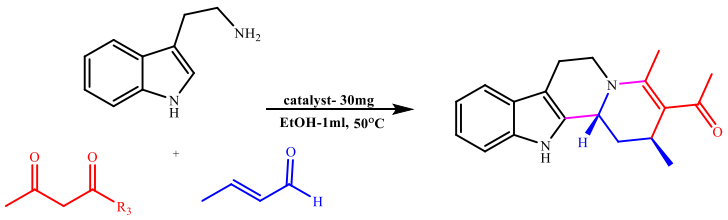
[a]**Reaction conditions:** Cyclohexanone (2.0 mmol), malononitrile (4 mmol), aldehyde (2 mmol) were performed in DMSO (5 ml), under the catalysis of PPL (180 U) at 40°C. [b]Isolated Yields

Scheme 1.19: Scope of substrates for the synthesis of *ortho*-aminocarbonitrile derivatives

1.2.14. α-Amylase catalyzed synthesis of substituted indoloquinolizines

Recently, Yu et. Al. developed the α-amylase-catalyzed one-pot three-component synthesis of highly substituted indoloquinolizine derivatives (**57**) using tryptamines (**54**), β-ketoesters (**55**), and α-β unsaturated aldehydes (**56**) as substrates (Scheme 1.20). They first tested several enzymes to catalyze this reaction but obtained the highest yield in the case of α-amylase from *Porcine pancreatic*. (Table 1.14, entry, 1). When BSA was used as a catalyst, the desired product was obtained in 6% yield only, and equivalent findings were attained when reaction was run for 48 h without the addition of any catalyst. Furthermore, the effect of aprotic solvents on the model reaction was investigated, and a very low yield was obtained; however, when protic solvents such as *i*-PrOH, EtOH, and MeOH were used, the yield increased from 30% to 45% at 50°C in less than 24 hours. [48] This reaction was tested in ethanol with 5% water content to increase the conversion yield, and the highest yield of 74% was detected after 48 hours at 50°C. They also tested the effect of various substitutions on the model substrates and obtained the corresponding products in 44–92 % yield (Scheme 1.20). However, bulky β-ketoesters, such as *tert*-butyl acetoacetate and ethyl butyrate, gave very low product yields due to the low reactivity generated by the increased steric hindrance.

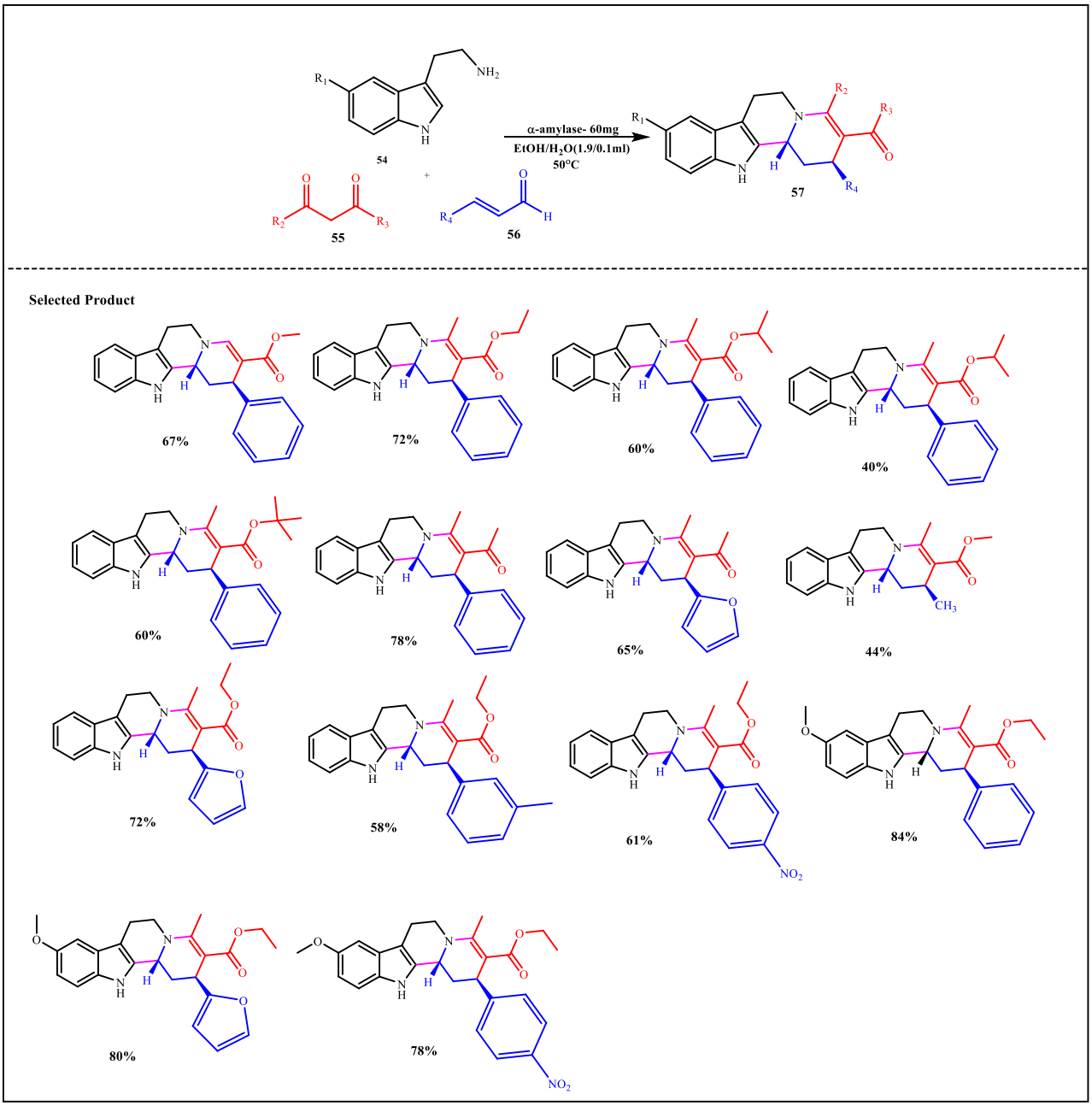
Table 1.14: α-Amylase catalyzed one-pot reaction[a]



Entry	Enzyme	%Yield[b]
1	α-Amylase (*Hog pancreas*)	43%
2	α-Amylase (*Bacillus subtilis*)	6%
3	α-Amylase (*Aspergillus oryzae*)	6%
4	Trypsin (*Porcine pancreas*)	27%
5	Amano lipase AK (*Pseudomonas* fluorescens)	22%
6	Lipase (*Porcine pancreas*)	25%

7	Lipase (*Candida antarctica B*)	9%
8	Amano lipase M (*Mucor javanicus*)	6%
9	BSA	6%

[a]**Reaction conditions:** Tryptamine (0.1 mmol), ethyl acetoacetate (0.2 mmol), cinnamaldehyde (0.1 mmol) and catalysts (30 mg) in ethanol (1 mL) at 50°C for 24 h. [b] Isolated yields were determined by HPLC.



[a]**Reaction conditions:** Tryptamine (0.2 mmol), ethyl acetoacetate (0.3 mmol), cinnamaldehyde (0.3 mmol), α-Amylase (60 mg), ethanol (1.9 mL), H_2O (0.1 mL), 200 rpm, 50°C. [b]Isolated yields were isolated yields.

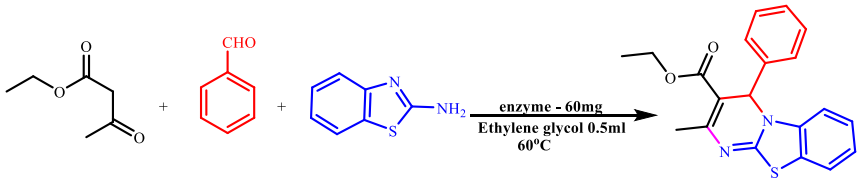
Scheme 1.20: α-Amylase catalysed one-pot reaction to synthesize substituted indoloquinolizines

1.2.15. Enzyme-catalysed one-pot Biginelli reaction

In 2020, N. Wang and co-workers demonstrated Biginelli reaction between aldehyde (**58**), β-ketoester (**59**) and 2-amino benzothiazole (**60**) to construct the 4H-pyrimido[2,1-b] benzothiazole derivatives (**61**) (Scheme 1.21). Initially, several enzymes were used in the model reaction including amylase enzyme. However, BSA and Lipase from *Mucor javanicus* were found inactive for this transformation while CRL, CCL, pepsin, BPL and trypsin gave better yields (Table 1.15, entry 1,4,6 &7-8& 11,1).[29] Among these enzymes, only trypsin provided an affordable yield i.e 42%. Further, thecontrol experiments showed that deactivated-trypsin has no activity. On the other hand, attempts were made to catalyze the reaction using individual amino acids and obtained no productwhich suggested that the structure of the enzyme is important for the catalytic activity of the enzyme.

Next, the influence of substrate molar ratio, enzyme concentration, and temperature on the tandem reaction was examined. A panel of β-dicarbonyl compounds and substituted aromatic aldehydes were reviewed to assess the generality of this reaction. The β-dicarbonyl compounds with either a linear substituent or a larger ester group at R_1 could afford the corresponding products in good yield. However, increasing the size of the R_2 substituent results in comparatively lower yieldof the products (Scheme 1.21).

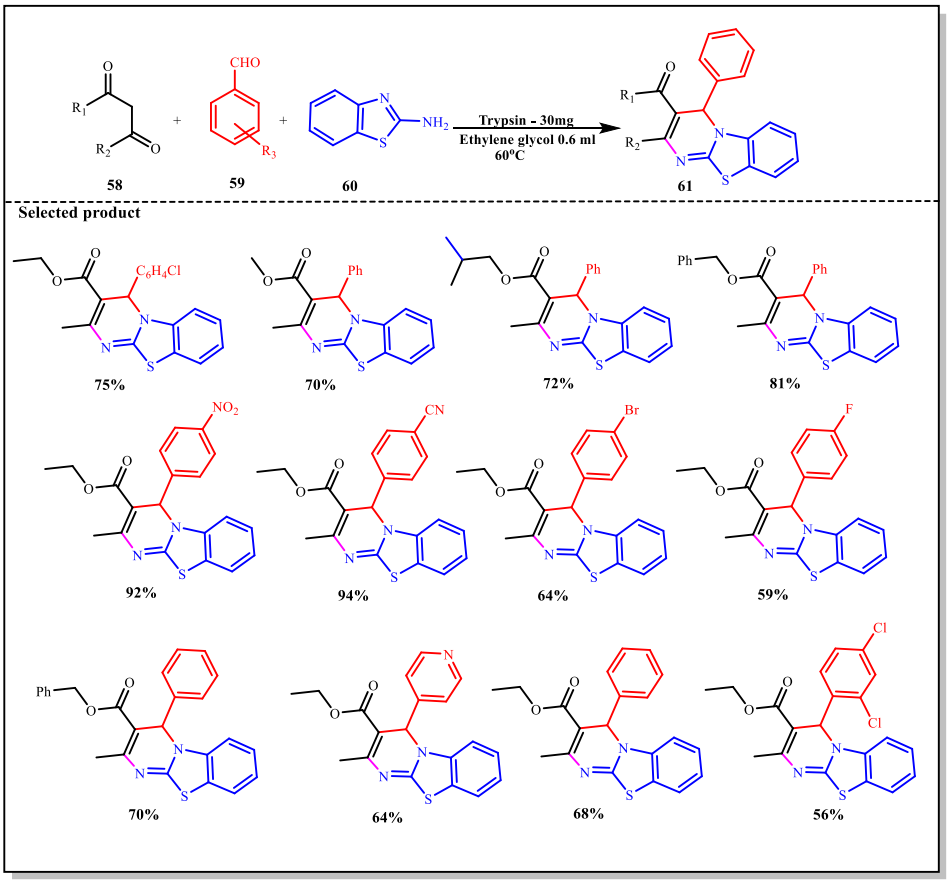
Table 1.15: Impact of enzymes on Biginelli reaction to synthesized the *4H*-pyrimido[2,1-*b*]benzothiazole derivatives[a]



Entry	Enzyme	%Yield[b]
1	Trypsin (*Bovine pancreas*)	42%
2	Lipase (*Porcine pancreas*)	19%

3	Lipase B (*Candida antarctica*)	20%
4	BSA	8%
5	Pepsin	24%
6	Lipase (*M. javanicus*)	7%
7	Lipase (*Candida rugosa*)	36%
8	Lipase (*Bacillus Pumilu*)	26%
9	α-Amylase (*Hog pancreas*)	20%
10	Lipase (human pancreas)	19%
11	Lipase (*Candida cylindracea*)	39%
12	Blank	6%
13	Ser+His + Asp	5.7%

[a]**Reaction conditions:** 2-Amino benzothiazole (0.2 mmol), ethyl acetoacetate(1.0 mmol), vinyl acetate (0.5 mL), and enzyme (60 mg) in ethylene glycol (0.5 mL) at 60°C for 2 d. [b] Isolated yield determined by HPLC.



[a]**Reaction conditions:** 2-Amino benzothiazole (0.6 mmol), ethyl acetoacetate(0.2 mmol), vinyl acetate (0.4 mL), and trypsin (30 mg) in ethylene glycol (0.6 mL) at 60°C for 2 d. [b] Isolated yield determined by HPLC.

Scheme 1.21: One-pot Biginelli reaction to synthesize the *4H*-pyrimido[2,1-*b*] benzothiazole derivatives

1.3. Conclusion:

In this chapter, we have summarized the examples of α-amylase-catalyzed promiscuous transformations. In this context, α-amylase catalyzed carbon-carbon or carbon-heteroatom formation reactions such as Michael-addition, Mannich reaction, aldol condensation, Knoevenagel reaction, and multicomponent reactions have been demonstrated. The development of enzyme-catalyzed promiscuous reactions could lead to new efficient and stable biocatalysts with alternative activities and offer more promising and environmentally friendly synthetic approaches to organic synthesis.

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1. Gaps in the study:

On the basis of the literature survey following gaps have been identified:

(i) Existing catalysts used for aza-Michael addition reaction of aromatic amines to enones have various drawbacks such as the need for high temperatures, longer reaction time, higher catalyst loading, low tolerance with different functional groups, and generation of toxic transition metal waste in some cases.

(ii) Currently available biocatalysts for aza-Michael addition such as lipases can only catalyze the Michael reaction of aliphatic amines to enone. As a result, no biocatalyst is available to catalyze the aza-Michael addition reaction of less nucleophilic aromatic amines to enones to this date.

(iii) There is no report available on using biocatalytic aza-Michael addition reaction in cascade or domino reactions.

(iv) The catalytic efficiency of α-amylase in non-natural organic transformations is not much explored yet.

(v) There is no report using α-amylase in chemo-enzymatic synthesis.

2. Objectives:

By encouraging the application of α-amylase in the area of organic chemistry, Herein, we proposed the following objectives:

i. Development of a highly efficient α-amylase-based biocatalyst for aza-Michael addition reaction of an aromatic amine with enone.

ii. Development of α-amylase-based biocatalyst for domino aza-Michael addition/Aldol condensation reaction to synthesize substituted quinolines.

iii. To find the application of α-amylase in chemo-enzymatic synthesis.

1. Chemicals and Enzymes:

The α-amylase enzyme from *A. oryzae* (E.C: 3.2.1.1, powder, ~ 30 U/mg,), α-amylase from *Bacillus sp.*(E.C: 3.2.1.1, powder, yellow-brown, ~380U/mg,), α-amylase from *B. amyloliquefaciens* (E.C:3.2.1.1, Liquid>250U/g,), α-amylase from *H. pancreas*(E.C: 232-565-6, powder, 50U/mg,), β-amylase (E.C: 3.2.1.1, powder, 220-80U/mg,) were procured from Sigma-Aldrich. Further, aniline ($C_6H_5NH_2$), o-toluidine ($C_7H_7NH_2$), p-toluidine ($C_7H_7NH_2$), 4-nitroaniline ($C_6H_4NO_2NH_2$), 4-chloroaniline ($C_6H_4ClNH_2$), 2,4-dichloaniline ($C_6H_3Cl_2NH_2$), 2-nitroaniline ($C_6H_4NO_2NH_2$), crotonaldehyde as well as all copper salts were brought from Loba Chemie Ltd. (India). Next, 2-bromo aniline ($C_6H_4BrNH_2$), 4-bromo aniline ($C_6H_4BrNH_2$), 2-amino pyridine ($C_5H_6N_2$), amino acetophenone ($C_7H_7CONH_2$) and methyl vinyl ketone (C_3H_6 CO) were supplied from Spectrochem, India. All amino benzaldehyde and halogenated benzaldehyde were purchased from Chemscen LLC. All bases (NaOH, Cs_2CO_3, DBU, C_5H_5N, Et_3NH, KOH, K_2CO_3) and solvents such as n-hexane, ethylacetate, THF, DMSO, DMF, toluene, $CDCl_3$, 1,4-dioxane were brought from Spectrochem, India. All the chemicals and solvents were used without any additional purification. Silica gel 60-120 and Silica gel 200-400 mesh were brought from Spectrochem, India and used for column chromatography to purify the synthesized compounds. Further, Silica gel GF-254 was brought from Spectrochem, India.

2. Instrumentation and Techniques:

All reagents and solvents were purchased from commercial sources and used without further purification. The sterile distilled water was used for the preparation of all aqueous solutions. The NMR spectra were recorded on a Jeol-400 MHz or Bruker-400 MHz spectrometer in deuterated solvents such as $CDCl_3$ or DMSO-d_6 with TMS as an internal reference. The chemical shifts δ was in ppm while coupling constant *J* has been reported in Hz. The 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. All the synthesized compounds were purified using column chromatography and characterized using ^1H & ^{13}C NMR and mass spectrometry. For finding the percent conversion, high-performance liquid chromatography (HPLC) was used. To characterize the immobilized enzyme FT-IR, UV, HR-TEM etc. techniques were used.

Thin-layer Chromatography (TLC)

For preparing the TLC plate, a ready-made glass plates that are chemically inert and stable are utilized and a layer of silica gel (GF-254) as a stationary phase is placed on its surface. Whilst the mobile phase consists a mixture of solvents. The glass chamber which is responsible for maintaining a consistent atmosphere inside is used to run the TLC. To monitor the course of a reaction, a little portion of the reaction mixture was taken from the reaction tube using a glass capillary and work-up the mixture using H_2O and EtOAc or DCM. The organic layer and the aqueous layer were then separated. The organic layer was used for loading the reaction mixture over the TLC plates using using a glass capillary tube. Afterwards, the TLC plate was placed in the glass chamber which is already having mobile phase (mixture of organic solvents). After, running the mobile phase up-to top line, the TLC plates were immediately placed in the UV chamber to monitor the devolved spots[1]. Also, iodine chamber was used to check the position of the spots on TLC plates. .

Column chromatography:

The column is made up of a glass tube with such an adequate stationary phase. The stationary phase (Silica 60-120 mesh size or Silica 200-400 mesh size) is loaded only after the bottom part of the column is packed with cotton or an asbestos pad. After loading the column, a cotton pad is positioned on top to protect the solid phase from becoming disturbed during the incorporation of the specimen or mobile phase (Hexane/ethyl acetate or DCM/ Methanol). The unusual separation bands are influenced by disruptions in the solid phase (adsorbent layer). There are two types of loading methods for making preparations for the column: (1) Dry Packing Method (Fine dry powder is used) (2) Wet Packing Method (Slurry is prepared along with solvent). To monitor the separation process, organic compounds introduced onto the top portion of a column packed with just an adsorbent (stationary phase) transfer through into the column at various rates based on the adsorption of each compound for the adsorbent as well as the solvent or mixture of solvents, and are generally collected in solution as they flow from the column at various times. The components with lesser adsorption and ability to bind to the solid phase elute first, while those have higher adsorption and affinity to the solid phase elute last.

Fourier transformation infrared spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy (FTIR) was used to characterize immobilize enzymes like α-amylase@CuNPs catalyst. The scanning limit of the Perkin Elmer-Spectrum RX-I was

4000 cm^{-1} to 400 cm^{-1}. The sample was extensively mashed,[2] followed by addition of KBr pellet die, which is then compacted with a Qwick Handi-Press to produce a pellet. Afterwards, the pellet is positioned on the specimen holder for FT-IR detection. A comprehensive examination of FTIR spectra stretching from 4000 cm^{-1} to 400 cm^{-1} was done to find the different bonding interactions.

Fourier transform-nuclear magnetic resonance (FT-NMR)

FT-NMR spectra of different amino carbonyls, substituted quinolines, modified indoles, and biaryl compounds were acquired on a JEOL ECS-400 (400 MHz) or Bruker (400 MHz) spectrophotometer having tetra-methyl silane (TMS) as an internal reference. The chemical shifts (δ) of the NMR spectra were expressed in parts per million (ppm), with the coupling constant *J* in (Hz).[3] The following multiplicities are observed: singlet (s), doublet (d), double-doublet (dd), triplet (t), multiplet (m), and broad singlet (brs). All the NMR spectra were obtained either in $CDCl_3$ or DMSO-d6 with the specified acquisition parameters: The MestReNova software was utilized to analyze all of the spectra (6.0.2-5475). The relaxed period of the protons employed in the quantitative analysis was evaluated using the inversion recovery experiment at a 90° pulse angle and the JEOL equipment's Delta NMR software.

High-performance liquid chromatography (HPLC)

The reverse phase High-performance Liquid Chromatography (HPLC) was accomplished using Thermo Scientific UltiMate 3000 infinity series instrument designed with an Ultraviolet (UV) detector, an C-18 column (4.6 mm inner diameter, length 250 mm, 5µm particle size) as a stationary phase, and a mobile phase mixture as a gradient system. Samples (0.01M) for the investigation were made by dissolving the desired amount of the reaction mixture in a methanol/water (80/20, v/v) solvent system, and the solutions were then filtered by syringe filters to filter out any non-soluble particulates. A fixed volume (20 µL) of the standard solution was introduced into the HPLC column, and a constant solvents flow rate (0.5 or 1.0 mL/min) was retained all throughout study. The product conversion yield was calculated by dividing the percentage of the peak area associated to the internal standard by the overall area of the chromatogram[4]. The percent purity of the product was calculated by calculating the percentage age of the relative peak area.

Ultra Violet-Visible Spectrometer (UV-Visible)

Analytik Jena Specord plus 200 UV-visible spectrometer with a double beam deuterium lamp and 1.4 nm bandwidth was used[5]. This is especially true for the increased wavelength range from 185 to 1200 nm. Rectangular cuvettes up to 10 cm in length can be employed in the specord plus spectrophotometer. Additionally, a cuvette with adjustable diameters ranging from 11 to 16 mm was also used.

3. References:

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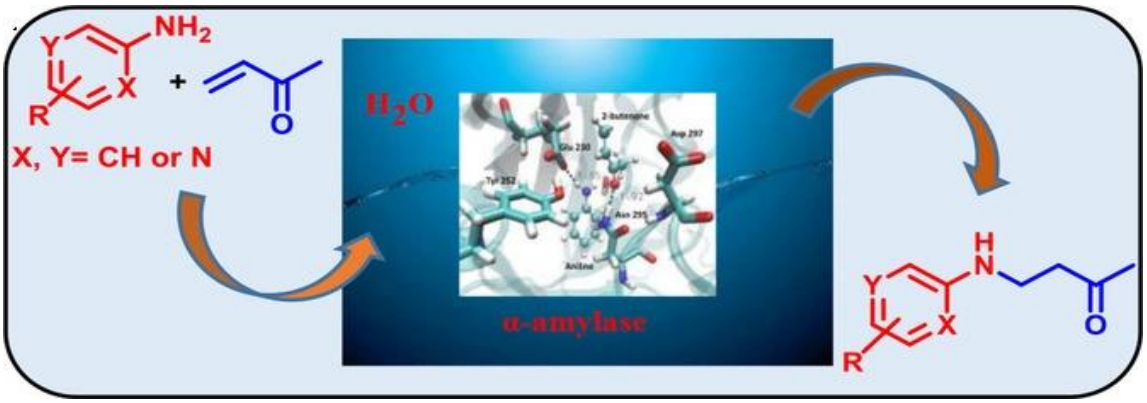
CHAPTER 2:

BIOCATALYTIC AZA-MICHAEL ADDITION OF AROMATIC AMINES TO ENONE USING α-AMYLASE IN WATER

Graphical abstract:



Abstract:

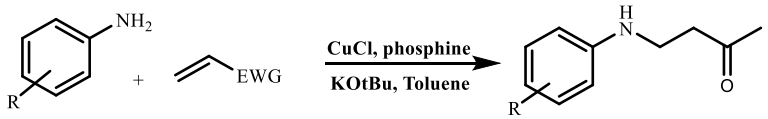
The Michael addition of amines with enones for synthesizing β-amino carbonyls constitutes a valuable transformation in organic chemistry. While various catalyst have been made available for catalyzing the Michael addition of aromatic amines to enones but there is no report of using α-amylase enzyme to 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. In addition, molecular docking and molecular dynamics (MD) simulation studies were 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.

2.1: Introduction:

The Michael addition of various nucleophiles to electron-deficient alkenes is one of the valuable transformations in the area of synthetic organic chemistry.[1-4] Aza-Michael addition leads to the formation of new carbon-nitrogen bonds to afford β-amino carbonyl compounds.[5-10] The carbonyls are imperative building blocks of various biologically active compounds.[11-14] Furthermore, they constitute versatile intermediates for the synthesis of amino alcohols and amino acids.[15-20] Under strongly acidic or basic conditions, aza-Michael addition reactions are commonly used to synthesize amino carbonyls.[21-23] Further Milder Lewis acids have also been reported to catalyze this reaction.[24] However, the conventional methods have certain drawbacks such as the requirement of high temperatures, long reaction times, poor compatibility with various substrate functional groups, and generation of side products. Moreover, novel catalysts such as β-cyclodextrin, bromo-dimethyl sulfonium bromide, and boric acid in water have been proven to be valuable to overcome the problems associated with conventional catalysts.[25-27] Despite this progress, these methods are limited only to catalyze the aza-Michael addition of aliphatic 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

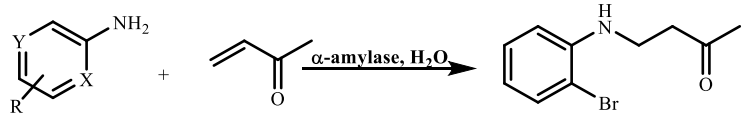
the Michael-addition of aromatic amines.[28-30] Very recently, Lee's group reported copper-catalyzed aza- Michael addition of aromatic amines to α, β-unsaturated olefins (Scheme 2.1a).[31-33] Although, this method has a few shortcomings such as use of strong bases, expensive and non-reusable ligands and generation of toxic transition-metal waste.

(a) Recent report by Lee's group: Copper-catalysed addition of aromatic amines to alkenes



EWG= CN, COMe, CO_2Me
R= aryl or heteroaromatics

(b) Our work:a-amylase catalyzed aza-Michael addition aromatic amine to enones



X, Y= CH, N
R= H, CH_3, OMe, NO_2, $COCH_3$, Cl, Br

Scheme 2.1: Aza-Michael addition of less nucleophilic aromatic amines to α, β-unsaturated olefins

On the other hand, the use of enzymes for catalyzing valuable organic transformations has exponentially grown in the current times.[34-39] Further, anumber of enzymes either in the free form or in immobilized form have been reported for catalyzing the aza-Michael addition reaction of amines with electron-deficient alkenes.[40-47] In this context, various lipase enzymes have been used as a catalyst.[48-52] Lin's group has been reported alkaline protease or hydrolase enzymes to catalyze the Michael addition of imidazole with acrylates. Very recently, Chen *et al.* used lipase enzyme to catalyze the aza-Michael addition of amines to acrylates in supercritical carbon dioxide.[53] Moreover, the α-amylase enzymes are known for catalyzing the hydrolysis of α-glucosidic linkages of polysaccharides such as starch or glycogenin nature.[54-56] Over the years, α-amylase has been used to catalyze various organic reactions.[57-62] We have already described in chapter first, that Guan and co-workers have reported a one-pot synthesis of nitrocyclopropane using an α-amylase enzyme as a catalyst.[63]

Additionally, Yu et al. developed α-amylase to catalyze the synthesis of highly substituted indoloquinolizines using tryptamines, β-ketoesters, and α, β-unsaturated aldehydes.[64] But, to the best of our knowledge there is no previous report of using α-amylase as a catalyst for the aza-Michael additionreaction of amines to enones. Herein, we report α-amylase from *Aspergillus oryzae* [E.C. 3.2.1.1] as an efficient biocatalyst for

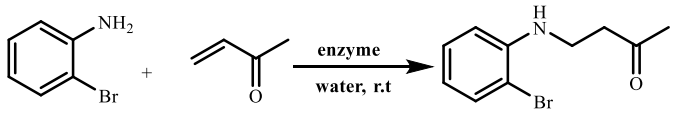
catalysing the aza-Michael addition of less nucleophilic aryl (hetero) amine derivatives to enone (methyl vinyl ketone).

2.2: Results and Discussion:

2.2.1: Screening of enzymes

We started our investigations by selecting the 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 2.1). To our surprise, only α-amylase gave the desired product (3h) in 28% conversion yield (entry 4,Table 2.1). Further, this reaction gave 45% conversion when performed at 40 °C using α-amylase as catalyst in water as a solvent (entry 5, Table 2.1).

Table 2.1: Screening of different enzymes for Michael addition of 2-bromoaniline to methyl vinyl ketone.[a]



1h 2 3h

Entry	enzymes	Conversion %[b]
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*	45%[c]
6	blank	NR[d]

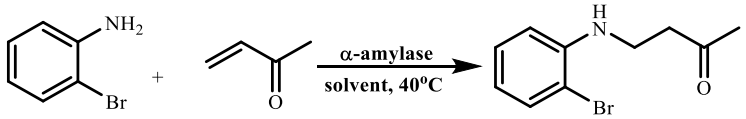
[a] **Reaction conditions:** 1.0 equiv. of 2-bromoaniline (0.58 mmol) and 1.2 equiv. of methyl vinyl ketone (0.69 mmol), 1 mL of an enzyme (1 mg/mL in H_2O) and 1 mL of H_2O were added to a glass tube having a Teflon cap and stirred at room temperature for overnight. [b] based on HPLC. [c] temperature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

2.2.2: Screening of solvents

After having the best enzyme in hand for this addition reaction, we moved toscreen various reaction conditions such as solvents, temperature, and amount of reagents and catalysts 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 2.2). Among the various solvents tested for this addition, reaction water remains the best choice. Also, we checked the different percentages of DMSO inwater as a solvent just to make both starting materials completely soluble during the reaction (entries 5–7, Table

2.2). We obtained maximum conversion when we used a 10% DMSO-water mixture (1:9 v/v) as a solvent (entry 6, Table 2.2).

Table 2.2: Screening of solvents for α-amylase-catalyzed addition of 2-bromoaniline to methyl vinyl ketone.[a]



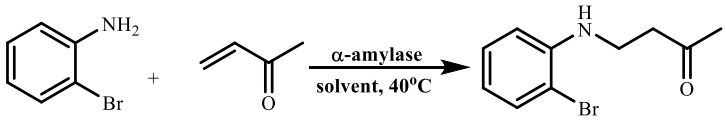
Entry	solvents	Conversion %[b]
1	H_2O	45%
2	DMSO	42%
3	THF	31%
4	Hexanes	15%
5	5% DMSO-water mixture	51%
6	10% DMSO-water mixture	65%
7	20% DMSO-water mixture	64%

[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) and1 mL of solvent were added to a glass tube having a Teflon cap and stirred at 40 °C for overnight. [b] based on HPLC.

2.2.3: Optimization of substrate's molar ratio and reaction temperature:

Further, we tested the effect of different molar ratios of 2-bromoanilne and methyl vinyl ketone on theconversion of this addition reaction (Table 2.3) and found that the 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 2.3). We also increased the temperature of this reaction up to 80 °C but found that 40 °C was the optimum temperature for getting the highest conversion.

Table 2.3: Optimization of the substrate's molar ratio and reaction temperature.[a]



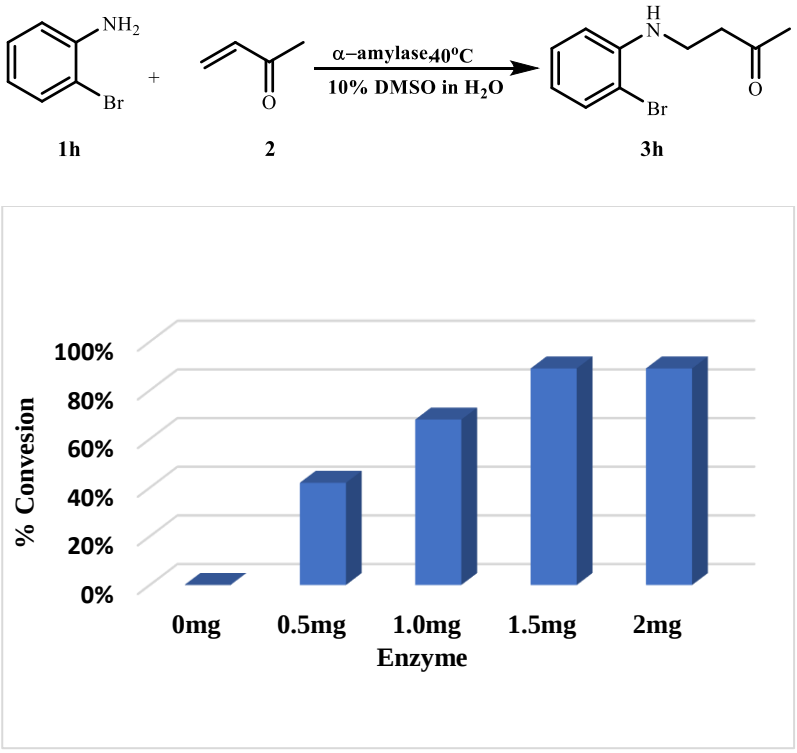
Entry	ratio of reagents (1a:2a)	temperature	[b]conversion
1	1:1	40°C	54%
2	**1:1.2**	**40°C**	**65%**
3	1:1.5	40°C	64%
4	1:0.5	40°C	34%

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 methylvinyl 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.

2.2.4: Optimization of enzyme concentration

Figure 2.1: Optimization of the concentration of α-amylase



[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%.

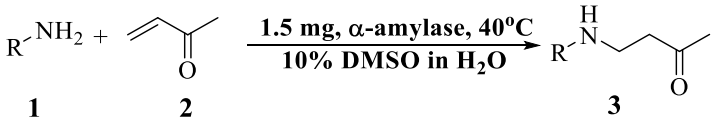
In the final phase of optimization, we increased the concentration of enzyme to improve the yield of this transformation (Figure 2.1). To our delight, high yield (89%) could be obtained even at low catalyst loading (1.5 mg/mL); this undoubtedly proved the efficiency of α-amylase as a catalyst for this addition reaction.

2.2.5: Substrate scope:

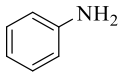
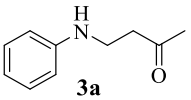
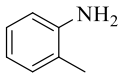
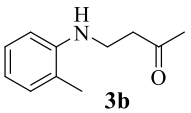
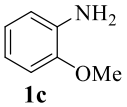
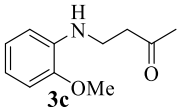
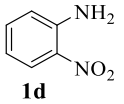
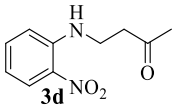
After having the optimized conditions for the aza-Michael addition of 2-bromo aniline (1 equiv.) and methyl vinyl ketone (1.2 equiv.) using α-amylase (1.5 mg/mL) as catalyst in 2 mL of 10% DMSO-water mixture as the solvent at 40 °C for overnight, we explored the

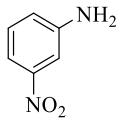
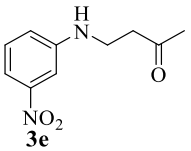
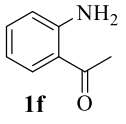
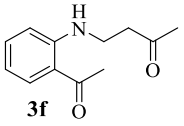
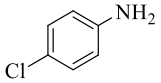
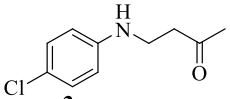
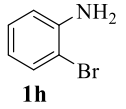
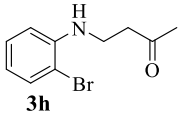
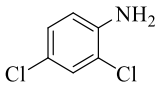
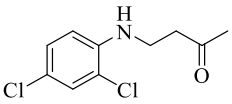
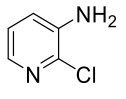
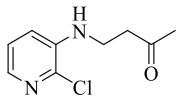
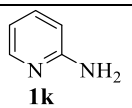
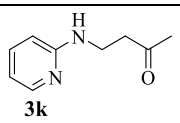
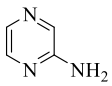
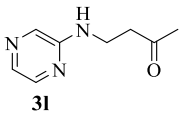
scope of different substitution on aryl (hetero) amines under the optimized reaction conditions (Table 2.4). The reaction of aniline with methyl vinyl ketone produced the Michael addition product (3a) in 74% yield (entry 1, Table 2.4). Then we tested the effect of electron-donating groups such as -Me and -OMe at the arene ring which allowed the reaction to afford the products in good yields (entries 2–3, Table 2.4). On the other hand, the presence of electron-withdrawing groups such as NO_2, and $COCH_3$ at the arene ring of aniline has a significant effect on the reactivity of the reaction and afforded the desired products in slightly lower yield (entry 4–6, Table 2.4). A much lower yield was obtained when the NO_2 group was at *ortho*-position (entry 4, Table 2.4). Moreover, the 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 2.4). Gratifyingly, the nitrogen-containing heteroaromatic amines also reacted well and gave the corresponding products in very good yield (entry 10–12, Table 2.4).

Table 2.4: 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.

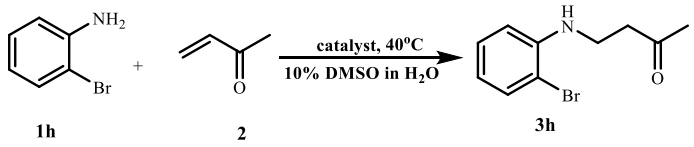
2.2.6: Immobilization of α-amylase at Cu-nanoparticles

In the second phase of our endeavour, 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.[65-68] Very recently Bäckvall's group reported a Pd(0)-CALB biohybrid catalyst and used it in a cascade reaction.[69] 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".[70-75] The characterization of the novel α-amylase@CuNPs was done using various techniques such as UV-visible, DLS, FTIR, TEM, and EDS.

Next, we tested the hybrid catalyst (α-amylase@CuNPs) for catalyzing the aza-Michael addition reaction of 2-bromoaniline with methyl vinyl ketone (Table 2.5). To our delight, we obtained very similar results when comparing the catalytic efficiency of this hybrid with the free enzyme (entry 1–2, Table 2.5). Subsequently, we tested α-amylase@CuNPs for reusability and the results are shown in Figure 3.2. These results indicated that hybrid catalysts can be used many times with high catalytic efficiency due to improved operational stability under the optimized reaction conditions.

Table 2.5: Comparison of the catalytic activities between α-amylase and α-amylase@CuNPs.[a]

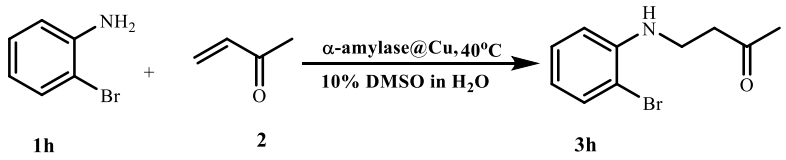


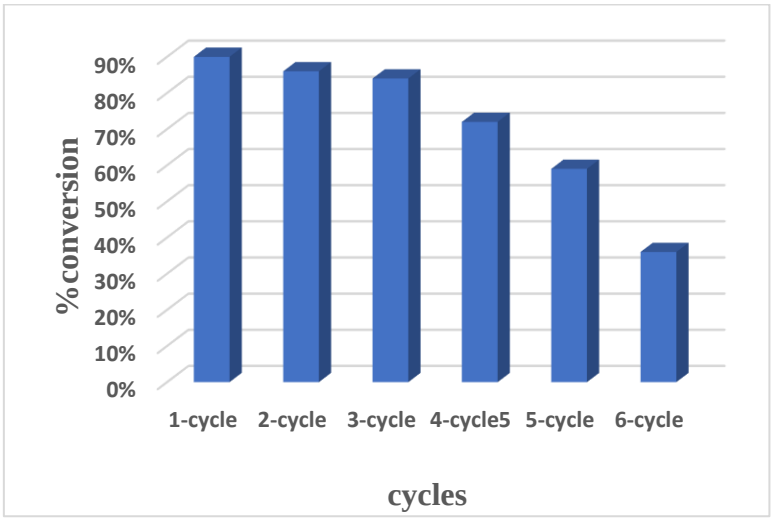
1h 2 3h

Entry	catalyst	Conversion %[b]
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 vernight. [b] based on HPLC.

2.2.7: Reusability of amylase@Cu-catalyst





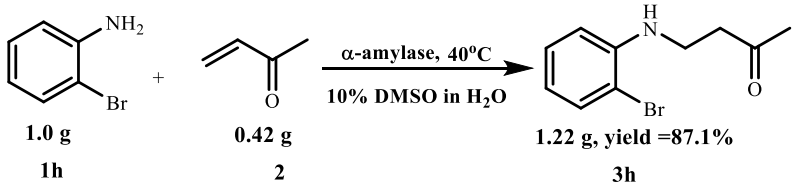
[a] **Reaction conditions:** 1.0 equiv. of 2-bromoaniline (0.58 mmol) and 1.2 equiv. of methyl vinyl ketone (0.69 mmol) in 200 µL of DMSO, 1.5 mL of α-amylase@CuNPs (1 mg/mL in H_2O) and 0.3 mL of H_2O were added to a glass tube having a teflon cap and stirred at 40 °C for overnight, [b] based on HPLC,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

Figure 2.2. Reusability test of amylase@Cu for the Michael addition of 2-bromo aniline with vinyl ketone.

2.2.8: Scale-up synthesis and calculation of green chemistry metrics

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 (3 h) using α-amylase (15 ml, 1 mg/ml in H_2O) was carried out (Table 2.6). The successful isolation of 1.22 g of (3 h) in 95% purity from this reaction proved the synthetic utility of α-amylase in the synthesis of β-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 2.6.

Table 2.6: Scale-up synthesis of β-amino carbonyl (3 h) and calculation of green chemistry metrics.[a]



Entry	metrics	results	Ideal value[109]
1	Isolated yield	87.1%	100%
2	selectivity	100%	100%
3	E-factor	0.16	0.13-0.5
4	PMI	1.16	1-1.5
5	Atom-economy	100 %	100%
6	Reaction mass efficiency	86.1%	100%

[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.

2.2.9: Computational investigation

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.[76-80] 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) 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.[81-82] Then we have carried out 50 ns Molecular Dynamics (MD) simulation to get the most preferable orientation of thesubstrates inside the binding site using GPU version64of the AMBER 16 package.[83] The details of the molecular docking and MD simulations are given in Appendix 1. The orientation of the substrate in the active site in one of the representative snapshotsfrom MD simulation and the key interactions of the substrates with nearby amino acids are shown in Figure 2.3. 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)[84] 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 3.4. 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.

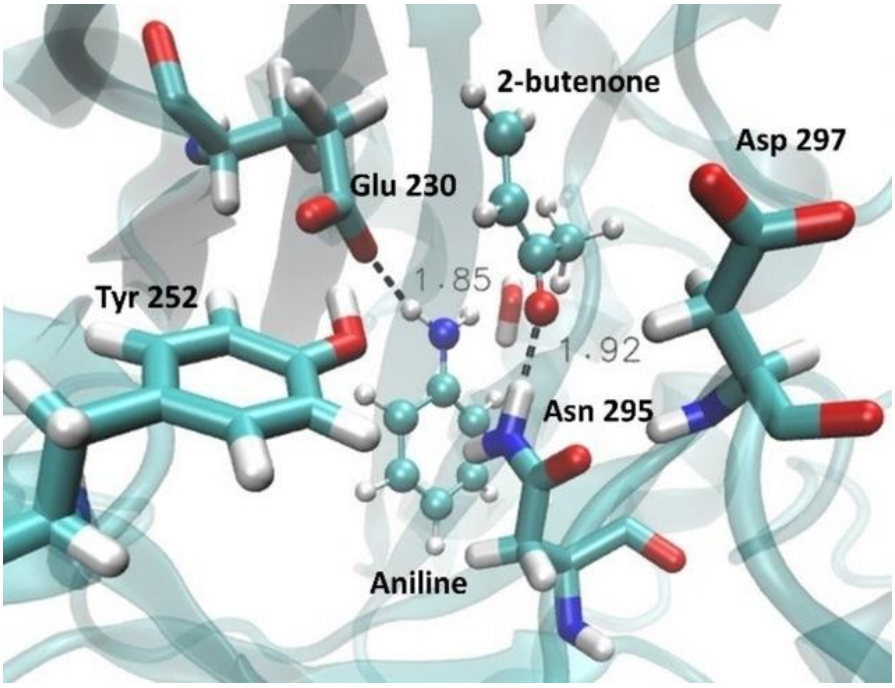


Figure 2.3: Overview of substrates (aniline and 2-butenone) accommodated in the active site of α-amylase. The representative snapshot was taken from molecular dynamic (MD) simulation, revealing substrates (aniline and 2-butenone) in the binding pocket of α-amylase and the surrounding residues.

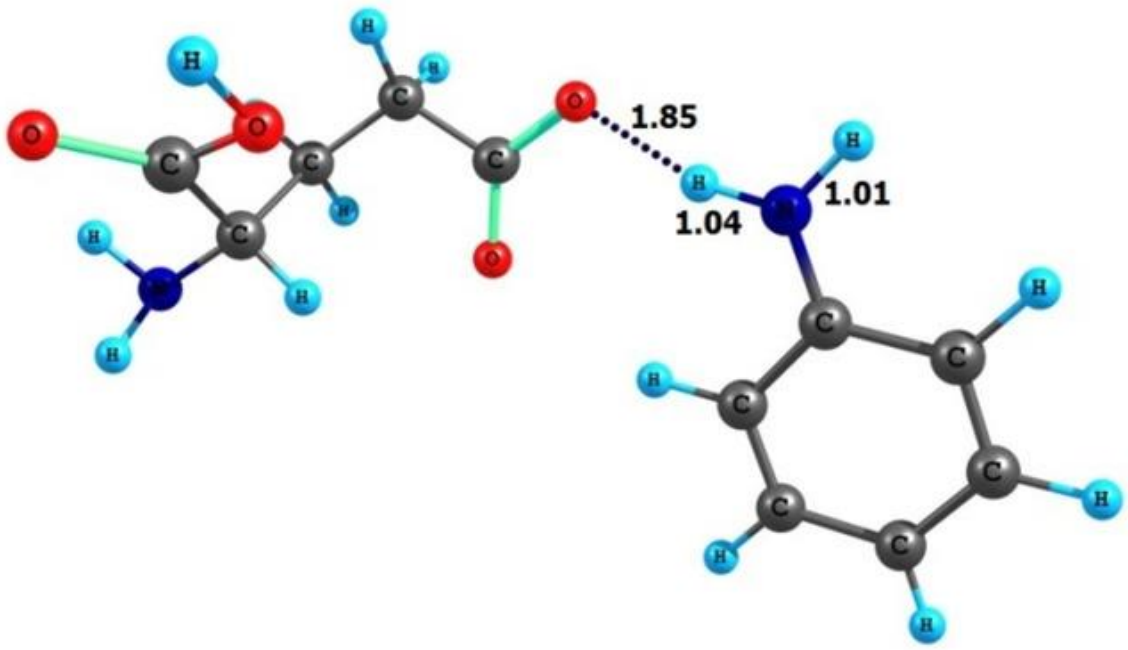
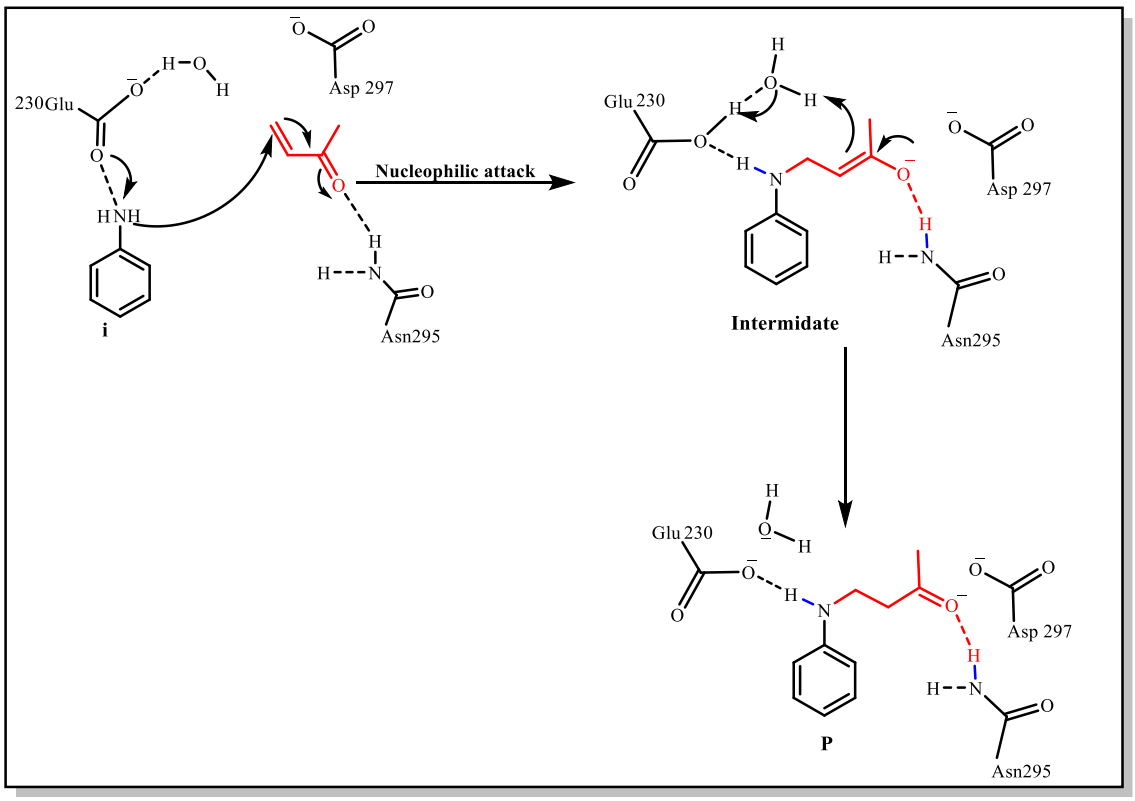


Figure 2.4: The optimized structure of model system comprised of aniline H-bonded to a glutamic acid. Relevent bond distances are given in Å

2.2.10: Proposed mechanism

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 2.3. 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, whichis 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 2.3. 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 2.2: 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.[85-87] The results showed that the addition of starch decreased the yield of the reaction from 88% to 50.9% 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. Next, we performed control experiments in the presence of equimolar amount of urea, with BSA and denatured α-amylase as a catalyst and obtained 22%, 27% and 19% conversion yield respectively. These experiments suggests that the active-site of α-amylase playing a role in this transformation. However, further investigation such as mutagenesis of active site are required to confirm the role of Glu230 in the catalysis.

2.3: Experimental

2.3.1: 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.

2.3.2: 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 (3 h) 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 procedure.

2.3.3: Synthesis and characterization of α-amylase@CuNPs:

The aqueous solution of L-Histidine (100 mM) was prepared by dissolving in sterile distilled water. To this solution added aqueous $CuSO_4$ solution (1 mM) slowly under soft stirring conditions and further stir the resulting solution at room temperature for 24 hrs to generate a homogenous mixture, also the dark blue colour of copper changes to a pale blue solution. Then to this mixture freshly prepared α-amylase solution was added slowly to achieve final concentration 2 mg/ml under constant stirring (50 rpm) at room temperature and allowed the reaction to proceed under same conditions for 2 hrs. The colour will change from pale blue to milky dark blue due to the formation of the hybrid nanocluster "α-amylase@CuNPs" of α-amylase with copper nanoparticles which characterized using various techniques such as UV-Visible, DLS, FTIR and TEM.

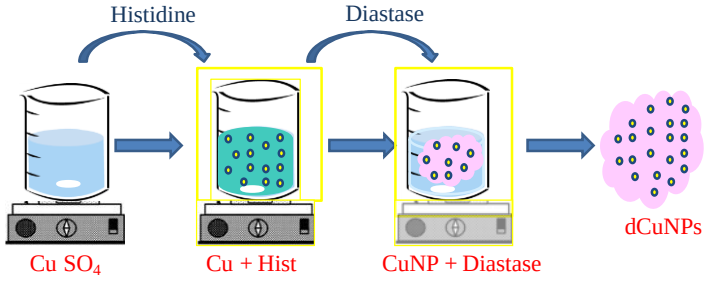


Figure 2.5: Schematic representation of the preparation of hybrid of α-amylase with Cu-nanaoparticles

The synthesis of α-amylase@CuNPs from CuNPs is a single step process characterized by the formation of single isosbestic point at 655 nm. Further due to the formation of α-amylase@CuNPs, the Histidine protected CuNPs got declustered and stabilized which was characterized by a hypsochromic shift of 45 nm in UV-Visible spectra (Figure 2.6). The CuNPs showed a characteristic absorption maximum at 812 nm which got shifted to 767 nm due to interaction with α-amylase (Figure 2.6). Further, the DLS study showed formation of ~ 140 nm particles when it is only histidine protected, but after stabilizing with α-amylase the size increased to ~ 3 μm (Figure 2.7).

The typical TEM images of the α-amylase@CuNPs have been shown in figure 3.9. The TEM image clearly shows that histidine reduced CuNPs are around 7-10 nm in diameter and are arranged in cluster/ agglomeration whereas upon immobilization with α-amylase individual nanoparticles got declustered remained scattered in all over the protein. The presence of amorphous layer around the CuNPs showed an association of protein. To confirm the association of Cu in the α-amylase@CuNPs cluster elemental analysis were done using EDS (Figure 2.10). The association of 26% weight percentage of copper was observed in the α-amylase@CuNPs clusters. Further, to understand the interaction between protein and Cu; FT-IR studies were performed (Figure 2.8). The FT-IR peak of the 540 cm^{-1} was observed due to formation of Cu-S bond, 555 cm^{-1} depicted interaction of Cu-N whereas peaks in the range 603-640 cm^{-1} showed formation of Cu-O bond due to interaction of CuNPs with α-amylase protein (Figure 2.2e), rest of the bond involved in the interaction depicted by FT-IR is presented in the Table 2.7.

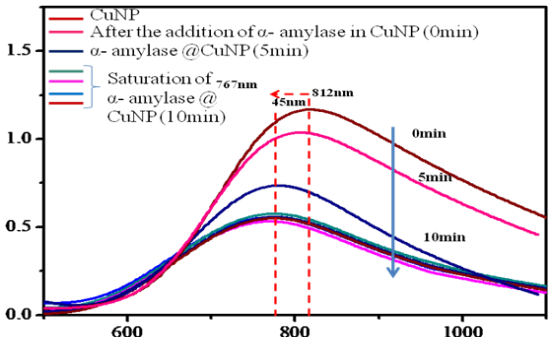


Figure 2.6. The SPR spectra of formation of Histidine protected CuNPs and further stabilization with α-amylase. The characteristics blue shift is showing declustering of CuNPs and stabilization in presence of α-amylase.

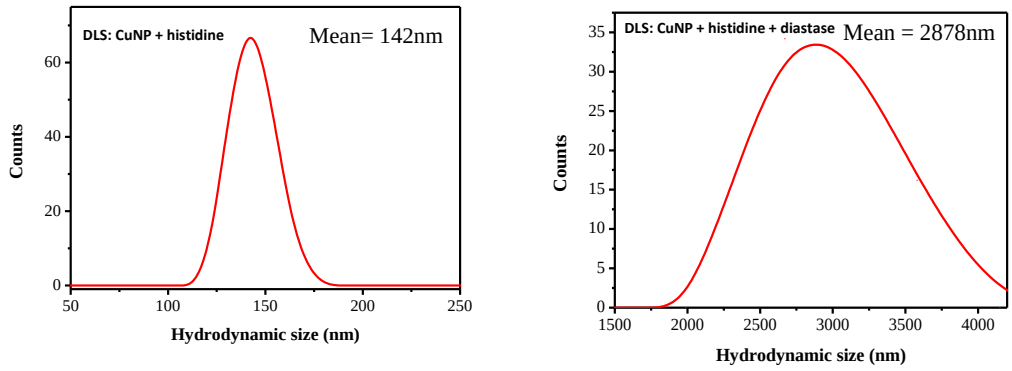


Figure 2.7. The DLS spectra showing hydrodynamic diameter of Histidine protected CuNPs and α-amylase@CuNPs.

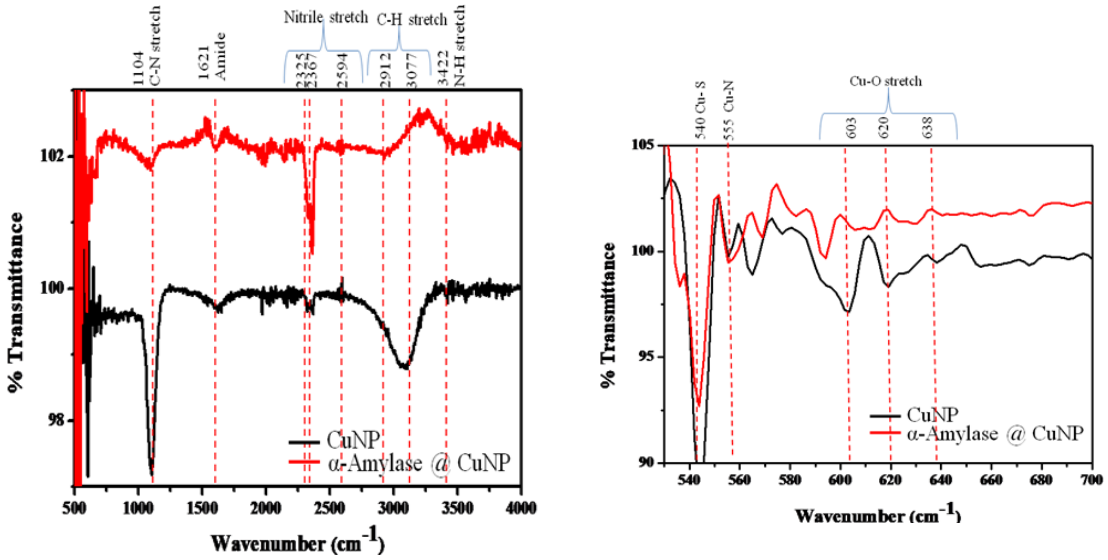


Figure 2.8. The FTIR spectra of CuNPs and α-amylase@CuNPs between 400-4000 cm^{-1}showing interaction between copper nanoparticles and the protein. The detailed study of

FTIR spectra between 530 cm^{-1} to 700 cm^{-1} showed interaction between Cu-S, Cu-N and Cu-O and were characterized absorption peaks at 540, 555 and 603-640 cm^{-1} respectively.

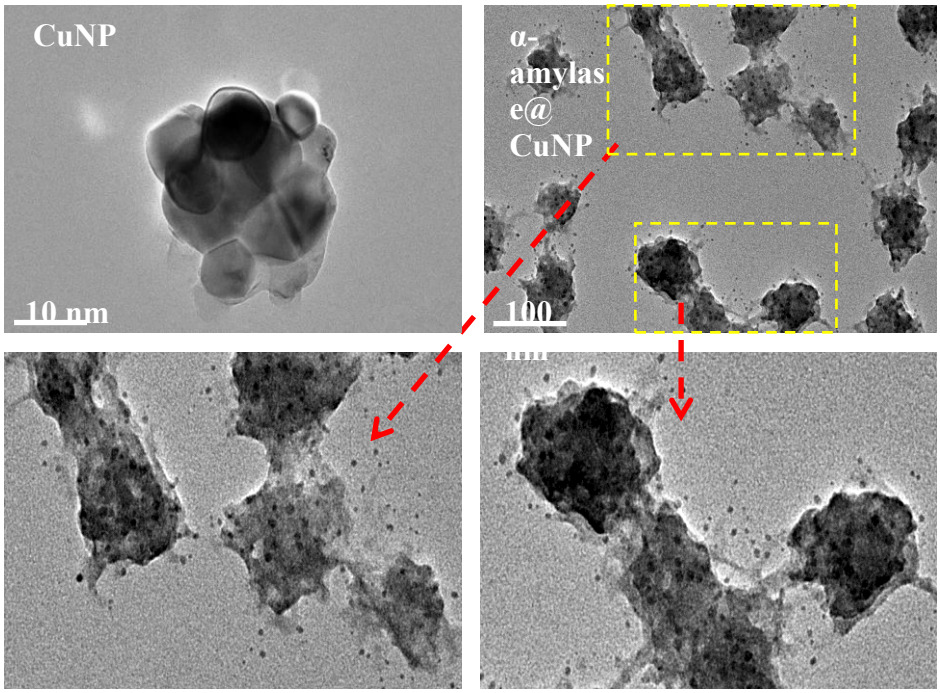


Figure 2.9. The TEM images of CuNPs and α-amylase@CuNPs. Image of CuNPs showed formation of CuNPs with an average size 7-10 nm which are arranged in clusters. Whereas upon interaction with α-amylase the CuNPs got declustered and immobilized in the protein.

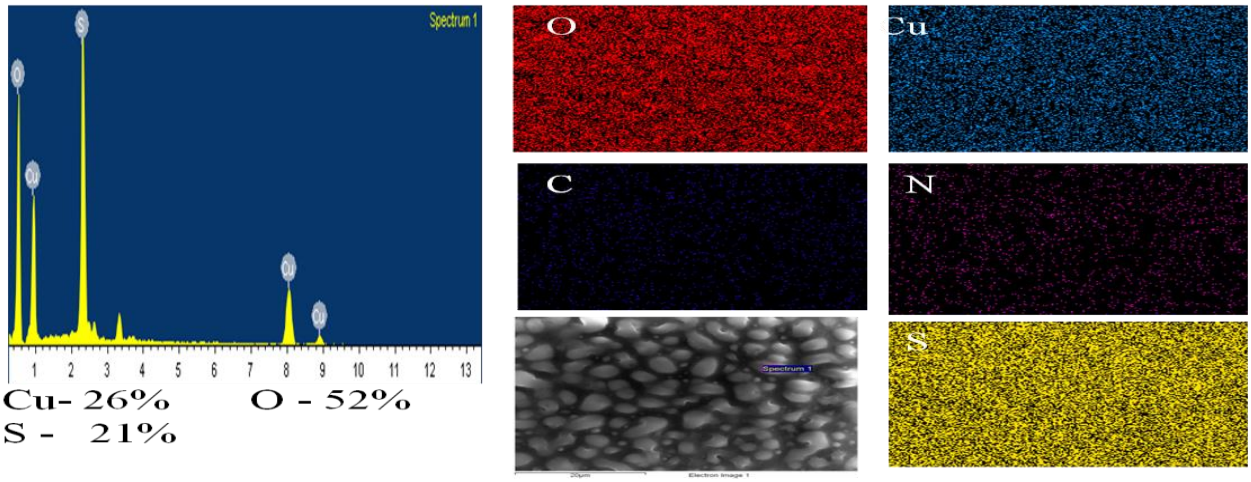


Figure 2.10. The EDS spectra and elemental mapping of α-amylase@CuNPs. The data showed association of 26% of Cu w/w in α-amylase@CuNPs.

Table 3.7: The FT-IR peak observed for the α-amylase@CuNPs

Functional Group	Absorption (cm^{-1})
555	Cu-N stretch
564, 569, 593	Cu-Cl stretch
603, 618, 638	Cu- O stretch
1104	C-N stretch
1621	C=O Amide
2325, 2367, 2594	Nitrile stretch
2912, 3077	C-H stretch
3422	N-H stretch

2.3.4: General details of computational investigation:

2.3.4.1: System preparation and setup

The PDB structure for the enzymes was downloaded from RCSB protein data bank.[7] The initial structure of substrate bound enzyme was generated by docking the substrates into the well-known binding site of enzyme using AutoDock Vina software.[88] The protonation states of the treatable residues were assigned based on pKa values obtained from the PROPKA software.[89] Missing hydrogen atoms are added by the Leap module of AMBER 16.[90] The general AMBER force field[91] was used for the substrates, while the missing parameters and the partial atomic charges for these substrates were obtained from the RESP model,[92-93] using B3LYP/6–31G* level of theory. The total charge of the system was neutralized by adding twenty four Na+ ions. Finally, the resulting system was solvated in a rectangular box of TIP3P[94] water extending up to a minimum cutoff of 10 Å from the protein boundary. The Amber ff14SB force field[95] was employed for the protein in all the MD simulations.

2.3.4.2: Molecular Dynamics Simulations

After proper setup, the energy of the system was minimized by 5000 steps of steepest descent and 15000 steps of conjugate gradients. Subsequently, it was gently annealed from 10 to 300 K under the canonical ensemble for 50 ps with a weak restraint of 5 kcal $mol^{-1}Å^{-1}$ on the protein and substrates. To achieve a uniform density after the heating dynamics, 1 ns of density equilibration with a weak restraint of 5 kcal $mol^{-1}Å^{-1}$ on the protein and substrates was performed under isothermal–isobaric ensemble at the target temperature and pressure of 300K

and 1.0 atm using the Langevin-thermostat[96] and the Berendsen barostat[97] respectively, with collision frequency of 2 ps and pressure-relaxation time of 1 ps. Thereafter, we removed all restraints except from the second substrate (2-butenone) and further equilibrated the systems for 5 ns, in which the pressure and temperature equilibrate. Finally, a production MD run was conducted for 50 ns. A weak restraint of 5 kcal $mol^{-1}Å^{-1}$ on the second substrate was kept to keep it near the aniline during the equilibration and production MD run. During all MD simulations, the covalent bonds containing hydrogen atoms were constrained using SHAKE,[98] and particle mesh Ewald (PME)[99] was used to treat long-range electrostatic interactions. We used an integration step of 2 fs during the entire simulations. All MD simulations were performed with GPU version of the Amber 16 package.[100]

2.3.4.3: DFT Calculation details

Density functional theoretical calculations were performed on the model systems comprised of aniline or 2-bromoaniline H-bonded to glutamic acid at the B3LYP/Def2-TZVP//B3LYP/6-31+G** level [101-106] of theory using Gaussian16 software. During the optimization of the model systems we have kept all atoms except the COO^- part of glutamic acid fixed with positional restraint.

2.3.5: Calculation of green chemistry metrics:

The Green chemistry metrics for enzymatic reaction such as E-factor, Process mass index (PMI), atom economy and reaction mass efficiency were calculated using following formulas to display the high greenness of this protocol.

E-Factor=Amount of waste/Amount of product

PMI (Process mass index = E-factor +1)

AE (Atom economy) = MW of product ÷ Σ (MW of stoichiometric reactants) × 100]

RME (Reaction mass efficiency) = mass of product /Σ(mass of stoichiometric reactants) × 100]

E-Factor=Amount of waste/Amount of product

Amount of reactants: 2-bromoaniline (1k) = 1.0 gm and methyl vinyl ketone (2a) = 0.426g

Total amount of reactants (1k+2a)= 1.426g

Amount of product (3k) = 1.225 g

Amount of waste = 1.426 – 1.225 = 0.201g

E-factor = 0.201/ 1.22 = 0.16

Process mass index (PMI): PMI = E-factor +1= 0.16+1 =1.16

Atomic economy (AE):

Atom economy (AE) = MW of product ÷ Σ (MW of stoichiometric reactants) × 100

Molecular weight of product (3k) = 242.02g/mol

Molecular weight of stoichiometric reactants (1k+2a) =172.02 + 70 =242.02g/mol

Atom economy (AE) = 242.02×100/242.02 = 100%

Reaction mass efficiency (RME):

Reaction mass efficiency = mass of product /Σ(mass of stoichiometric reactants) × 100

Mass of product (3k) =1.225g

Total mass of reactants (1k+2a) = 1.426g

RME= 1.225÷1.426×100 = 86.1%

2.3.6: Characterization data of compounds

^{1}H and ^{13}C NMR Spectrum are attached in appendix of Chapter 2

4-(phenylamino)butan-2-one (3a) [107,108]

Semi-solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.22 (t, *J* =7.6 Hz, 2H), 6.76 (t, *J* =7.2 Hz, 1H), 6.72 (d, *J* = 8.0 Hz, 2H), 4.01 (brs, 1H), 3.45 (t, *J* = 6.4 Hz, 2H), 2.77 (t, *J* = 6.0 Hz, 2H), 2.18 (s,3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.0, 147.7, 129.3, 117.6, 113.0, 42.6, 38.4, 30.2 ppm.

4-(o-tolylamino)butan-2-one (3b) [108]

Semi-solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.17 (t, J= 7.6 Hz, 1H), 7.08 (d, J= 7.2 Hz, 1H), 6.71 (m, 2H), 3.92 (brs, 1H), 3.51 (t, *J* = 6.4 Hz, 2H), 2.82(t, *J* = 6 Hz, 2H), 3.06 (s, 3H), 2.13 (s,3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), δ 208.1, 145.6, 130.2, 127.0, 122.5, 117.2, 109.6, 42.6, 38.3, 30.3, 17.4 ppm.

4-((2-methoxyphenyl)amino)butan-2-one (3c) [108]

Semi-solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 6.89 (t, *J* = 4.0 Hz, 1H), 6.75 (d, *J* = 4.0 Hz, 1H), 6.69(t, *J* = 4.0 Hz, 1H), 6.63(d, *J* = 4.0 Hz, 1H), 4.42 (brs, 1H), 3.81 (s, 3H), 3.44 (t, *J*= 8.0 HZ, 2H), 2.78 (t, *J*= 8.0 HZ, 2H), 2.16 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), δ 207.4, 147.1, 137.7, 121.2, 116.7, 109.8, 109.6, 55.4, 42.9, 38.1, 30.2 ppm.

4-((2-nitrophenyl)amino)butan-2-one (3d) [107,108]

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 8.17 (d, *J*= 8.0 Hz, 1H), 8.15 (brs, 1H), 7.44 (t, *J*= 4.0 Hz, 1H), 6.87 (d, *J*= 8.0 Hz, 1H), 6.67 (t, *J*= 8.0 Hz, 1H), 3.62-3.57 (m, 2H), 2.87 (t, *J*= 4.0 Hz, 2H), 2.21 (s, 3H) ppm, ^{13}C NMR (CDCl3, 100 MHz), δ 213.5, 144.9, 136.1, 126.7, 115.4, 113.6, 42.6, 37.2, 30.0 ppm.

4-((3-nitrophenyl)amino)butan-2-one (3e) [108]

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ7.49-7.46 (m, 1H), 7.35 (d, *J*= 2.0 Hz, 1H), 7.25 (t, *J*= 8 Hz, 1H), 6.84 (d, *J*= 2.4,10.4 Hz, 1H), 4.46 (brs, 1H), 3.44 (t, *J*= 5.6 HZ, 2H), 2.78 (t, *J*= 6.0 Hz, 2H), 2.17 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.6, 149.4. 148.5, 129.7, 119.1, 112.1, 106.1, 42.1, 38.1, 30.3 ppm.

4-((2-acetylphenyl)amino)butan-2-one (3f)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 8.87 (brs, 1H), 7.74 (d, *J*= 9.6 Hz, 1H), 7.35 (t, *J*= 1.6 Hz, 1H), 6.71 (d, *J*= 8.8 Hz, 1H), 6.61 (t, *J*= 8.0 Hz, 1H), 3.51-3.46 (m, 2H), 2.82 (t, *J*= 6.8 HZ, 2H), 2.55 (s, 3H), 2.33(s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 206.8, 200.8, 150.6, 135.0, 132.8, 117.7, 114.2, 111.4, 42.9, 37.1, 30.3, 27.8 ppm, HRMS (ESI) Calcd. for $C_{12}H_{16}NO_2$ $[M+H]^+$ 206.2613; Found 206.2571.

4-((4-chlorophenyl)amino)butan-2-one (3g) [107,108]

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.28 (d, *J*= 8.8 Hz, 2H), 6.54 (d, *J*= 8.8 Hz, 2H), 4.03 (brs, 1H), 3.41 (t, *J*= 6 Hz, 2H), 2.76 (t, *J*= 6 HZ, 2H), 2.18 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), δ 207.8, 146.3, 129.1, 112.2, 114.8, 42.4, 38.5, 30.3 ppm.

4-((2-bromophenyl)amino)butan-2-one (3h)[107,108]

Semi-solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.40 (d, *J*= 8.2 Hz, 1H), 7.24 (t, *J*= 8.4 Hz, 1H), 6.64 (d, *J*= 8.0 Hz, 1H), 6.57 (t, *J*= 8.1 Hz, 1H), 4.55 (brs, 1H), 3.46 (t, *J*= 6.0 Hz, 2H), 2.78 (t, *J*= 6.0 Hz, 2H), 2.16 (s, 3H) ppm, ^{13}C NMR($CDCl_3$, 100 MHz), δ 207.3, 144.5, 132.6, 128.4, 117.9, 111.1, 110.1, 42.6, 38.8, 30.4 ppm.

4-((2,4-dichlorophenyl)amino)butan-2-one (3i)[108]

Semi-solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.2 (s, 1H), 7.05(d, *J*= 8.0 Hz, 1H), 6.56 (d, *J*= 8.0 Hz, 1H), 4.53 (brs, 1H), 3.43-3.38 (m, 2H), 2.76 (t, *J*= 8.0 Hz, 2H), 2.16 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), δ 207.1, 142.4, 128.8, 127.7, 121.3, 119.7, 111.6, 42.3, 38.1, 30.3 ppm, HRMS (ESI) Calcd. for $C_{10}H_{12}Cl_2NO$ $[M+H]^+$ 233.1120; Found 233.1041.

4-((2-chloropyridin-3-yl)amino)butan-2-one (3j)

Semi-solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.61 (s,1H), 7.03 (d, *J*= 8.0 Hz, 1H), 6.84 (d, *J*= 8.0 Hz, 1H), 4.59 (brs, 1H), 3.38 (t, *J*= 8.0 Hz, 2H), 2.73 (t, *J*= 8.0 Hz, 2H), 2.11 (s, 3H) ppm

^{13}C NMR($CDCl_3$, 100 MHz), δ 207.1, 140.4, 136.4, 136.3, 123.4, 117.3, 42.1, 37.6, 30.2 ppm, HRMS (ESI) Calcd. for $C_9H_{12}ClN_2O$ $[M+H]^+$ 199.6576; Found 199.6541.

4-(pyridin-2-ylamino)butan-2-one (3k) [109]

Semi-solid, ^{1}H NMR (CDCl3, 400 MHz), δ 8.30 (d, *J*= 4.8 Hz, 2H), 6.53 (t, *J*= 4.8 Hz, 1H), 5.68 (brs, 1H), 3.70 (m, 2H), 2.79 (t, *J*= 6.0 Hz, 2H), 2.16 (s, 3H) ppm, , ^{13}C NMR (CDCl3, 100 MHz), δ 207.93, 162.1, 158.2, 158.0, 110.6, 43.1, 35.9, 30.2 ppm.

4-(pyrazin-2-ylamino)butan-2-one (3l)

Semi-solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.88 (s, 1H), 7.77 (d, *J*= 1.2 Hz, 1H), 7.68 (d, *J*= 3.2 Hz, 1H), 5.40 (brs, 1H), 3.59-3.54 (m, 2H), 2.74 (t, *J*= 6.0 Hz, 2H), 2.10 (s, 3H) ppm, ^{13}C NMR($CDCl_3$, 100 MHz), δ 208.2, 154.4, 141.6, 132.8, 132.2, 42.46, 35.6, 30.2 ppm, HRMS (ESI) Calcd. for $C_8H_{12}N_3O$ $[M+H]^+$ 166.2040; Found 166.2015.

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2.4: 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 β-aminocarbonyl derivative (3 h) 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. 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 enone through strong hydrogen bonding. Finally, this work expands the application of α-amylase to catalyze the valuable transformations in the organic chemistry.

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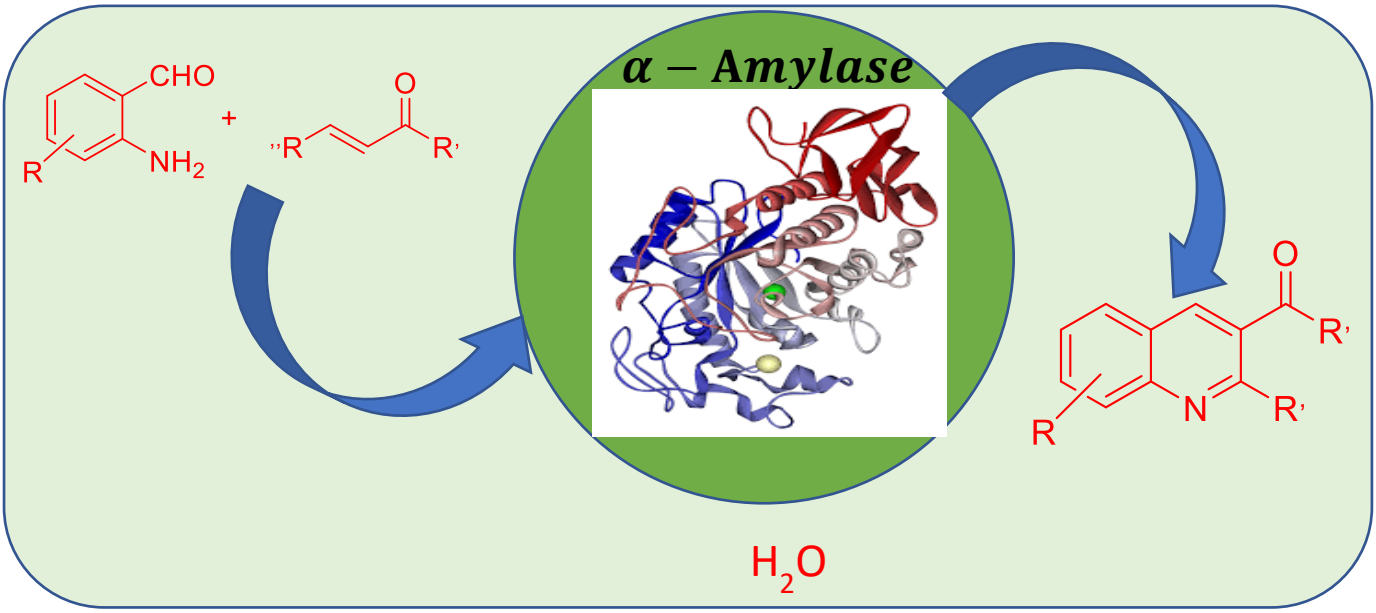
CHAPTER 3:
BIOCATALYTIC SYNTHESIS OF QUINOLINE DERIVATIVES VIA α-AMYLASE CATALYSED ONE-POT DOMINO AZA MICHAEL/ALDOL/AROMATIZATION REACTIONS

Graphical abstract:



Abstract

Quinoline moiety is a part of various drug molecules and natural products. Previously, a number of catalysts have been made available to synthesize substituted quinolines. Herein, we have developed α-amylase catalysed synthesis of substituted quinolines via one-pot domino aza-Michael/Aldol/aromatization reactions. Moreover, the α-amylase enzyme from *Aspergillus oryzae* was found to catalyse the cascade reaction of various 2-amino benzaldehyde with α, β-unsaturated carbonyls in high catalytic efficiency.

3.1: Introduction:

The quinoline ring system is a part of a number of pharmaceuticals and has great significance in drug discovery (Figure 3.1).[1-4] Furthermore, the quinoline derivatives display a broad range of biological activities comprising antimalarial, anticancer, antiviral, antifungal, and antituberculosis activities.[5-10] The synthesis of quinoline derivatives has seen tremendous progress in the past and a number of traditional methods such as Combes synthesis, the Skraup synthesis, Friedlander synthesis and Larock quinoline synthesis have been developed.[11-17] Besides, various transition-metal catalyzed approaches to synthesize C-3 functionalized quinolines have been reported.[18-20]

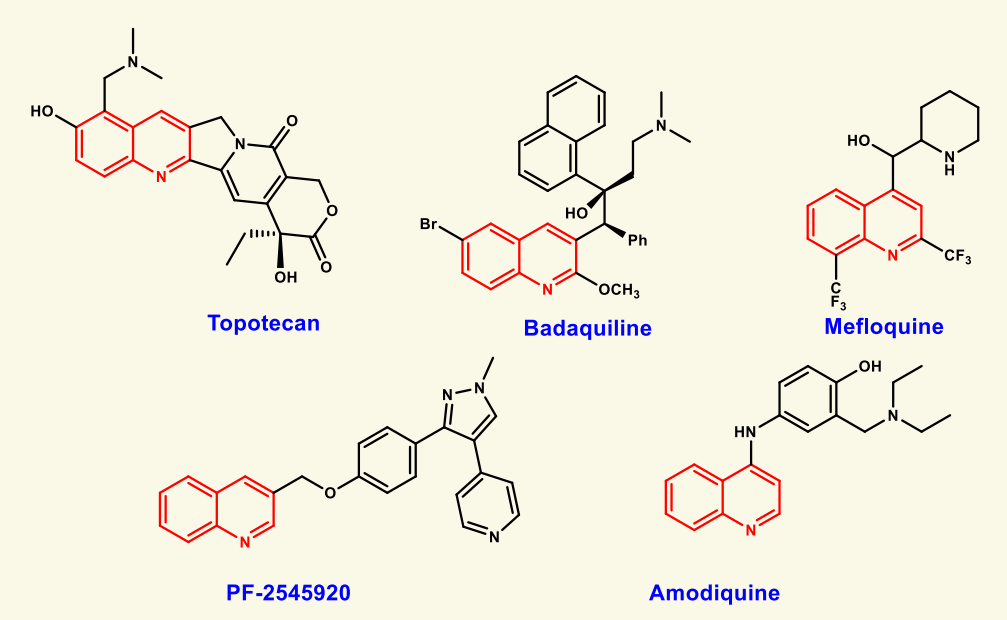
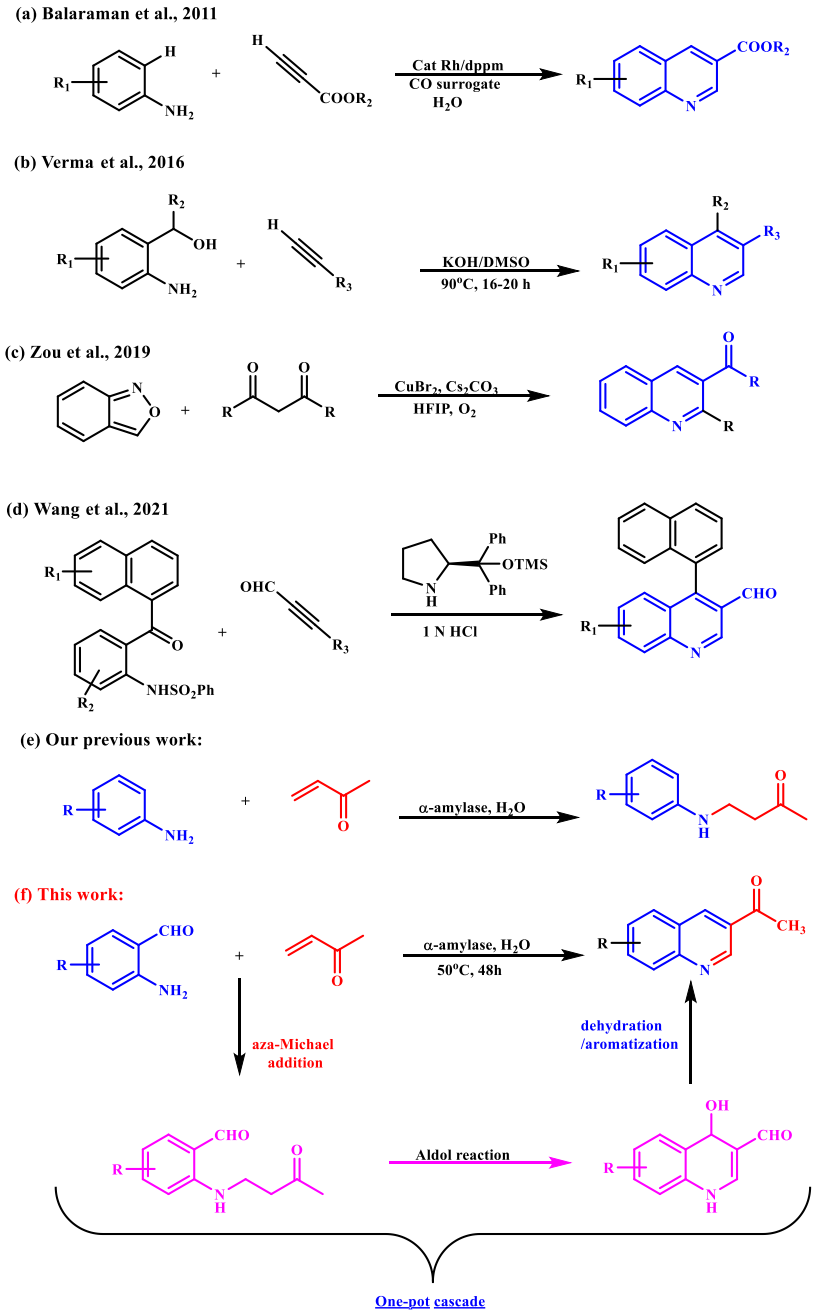


Figure 3.1. Examples of quinoline containing drug molecules

In this context, Balaraman et al. reported the synthesis of C-3 substituted quinolines via Rh/dppm catalyzed C-H activation reaction (Scheme 3.1a).[21] Later, Verma and co-workers have developed the synthesis of C-3 functionalized quinolines via cycloaddition reaction of azadienes with terminal alkynes (Scheme 3.1b).[22] Zhou et al. reported the synthesis of

quinoline derivatives via ring-opening/reconstruction of anthranils with oxo-compounds (Scheme 3.1c).[23] On the other hand, cascade or tandem reactions are being increasingly applied in synthetic chemistry to construct clinically significant molecules due to several advantages over the step-wise synthesis such as multiple bond can form in one-pot, lower waste generation as well as lesser time and work required along with high atom economy.[24-28]



Scheme 3.1: Recent approaches to synthesize quinoline derivatives

Among various cascade reactions, Michael addition-Aldol condensation cascade has played a noteworthy role in the synthesis of quinoline derivatives in the previous years.[29-33] In this context, Cordova's group reported organocatalytic domino Aza-Michael/Aldol reaction to synthesize 1,2-dihydroquinolidines.[34] Sun et al. reported organocatalyzed stereoselective synthesis of aminoindanols via an efficient cascade aza Michael–aldol reaction.[35] Very recently, Wang group described aza-Michael/Aldol reaction for atroposelective construction of 4-naphthylquinoline-3-carbaldehydes (Scheme 3.1d).[36]

Biocatalysis is the use of nature's catalytic repertoire like enzymes or whole cells in a chemical reaction and has become one of the most powerful technology in synthetic chemistry to produce fine chemicals and pharmaceuticals.[37-40] This technology offers a number of advantages over traditional metal catalysts such as exquisite regio-, chemo-, and stereoselectivities, generation of less number of by-products as a result higher yield of the purer product.[41-43] Moreover, enzyme catalysed processes are far more cost-effective and eco-friendly since they are originally derived from natural products and perform best under mild reaction conditions. Despite of various advantages over the conventional chemical catalysis, enzymes mainly are available to catalyse natural procedures in industry which limits their applications in the chemical synthesis. However, the development of various technologies in recent years such as recombinant technology have played an important role in expanding the potential of enzymes for non-natural organic transformations.[44-45] Among different classes of enzymes, hydrolase enzymes (lipase, amylase, amidase etc.) were the first class of biocatalyst used for organic synthesis.[46-47] A number of benefits like easy availability in the market, low-cost, reusability, high stability in organic solvent as well as thermostability still makes this class of enzymes an ideal choice to explore novel abiotic organic transformations.[48-49] Recently, we have reported α-amylase catalysed aza-Michael addition reaction of less nucleophilic aromatic amines with methyl vinyl ketones (Scheme 3.1e).[50] Further, lipase catalysed GBB-multicomponent reaction to synthesize imidazo[1,2-*a*]pyridine derivatives was developed.[51] In continuation of our efforts to find novel abiotic transformations using hydrolase enzymes, herein, we report α-amylase catalysed aza-Michael/Aldol/aromatization cascade to synthesize C-3 substituted quinoline derivatives.

3.2: Results and discussion:

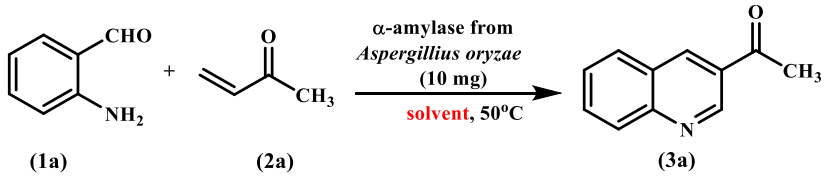
In our previous study, we have found α-amylase from *Aspergillius oryzae* as a best catalyst for aza-Michael addition of less nucleophilic aromatic amine with methyl vinyl ketone. Subsequently, we started this study with α-amylase from *Aspergillius oryzae* and selected 2-

amino benzaldehyde (**1a**) and methyl vinyl ketone (**2a**) as model substrates to optimize the reaction conditions.

3.2.1: Effect of solvent on model reaction

At first, the reaction was executed using equimolar amount of model substrates with 5 mg/ml of α-amylase in 2 mL of H_2O as a solvent at 50 °C which provided the product (**3a**) in 65% conversion yield. Next, we screened different solvent such as THF, hexane, toluene, ethanol and methanol but found no improvement in the conversion yield of product (entries 2–6, Table 3.1). Then, we tried solvent combinations like different percentage of DMSO or ethanol in

Table 3.1: Screening of different solvents for the synthesis of 3-acetyl quinoline[a]



(1a) (2a) (3a)

Entry	solvent	[b]conversion
1	H_2O	65%
2	THF	32%
3	Hexane	12%
4	Toluene	24%
5	Ethanol	48%
6	Methanol	35%
7	5% DMSO in H_2O (v/v)	68%
8	10% DMSO in H_2O (v/v)	74%
9	**20% DMSO in H_2O (v/v)**	**81%**
10	10% Ethanol in H_2O (v/v)	71%
11	10% Methanol in H_2O (v/v)	64%
12	20% Ethanol in H_2O (v/v)	74%
13	DMSO	21%

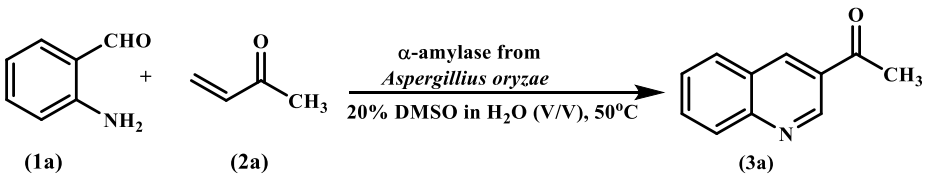
[a]**Reaction conditions:** 1.0 equiv. of 2-aminobenzaldehyde (0.825 mmol) and 1.2 equiv. of methyl vinyl ketone (0.990 mmol), α-amylase 10 mg and 2 mL of solvent were added to a glass tube having a teflon cap and stirred at 50 °C for overnight, [b]based on HPLC.

water to make sure that substrates are completely soluble during the reaction (entries 7–12, Table 3.1). Gratifyingly, we obtained product (**3a**) in 81% conversion yield, when used 2 mL of 20% DMSO in water (v/v) as a solvent (entry 9, Table 3.1). While, in case of DMSO

or ethanol only, the product (**3a**) was obtained in 21% and 48% conversion yield respectively (entries 5&13, Table 3.1).

3.2.2: Impact of enzyme concentration, substrates molar ratio and reaction temperature: After having best solvent for enzymatic reaction, we checked the effect of enzyme concentration, substrate molar ratio and reaction temperature on the conversion of model reaction. First, enzyme concentration was increased from 5 mg/mL to 7.5 mg/mL then 10 mg/mL, however, best conversion was obtained with 7.5 mg/mL concentration of α-amylase (entries 2–4, Table 3.2). Also, inferior conversion was obtained when enzyme concentration was decreased from 5 mg/mL to 2.5 mg/mL (entry 1, Table 3.2). Further, the molar ratio of model substrates was screened (entries 3 & 5–6, Table 3.2) and found that 1:1.2 for 1a:2a is a best combination (entry 5, Table 3.2). We also tested the reaction temperature (entries 7–8, Table 3.2) and observed that 50 °C is the optimal temperature to archived maximum conversion in this reaction (entry 5, Table 3.2).

Table 3.2: Screening of enzyme concentration, substrate molar ratio and reaction temperature[a]



(1a) (2a) (3a)

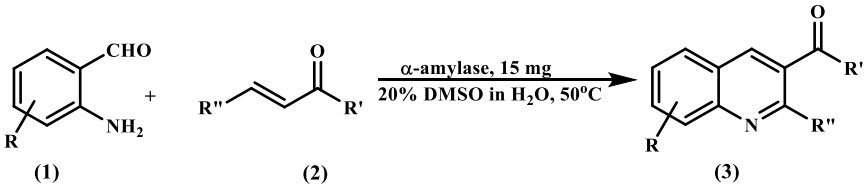
Entry	enzyme loading	substrate ratio (1a:2a)	temperature	[b]conversion
1	5 mg	1:1	50°C	59%
2	10 mg	1:1	50°C	81%
3	15 mg	1:1	50°C	88%
4	20 mg	1:1	50°C	89 %
5	**15 mg**	**1:2**	**50°C**	**91%**
6	15 mg	1:5	50°C	90%
7	15 mg	1:1	40°C	85%
8	15 mg	1:1	60°C	89%

[a]**Reaction conditions:** 2-aminobenzaldehyde (100 mg), methyl vinyl ketone, α-amylase and 2 mL of 20% DMSO in H_2O (v/v) were added to a glass tube having a teflon cap and stirred for overnight, [b]based on HPLC.

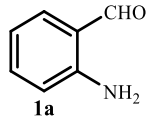
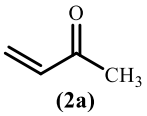
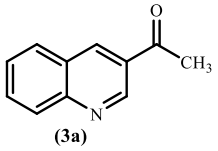
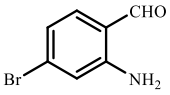
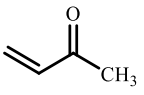
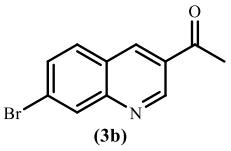
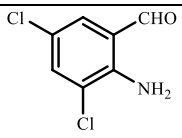
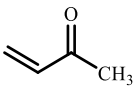
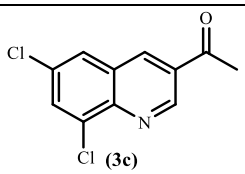
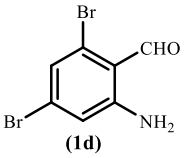
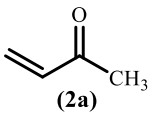
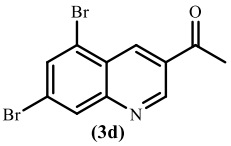
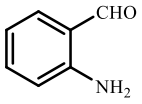
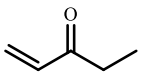
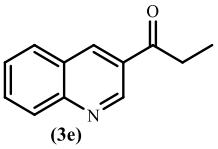
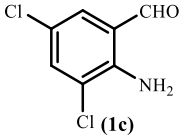
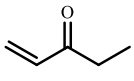
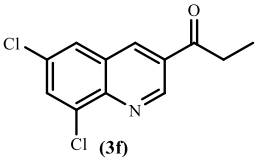
3.2.3: Substrate scope:

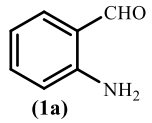
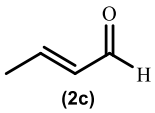
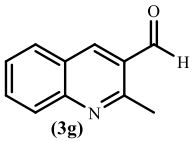
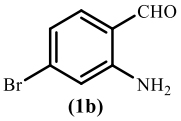
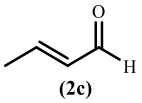
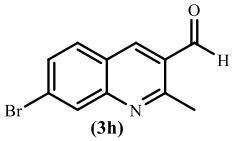
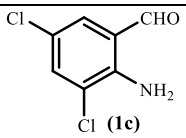
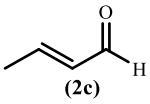
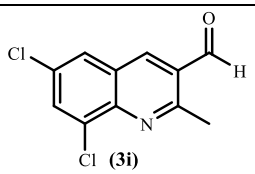
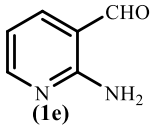
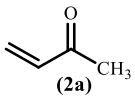
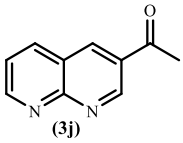
After having optimal reaction conditions in hand (entry 5, Table 3.2), we demonstrated the generality and synthetic utility of this transformation by set-up the reactions of substituted 2-amino benzaldehyde with different α, β-conjugated carbonyls and presented the results in Table 3.3. In this context, the reaction of 4-bromo substituted 2-aminobenzaldehyde (**1b**) with methyl vinyl ketone (**2a**) afforded product (**3b**) in 68% isolated yield (entry 2, Table 3.3), however, yield of (**3b**) was lesser than the yield of product (**3a**) which formed by the reaction of 2-aminobenzaldehyde and methyl vinyl ketone (entry 1, Table 3.3). Further, we tested the reaction of methyl vinyl ketone with 5-chloro substituted 2-aminobenzaldehyde (**1c**) and obtained the product (**3c**) in 56% isolated yield (entry 3, Table 3.3). However, in the case of 3,5-dibromo substituted 2-aminobenzaldehyde (**1d**), corresponding product (**3d**) was obtained only in trace amount (entry 4, Table 3.3). All these experiments (entries 1–4, Table 3.3) suggested that electronic effect/steric hindrance of halide substituents on 2-aminobenzaldehyde playing a role in this transformation, as a result the yield of isolated products either got decreased or no reaction observed. In the next phase, we employed ethyl vinyl ketone (**2b**) and crotonaldehyde (**2c**) in place of methyl vinyl ketone with different 2-aminobenzaldehyde (entries 5–9, Table 3.3). When ethyl vinyl ketone was reacted with 2-aminobenzaldehyde or 5-chloro substituted 2-aminobenzaldehyde, reaction provided the products (**3e**) and (**3f**) in 73% and 62% yield respectively (entries 5–6, Table 3.3). Further, we employed crotonaldehyde with 2-aminobenzaldehyde and got the product (**3g**) in 75% isolated yield (entry 7, Table 3.3). However, in case of 5-chloro substituted 2-aminobenzaldehyde or 4-bromo substituted 2-aminobenzaldehyde, reaction of crotonaldehyde furnished the products (**3h**) and (**3i**) in 68% and 60% yield respectively (entries 8–9, Table 3.3). Unexpectedly, no product formation was observed in the reaction of 2-aminonicotinaldehyde and methyl vinyl ketone (entry 10, Table 3.3). Also, we employed 4-methoxy or 4-trifluoromethyl substituted 2-aminobenzaldhyde to test the electronic effect of electron-donating and electron-withdrawing groups on this transformation, however, we were unsuccessful due to very low stability of 4-methoxy or 4-trifluoromethyl substituted 2-aminobenzaldhyde.

Table 4.3: Substrate scope of α-amylase catalysed synthesis of substituted quinoline[a]



(1) (2) (3)

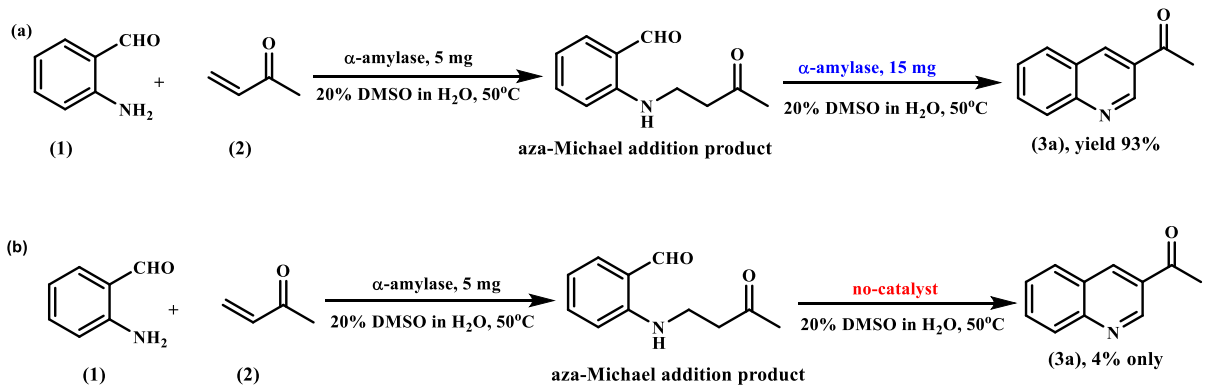
Entry	amino aldehyde (1)	vinyl ketone (2)	product (3)	Isolated yield
1.	 1a	 (2a)	 (3a)	86%
2.	 (1b)	 (2b)	 (3b)	68%
3.	 (1c)	 (2a)	 (3c)	56%
4.	 (1d)	 (2a)	 (3d)	NR
5.	 (1a)	 (2b)	 (3e)	73%
6.	 (1c)	 (2b)	 (3f)	62%

7.	 **(1a)**	 **(2c)**	 **(3g)**	**75%**
8.	 **(1b)**	 **(2c)**	 **(3h)**	**68%**
9.	 **(1c)**	 **(2c)**	 **(3i)**	**60%**
10.	 **(1e)**	 **(2a)**	 **(3j)**	**NR**

[a]**Reaction conditions:** 1.0 equiv. of substituted 2-aminobenzaldehyde,1.2 equiv. of α,β-unsaturated carbonyls, α-amylase 15 mg and 2 mL of 20% DMSO in H_2O (v/v) were added to a glass tube having a teflon cap and stirred at 50 °C for overnight, [b]isolated yield.

3.3: Control experiments:

Next, we have performed experiments to confirm the role of enzyme in the Aldol condensation reaction as depicted in scheme 3.2. First, we executed aza-Michael addition of 2-aminobenzaldehyde with methyl vinyl ketone using previously reported reaction conditions. After, completion of the reaction, aza-Michael addition product was purified and used for Aldol condensation under the aforementioned optimized reaction conditions. Interestingly, we obtained the product **(3a)** in 93% isolated yield in the step-wise synthesis (Scheme 3.2a). However, when aza-Michael addition product was used in the absence of α-amylase enzyme, the product (**3a**) was obtained only in 4% yield as a result aza-Michael addition product was mainly recovered from this reaction (Scheme 3.2b). Additionally, we have set-up few control experiments like adding equimolar amount of urea, use of BSA or denatured amylase as a catalyst and obtained only 9%, 16% and 21% yield respectively. This proved the importance of α-amylase enzyme in the Aldol condensation during the cascade reaction.

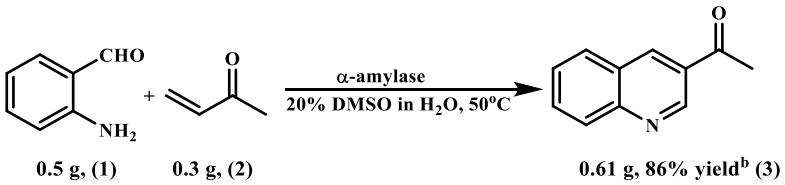


Scheme 3.2: Control experiments to prove the role of α-amylase in cascade reaction

3.4: Scale-up synthesis of 3-acetylquinoline

In the final phase of our work, we carried out a gram level reaction of 2-aminobenzaldehyde (0.5 g, 4.12 mmol) and methyl vinyl ketone (0.34 g, 4.94 mmol) using 75 mg of α-amylase in 10 mL H_2O having 20% DMSO (v/v) to prove the scalability of this enzymatic reaction and calculated the green chemistry parameters for this reaction (Table 3.4). The successful isolation of (0.61 g) of (**3a**) in 86% isolated yield proved the synthetic utility of this protocol. Further, the values of green chemistry metrics such as E-factor, PMI, atom-economy and reaction mass efficiency demonstrate the high greenness of this protocol.

Table 3.4: Scale-up synthesis of 3-acetylquinoline (3a) and calculation of green chemistry metrics.[a]



Entry	metrics	results
1	Isolated yield	86%
2	Selectivity	100%
3	E-factor	0.4
4	PMI	1.4
5	atom-economy	89.5%
6	reaction mass efficiency	67.5%

[a]**Reaction conditions:** 2-aminobenzaldehyde (0.5 g, 4.12 mmol), methyl vinyl ketone (0.34 g, 4.94 mmol), α-amylase 75 mg and 10 mL of 20% DMSO in H_2O (*v/v*) were added to a glass tube having a teflon cap and stirred for overnight at 50°C, [b]isolated yield.

3.5: Experimental:

3.5.1: General procedure for the synthesis of substituted quionolines (3a-3i)

In a 10 mL glass tube having a magnetic stirrer bar added 15 mg of α-amylase (7.5 mg/mL in H_2O) and 2 mL of 20% DMSO in H_2O (v/v). Then, added 2-aminobenzaldehyde (100 mg, 1 equiv., 0.825 mmol) and methyl vinyl ketone (69.341 mg, 1.2 equiv., 0.990 mmol) and stirred

the reaction mixture for 48 h at 50 °C and monitored the reaction using TLC plates. After completion of the reaction as indicated by TLC, the reaction mixture was filtered via a small pad of celite followed by washing of the celite with ethyl acetate. Next, the solvents were evaporated under reduced pressure and crude product was purified by column chromatography on silica gel (eluent: hexane/ EtOAc) to afforded the corresponding products (**3a-3i**) in 56–86% isolated yields.

3.5.2: Characterization data of compounds

^{1}H and ^{13}C NMR Spectrum are attached in appendix of Chapter 3

1-(quinoline-3-yl)ethan-1-one (3a)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 9.43 (d, J = 2.3 Hz, 1H), 8.71 (d, J = 1.8 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.86 (t, J= 7.6 Hz 1H), 7.65 (t, J= 8.0 Hz, 1H), 2.75 (s, 3H), ($CDCl_3$, 100 MHz), δ 196.7, 149.9, 149.2, 137.6, 132.2, 129.5, 129.4, 129.3, 127.7, 126.9, 26.2 ppm.

1-(7-bromoquinolin-3-yl)ethan-1-one (3b)

Solid, ^{1}HNMR ($CDCl_3$, 400 MHz), δ 9.41 (s 1H), 8.67 (s, 1H), 8.34 (s, 1H), 7.82 (d, J= 9.2 Hz, 1H), 7.73 (d, J= 9.2 Hz, 1H), 2.73 (s, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz), δ 196.0, 150.3, 137.3, 132.0, 131.4, 130.6, 130.5, 129.5, 126.7, 125.6, 26.9 ppm.

1-(6,8-dichloroquinolin-3-yl)ethan-1-one (3c)

Solid, ^{1}HNMR ($CDCl_3$, 400 MHz), δ 9.40 (d, J= 2.8 Hz, 1H), 8.62 (d, J= 2.4 Hz, 1H), 8.11 (d, J= 9.6 Hz, 1H), 7.93 (d, J=8.0 Hz, 1H), 7.77 (dd, J= 2.8 Hz, 1H), 2.74 (s, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz), δ 195.6, 149.3, 148.3, 147.1, 135.5, 132.5, 133.0, 131.9, 128.9, 126.9, 126.6, 28.6 ppm.

1-(quinoline-3-yl)propan-1-one (3e)

Solid, ^{1}HNMR ($CDCl_3$, 400 MHz), δ 9.43 (d, J= 2.8 Hz 1H), 8.72 (d, J= 3.2 Hz 1H), 8.16 (d, J= 9.6 Hz, 1H), 7.96 (d, J= 1.2 Hz, 1H), 7.85 (t, J= 1.9 Hz 1H), 7.64 (t, J= 7.6 Hz, 1H), 3.18 (q, J=7.6 Hz, 2H), 1.31 (t, J=7.6 Hz, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz), δ 199.6, 149.8, 149.2, 136.9, 132.0, 129.5, 129.4, 129.1, 127.6, 127.0, 32.8, 8.5 ppm.

1-(6,8-dichloroquinolin-3yl)propan-1-one (3f)

Solid, ^{1}HNMR ($CDCl_3$, 400 MHz), δ 9.41 (d, J= 2.4 Hz, 1H), 8.62 (d, J= 2.8 Hz, 1H), 8.10 (d, J= 9.6 Hz, 1H), 7.93 (d, J=2.8Hz , 1H), 7.77 (dd, J= 3.2, 2.4 Hz, 1H), 3.16 (q, J=7.6 Hz, 2H), 1.31 (t, J=7.6 Hz, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz), δ 198.4, 148.4, 147.2, 134.9, 132.5, 131.9, 130.1, 127.8, 126.9, 31.5, 8.4 ppm.

2-methylquinoline-3-carbaldehyde (3g)

Solid, ^{1}HNMR ($CDCl_3$, 400 MHz), δ 10.36 (s, 1H), 8.58 (s, 1H), 8.08 (d, J= 9.2 Hz, 1H), 7.94 (d, J= 8.4 Hz, 1H), 7.85 (t, J= 6.8 Hz, 1H), 7.59 (t, J= 8.0 Hz, 1H), 3.02 (s, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz), δ 191.7, 158.6, 149.2, 142.8, 132.9, 129.3, 128.7, 128.0, 127.0, 126.1, 22.8 ppm.

7-bromo-2-methylquinoline-3-carbaldehyde (3h)

Solid, ^{1}HNMR ($CDCl_3$, 400 MHz), δ 10.35 (s 1H), 8.55 (s, 1H), 8.25 (s, 1H), 7.80 (d, J= 9.2 Hz, 1H), 7.67 (dd, J= 2.4, 9.2 Hz, 1H), 3.01 (s, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz), δ 190.2, 158.7, 141.2, 130.5, 129.8, 129.2, 127.3, 126.5, 123.8, 23.2 ppm.

6,8-dichloro-2-methylquinoline-3-carbaldehyde (3i)

Solid, ^{1}HNMR ($CDCl_3$, 400 MHz), δ 10.37 (s, 1H), 8.50 (s, 1H), 8.01 (d, J= 9.2 Hz, 1H), 7.91 (d, J=2.4 Hz, 1H), 7.77-7.74 (m, 1H), 3.01 (s, 3H) ppm. ^{13}CNMR ($CDCl_3$, 100 MHz), δ 191.3, 158.9, 147.6, 141.3, 133.7, 132.6, 130.4, 128.6, 127.5, 126.7, 24.0 ppm.

3. 6: Conclusion:

In summary, we are describing a biocatalytic synthesis of quinoline derivatives via α-amylase catalysed one-pot domino aza-Michael/Aldol/aromatization reactions. Further, this methodology could be applied to different 2-aminobenzaldehydes and α,β-unsaturated carbonyls to construct substituted quinolines derivatives in good yields (56–86%). The scalability of this protocol was exhibited by set-up a gram level reaction. Also, the green chemistry metrics such as E-factor, PMI, atom-economy and reaction mass efficiency were calculated for this reaction.

3.7: References:

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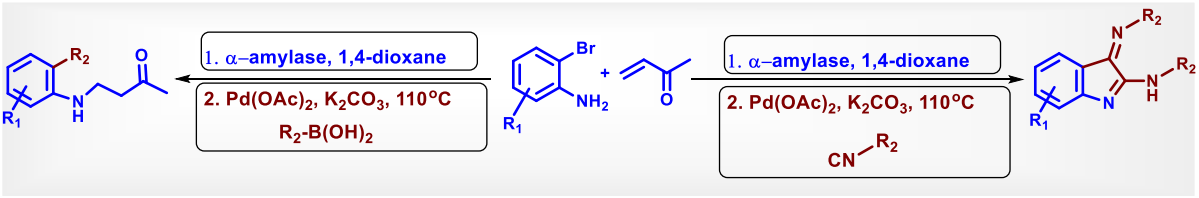
CHAPTER 4:

SYNTHESIS AND SUBSTRATE CONTROLLED MODIFICATION OF β-AMINOCARBONYLS USING α-AMYLASE ENZYME AND PD-CATALYST IN ONE-POT

Graphical abstract:



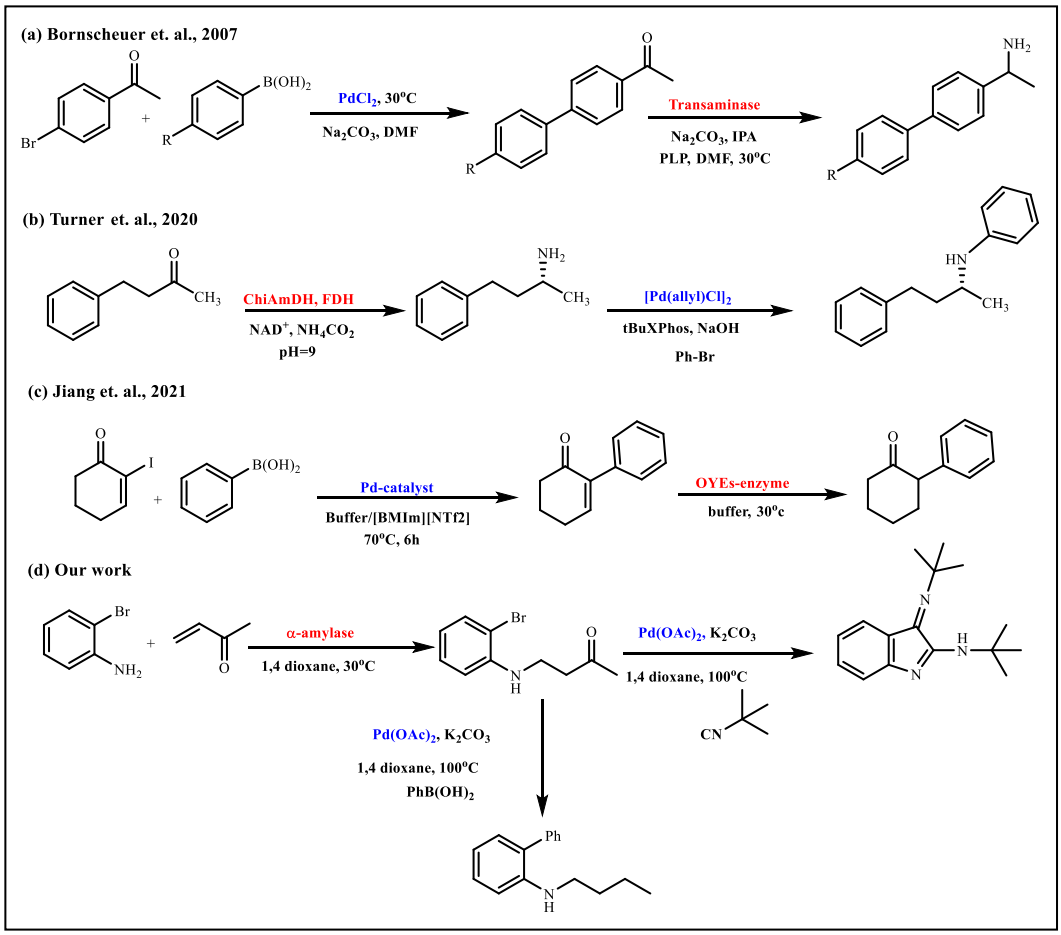
CHAPTER 4

Abstract:

The area of combining an enzyme and transition metals to catalyse the sequence of reactions in one-pot which improve total conversion and operational stability has seen tremendous growth in recent years. Herein, we describe the synthesis and substrate-controlled modification of β-aminocarbonyl using α-amylase enzyme and Pd-based catalyst in the one pot. The chemo-enzymatic approach provides substituted indole derivatives unprecedentedly when isocyanide was used in the reaction. In the second phase, the isocyanide was replaced by phenylboronic acid which in turn provided the substituted amino biaryls in good yields. Further, the one-pot chemo-enzymatic approach worked well when substituted 2-bromo aniline and isocyanide/phenyl boronic acid were employed in the reaction and provides the corresponding products in 56-84% isolated yields.

4.1: Introduction:

In the last few decades, the use of enzyme in the synthesis/modification of organic molecules has attracted a great attention by chemical community.[1-8] A wide range of compounds or intermediates in pharmaceutical and chemical industries have been synthesized using different class of enzymes such as reductases, hydrolases, oxygenases or dehalogenases.[9-19] Further, the use of enzymes in synthetic chemistry provide an environmental friendly alternative of traditional chemical approaches due to mild and less waste intensive operational conditions.[20-26] Notwithstanding, the application of enzymes in synthetic chemistry still lagging behind due to certain limitations such as lower self-life, narrow substrate scope when using non-natural compounds, instability at extreme temperatures and pH values, longer development procedure etc.[27-30] To boost the application of enzymes in synthetic chemistry, the area of combining an enzyme with chemical catalyst has seen exponential growth in the last few years.[31-38] This approach allows chemists to execute the overall transformations in higher efficiency which was not achievable using individual catalyst. In this context, various chemo-enzymatic approaches involving at least one enzyme and transition metal in the same-pot to catalysed sequential reactions have been developed.[39-47] Recently, Bornscheuer et al. developed a combination of palladium-catalysed Suzuki–Miyaura reaction and enzyme catalysed transamination reaction in one-pot to provide chiral amines with excellent conversion as well as optical purity (Scheme 4.1a).



Scheme 4.1: Recent chemo-enzymatic approaches using enzyme and transition metal in one-pot

Further, Turner and co-workers reported the synthesis of chiral *N*-arylamines by combining biocatalytic reductive amination with Buchwald-Hartwig cross-coupling reaction (Scheme 4.1b).[48] Moreover, a one-pot chemoenzymatic approach combining Pd-catalyzed C–C formation and enzymatic C=C asymmetric hydrogenation was developed by Jiang and group members (Scheme 4.1c).[49]

On the other hand, β-aminocarbonyls unit is found in a number of clinically important molecules and natural products (Figure 4.1).[50-57] Besides, β-aminocarbonyls are important precursors for synthesizing amino alcohols, diamines, β-amino acid derivatives and other nitrogen-containing molecules.[58-61] In addition, the synthesis and further conversion/modification of β-aminocarbonyls to produced clinically important molecules have been used significantly in the past years. A wide range of methods have been established to synthesize β-amino carbonyl compounds.[62-70] In this context, aza-Michael addition reaction has attracted significant attention from organic chemist to develop a variety of β-aminocarbonyls by the addition of an amine to an electron deficient alkene.[71-76] Recently, we have reported α-

amylase catalysed aza-Michael addition of less nucleophilic aromatic amines to enone using water as a solvent.[77] Further, combining the aza-Michael addition reaction with other transformations in the same-pot provides a strategy to synthesize clinically valuable heterocycles in higher efficiency.[78-81]

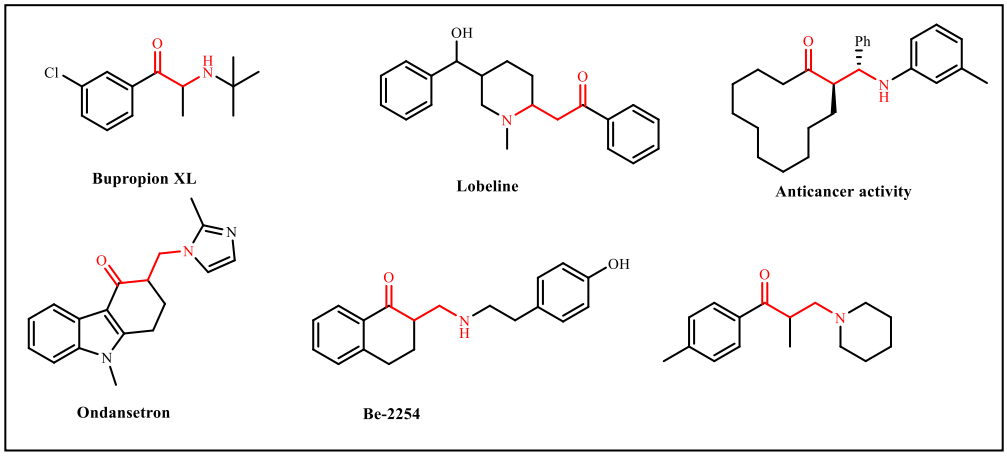


Figure 4.1: Biologically active amino-carbonyls

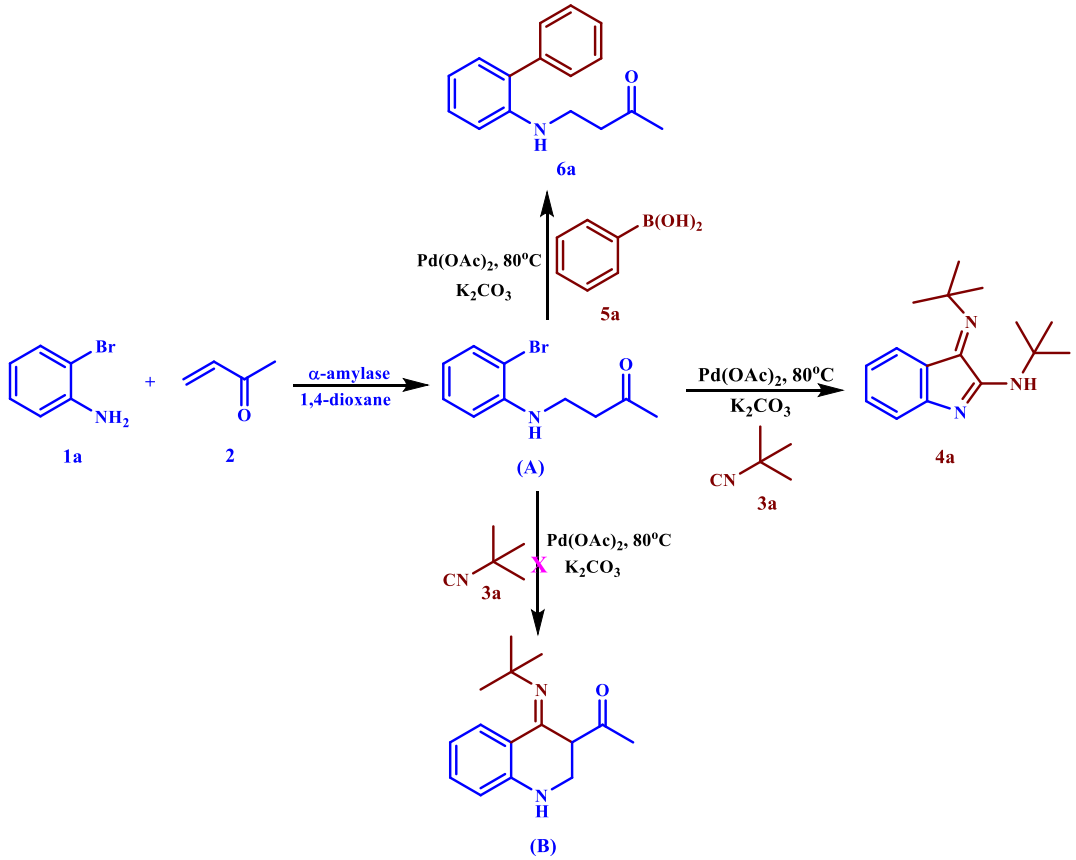
Recently, a palladium-catalyzed arylation/aza-Michael addition cascade reaction of β-substituted cyclic enones and 2-haloanilines to construct complex and useful spiro- and bridged-heterocycles from readily available starting materials was developed by Liu and co-workers.[82] Also, we have developed a one-pot approach to synthesize quinoline derivatives via aza-Michael/Aldol/aromatization reactions cascade in the one-pot.[83] With the interest of our group in the development of novel enzymatic approaches[84], herein, we have developed a one-pot chemo-enzymatic approach combining α-amylase catalysed synthesise of β-aminocarbonyls followed by Pd-catalysed substrate controlled modification to produce indole derivatives or amino-biaryls.

4.1.1: Our hypothesis:

We hypothesized to perform α-amylase catalysed aza-Michael addition reaction followed by the insertion of isocyanide using transition metal based catalyst in one-pot to produce tetrahydroquinoline derivative (**B**) as depicted in scheme 4.2. To realized our hypothesis, we started a reaction of 2-bromoaniline (**1a**) and methyl vinyl ketone (**2**) using 1,4-dioxane as a solvent and α-amylase enzyme as a catalyst. After completion of the enzymatic reaction as indicated by TLC, isocyanide (**3a**), $Pd(OAc)_2$, and K_2CO_3 were added in the same-pot. However, formation of indole derivatives (**4a**) was unexpectedly observed in this reaction instead of tetrahydroquinoline derivative (**B**) (Scheme 4.2). Next, we replaced $Pd(OAc)_2$ by different metal catalysts to investigate their effect on the course of the reaction. As a result,

$Pd(Ac)_2$ and $RuCl_2$ gave indole derivative (**4a**) while in case of $Rh_2(OAc)_4$, CuI and $FeCl_2$, only the formation of retro-Michael addition product was observed.

To further investigate, we changed *tert*-butyl isocyanide by phenyl boronic acid under the same reaction conditions and interestingly obtained desired product (**6a**) (Scheme 4.2). These results suggested, substrate selective modification of aza-Michael addition product in the one-pot chemo-enzymatic transformation. Interestingly, we set-up this chemo-enzymatic approach in sequential fashion too after purifying the aza-Michael product (**A**), however no change in the outcome of the reaction was observed.



Scheme 4.2: Possible structures under one-pot chemo-enzymatic approach

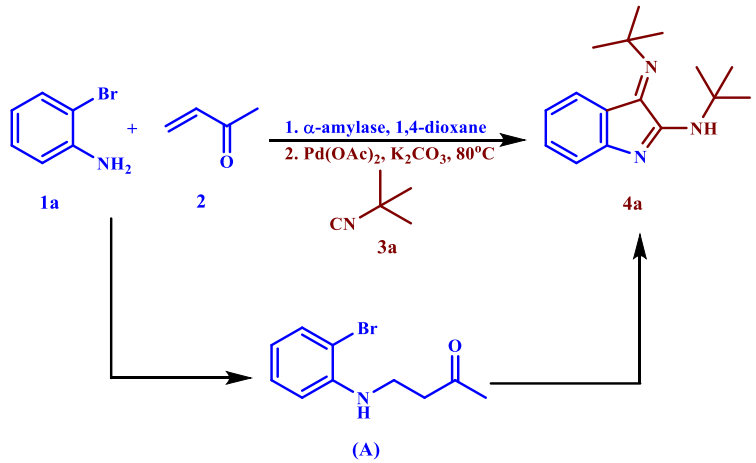
4.2: Results and discussion:

4.2.1: Optimization of solvents and metal based catalysts:

Drawing motivation from the initial results and advantages of the chemo-enzymatic approach in organic synthesis, we decided to further explore the preliminary findings. The model reaction provided desired product (**4a**) in 67% yield after applying standard reaction conditions (entry 1, Table 4.1). To further improve the yield of the model reaction, different solvents such as toluene, THF, CH_3CN, DMF were screened, however, no increment in the yield was observed

(entries 1-5, Table 4.1). Next, we replaced K_2CO_3 by KO*t*Bu, Cs_2CO_3 and DBU to check the effect of base on the outcome of the reaction, but K_2CO_3 remains as the best choice (entries 6-8, Table 4.1). The model reaction gave product (**4a**) in 75% yield, when temperature was increased from 80°C to 110°C (entry 9, Table 4.1). Subsequently, different salts of palladium such as $Pd(PPh_3)_4$ and $PdCl_2$ were used but no increment in the isolated yield of product was obtained (entries 10-11, Table 4.1).

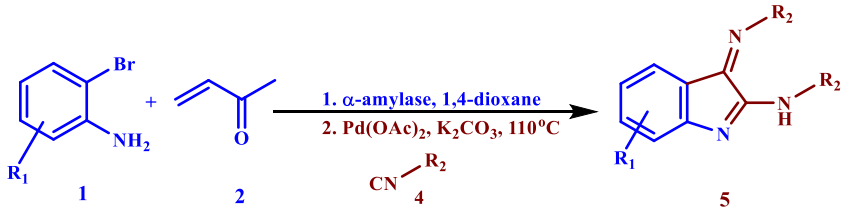
Table 4.1: Optimization of the reaction conditions for one-pot chemo-enzymatic approach

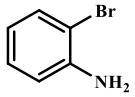
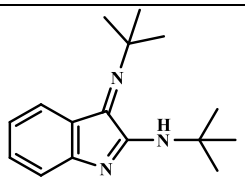
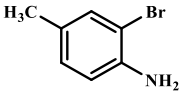
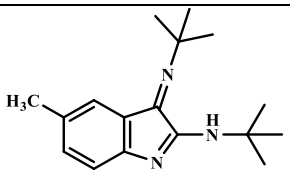
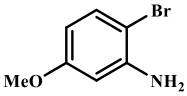
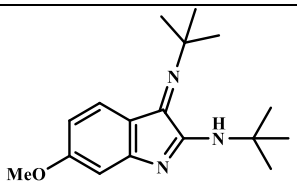
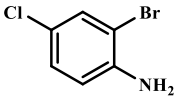
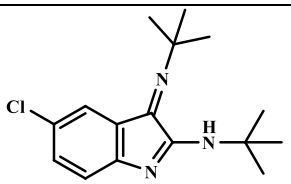
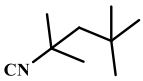
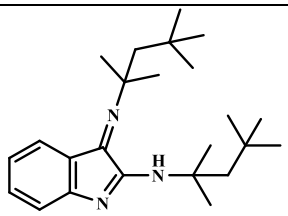
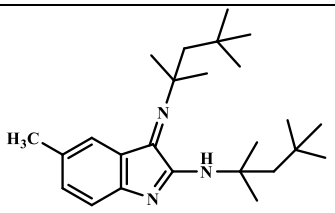


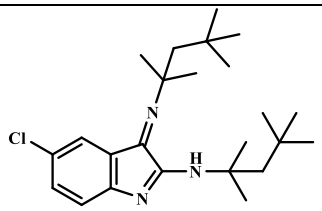
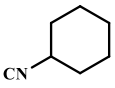
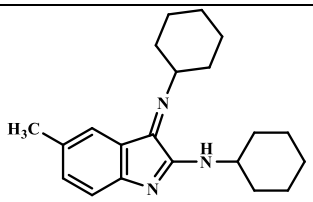
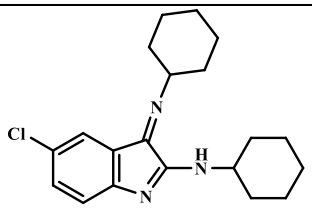
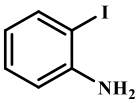
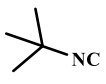
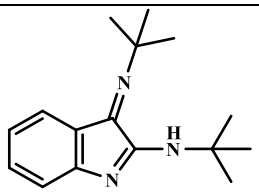
Entry	[a]Deviation from the standard conditions	[b]yield (4a)
1	None	67%
2	Toluene instead of 1,4-dioxane	55%
3	THF instead of 1,4-dioxane	36%
4	CH_3CN instead of 1,4-dioxane	39%
5	DMF instead of 1,4-dioxane	52%
6	KOtBu instead of K_2CO_3	46%
7	$CsCO_3$ instead of K_2CO_3	61%
8	DBU instead of K_2CO_3	37%
9	**Temperature 110°C instead of 80°C**	**75%**
10	$Pd(PPh_3)_4$ instead of $Pd(OAc)_2$	52%
11	$PdCl_2$ instead of $Pd(OAc)_2$	39%
12	$Pd(OAc)_2$ used 5 mol%	57%
13	$Pd(OAc)_2$ used 15 mol%	71%
14	α-amylase added 5 mg instead of 10 mg	38%
15	α-amylase added 15 mg instead of 10 mg	63%

[a]**Standard conditions:** 2-Bromo aniline (1.0 equiv.), methyl vinyl ketone (1.2 equiv), tert-butylisocyanide (1.2 equiv), α-amylase (10 mg), 1,4-dioxane (5 ml), $Pd(OAc)_2$ (10 mol%), K_2CO_3 (1.5 equiv.), temperature = 80°C, [b]isolated yield.

Table 4.2: Substrate scope of one-pot aza-Michael addition and isocyanide insertion reaction



Entry	Aniline	Isocyanide (4)	Products
1	 1a	 4a	 5a, 75%
2	 1b	4a	 5b, 78%
3	 1c	4a	 5c, 80%
4	 1d	4a	 5d, 69%
5	1a	 4b	 5e, 81%
6	1b	4b	 5f, 73%

7	1d	4b	 5g, 79%
8	1b	 4c	 5h, 56%
9	1d	4c	 5i, 61%
10	 1e	 4a	 5a, 73%

[a]**Reaction conditions:** Aniline (1.0 equiv.), methyl vinyl ketone (1.2 equiv), isocyanide (1.2 equiv), α-amylase (10 mg), 1,4-dioxane (5 ml), $Pd(OAc)_2$ (10 mol%), K_2CO_3 (1.5 equiv.), temperature = 110°C, [b]isolated yield.

Also, there was no improvement observed in the outcome of the model reaction either increasing or decreasing the loading of $Pd(OAc)_2$ as a catalyst (entries 12-13, Table 4.1). Besides, we obtained product (**4a**) in inferior yield after changing the amount of α-amylase in the chemo-enzymatic transformation (entries 14-15, Table 4.1).

4.2.2: Substrate scope of one-pot chemo-enzymatic reaction

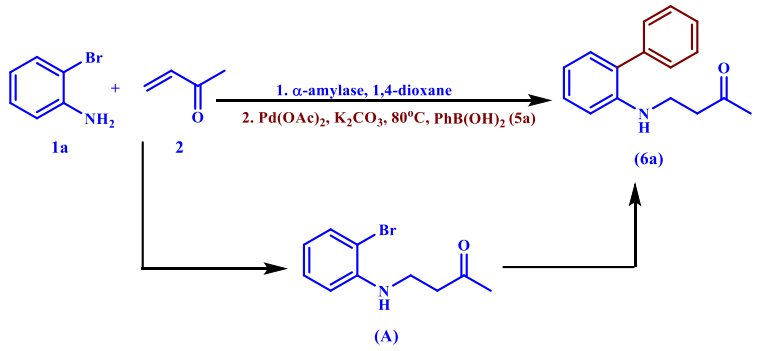
Having the best reaction conditions in hand, we screened the scope of substrates for the chemo-enzymatic reaction. First, we screened the effect of substituents such as -OMe, $-CH_3$ and -Cl on 2-bromoaniline and obtained the corresponding products in the range of 69-80% yield (entries 1-4, Table 4.2). This proved that substituent on 2-bromoaniline does not have significant effect on the outcome of this transformation. Next, we replaced *tert*-butyl isocyanide with 1,1,3,3-*tetra*methylbutyl isocyanide and cyclohexyl isocyanide (entries 5-9, Table 4.2). In this context, when 1,1,3,3-tetramethylbutyl isocyanide employed in the chemo-enzymatic transformation, no significant variation in the yield of the corresponding products

was observed (entries 5-7, Table 4.2). While, reaction with cyclohexyl isocyanide provided slightly lower yield in comparison of *tert*-butyl isocyanide (entries 8-9, Table 4.2).

4.2.3: Screening of metal-catalyst, solvents and bases for one-pot aza-Michael addition and Suzuki-Miyura coupling reaction

In the next phase of our work, we optimized the reaction conditions for the chemo-enzymatic reaction consisting one-pot aza-Michael addition and Suzuki-Miyura coupling reaction to produced amino substituted biaryls. In this context, we replaced $Pd(OAc)_2$ with $Pd(PPh_3)_4$ and $PdCl_2$, but no improvement in the yield of the reaction was observed (entries 2-3, Table 4.3). Next, different solvents and their combination with water were screened and found CH_3CN in H_2O (1:1, v/v) as the best choice for getting higher yield (entries 4-8, Table 4.3). Also, we screened various bases but K_2CO_3 remained as the best choice (entries 9-11, Table 4.3). There was no significant improvement in the yield of the model reaction, when temperature was increased from 80°C to 110°C (entry 12, Table 4.3). Further, changing the loading of Pd-salt as well as α-amylase did not show significant impact on the outcome of this one-pot transformation (entries 13-16, Table 4.3).

Table 4.3: Optimization of reaction conditions for one-pot aza-Michael addition and Suzuki-coupling reaction



Entry	[a]deviation from the standard conditions	yield (6a)
1	None	54%
2	$Pd(PPh_3)_4$ instead of $Pd(OAc)_2$	51%
3	$PdCl_2$ instead of $Pd(OAc)_2$	16%
4	CH_3CN instead of 1,4-dioxane	61%
5	THF instead of 1,4-dioxane	47%
6	H_2O instead of 1,4-dioxane	13%
7	DMF instead of 1,4-dioxane	49%
8	**CH_3CN+ H_2O (1:1, v/v) instead of 1,4-dioxane**	**79%**
9	DBU instead of K_2CO_3	47%
10	NaOH instead of K_2CO_3	31%

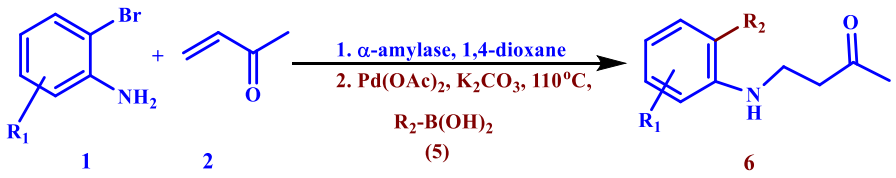
11	$CsCO_3$ instead of K_2CO_3	71%
12	Temperature 100°C instead of 80°C	77%
13	$Pd(OAc)_2$ used 5 mol%	61%
14	$Pd(OAc)_2$ used 15 mol%	75%
15	α-amylase added 5 mg instead of 10 mg	56%
16	α-amylase added 15 mg instead of 10 mg	76%

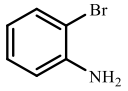
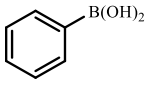
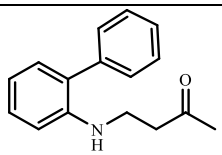
[a]**Standard conditions:** 2-Bromo aniline (1.0 equiv.), methyl vinyl ketone (1.2 equiv), phenyl boronic acid (1.2 equiv), α-amylase (10 mg), 1,4-dioxane (5 ml), $Pd(OAc)_2$ (10 mol%), K_2CO_3 (1.5 equiv.), temperature = 80°C, [b]isolated yield.

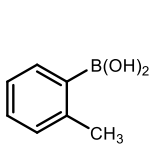
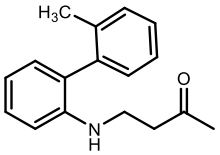
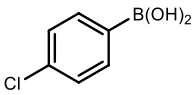
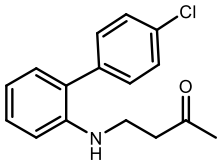
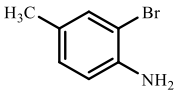
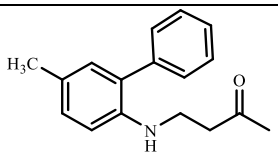
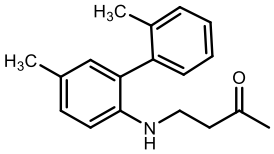
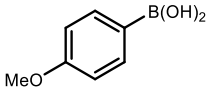
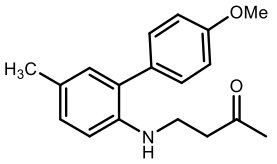
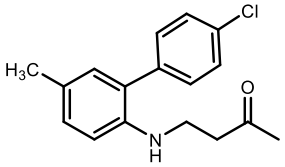
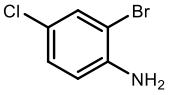
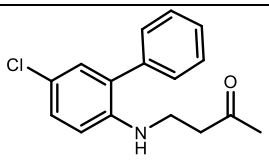
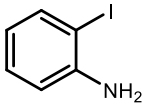
4.2.4: Substrate scope for one-pot aza-Michael addition and Suzuki-coupling reaction:

With the optimal conditions in hand (entry 8, Table 4.3), first we tested the scope of phenyl boronic acid (**5**) to prove the feasibility and generality of this reaction. Notably, 2-methyl boronic acid (**5b**) was tolerated well and provided the corresponding product (**6b**) in 81% isolated yield (entry 2, Table 4.4). Further, the one-pot procedure gave the desired product (**6c**) in 73% isolated yield when 4-chloro phenyl boronic acid was employed in the reaction (entry 3, Table 4.4). Next, the scope of the one-pot chemo-enzymatic approach was tested for substituted 2-bromo anilines (entries 4-8, Table 4.4). In this context, 4-CH_3 or 4-Cl substituted 2-bromo aniline were employed in the reaction in a combination of substituted phenyl boronic acid and delightfully obtained the corresponding products in the range of 59-81% isolated yield which proved the higher efficiency of one-pot chemoenzymatic approach. Also, 2-bromo aniline was replaced with 2-iodo aniline but the efficiency of this one-pot transformation was remained intact.

Table 4.4: Substrate scope of one-pot aza-Michael addition and Suzuki-coupling reaction



Entry	Aniline(1)	acid (5)	Products(6)
1	 1a	 5a	 6a, 79%

2	 **1a**	**5b**	 **6b, 81%**
3	 **1a**	**5c**	 **6c, 73%**
4	 **1b**	**5a**	 **6d, 81%**
5	**1b**	**5b**	 **6e, 69%**
6	**1b**	 **5d**	 **6f, 71%**
7	**1b**	**5c**	 **6g, 73%**
8	 **1c**	**5a**	 **6h 59%**
9	 **1d**	**5a**	**6a 81%**

[a]**Reaction conditions:** Aniline (1.0 equiv.), methyl vinyl ketone (1.2 equiv), phenyl boronic acid (1.2 equiv), α-amylase (10 mg), CH_3CN+ H_2O (1:1, v/v) (5 ml), $Pd(OAc)_2$ (10 mol%), K_2CO_3 (1.5 equiv.), temperature = 80°C, [b]isolated yield.

4.3: Experimental:

4.3.1: General procedure for the one-pot synthesis of substituted indole derivatives (5a-5i):

In a sealed glass tube having a stirrer bar added 10 mg of α-amylase. Then, 2-bromo aniline (100 mg, 1 equiv., 0.581 mmol) and methyl vinyl ketone (48.8 mg, 1.2 equiv., 0.698 mmol) were dissolved in 5 ml of 1,4 dioxane and added to the sealed tube. The progress of the reaction was monitored using TLC and after the completion of aza-Michael addition reaction, K_2CO_3, $Pd(OAc)_2$ (10 mol%, 13.021 mg, 0.058 mmol) and tert-butyl isocyanide (57.993 mg, 2.0 equiv., 0.698 mmol) were added in the same glass tube and stirred the reaction mixture at 100°C for another 12h. After completion of the reaction, the reaction mixture was transferred into water and extracted with ethyl acetate (3 x 10 mL). Next, the solvents were evaporated under reduced pressure and the crude product was purified by column chromatography using silica gel as stationary phase and the gradient of hexane and EtOAc as a mobile phase and obtained the corresponding products (**5a-5i**) in 56–81% isolated yields.

4.3.2: General procedure for one-Pot synthesis of substituted amino biaryls derivatives (6a-6h)

In a sealed glass tube having a stirrer bar added 10 mg of α-amylase. Then, 2-bromo aniline (100 mg, 1 equiv., 0.581 mmol) and methyl vinyl ketone (48.8 mg, 1.2 equiv., 0.698 mmol) were dissolved in 5 ml of 1,4 dioxane and added to the sealed tube. The progress of the reaction was monitored using TLC and after the completion of aza-Michael addition reaction, K_2CO_3, $Pd(PPh_3)_4$ (3 mol%, 20.152 mg, 0.0174 mmol) and phenyl boronic acid (106.321 mg, 1.5 equiv., 0.872 mmol) were added in the same glass tube and stirred the reaction mixture at 100°C for overnight. After completion of the reaction, the mixture was transferred into water and extracted with ethyl acetate (3 x 10 mL). Next, the solvents were evaporated under reduced pressure and the crude product was purified by column chromatography using silica gel as stationary phase and the gradient of hexane and EtOAc as a mobile phase to afford the corresponding products (**6a-6h**) in 59–81% isolated yields.

4.3.3: Characterization data of compounds

^{1}H and ^{13}C NMR Spectrum are attached in appendix of Chapter 4

N-(tert-butyl)-3-(tert-butylimino)-3H-indol-2-amine (5a)[1]

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.53 (d, *J* =7.6 Hz, 1H), 7.23 (t, *J* =7.6 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.82 (t, *J* =7.2 Hz, 1H), 6.07 (brs, 1H), 1.48 (d, *J* =3.6 Hz, 18H) ppm, ^{13}C

NMR ($CDCl_3$, 100 MHz), δ 165.5, 160.8, 157.3, 133.2, 128.4, 120.9, 119.5, 118.3, 56.0, 51.2, 29.2, 28.8 ppm.

N-(tert-butyl)-3-(tert-butylimino)-6-methyl-3H-indol-2-amine (5b)[1]

Solid, ^1H NMR ($CDCl_3$, 400 MHz), δ 7.32 (s, 1H), 7.03 (d, *J* =7.6 Hz, 1H), 6.99-6.83 (m, 1H), 5.99 (brs, 1H), 2.27 (s, 3H), 1.47 (d, *J* =4 Hz, 18H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 162.9, 160.5, 157.4, 133.7, 129.9, 129.1, 119.5, 117.9, 55.9, 51.3, 29.2, 28.8, 21.2 ppm.

N-(tert-butyl)-3-(tert-butylimino)-5-methoxy-3H-indol-2-amine (5c)

Solid, ^1H NMR ($CDCl_3$, 400 MHz), δ 7.42 (d, *J* =8.4 Hz, 1H), 6.67 (d, *J* =2.4 Hz, 1H), 6.32 (dd, *J* =2.4 Hz, *J* =8.4 Hz, 1H), 6.16 (brs, 1H), 3.80 (s, 3H), 1.47 (s, 9H), 1.44 (s, 9H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 168.1, 164.2, 162.2, 158.2, 129.6, 112.3, 106.8, 103.6, 55.5, 51.4, 29.3, 29.2, 28.8 ppm.

N-(tert-butyl)-3-(tert-butylimino)-6-chloro-3H-indol-2-amine (5d)[1]

Solid, ^1H NMR ($CDCl_3$, 400 MHz), δ 7.45 (d, *J* =2 Hz, 1H), 7.18 (dd, *J* =8 Hz, 1H), 6.99 (d, *J* =8.4 Hz, 1H), 6.05 (brs, 1H), 1.47 (d, *J* =2.4 Hz, 18 H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 163.9, 160.8, 156.2, 132.6, 128.1, 125.6, 120.5, 118.9, 58.3, 51.8, 29.4, 28.9 ppm.

N-(2,4,4-trimethylpentan-2-yl)-3-(2,4,4-trimethylpentan-2-yl)imino-3H-indol-2amine (5e)

Solid, ^1H NMR ($CDCl_3$, 400 MHz), δ 7.59 (d**,** *J* =7.2 Hz, 1H), 7.26 (t, *J* =7.6 Hz, 1H), 7.12 (d, *J* =7.2 Hz, 1H), 6.84 (t, *J* =8.4 Hz, 1H), 6.26 (brs, 1H), 1.90 (s, 2H), 1.86 (s, 2H), 1.56 (s, 6H), 1.53 (s, 6H), 1.04 (d, *J* =4.4 Hz, 18H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 166.1, 160.5, 156.3, 133.1, 128.0, 120.6, 119.5, 118.3, 59.9, 55.2, 55.1, 52.1, 32.1, 32.0, 31.8, 31.6, 29.1, 28.8 ppm.

6-methyl-N-(2,4,4-trimethylpenan-2-yl)-3-(2,4,4-trimethylpentan-2-yl)imino-3H-indol-2-amine (5f)

Solid, ^1H NMR ($CDCl_3$, 400 MHz), δ 7.39 (s, 1H), 7.07 (d, *J* =8 Hz, 1H), 7.01 (d, *J* =8 Hz, 1H), 6.19 (brs, 1H), 2.30 (s, 3H), 1.89 (s, 2H), 1.84 (s, 2H), 1.54 (s, 6H), 1.53 (s, 6H), 1.03 (d, *J* =2.8 Hz, 18H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 163.7, 160.2, 156.4, 133.5, 129.7, 128.9, 119.6, 117.8, 59.9, 55.2, 52.0, 32.1, 32.0, 31.8, 31.5, 29.8, 28.7, 21.2 ppm.

6-chloro-N-(2,4,4-trimethylpentan-2-yl)-3-(2,4,4-trimethylpentan-2-yl)imino-3H-indol-amine (5g)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.53 (d, *J* =2 Hz, 1H), 7.21 (dd, *J* =8.4 Hz, 1H), 7.03 (d, *J* =8 Hz, 1H), 6.24 (brs, 1H), 1.88 (s, 2H), 1.84 (s, 2H), 1.55 (s, 6H), 1.52 (s, 6H), 1.03 (s, 18H) ppm.

N-cyclohexyl-3-(cyclohexylimino)-6-methyl-3H-indol-2-amine (5h)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.28 (s, 1H), 7.08 (d, *J* =8 Hz, 1H), 7.01 (d, *J* =8 Hz, 1H), 5.83 (brs, 1H), 4.16-4.09 (m, 1H), 3.93-3.81 (m, 1H), 2.29 (s, 3H), 2.13-2.09 (m, 2H), 1.99-1.82 (m, 6H), 1.63-1.56 (m, 2H), 1.54-1.39 (m, 6H), 1.27-1.25 (m, 4H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 161.4, 160.3, 157.9, 133.7, 130.6, 126.6, 121.2, 117.5, 60.4, 50.5, 33.3, 33.2, 29.8, 25.7, 24.9, 24.7, 21.2 ppm.

6-chloro-N-cyclohexyl-3-(cyclohexylimino)-3H-indol-2-amine (5i)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.39 (s, 1H), 7.23 (d, *J* =8 Hz, 1H), 7.03 (d, *J* =8 Hz, 1H), 5.91 (brs, 1H), 4.12-4.03 (m, 1H), 3.91-3.82 (m, 1H), 2.12-2.01(m, 4H), 1.93-1.87 (m, 4H), 1.82-1.74 (m, 4H), 1.50-1.45 (m, 4H), 1.38-1.32 (m, 4H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 161.7, 159.5, 158.8, 137.5, 132.7, 126.1, 125.8, 118.6, 60.7, 50.7, 37.2, 33.3, 33.1, 32.0, 30.1, 29.8, 27.1, 25.6, 24.9, 24.5 ppm.

N-(tert-butyl)-3-(tert-butylimino)-3H-indol-2-amine (5j)[1]

Solid, NMR ($CDCl_3$, 400 MHz), δ 7.53 (d, *J* =7.6 Hz, 1H), 7.23 (t, *J* =7.6 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 6.82 (t, *J* =7.2 Hz, 1H), 6.07 (brs, 1H), 1.48 (d, *J* =3.6 Hz, 18H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), δ 165.5, 160.8, 157.3, 133.2, 128.4, 120.9, 119.5, 118.3, 56.0, 51.2, 29.2, 28.8 ppm.

4-(1,1-biphenyl-2-ylamino)butan-2-one (6a)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.49-7.44 (m, 2H), 7.41-7.36 (m, 3H), 7.29-7.25 (m, 1H), 7.13 (dd, *J* =7.2 Hz, 1H), 6.83-6.79 (m, 1H), 6.76 (d, *J* =8.4 Hz, 1H), 4.19 (brs, 1H), 3.45 (t, *J* =6 Hz, 2H), 2.74 (t, *J* =6.4 Hz, 2H), 2.15 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.8, 144.6, 139.3, 130.5, 129.4, 128.9, 128.8, 128.2, 127.4, 117.3, 116.1, 114.9, 110.4, 42.7, 38.5, 30.4 ppm.

4-(2-methyl-1,1-biphenyl-2-yl)aminobutan-2-one (6b)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.41-7.26 (m, 3H), 7.24-7.23 (m, 1H), 7.14-7.10 (m, 1H), 6.98 (d, *J* =7.6 Hz, 1H), 6.77 (t, *J* =7.6 Hz, 1H), 6.70 (d, *J* =8 Hz, 1H), 3.62 (brs, 1H), 3.39 (t, *J* =6.8 Hz, 2H), 2.66 (t, *J* =6.4 Hz, 2H), 2.09 (d, *J* =2.8 Hz, 6H) ppm, ^{13}C NMR ($CDCl_3$, 100

MHz), 207.8, 144.9, 138.3, 137.3, 130.4, 130.3, 130.0, 128.6, 127.9, 127.8, 126.4, 116.9, 109.9, 42.8, 38.4, 30.5, 19.8 ppm.

4-(4-chloro-1,1-biphenyl-2-yl)aminobutan-2-one (6c)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.42-7.38 (m, 2H), 7.32-7.28 (m, 2H), 7.26-7.21 (m, 1H), 7.05 (dd, *J* =8.8 Hz, 1H), 6.79 (t, *J* =7.2 Hz, 1H), 6.72 (t, *J* =8 Hz, 1H), 4.09 (brs, 1H), 3.40 (t, *J* =6.4 Hz, 2H), 2.71 (t, *J* =6.4 Hz, 2H), 2.13 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.9, 144.5, 137.8, 133.3, 130.8, 130.5, 129.5, 129.2, 129.1, 126.9, 117.4, 116.8, 110.6, 42.8, 38.5, 30.4 ppm.

4-(4-methyl-1,1-biphenyl-2-yl)aminobutan-2-one (6d)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.44-7.39 (m, 2H), 7.36-7.30 (m, 3H), 7.06 (dd, *J* =8 Hz, 1H), 6.91 (d, *J* =2.0 Hz, 1H), 6.64 (d, *J* =8 Hz, 1H), 4.02 (brs, 1H), 3.38 (t, *J* =6.4 Hz, 2H), 2.68 (t, *J* =6.4 Hz, 2H), 2.26 (s, 3H), 2.10 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.9, 142.3, 139.4, 131.3, 129.4, 129.3, 129.1, 128.9, 128.8, 128.4, 127.3, 126.5, 110.8, 42.8, 38.9, 30.4, 20.4 ppm.

4-(2,4-dimethyl-1,1-biphenyl-2-yl)aminobutane-2-one (6e)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.28-7.21 (m, 3H), 7.13 (d, *J* =6.4 Hz, 1H), 7.07 (dd, *J* =8 Hz, 1H), 6.81 (d, *J* =2.1 Hz, 1H), 6.63 (d, *J* =8.4 Hz, 1H), 3.46 (brs, 1H), 3.37 (t, *J* =6.4 Hz, 2H), 2.65 (t, *J* =6.4 Hz, 2H), 2.26 (s, 3H), 2.09 (d, *J* =4.8 Hz, 6H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.8, 142.4, 138.5, 137.1, 130.8, 130.3, 130.2, 128.9, 128.1, 127.8, 126.3, 126.2, 110.3, 42.9, 38.8, 30.4, 20.5, 19.7 ppm.

4-(4-methoxy-4-methyl-1,1-biphenyl-2-yl)aminobutan-2-one (6f)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.28-7.25 (m, 2H), 7.03 (dd, *J* =8.4 Hz, 1H), 6.97-6.94 (m, 2H), 6.89 (d, *J* =1.8 Hz, 1H), 6.62 (d, *J* =8 Hz, 1H), 3.99 (brs, 1H), 3.84 (s, 3H), 3.38 (t, *J* =6.4 Hz, 2H), 2.69 (t, *J* =6.4 Hz, 2H), 2.26 (s, 3H), 2.11 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.9, 158.8, 142.5, 131.6, 131.3, 130.4, 128.8, 128.1, 126.5, 115.8, 114.3, 114.2, 110.7, 55.4, 42.8, 38.9, 30.4, 20.4 ppm.

4-(4-chloro-4-methyl-1,1-biphenyl-2-yl)aminobutan-2-one (6g)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.41-7.37 (m, 2H), 7.35-7.25 (m, 2H), 7.07 (dd, *J* =8 Hz, 1H), 6.88 (d, *J* =2.7 Hz, 1H), 6.64 (d, *J* =8 Hz, 1H), 3.95 (brs, 1H), 3.37 (t, *J* =6.4 Hz, 2H),

2.70 (t, *J* =6.4 Hz, 2H), 2.26 (s, 3H), 2.12 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.9, 142.2, 137.9, 133.2, 133.1, 130.7, 129.5, 129.1, 127.1, 126.7, 110.9, 42.6, 38.8, 30.4, 20.4 ppm.

4-(5-chloro-1,1-biphenyl-2-yl)aminobutan-2-one (6h)

Solid, ^{1}H NMR ($CDCl_3$, 400 MHz), δ 7.45-7.39 (m, 2H), 7.37-7.30 (m, 3H), 7.18 (dd, J =2.8 Hz, *J* =8.8 Hz, 1H), 7.05 (d, *J* =2.5 Hz, 1H), 6.61 (d, *J* =8.8 Hz, 1H), 4.13 (brs, 1H), 3.37 (t, *J* =6.4 Hz, 2H), 2.68 (t, *J* =6.4 Hz, 2H), 2.11 (s, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz), 207.6, 143.3, 138.0, 130.1, 129.6, 129.2, 129.1, 128.3, 127.9, 121.9, 111.5, 42.5, 38.6, 30.4 ppm.

4.4: Conclusion:

The area of combining an enzyme with chemical catalyst has seen exponential growth in the last few years and it allows to chemists to execute the overall transformations in higher efficiency which was not achievable using individual catalyst. In this work, we have developed a chemo-enzymatic approach to synthesize substituted indoles or amino-biaryls using a combination of α-amylase enzyme and Pd-catalyst in one-pot. This approach consist the enzymatic synthesis of β-aminocarbonyls followed by substrate controlled modification using metal catalyst. Moreover, the substituted indoles were obtained in good yields when isocyanide was employed in the one-pot approach, however, amino substituted biaryls formed in case of phenyl boronic acids.

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Conclusions of the thesis:

Biocatalysts are natural substances such as enzymes, microorganisms or whole cells that speed up or initiate a chemical processes. Furthermore, enzymes are proteins produced by living organisms that assist in the conversion of organic molecules into high-value products without being modified. Enzymes have also been considered as a productive and environmentally friendly tool in chemical synthesis when employed as a catalyst for non-natural transformations. Enzymes are composed of 20 L-amino acids that are bound in specific ways to produce various enzymes. Enzymes are divided into seven categories, from which particular enzymes are entitled. These families are: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and translocases. Hydrolases are a type of enzyme that employs a water molecule to breakdown a chemical bond, resulting in the subdivision of a larger molecule into smaller units. Despite, lot of advantages of various enzymes in synthetic chemistry as green and sustainable catalyst, mostly of them are limited to catalyse mainly the natural reactions (the reactions known for particular enzyme in the nature). Thus, finding of an enzyme for catalysing a non-natural reactions is a longstanding challenge in the field of biocatalysis. In this context, α-amylase is a hydrolase enzyme that catalyse the hydrolysis of α-1,4-glycosidic bonds in starch, resulting in the production of glucose, maltose, and dextrin. In this regard, our goal is to evaluate major advances to the use of α-amylase for non-conventional processes during the previous eleven years in order to update the field's current situation.

In **chapter 3**, we developed the first example of biocatalytic aza-Michael addition of less nucleophilic aromatic amines to enone using α-amylase as a catalyst. This strategy was 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 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. In addition, an analysis of the 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 activate the enols through strong hydrogen bonding.

In **chapter 4** we described a biocatalytic synthesis of quinoline derivatives via α-amylase catalysed one-pot domino aza-Michael/Aldol/aromatization reactions. Further, this methodology was applied to different 2-aminobenzaldehydes and α,β-unsaturated carbonyls to

construct substituted quinolines derivatives in good yields (56–86%). The scalability of this protocol was exhibited by set-up a gram level reaction. Also, the green chemistry metrics such as E-factor, PMI atom-economy and reaction mass efficiency were calculated for this reaction.

In **chapter 5**, we reported the application of α-amylase in chemoenzymatic synthesis. The area of combining an enzyme with a chemical catalyst has seen exponential growth in the last few years and it allows to chemists to execute the overall transformations with higher efficiency which is not achievable using an individual catalyst. In this work, we have developed a chemo-enzymatic approach to synthesize substituted indoles or amino-biaryls using a combination of α-amylase enzyme and Pd-catalyst in one-pot. This approach consists of the enzymatic synthesis of β-aminocarbonyl followed by substrate-controlled modification using a metal catalyst. Moreover, the substituted indoles were obtained in good yields when isocyanide was employed in the one-pot approach, however, amino-substituted biaryls formed in the case of phenylboronic acids.

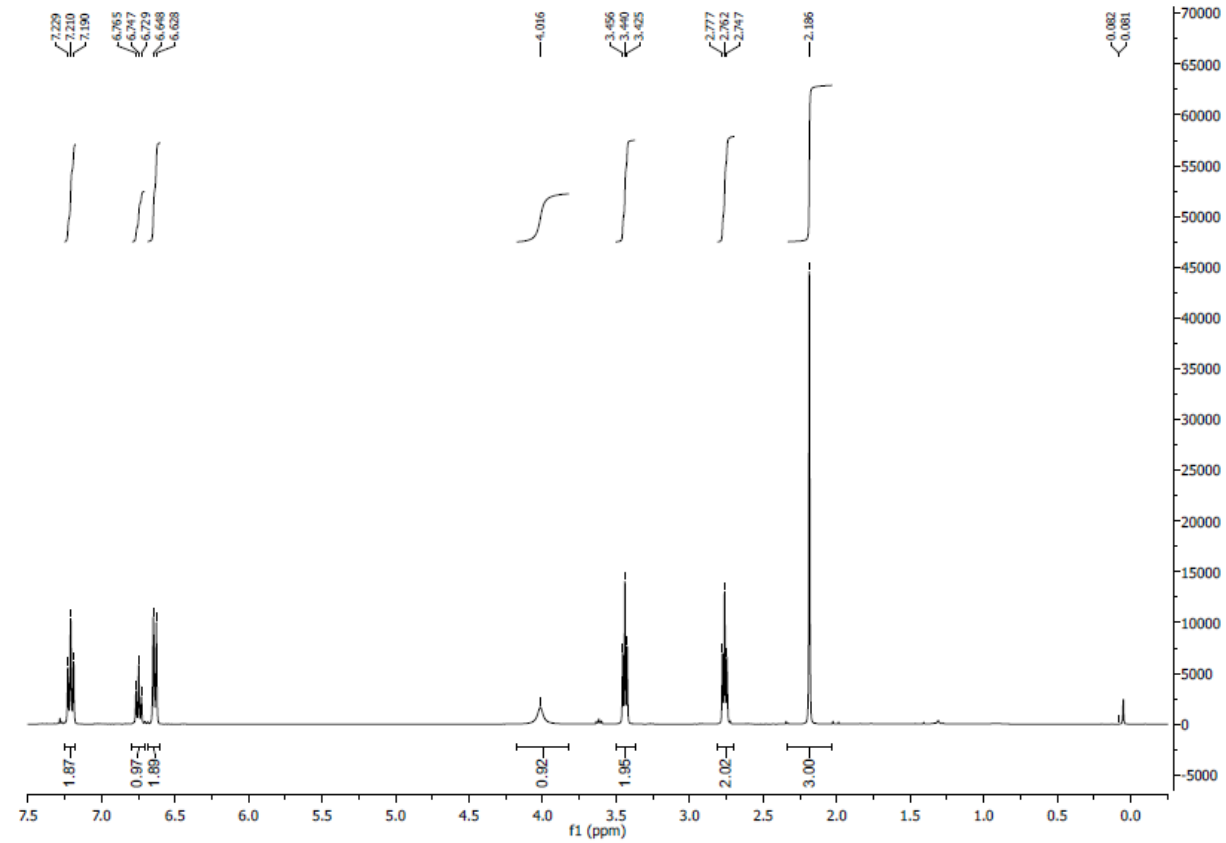
Outlook:

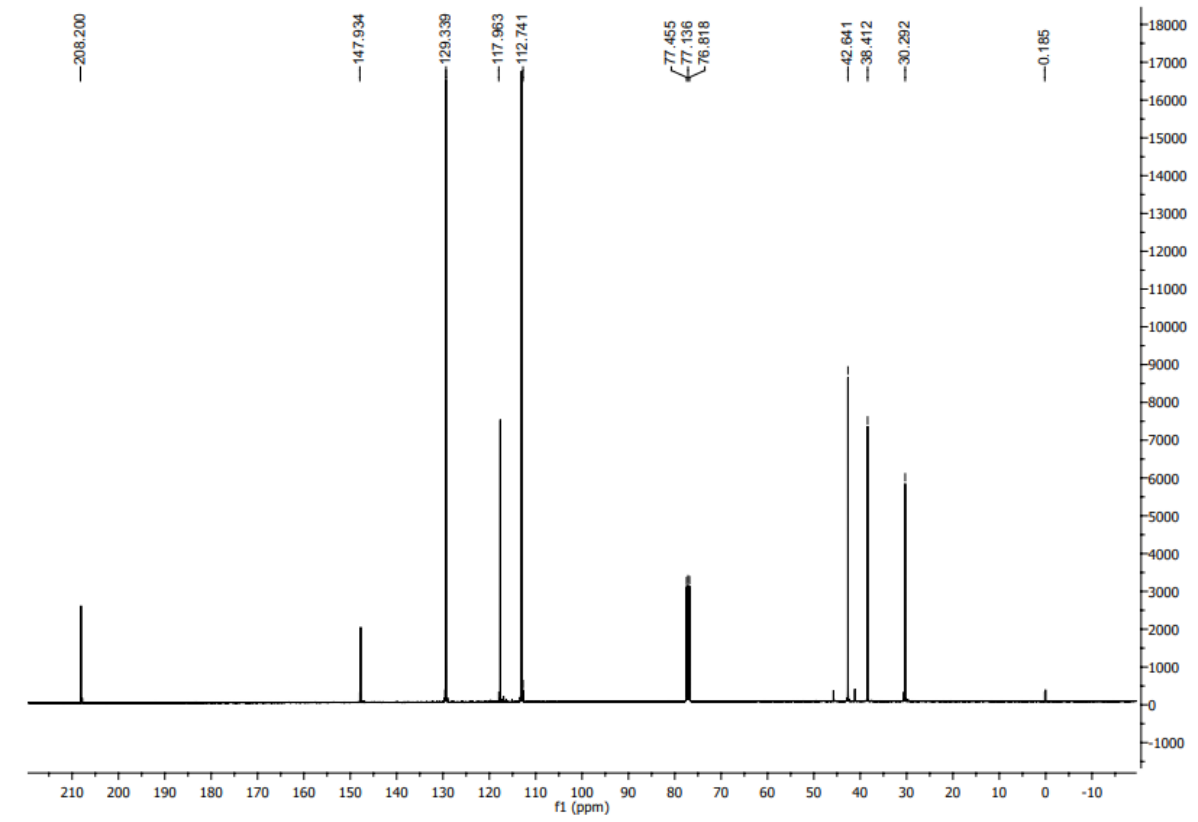
1. The aza-Michael addition reaction of aromatic amine to enone, which is catalysed by α-amylase from *Aspergillus Oryzae*, has certain limitation such as lower tolerance for few substrates and required longer reaction time. These limitation can be overcome by mutating the active-site of enzyme (mutagenesis) in future work.
2. Currently available biocatalyst for aza-Michael addition such as α-amylase and lipases can catalyse the intermolecular aza Michael addition reaction of aromatic amines to enone. As a result, no biocatalyst has been reported so far to catalyse the intramolecular aza-Michael addition reaction of amines to enones.
3. There is no biocatalyst available to catalyze the aza-Michael addition reaction in an enantioselective manner which can be explored further using enzyme engineering (mutagenesis).
4. The thia-Michael addition reaction of aromatic amines to enone has not yet been explored with α-amylase.
5. A domino thia-Michael/aldol/aromatization reaction to synthesize C-3 substituted thio-chromones using α-amylase enzyme as a catalyst can be explored in future.
6. We have described the aza-Michael/Aldol/aromatization cascade catalysed by α-amylase enzyme to synthesize C-3 substituted quinoline derivatives, however, we observed that some substrates provided very poor isolated yields. To improve the yield of substituted

quinoline derivatives, a futuristic study is required with exploring the mutated α-amylase enzymes as a catalyst.

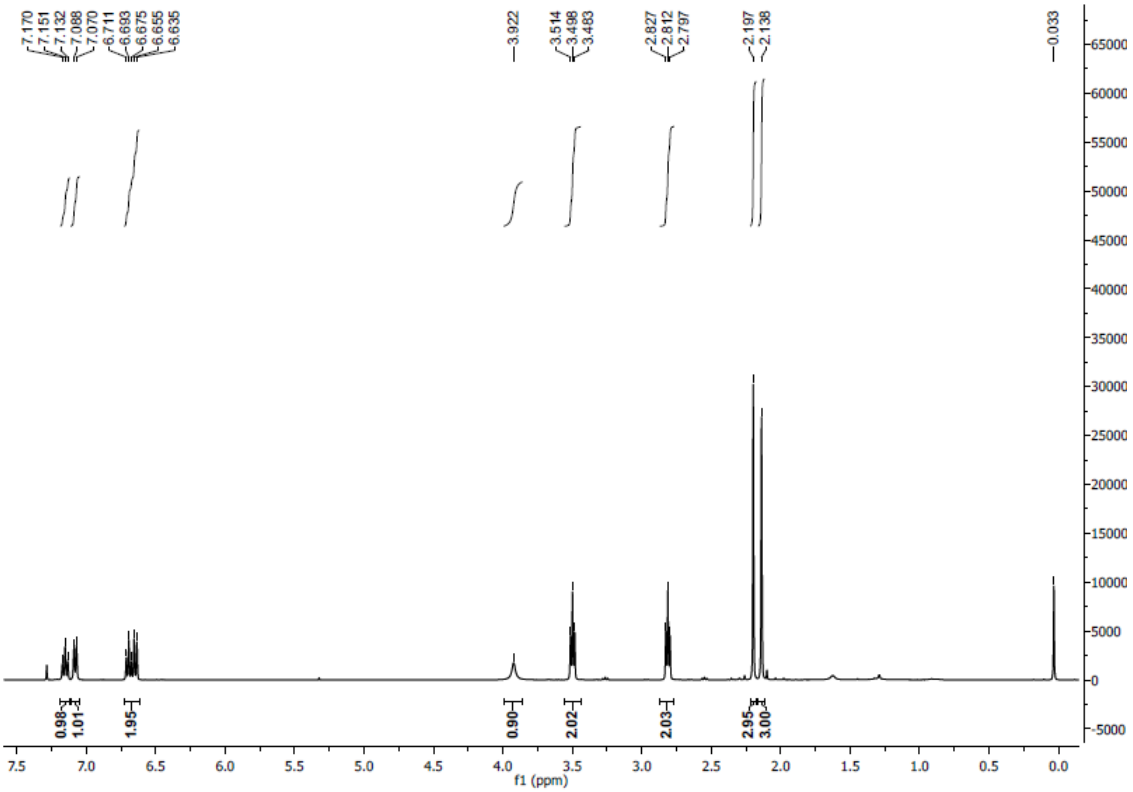
7. During the exploration of chemo-enzymatic synthesis, we observed that α-amylase enzyme shows good tolerance with transition metal catalysts and reaction conditions. As a result, more chemo-enzymatic approaches can be explored using α-amylase enzyme as biocatalyst in future.

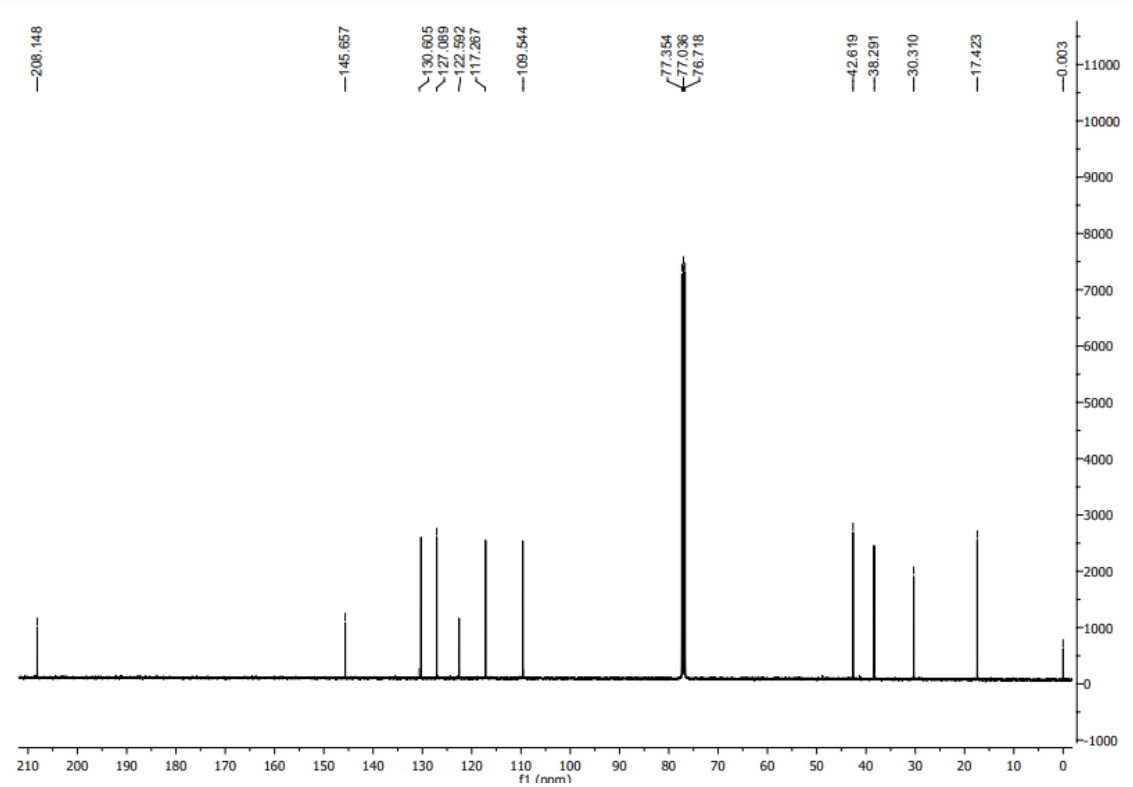
^{1}H and ^{13}C-NMR spectra of 3a



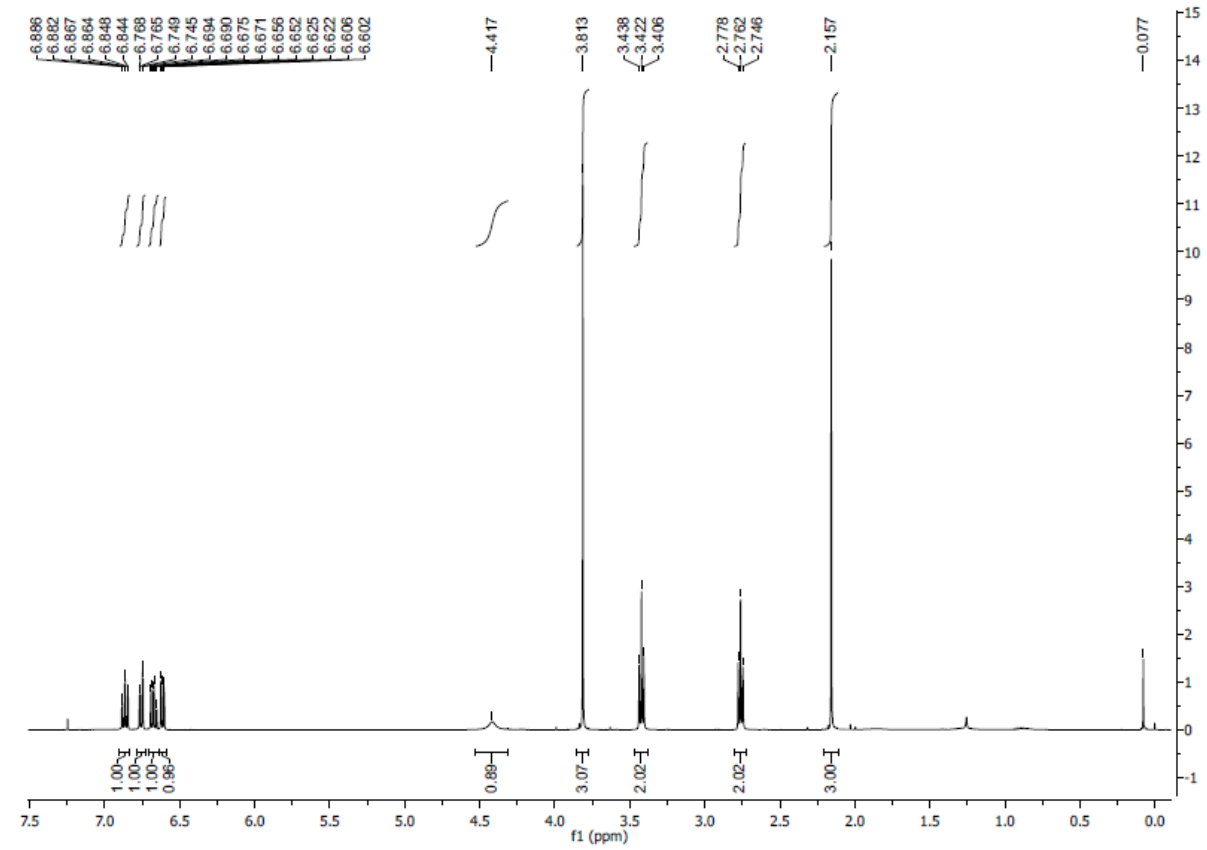


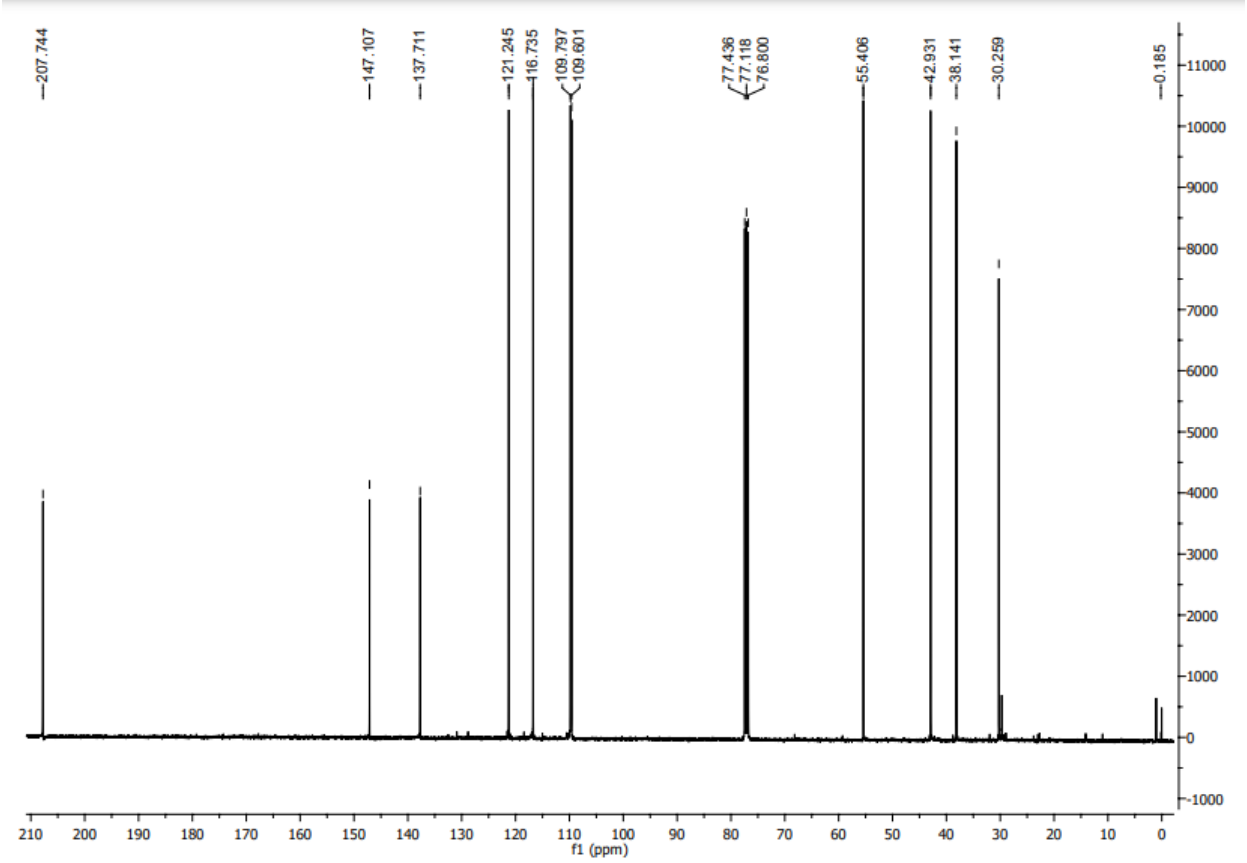
^{1}H and ^{13}C-NMR spectra of 3b



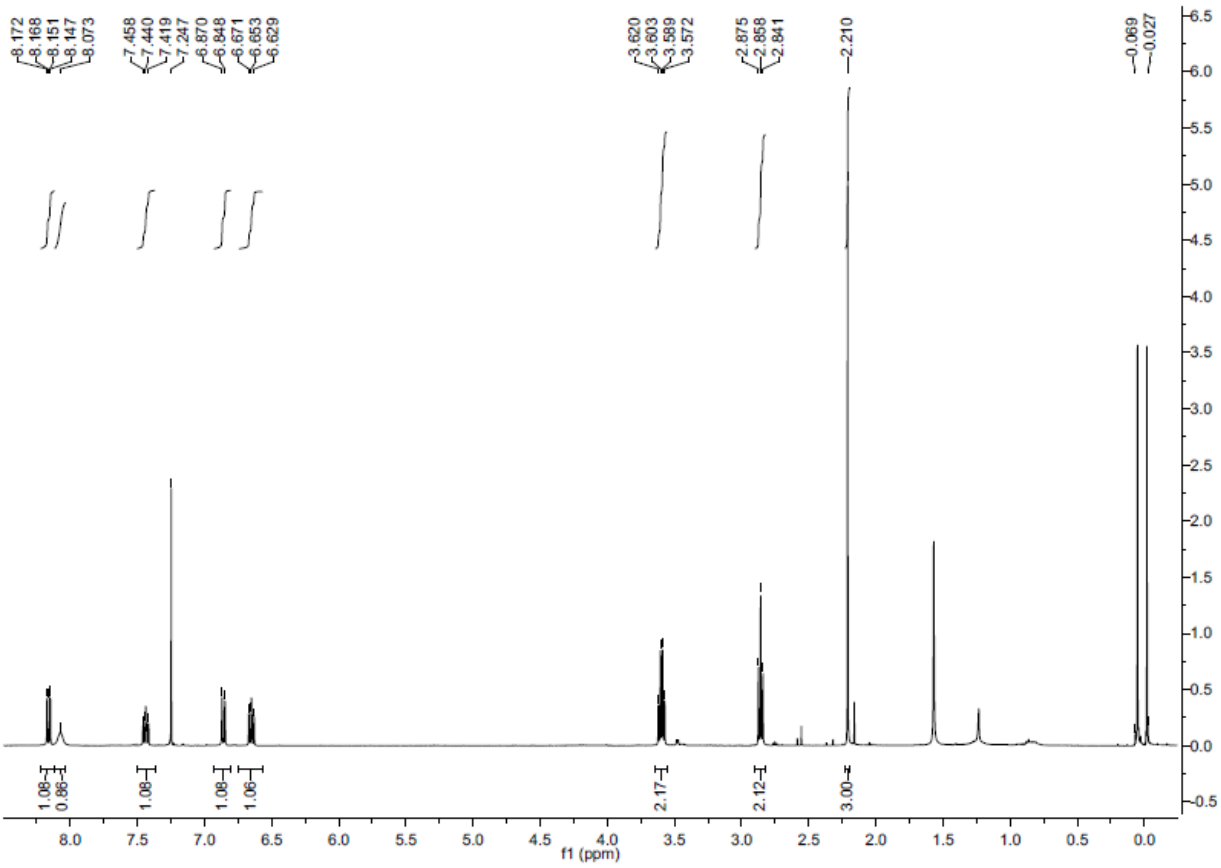


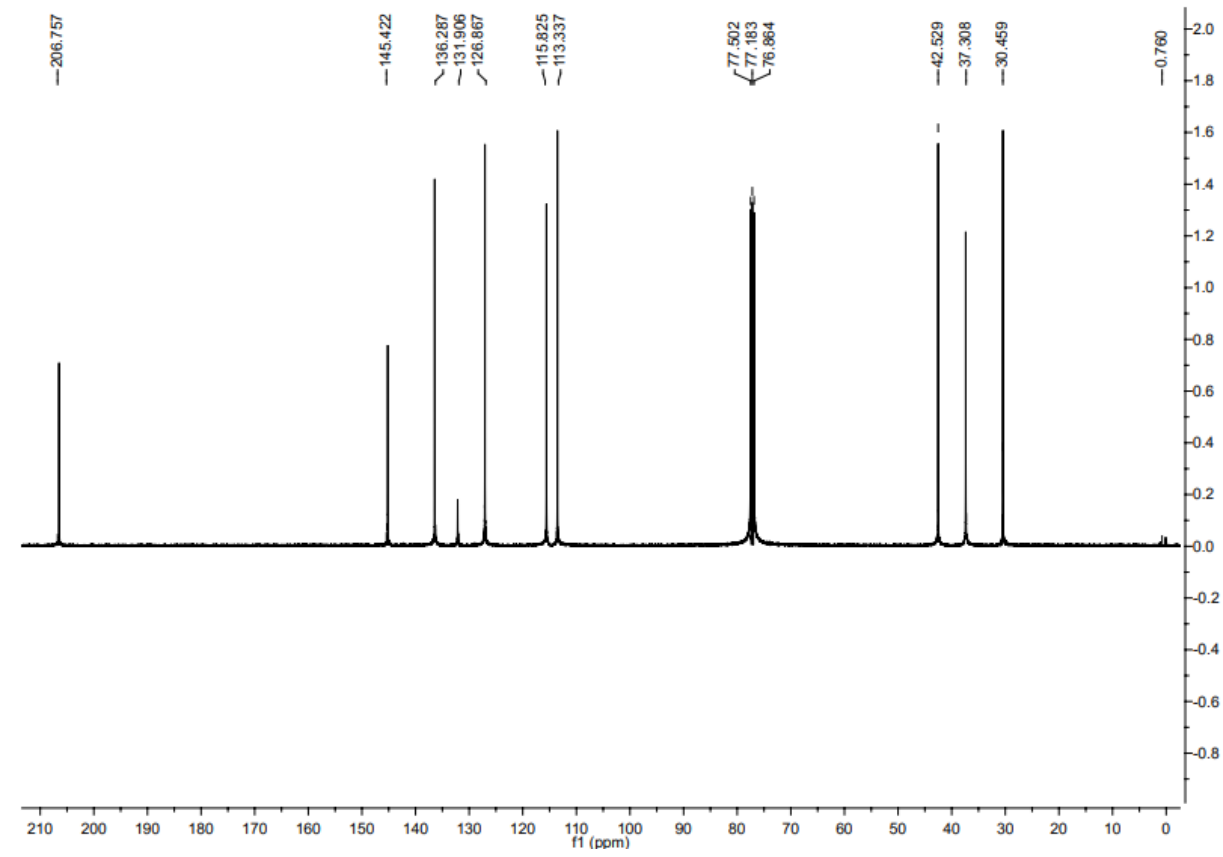
^{1}H and ^{13}C-NMR spectra of 3c



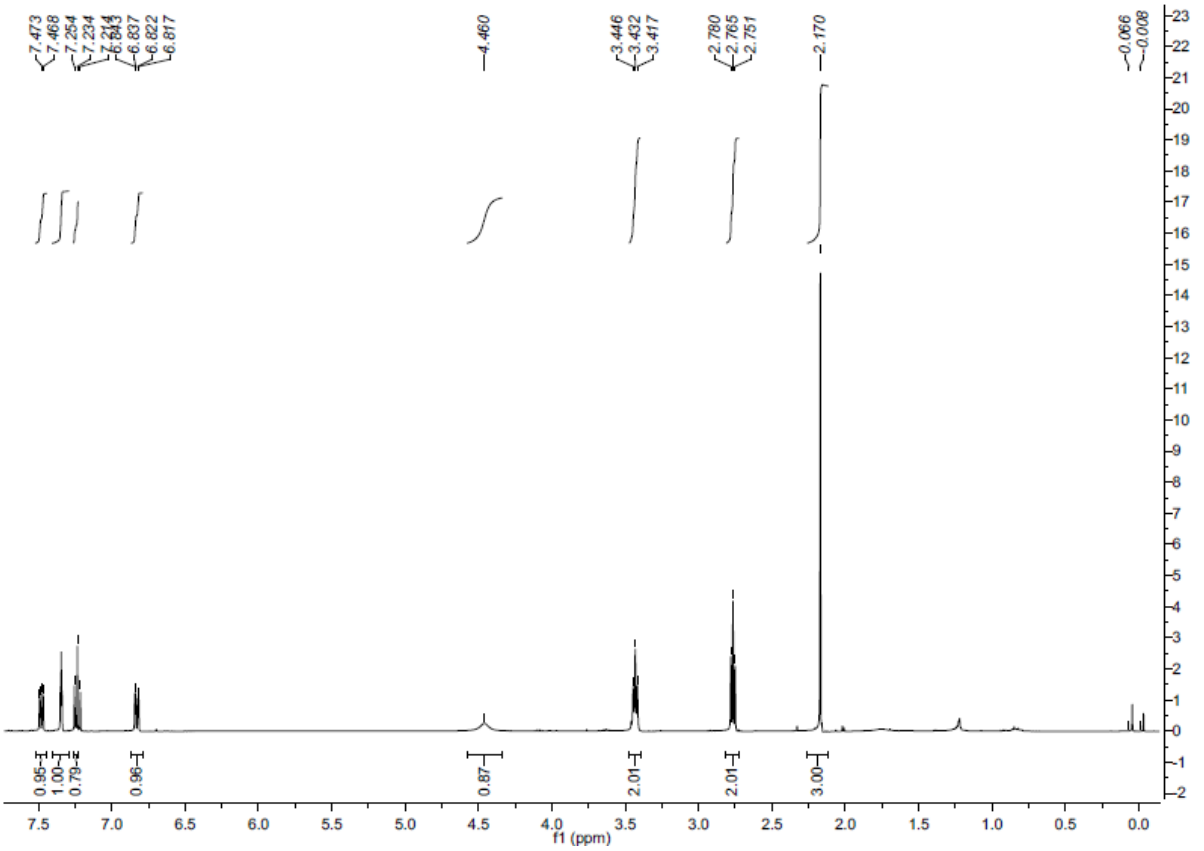


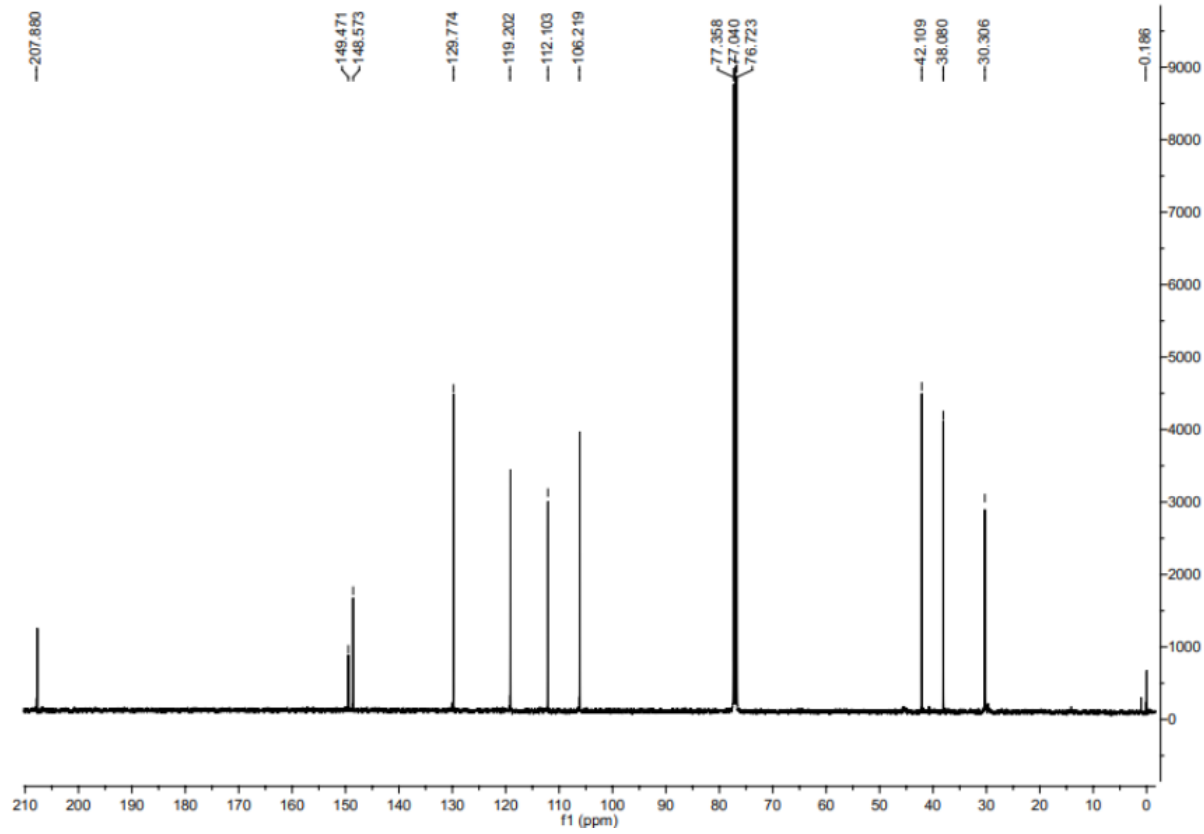
^{1}H and ^{13}C-NMR spectra of 3d





^{1}H and ^{13}C-NMR spectra of 3e





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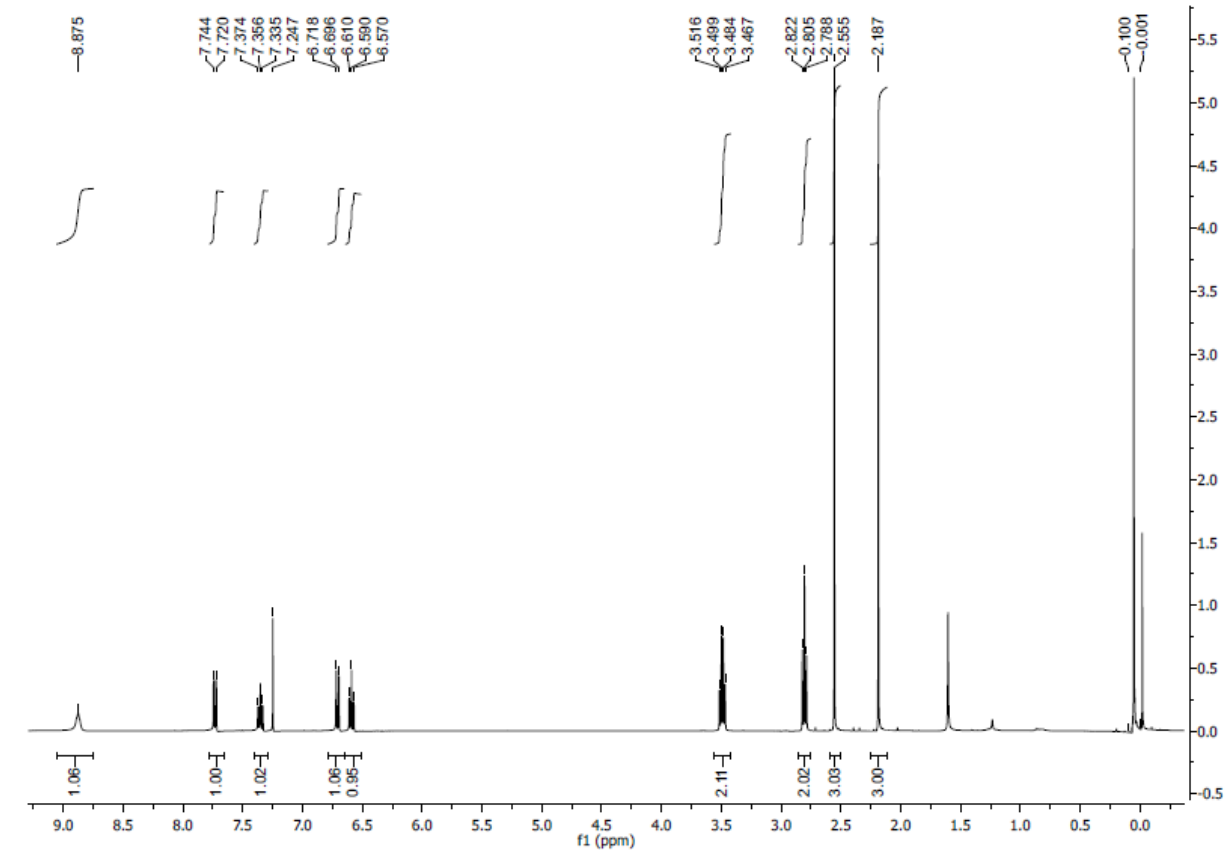

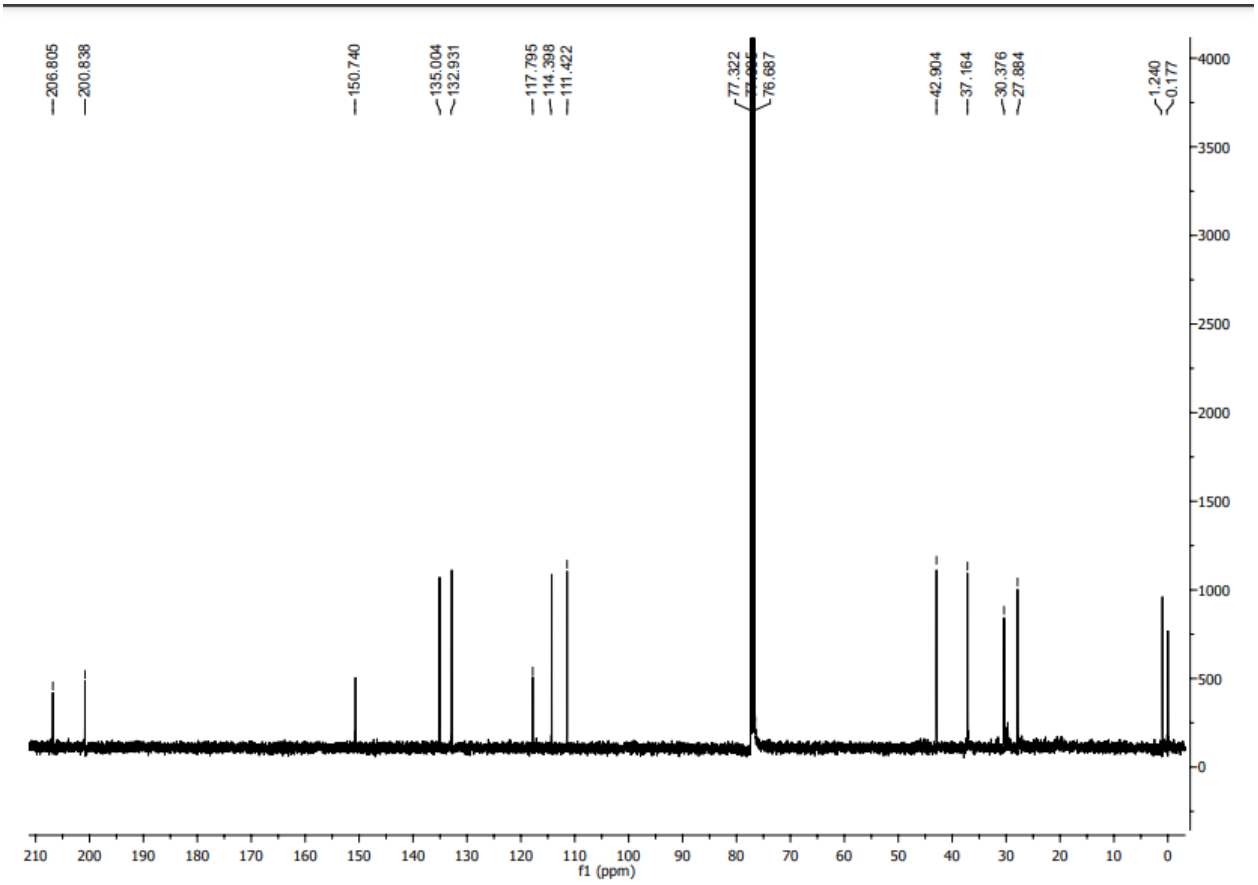

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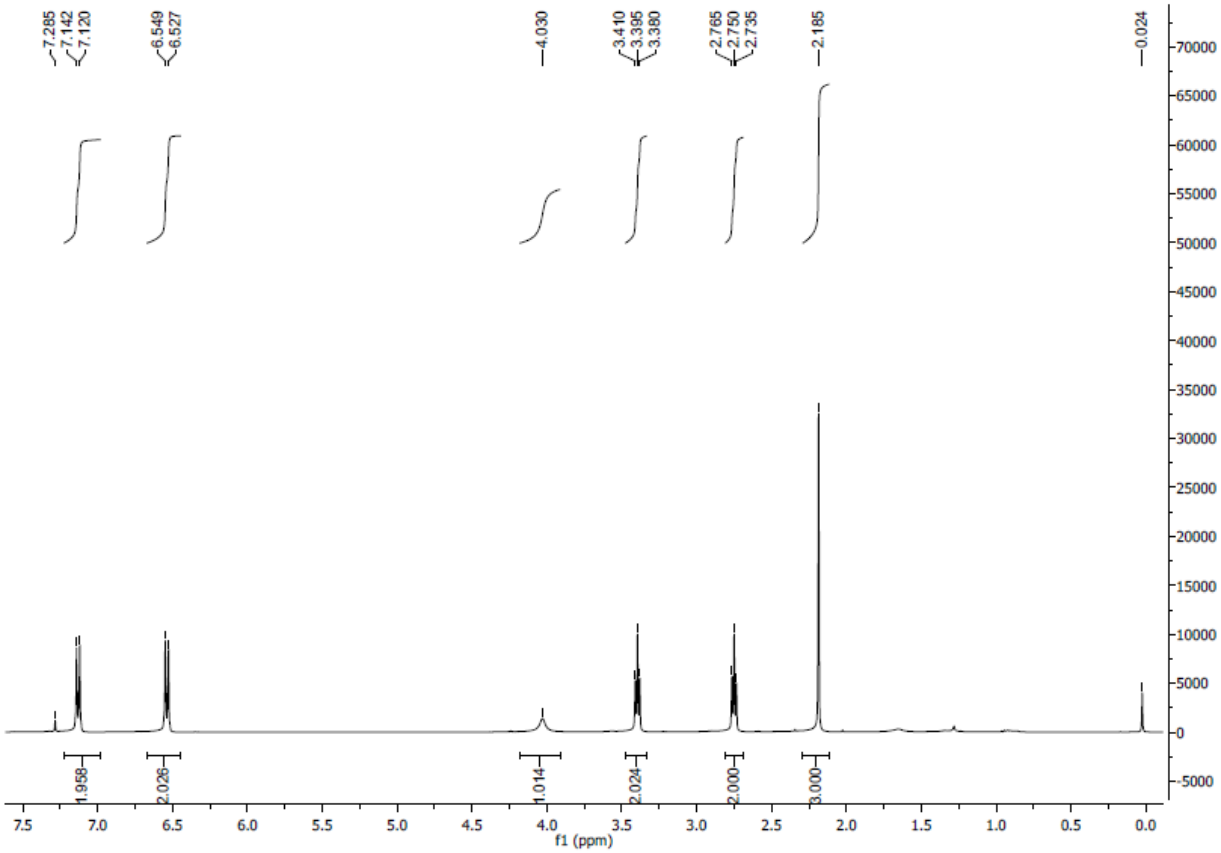
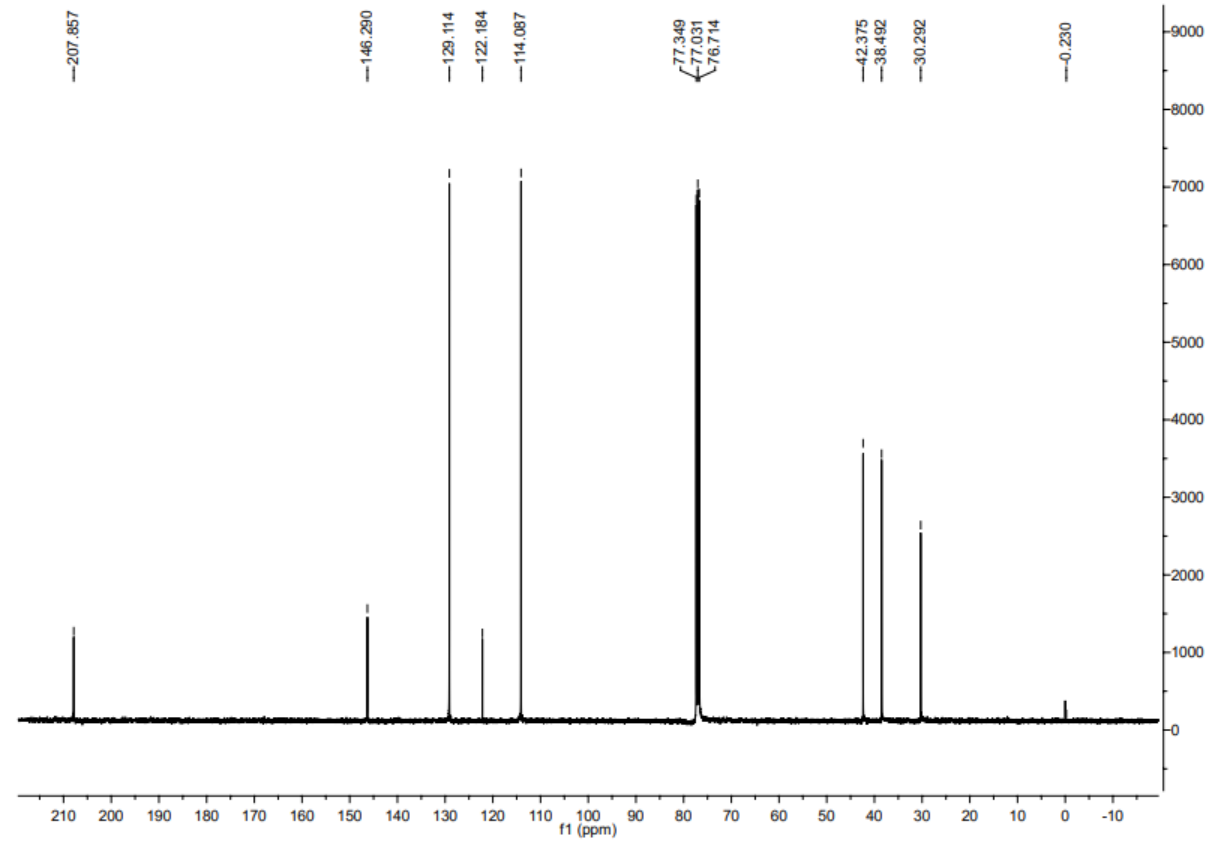
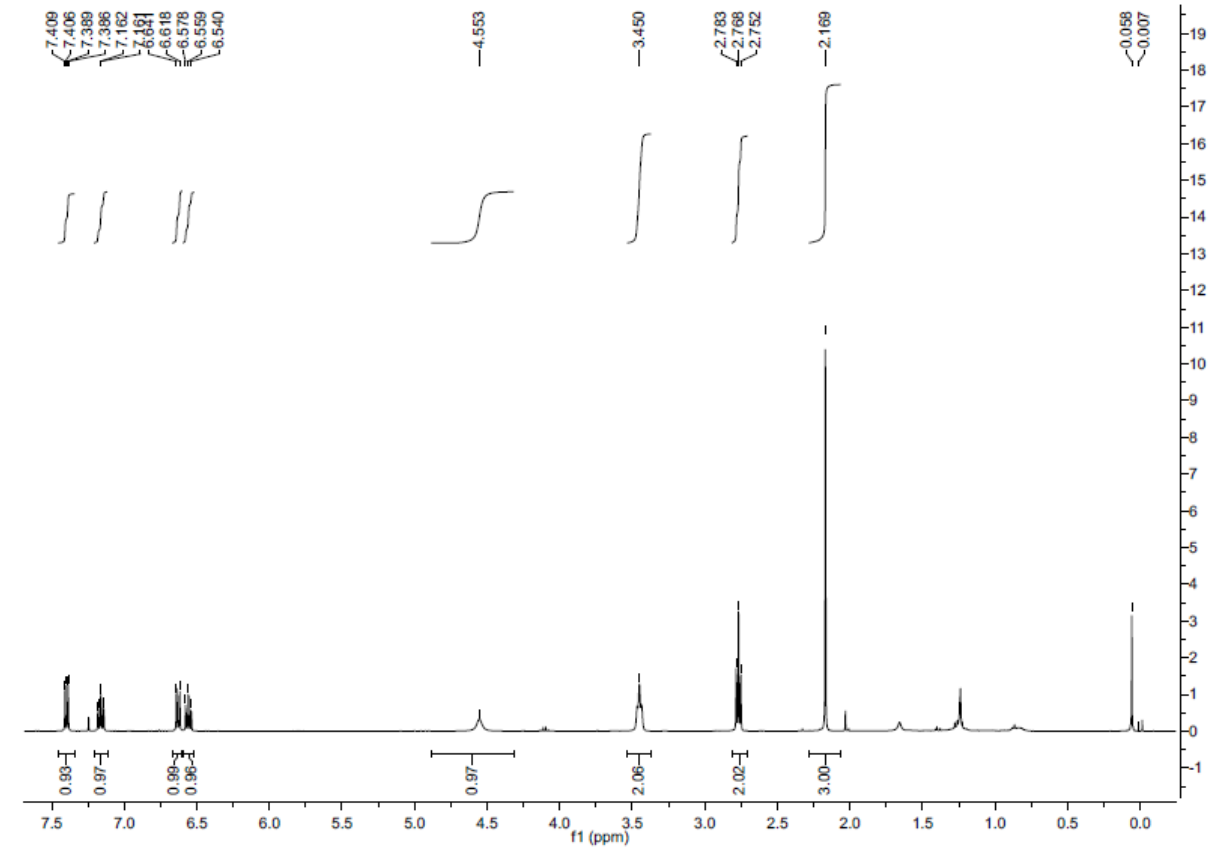
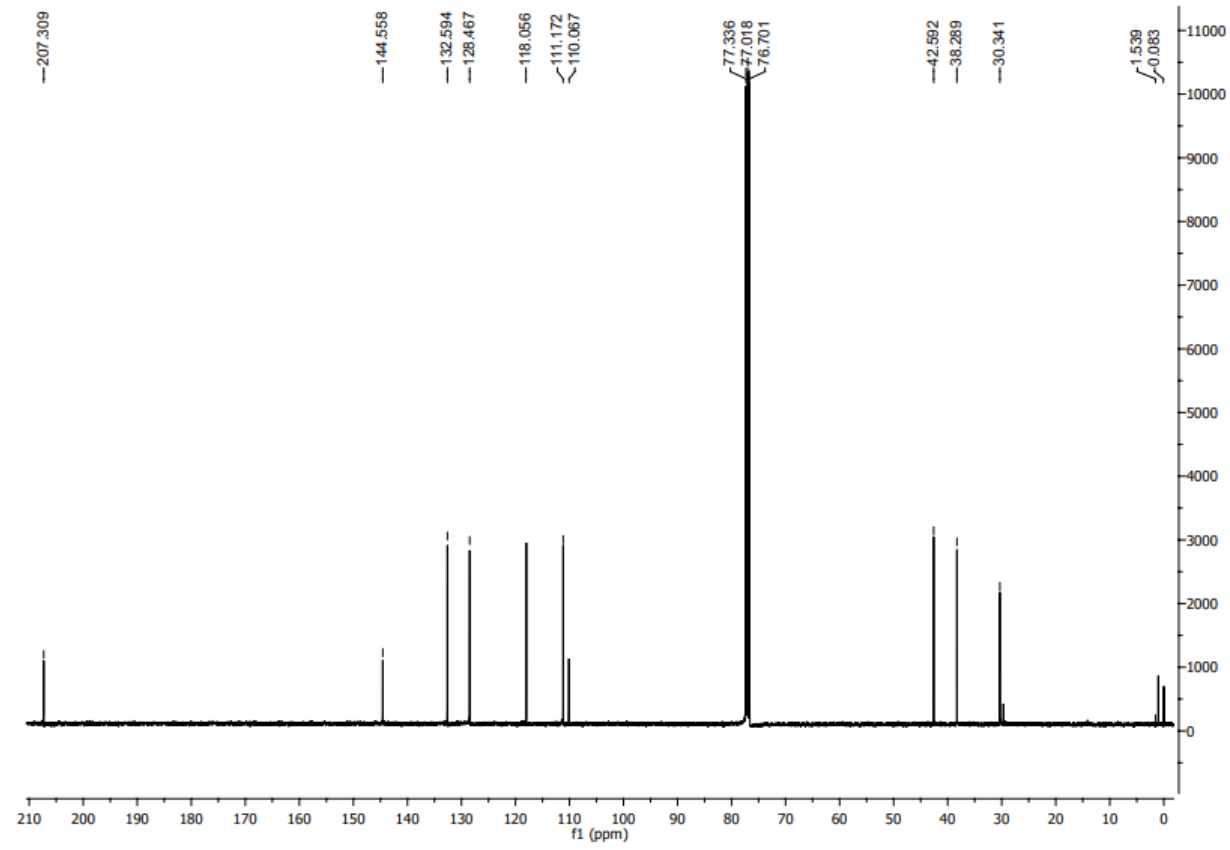


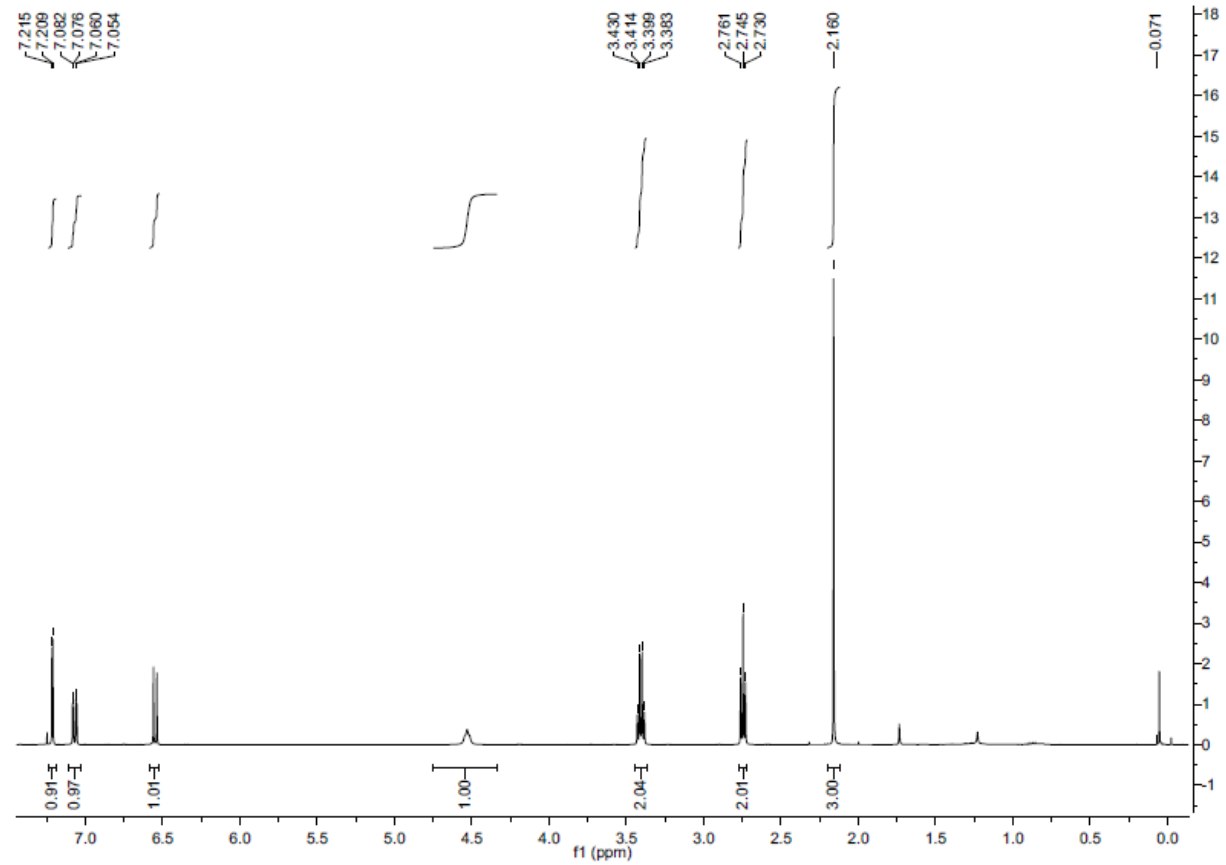


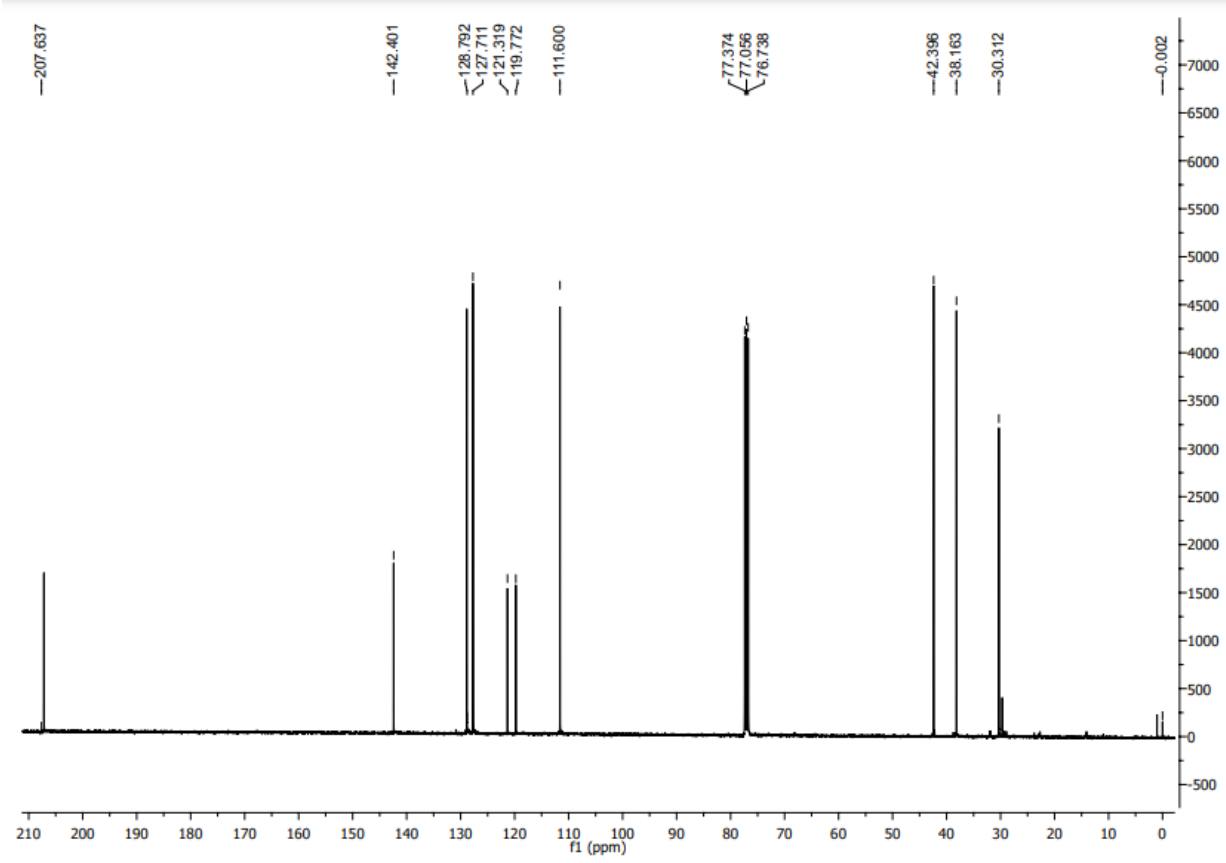
^{1}H and ^{13}C-NMR spectra of 3h



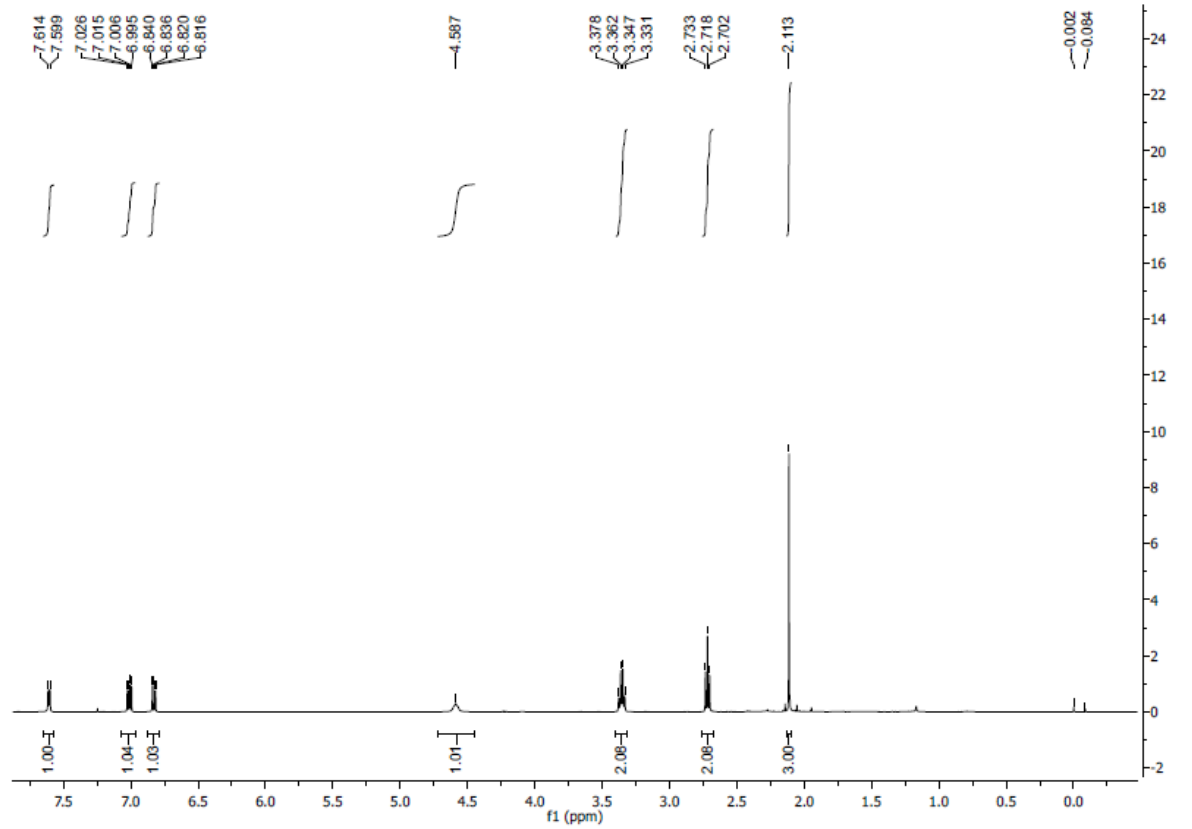


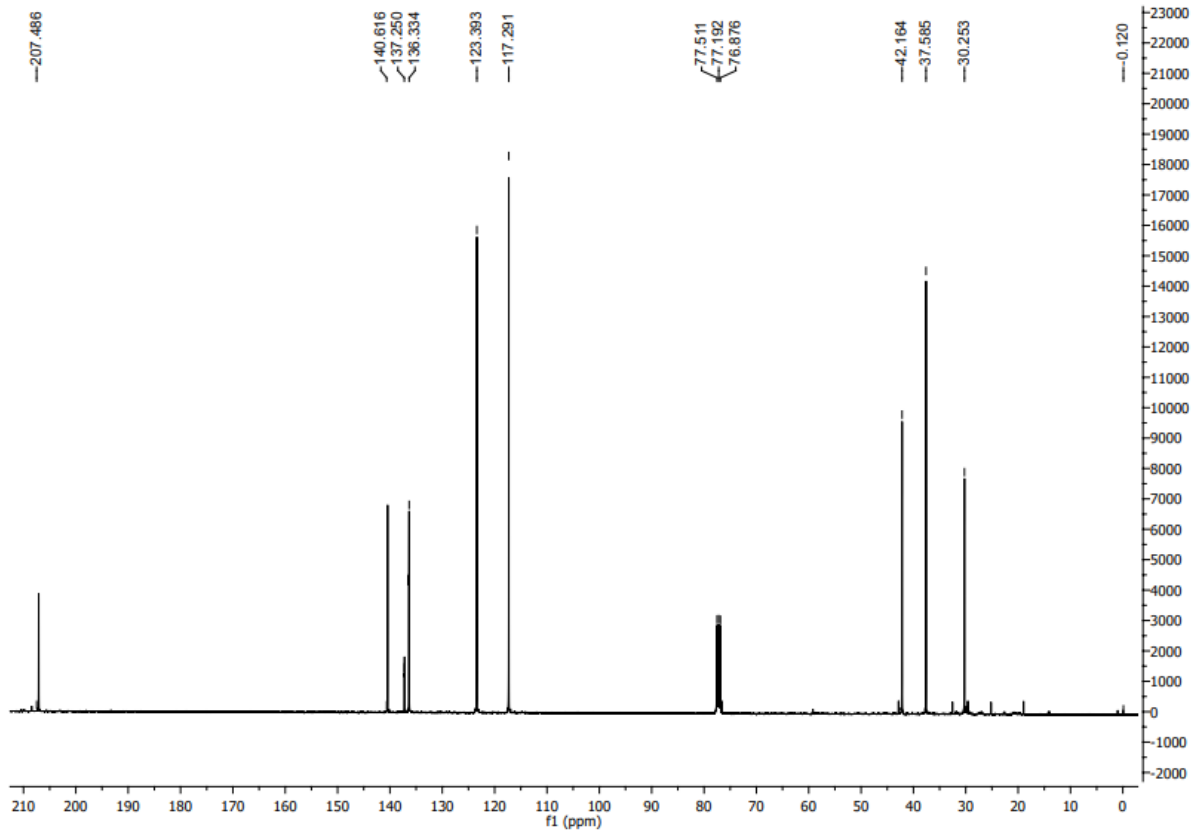
^{1}H and ^{13}C-NMR spectra of 3i



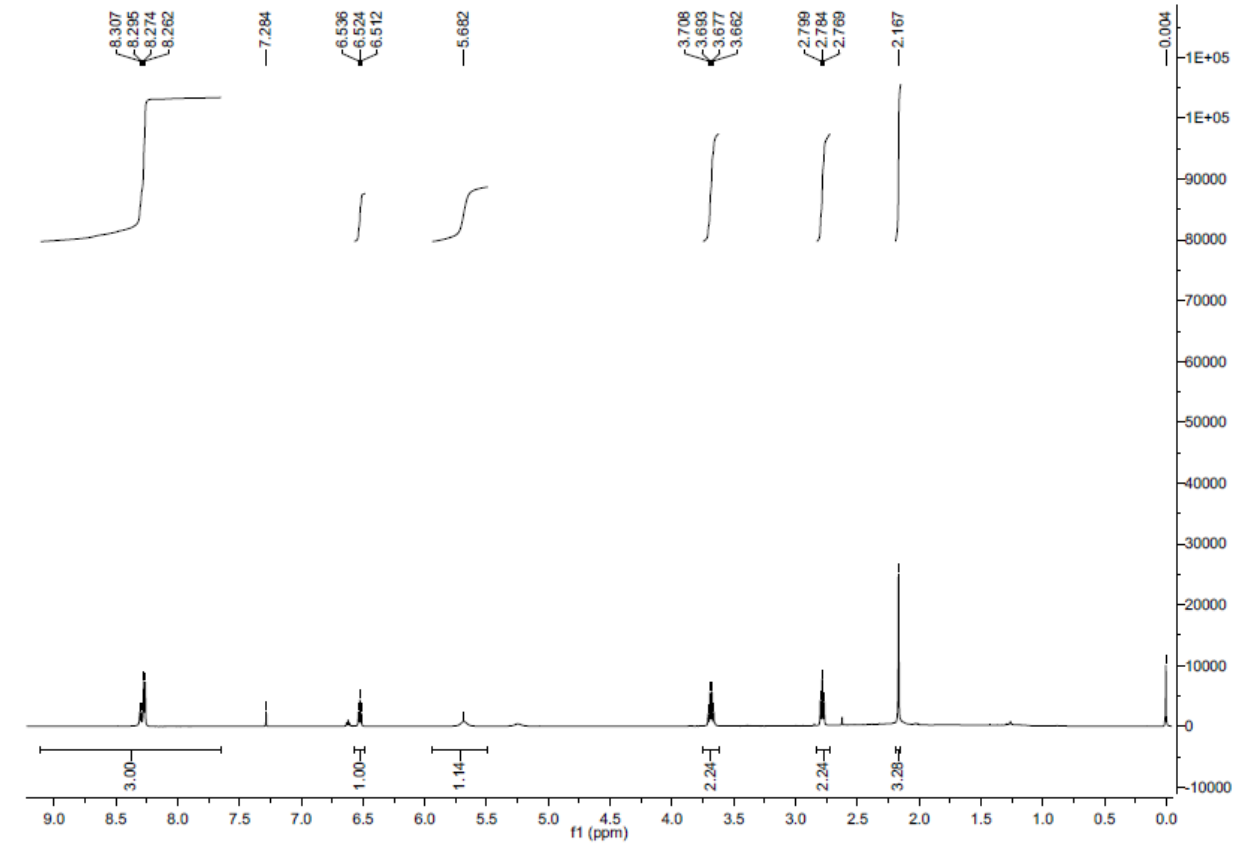


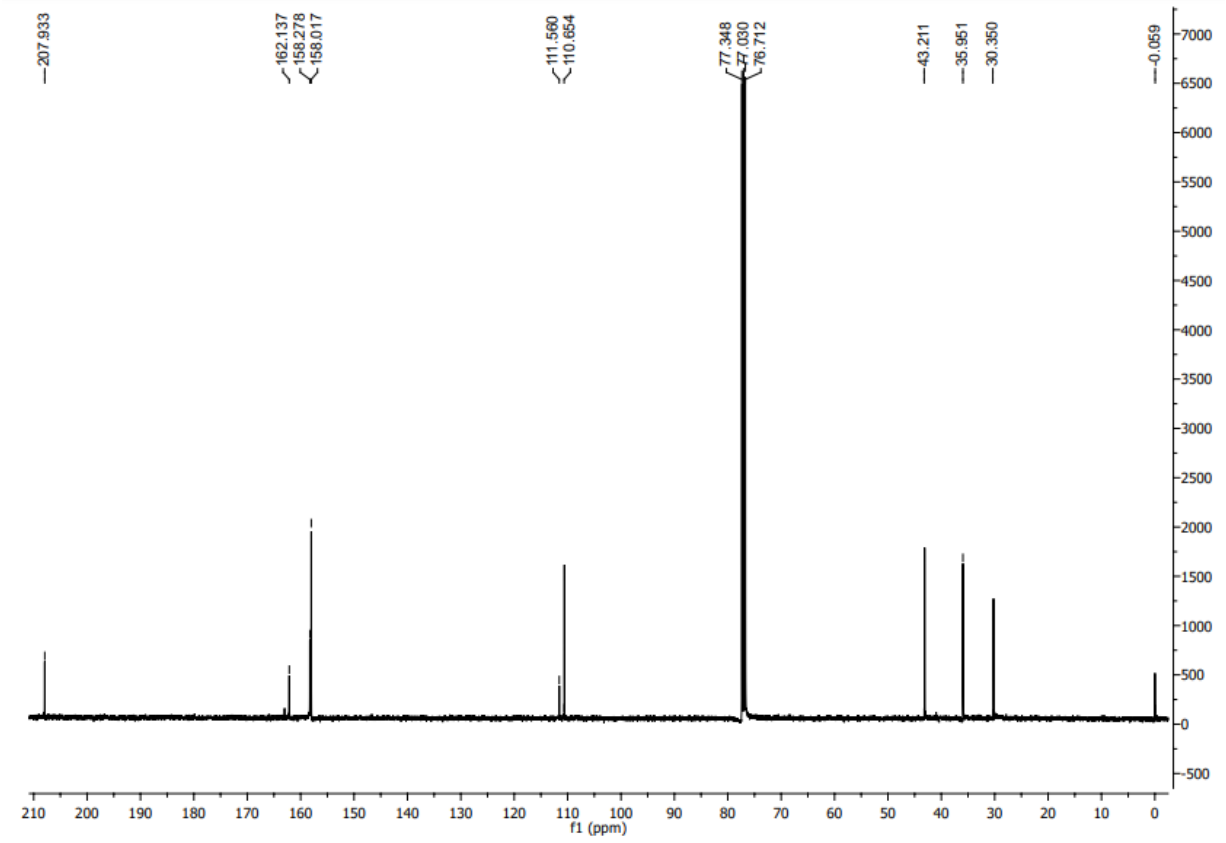
^{1}H and ^{13}C-NMR spectra of 3j



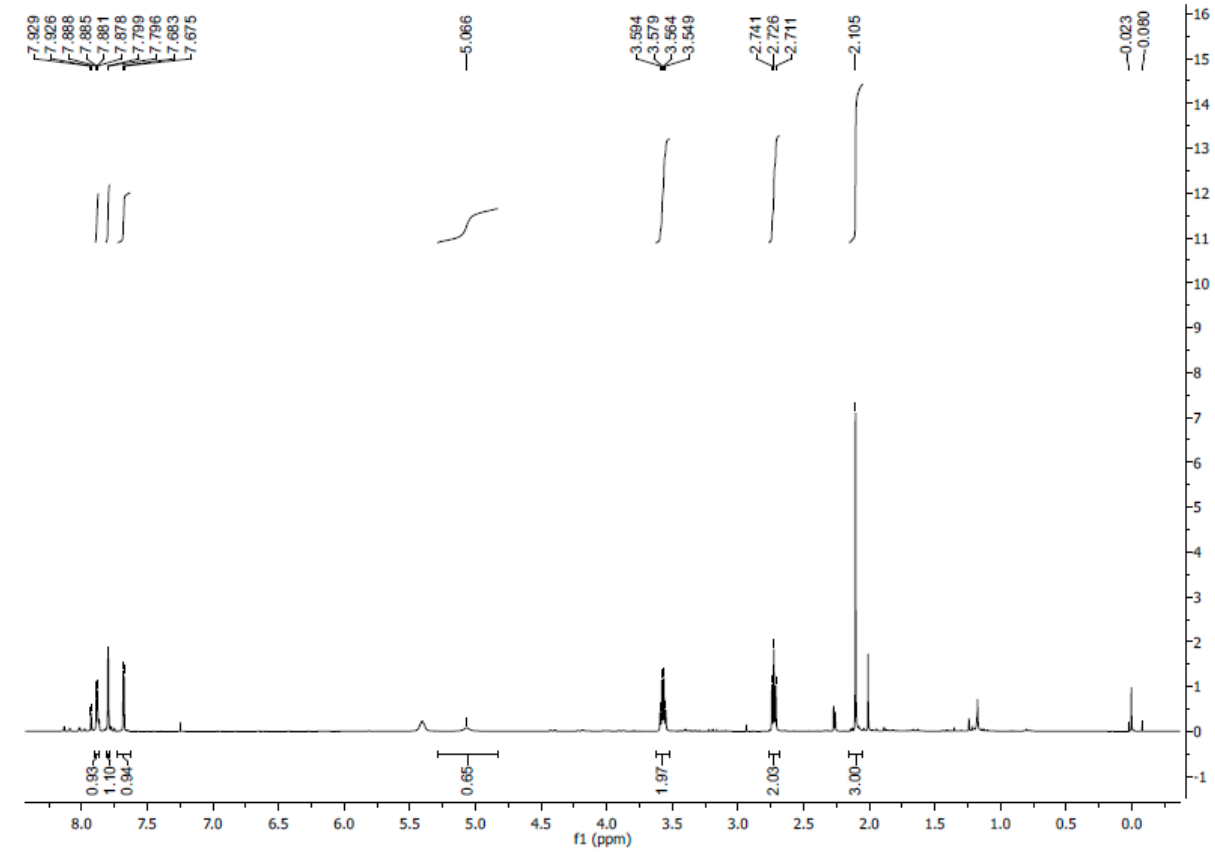


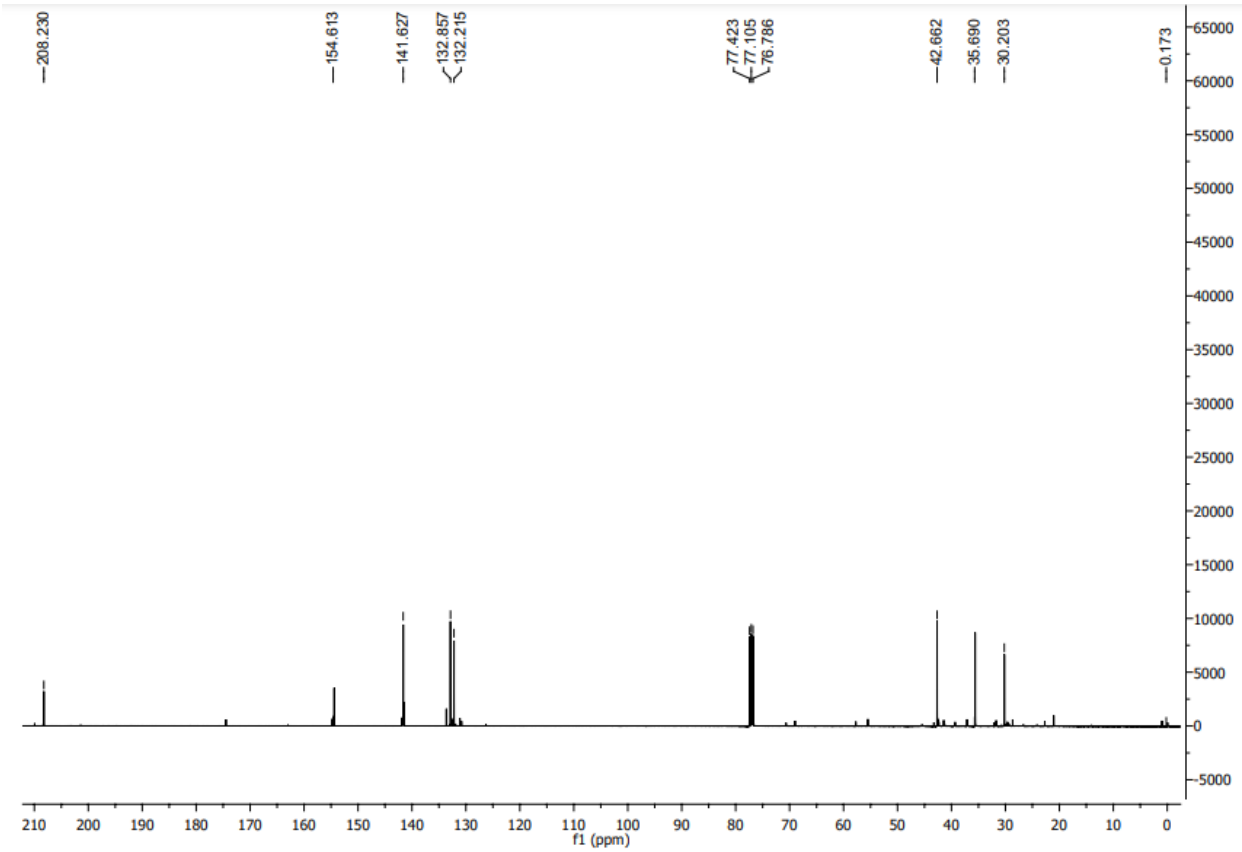
^{1}H and ^{13}C-NMR spectra of 3k





^{1}H and ^{13}C-NMR spectra of 3l





APPENDIX OF CHAPTER 2

Molecular Dynamics Simulations:

Variation of key H-bond distances during MD Simulation:

The variation in hydrogen bond distances between hydrogen atom of aniline and the oxygen atom of the carboxylic group of the residue Glu230 is shown Figure 1.

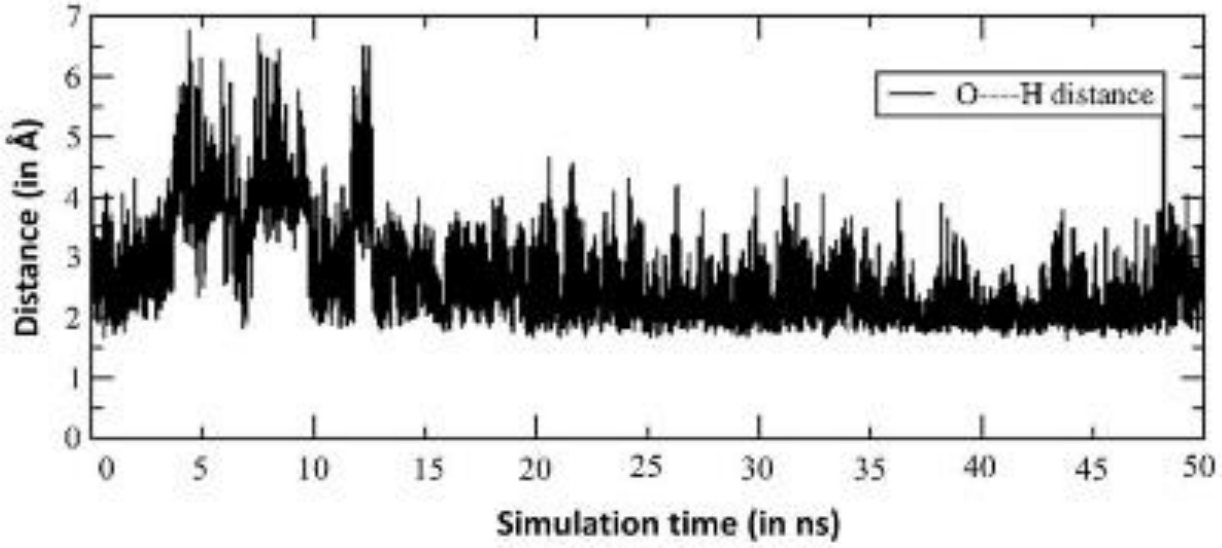


Figure 1: The variation of the Glu-O---HN distances vs. simulation time.

The variation in hydrogen bond distances between hydrogen atom of NH_2 group of the residue Asn295 and the oxygen atom of 2-butenone is shown Figure 2.

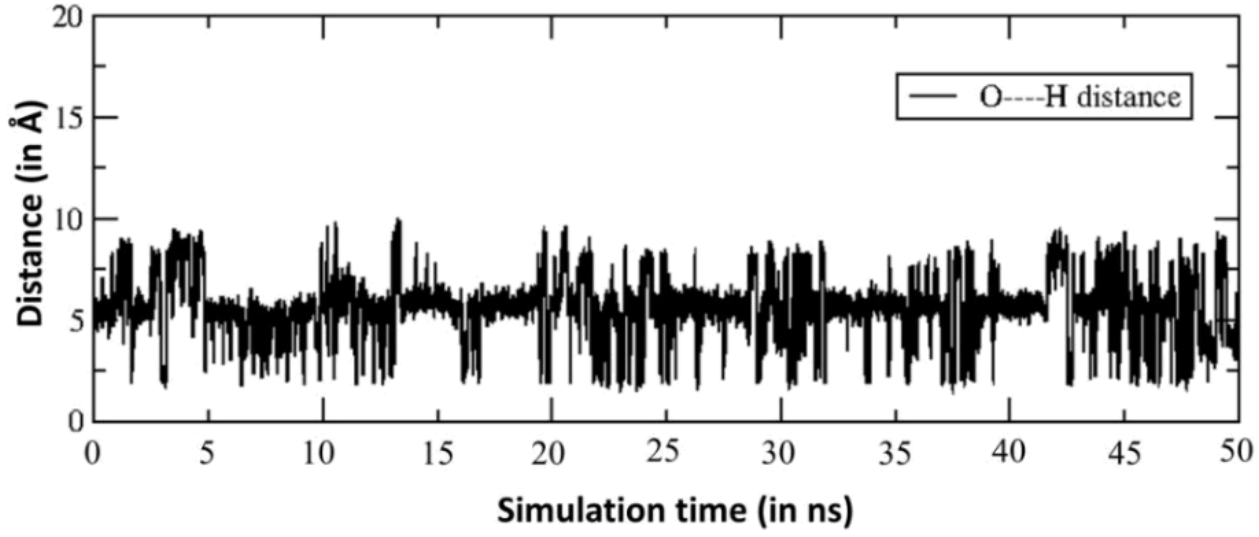


Figure 2: The variation of the **C**=O---H_2N-Asn distances vs. simulation time.

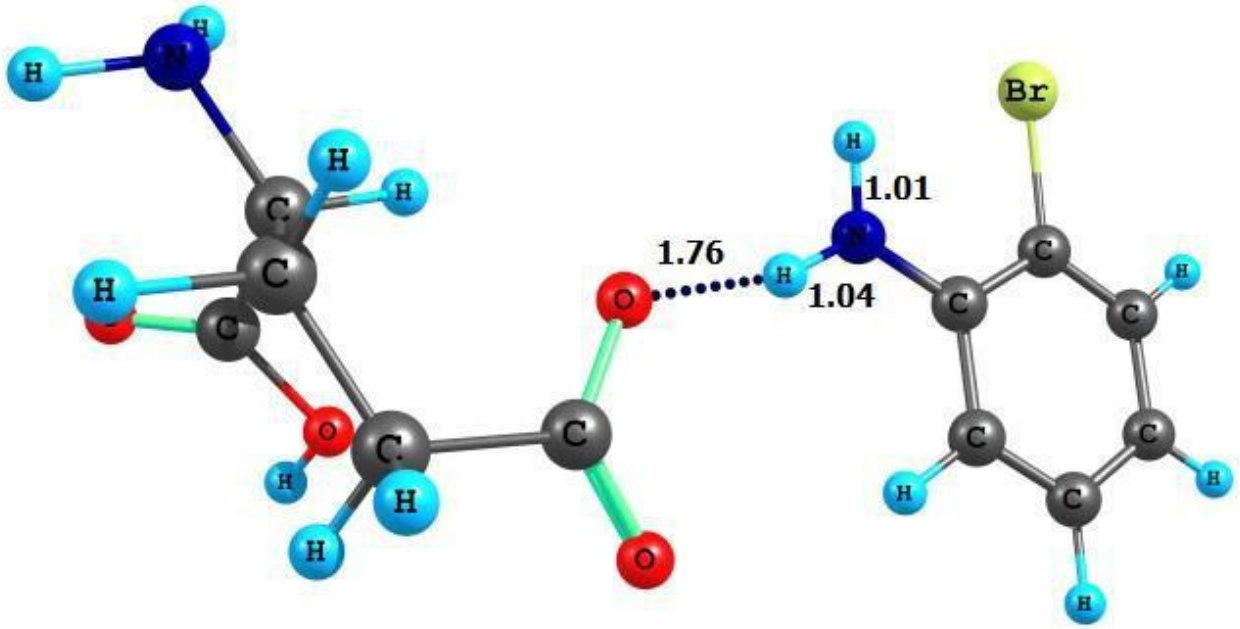
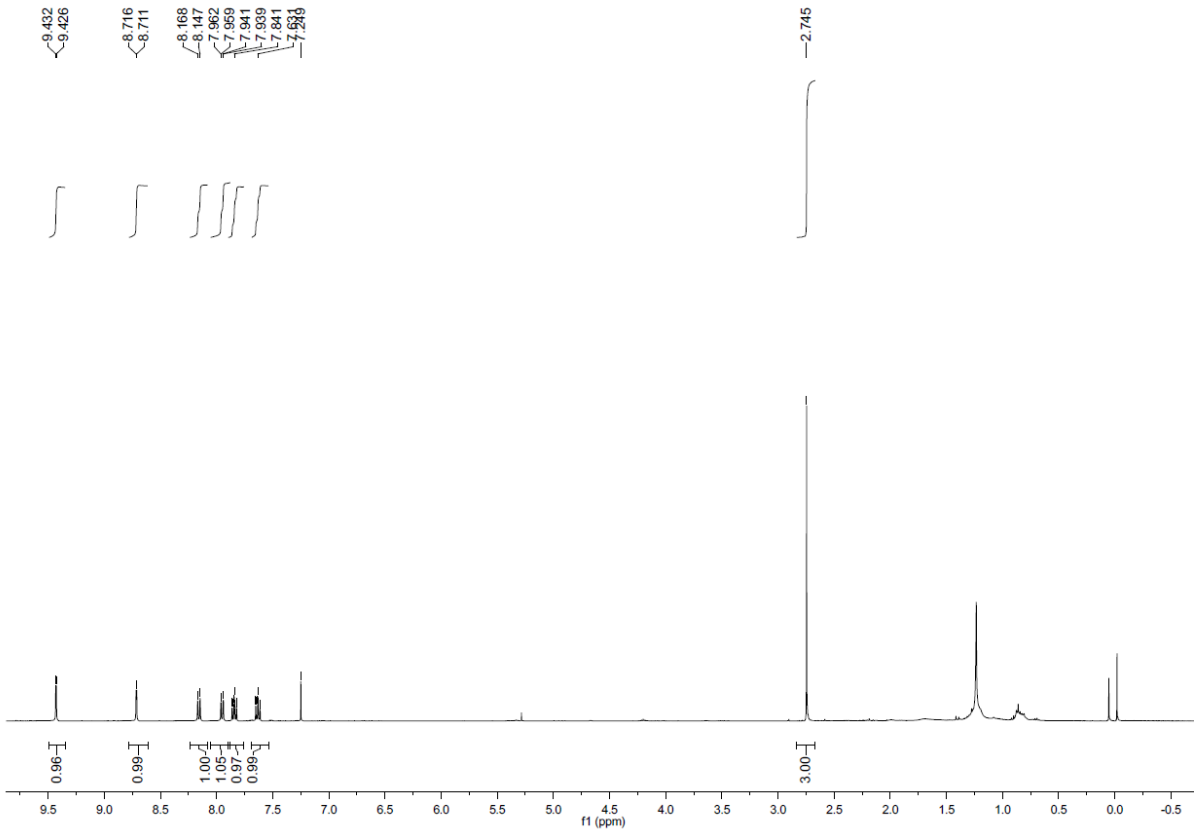
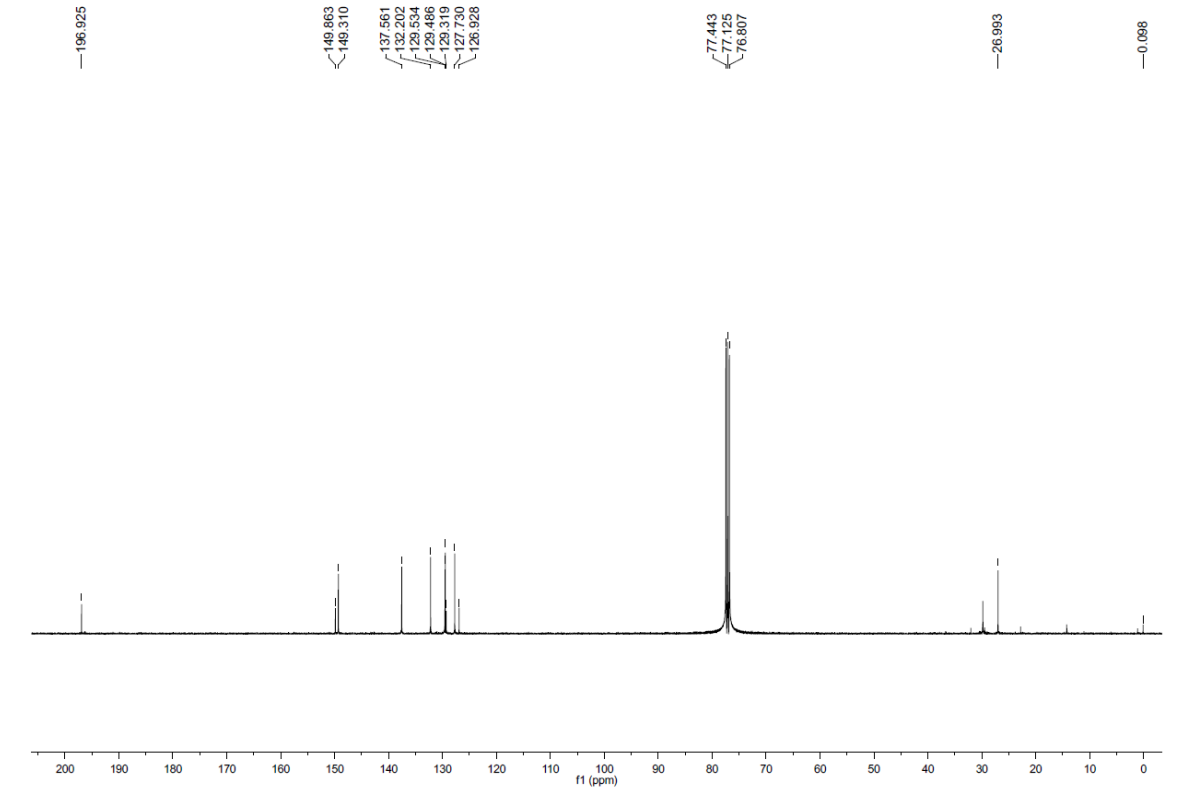


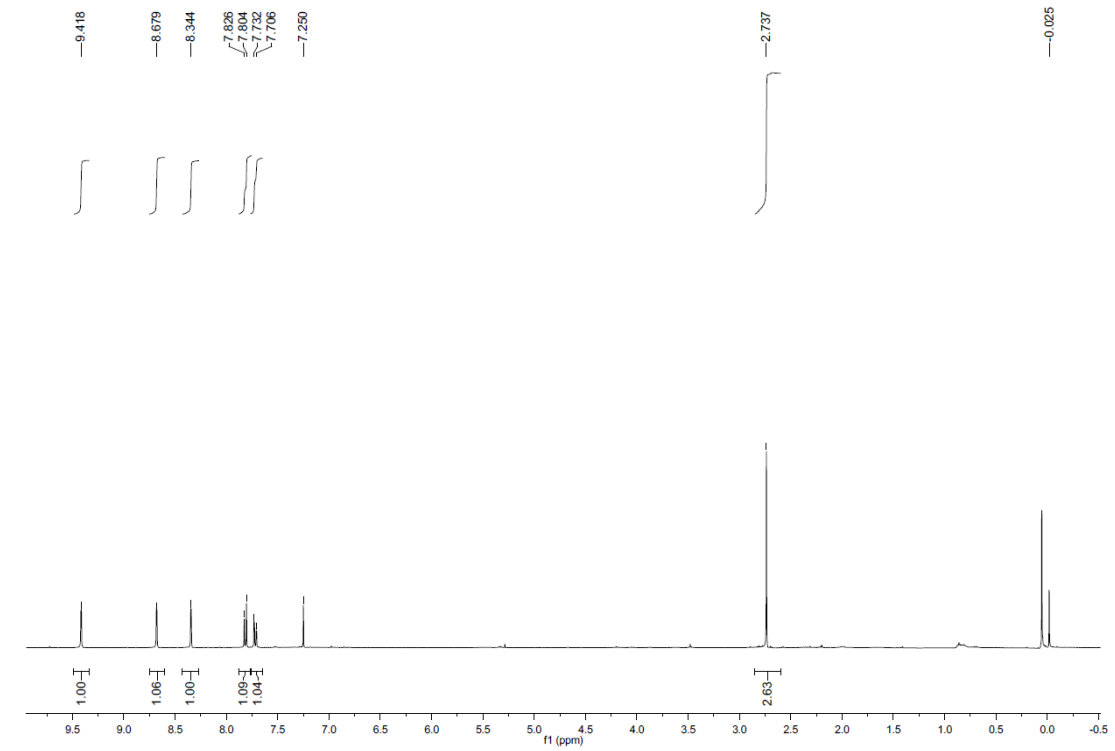
Figure 3: The optimized structure of model system comprised of 2-bromoaniline H-bonded to a glutamic acid. Key bond distances are given in Å. 2-bromoaniline aquires a negative charge density of -0.062 due to formation of strong H-bonding with the glutamic acid residue.

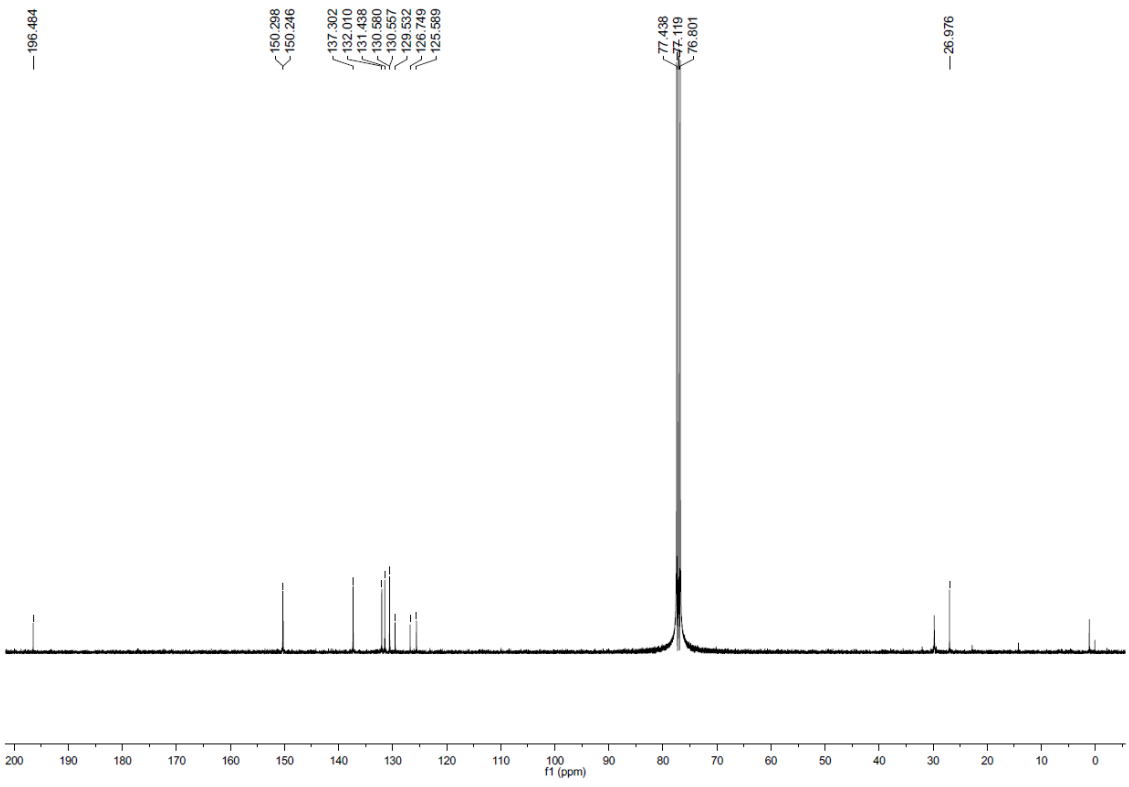
^{1}H and ^{13}C-NMR spectra of 3a



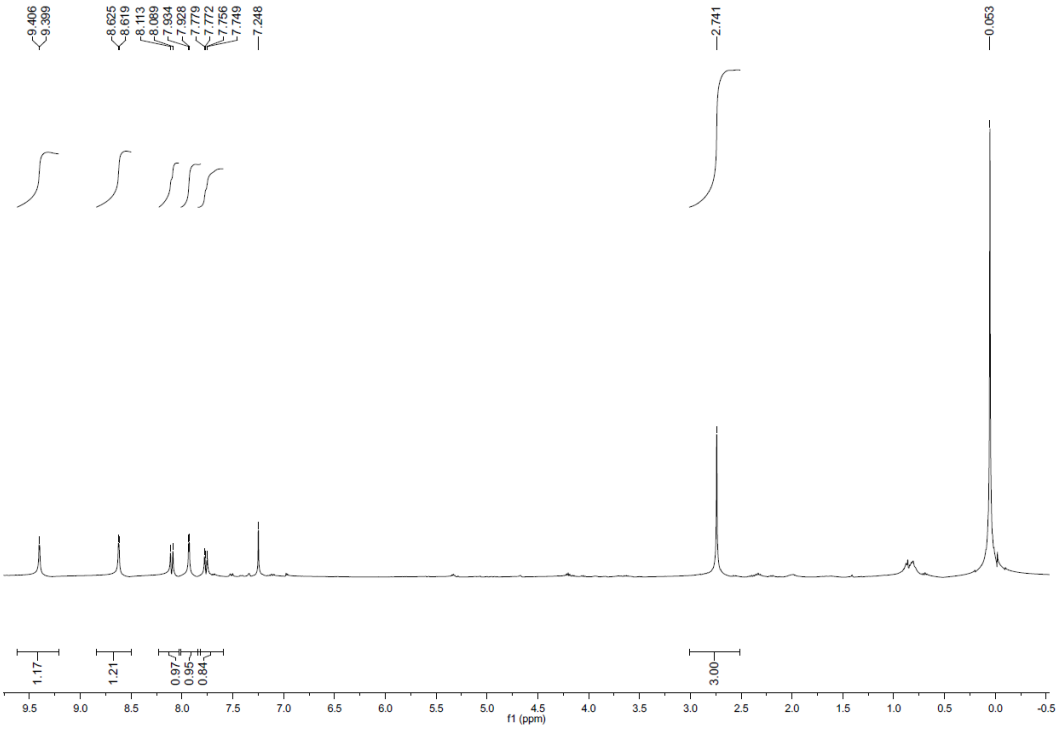


^{1}H and ^{13}C-NMR spectra of 3b

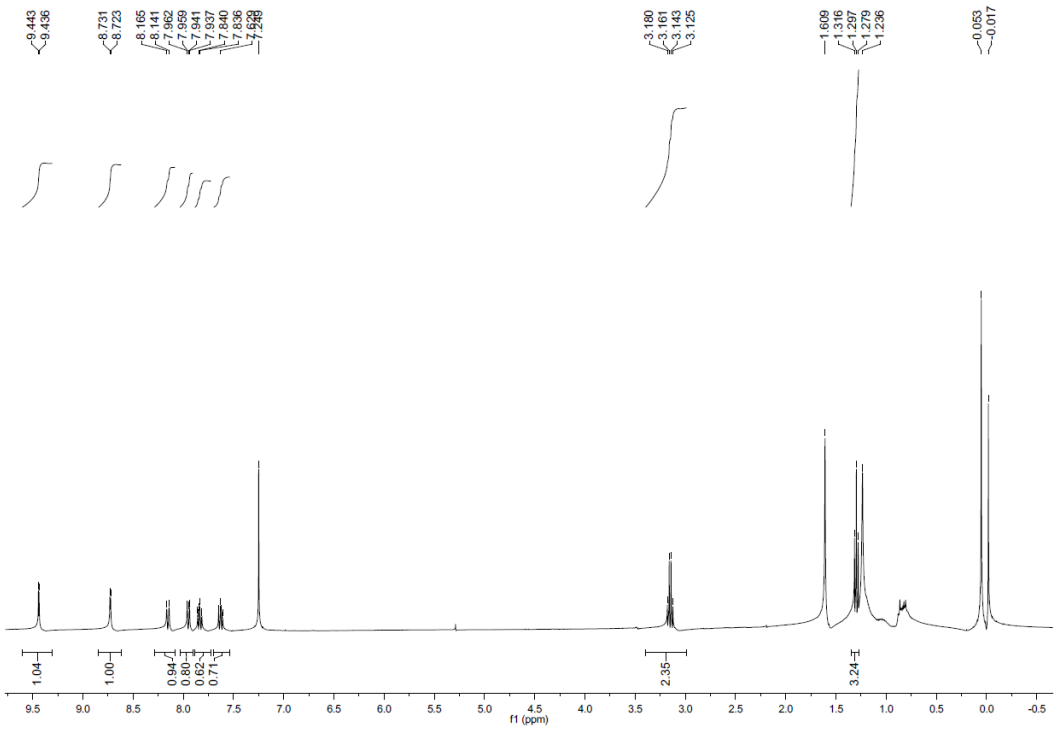




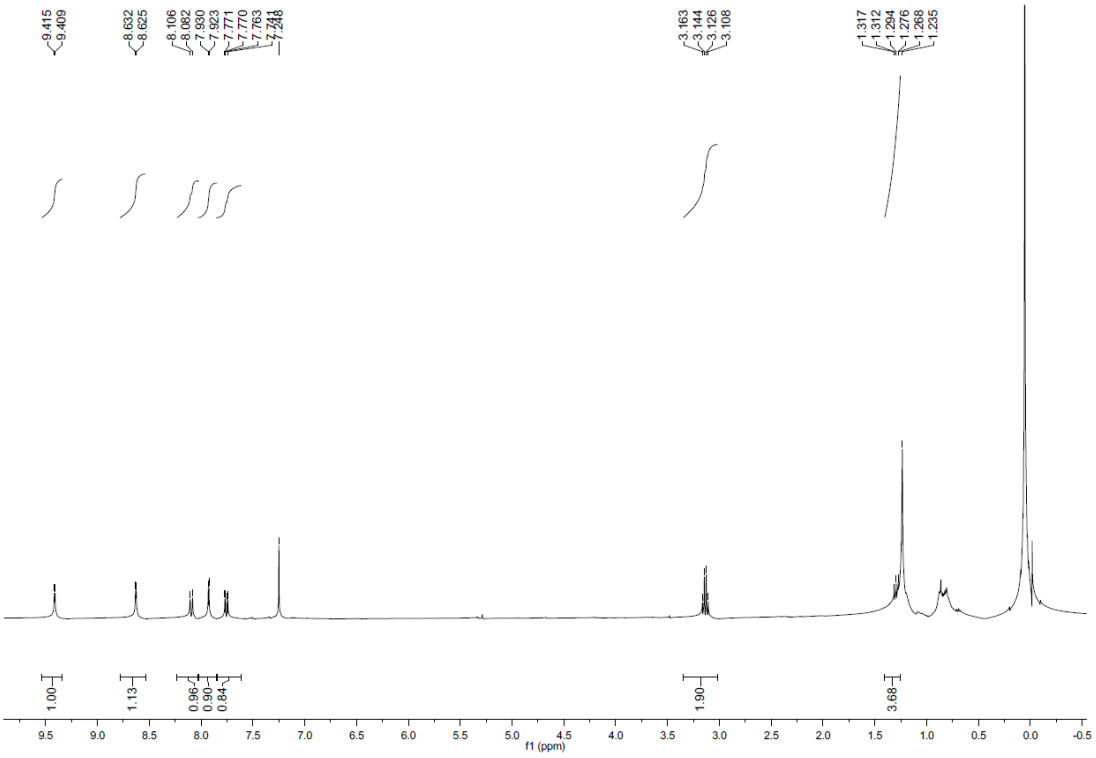
^{1}H spectra of 3c



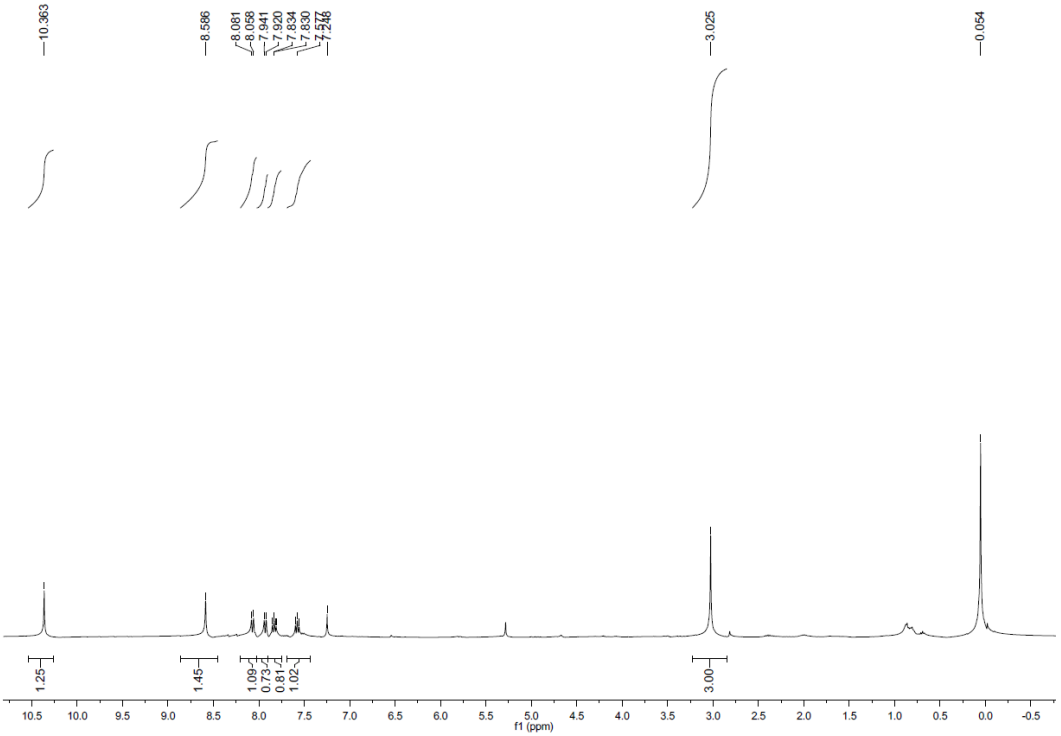
^{1}H spectra of 3e



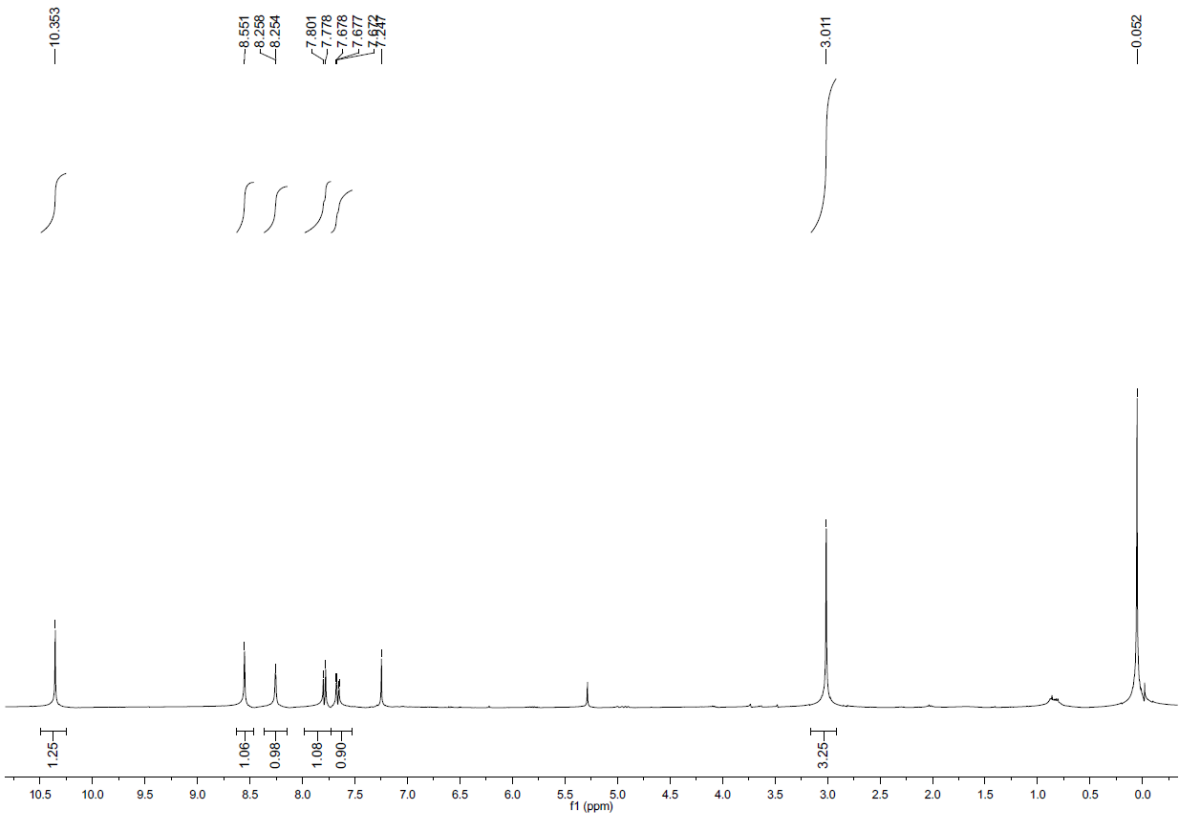
^{1}H spectra of 3f



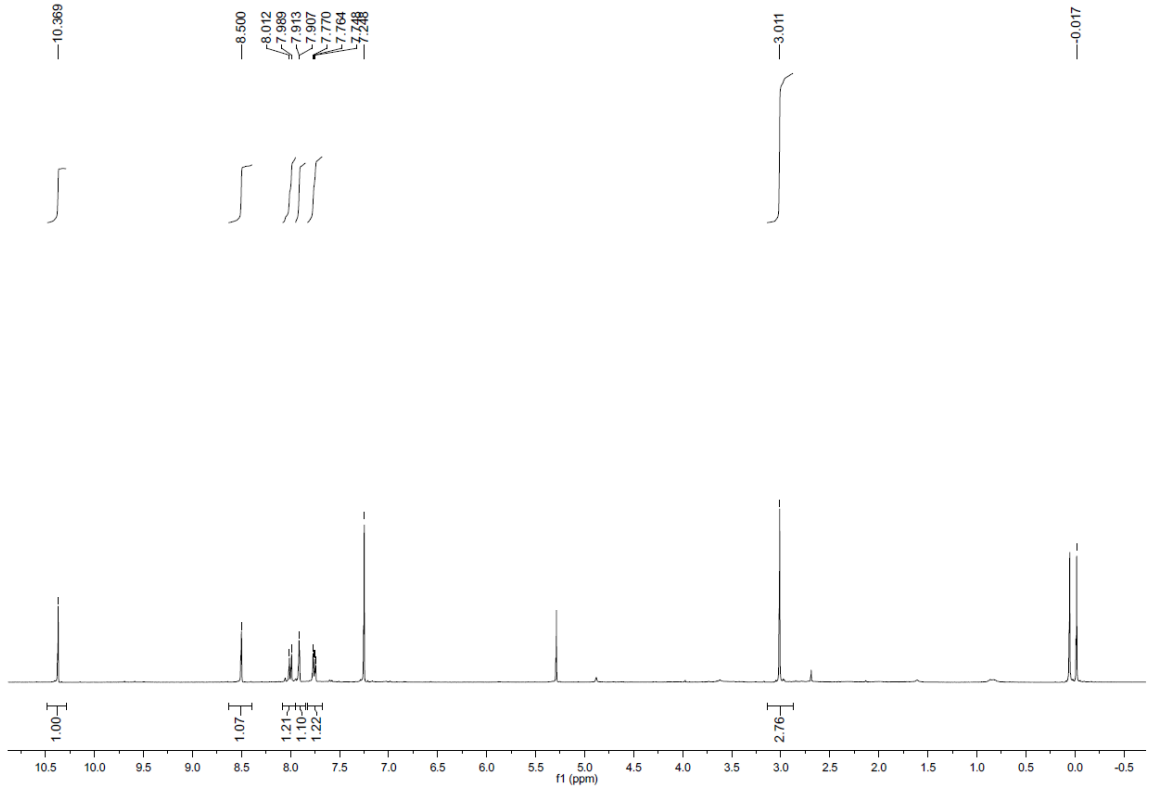
^{1}H spectra of 3g

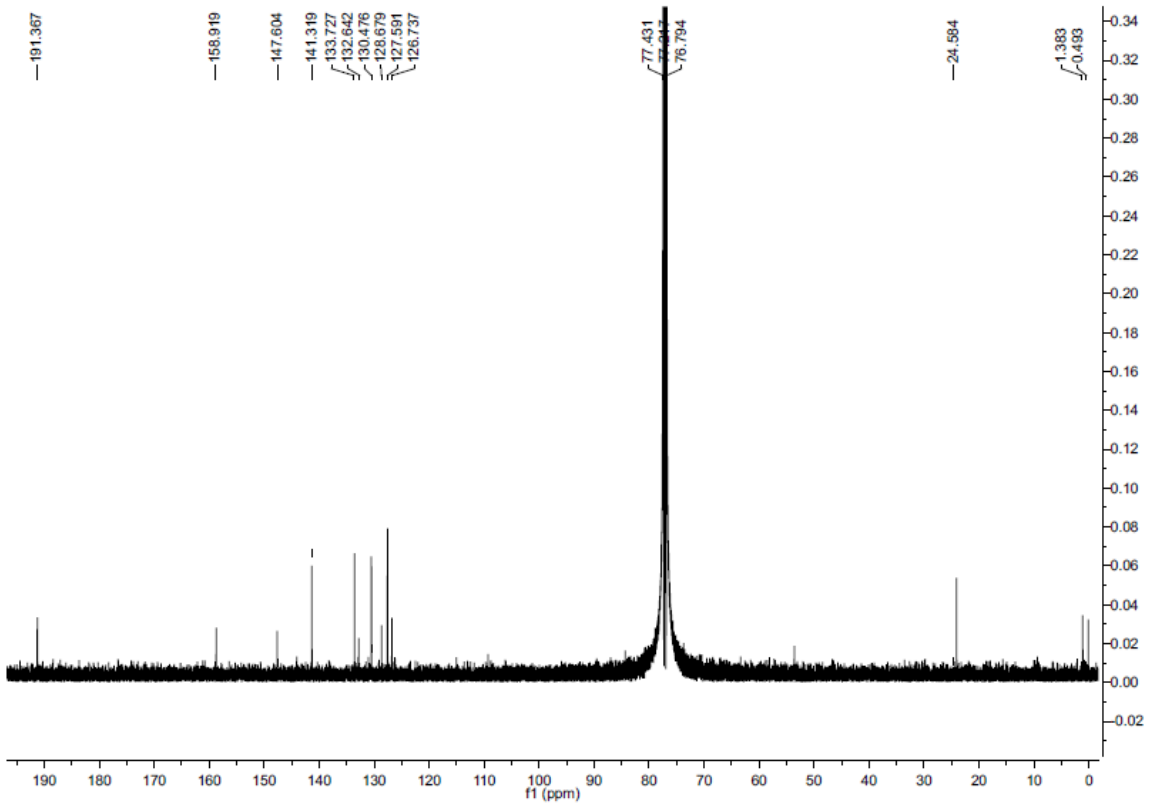


^{1}H spectra of 3h



^{1}H and ^{13}C spectra of 3i

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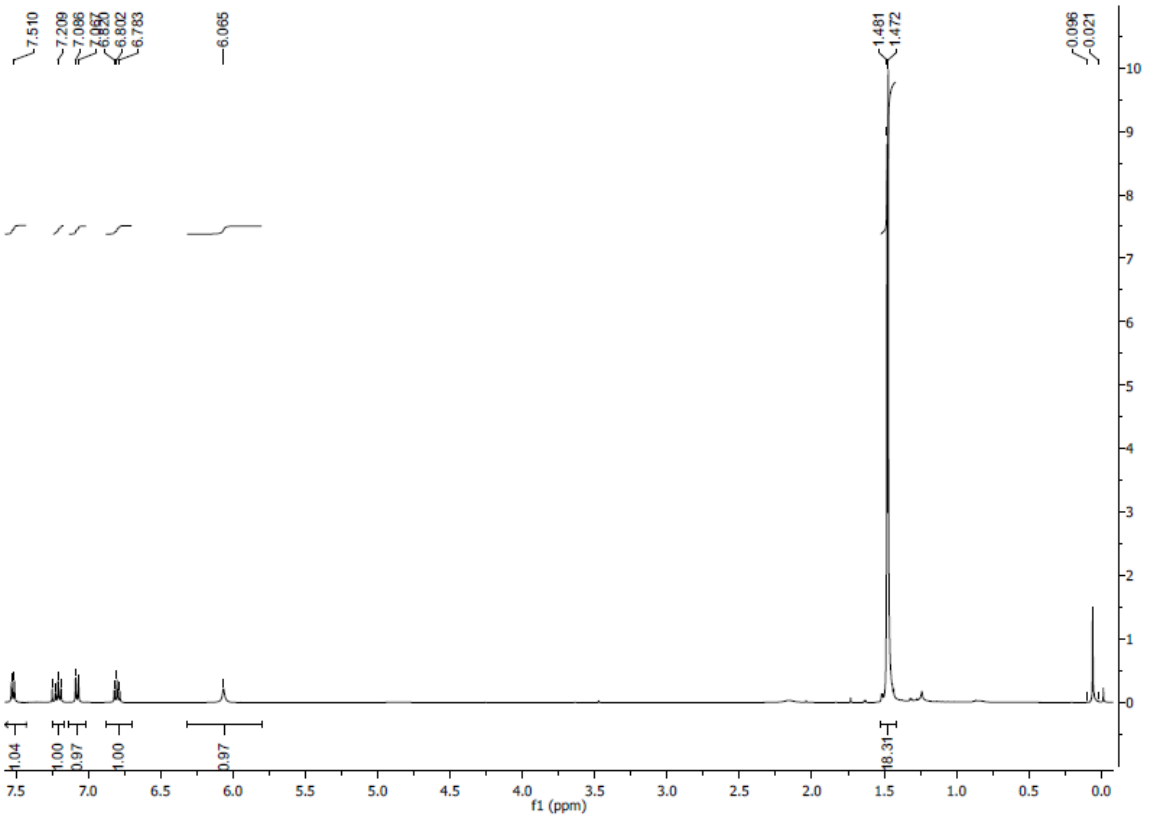
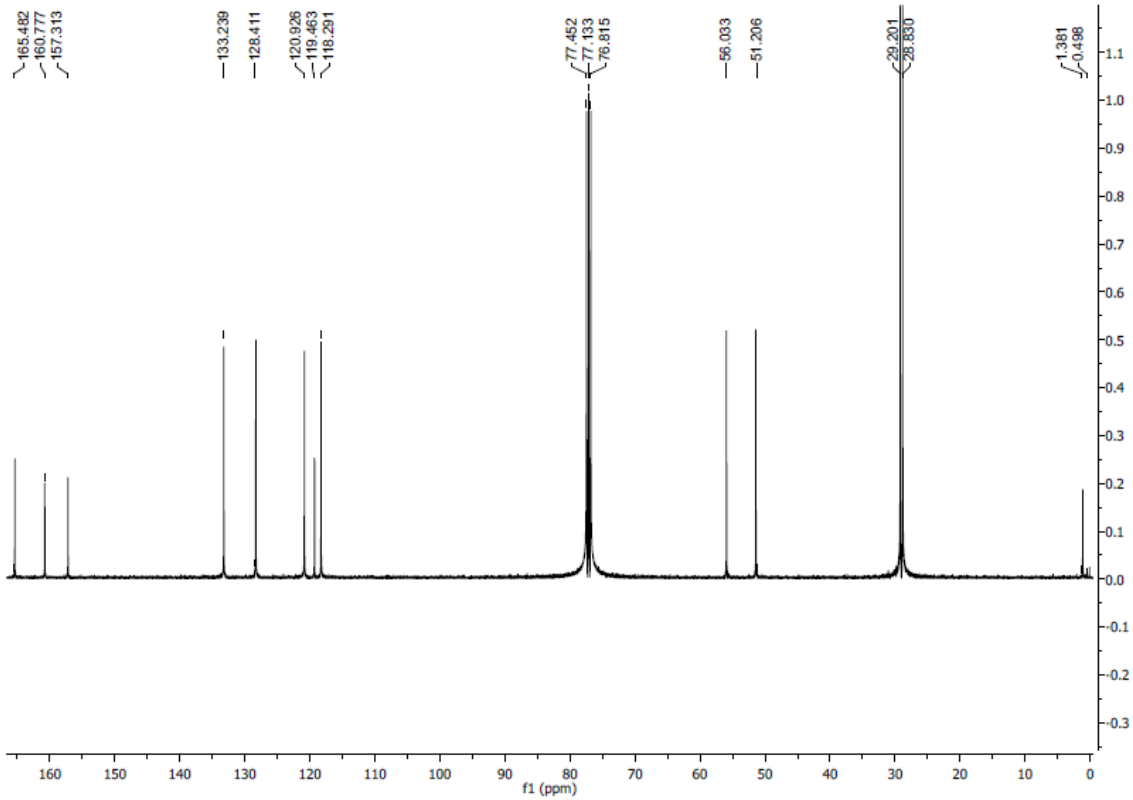
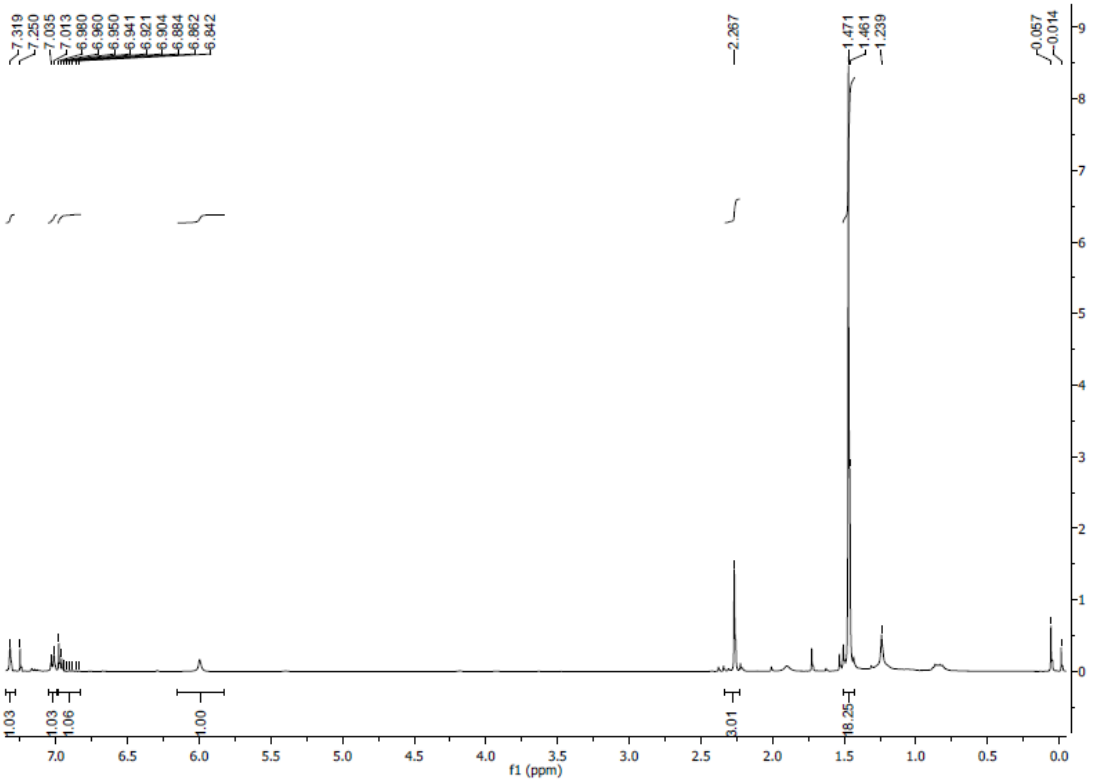
158.919
147.604
141.319
133.727
132.642
130.476
128.679
127.591
126.737
77.431
77.047
76.794
24.584
1.383
0.493
f1 (ppm)

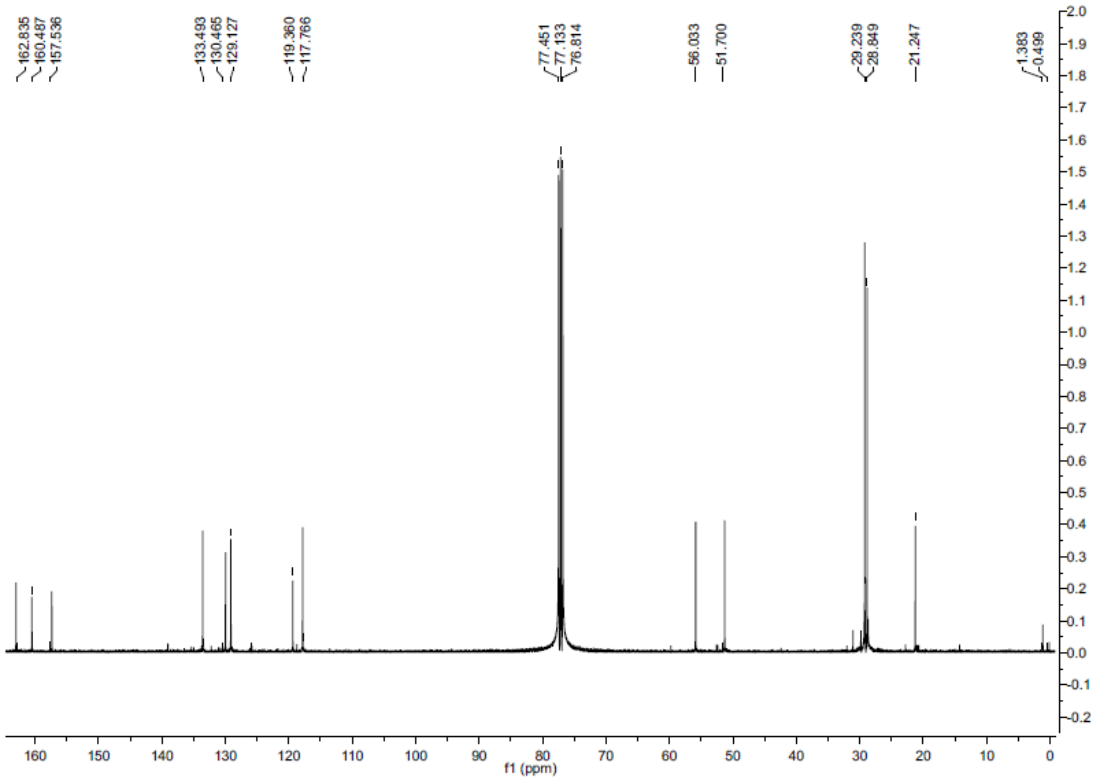
^{1}H and ^{13}C-NMR spectra of 5a



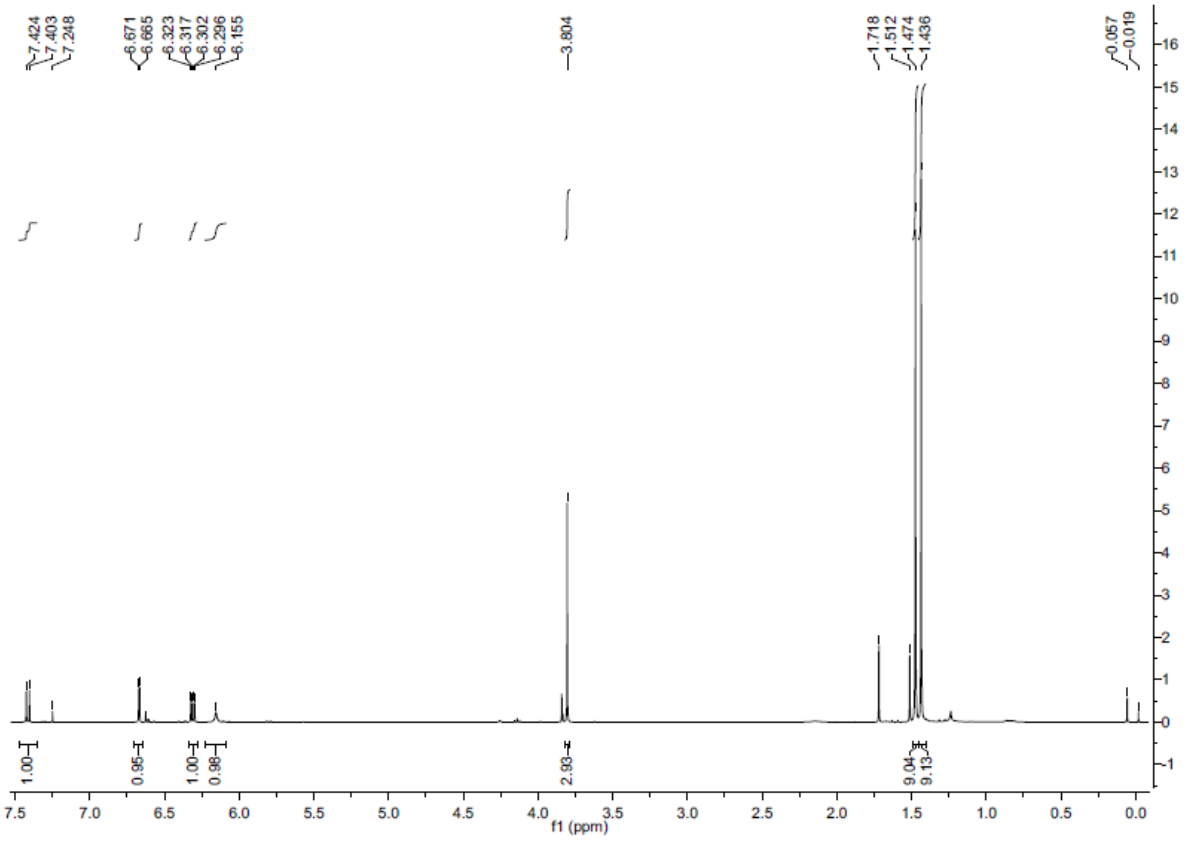


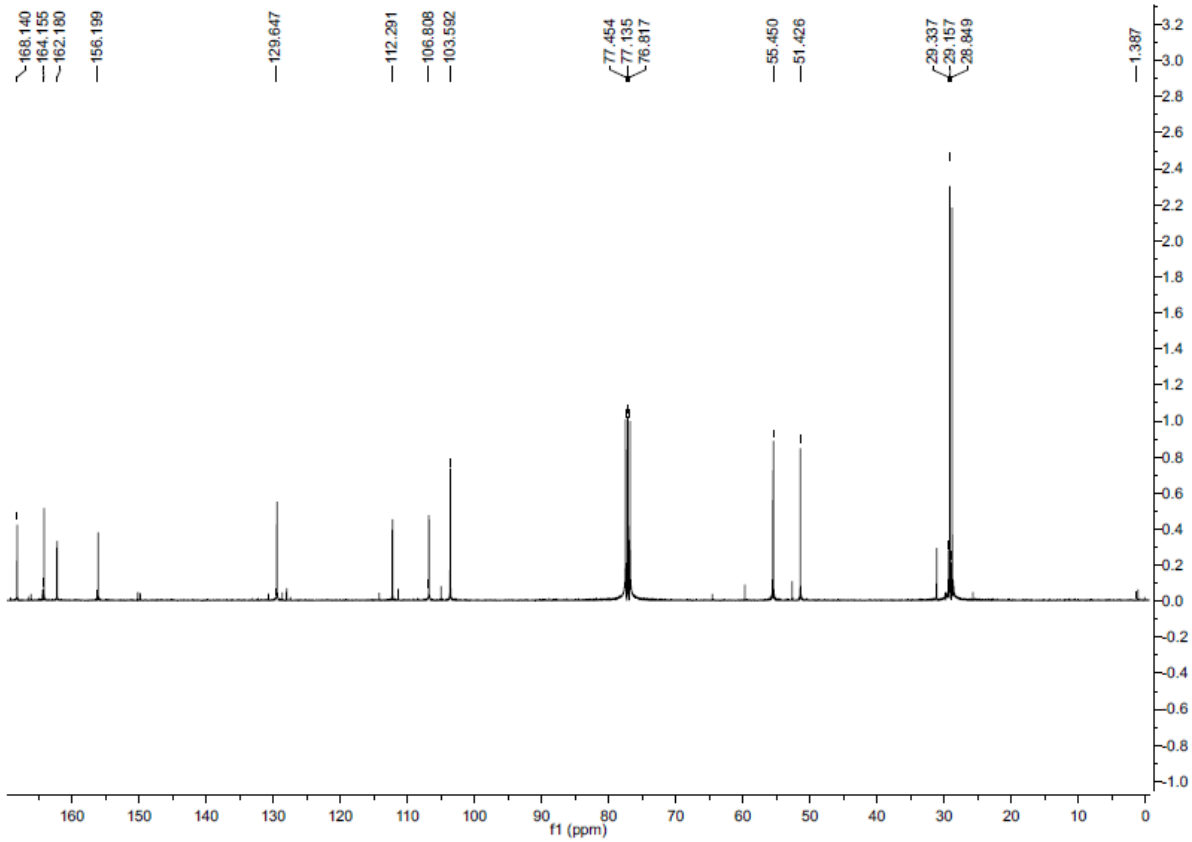
^{1}H and ^{13}C-NMR spectra of 5b



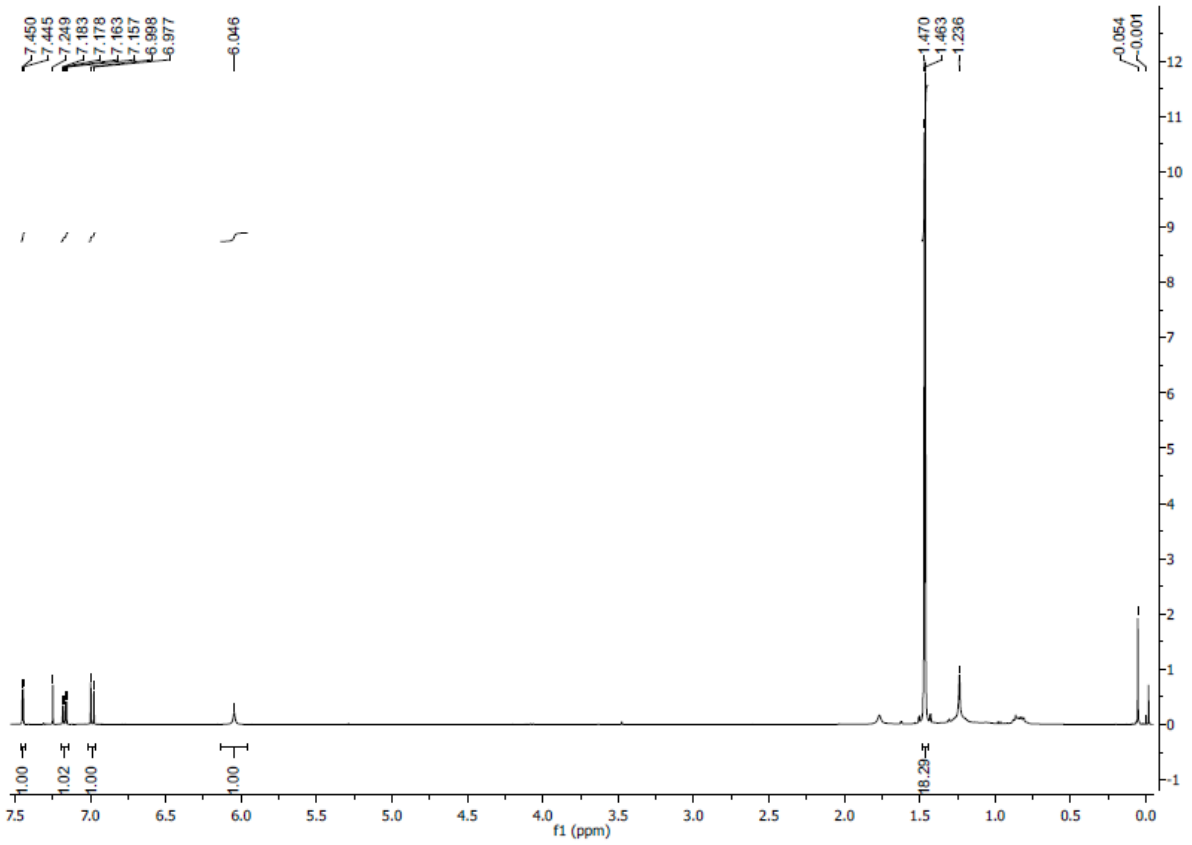


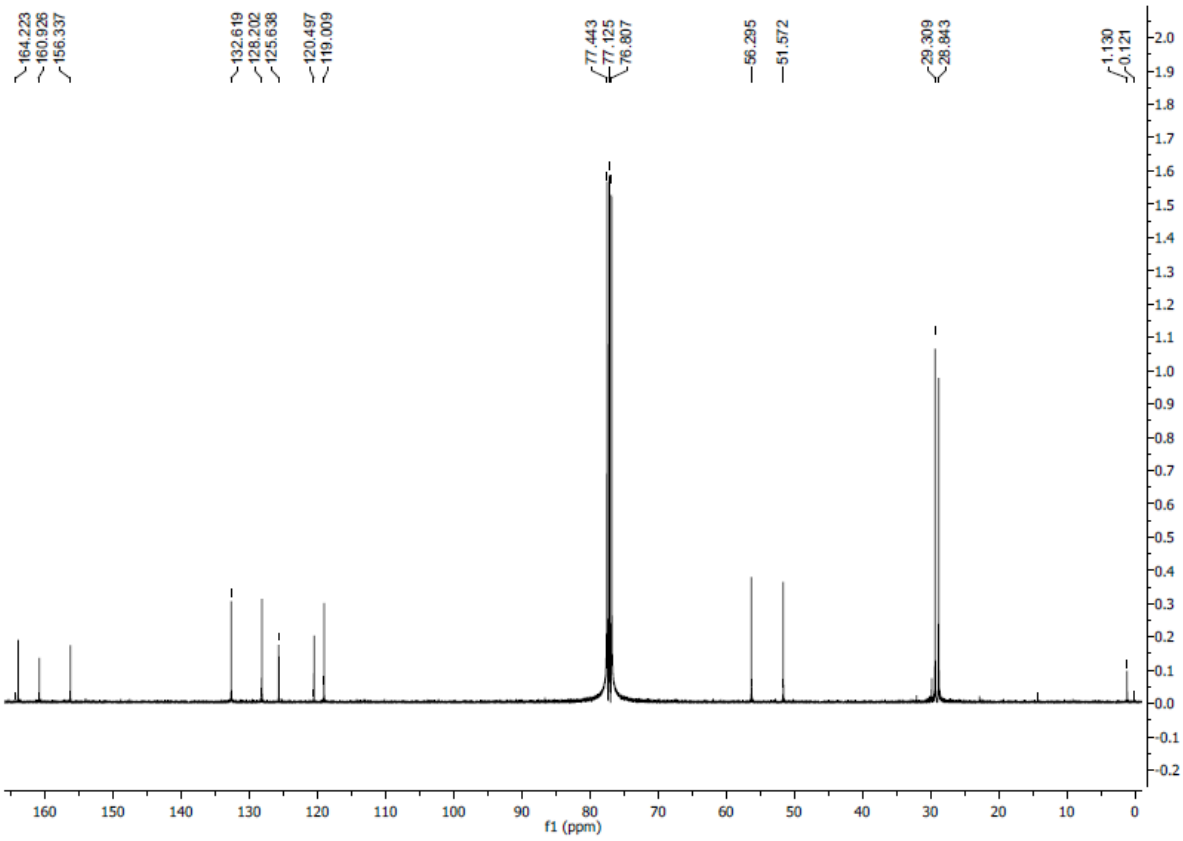
^{1}H and ^{13}C-NMR spectra of 5c



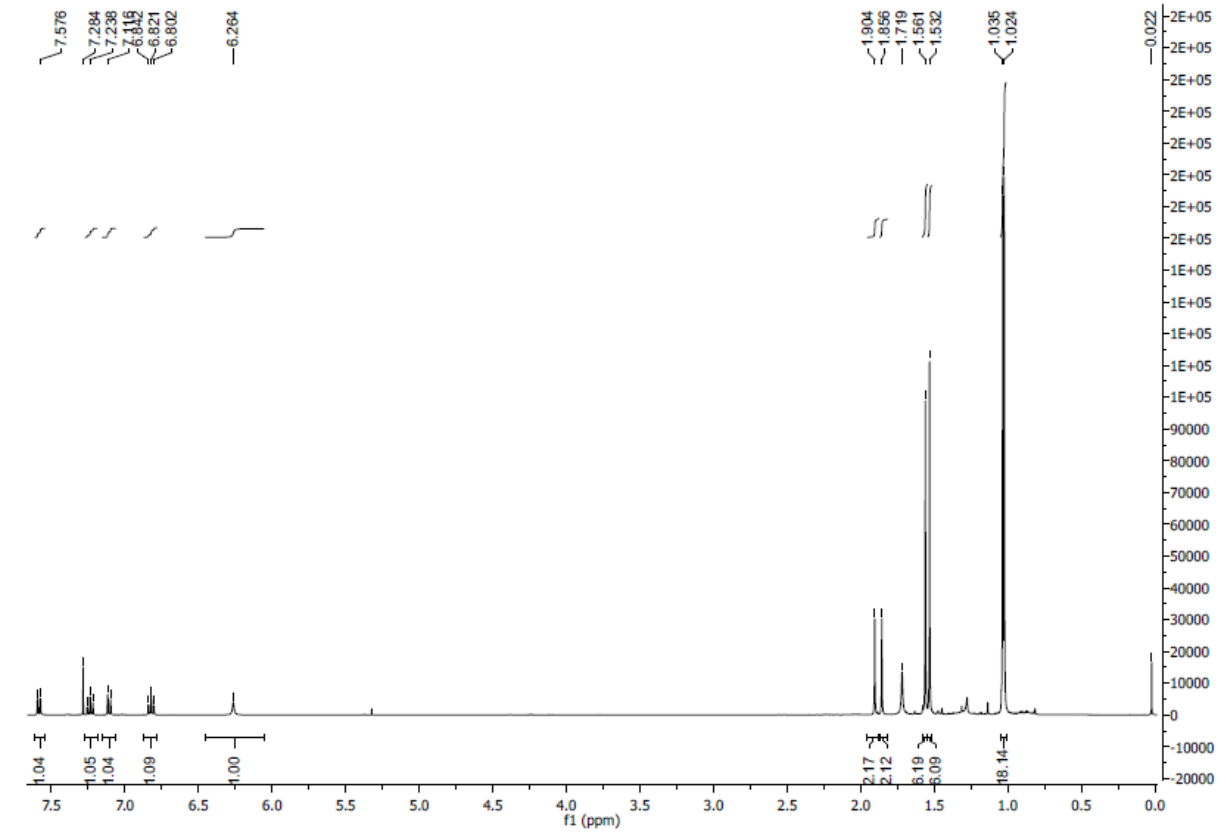


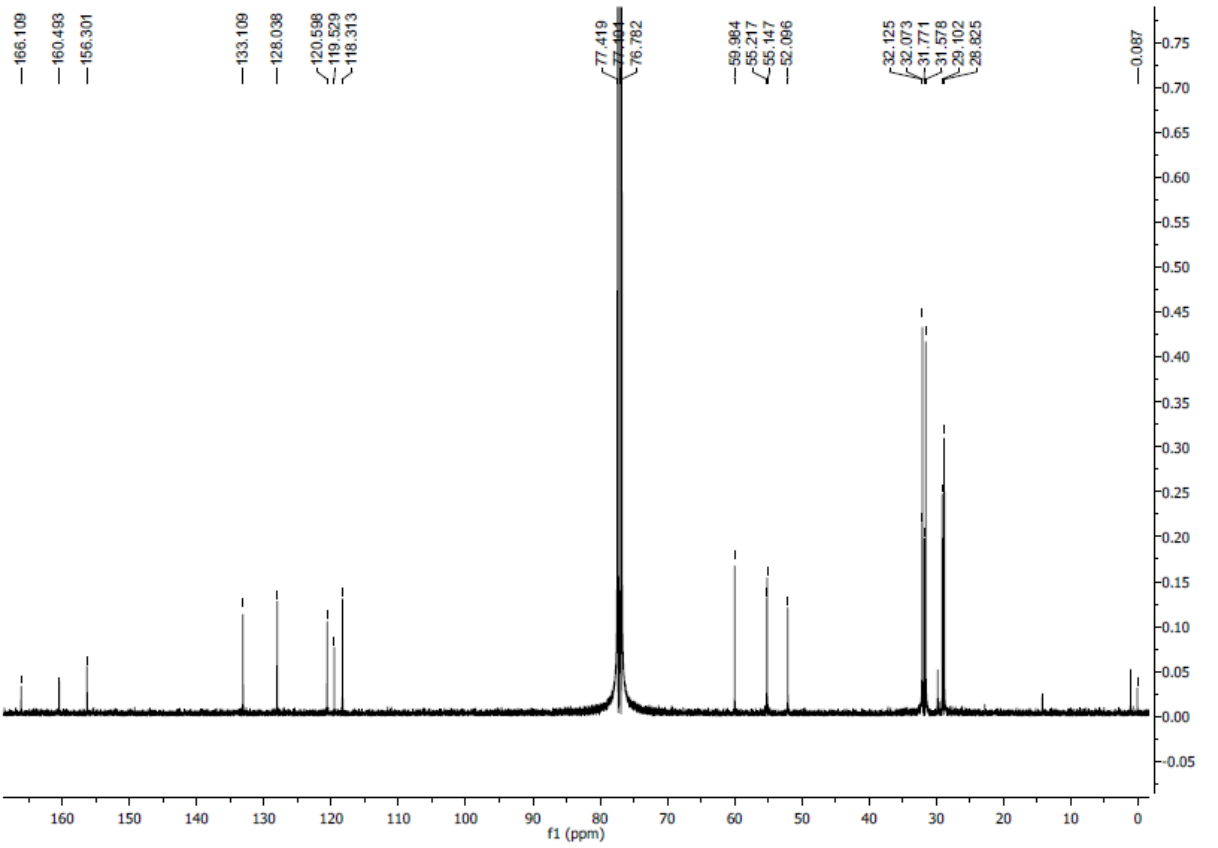
^{1}H and ^{13}C-NMR spectra of 5d



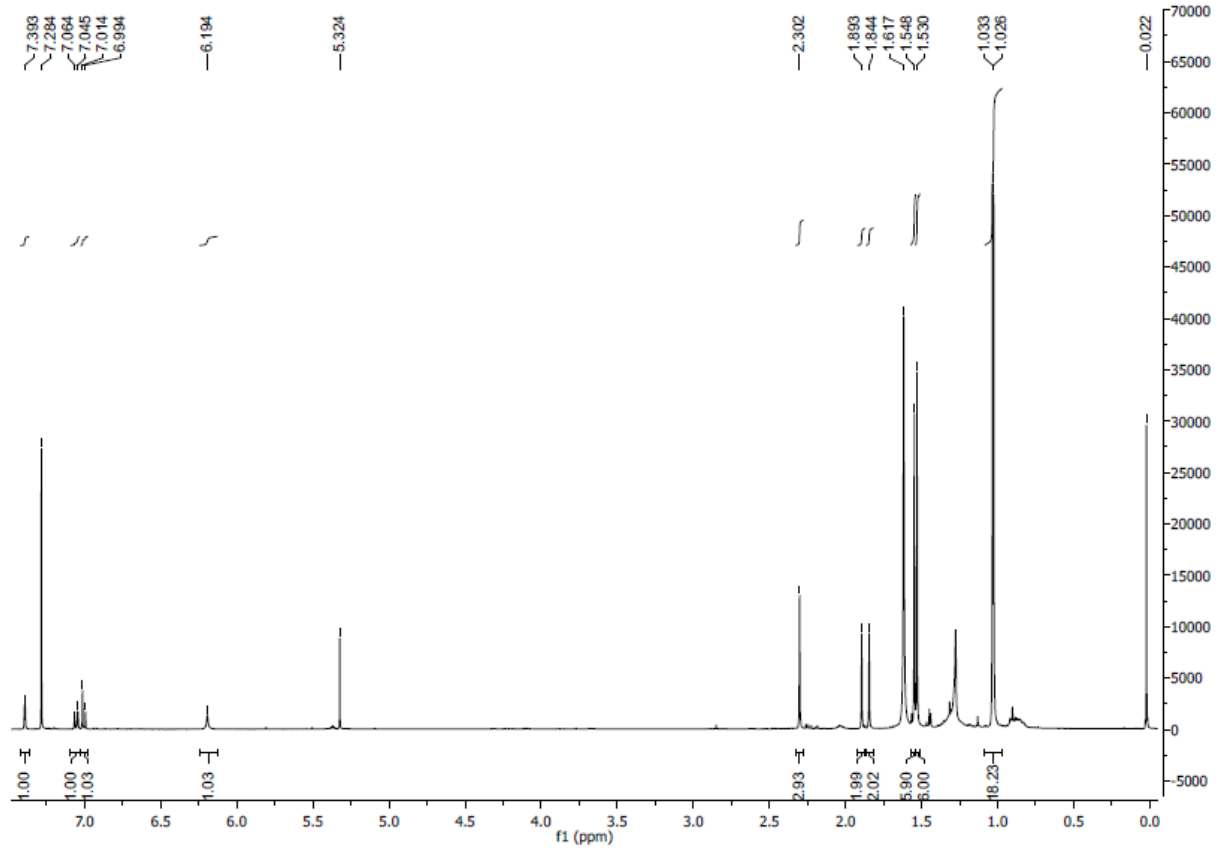


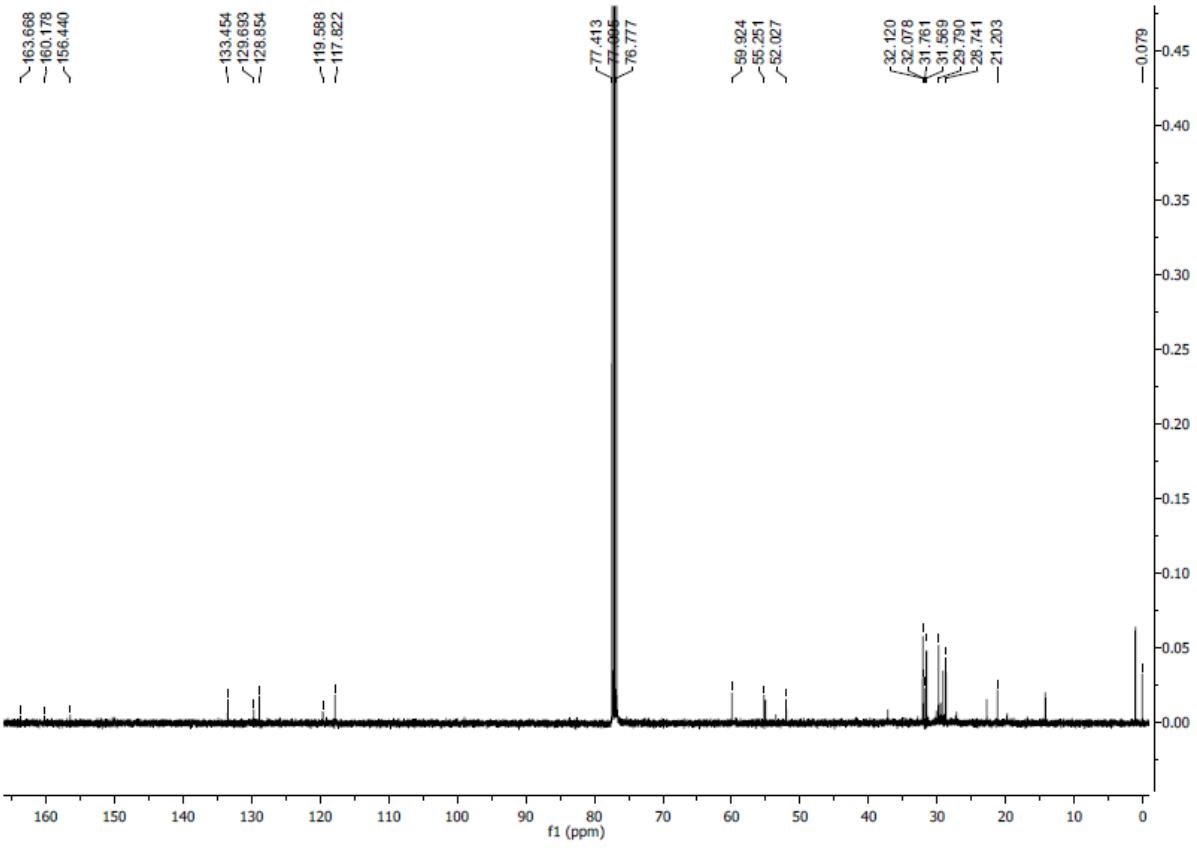
^{1}H and ^{13}C-NMR spectra of 5e



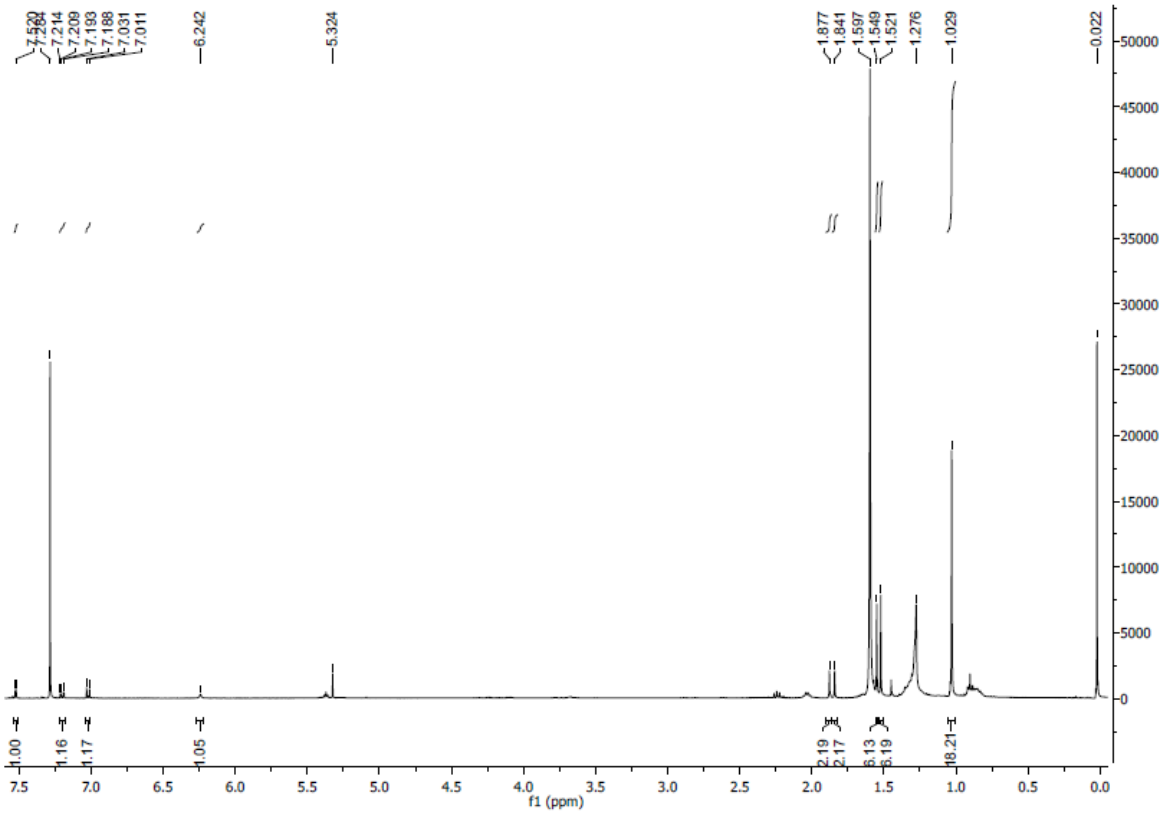


^{1}H and ^{13}C-NMR spectra of 5f

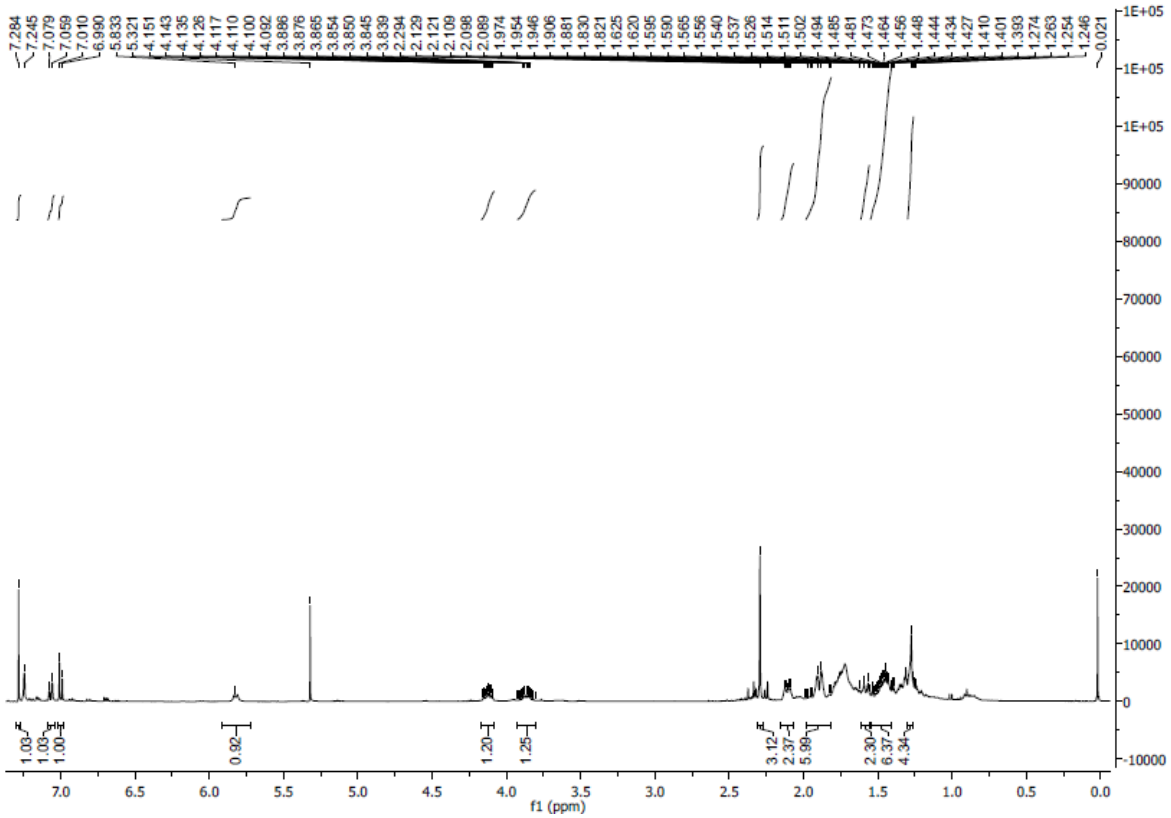


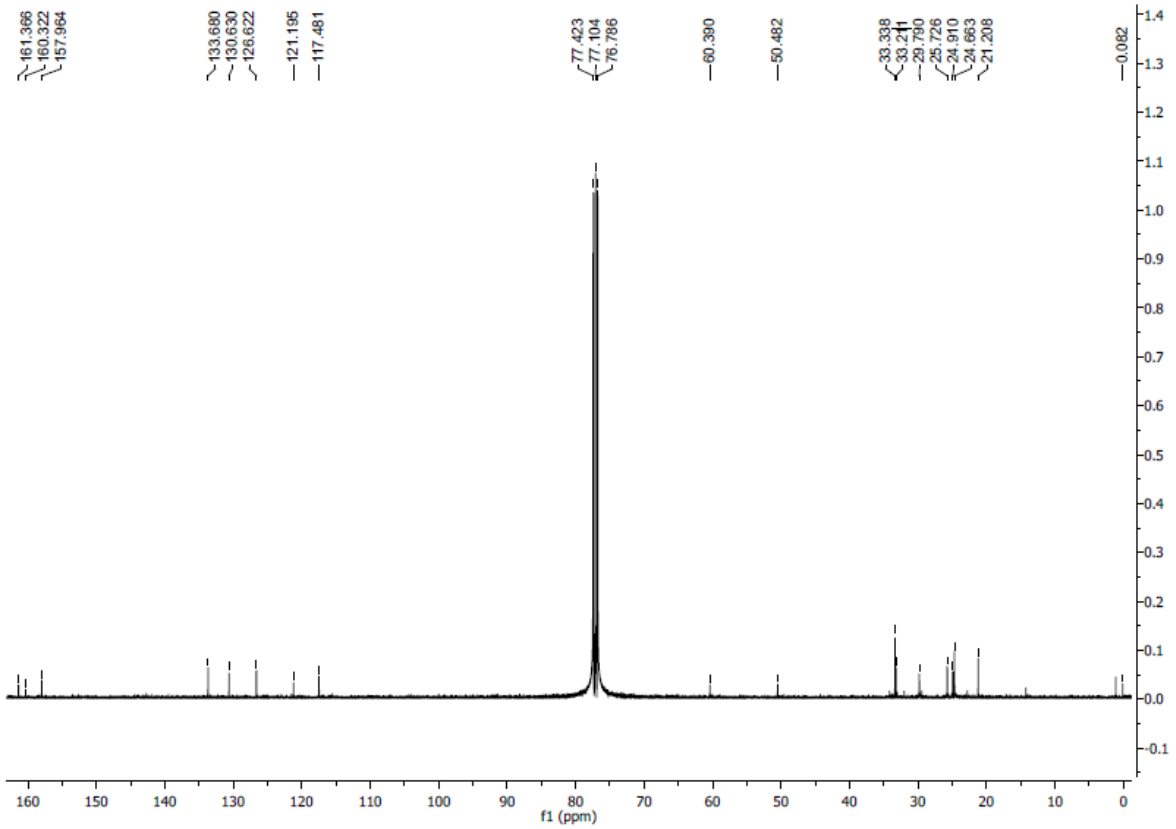


^{1}H spectra of 5g

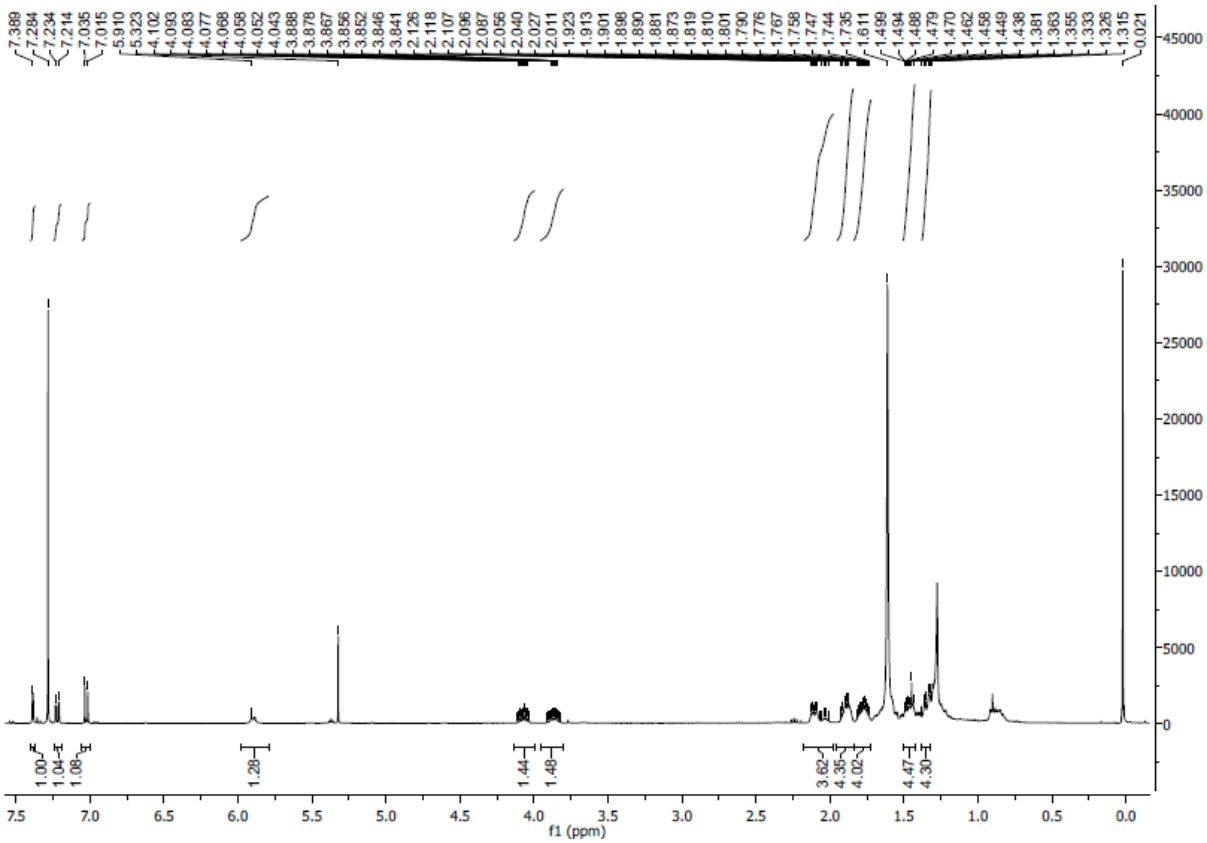


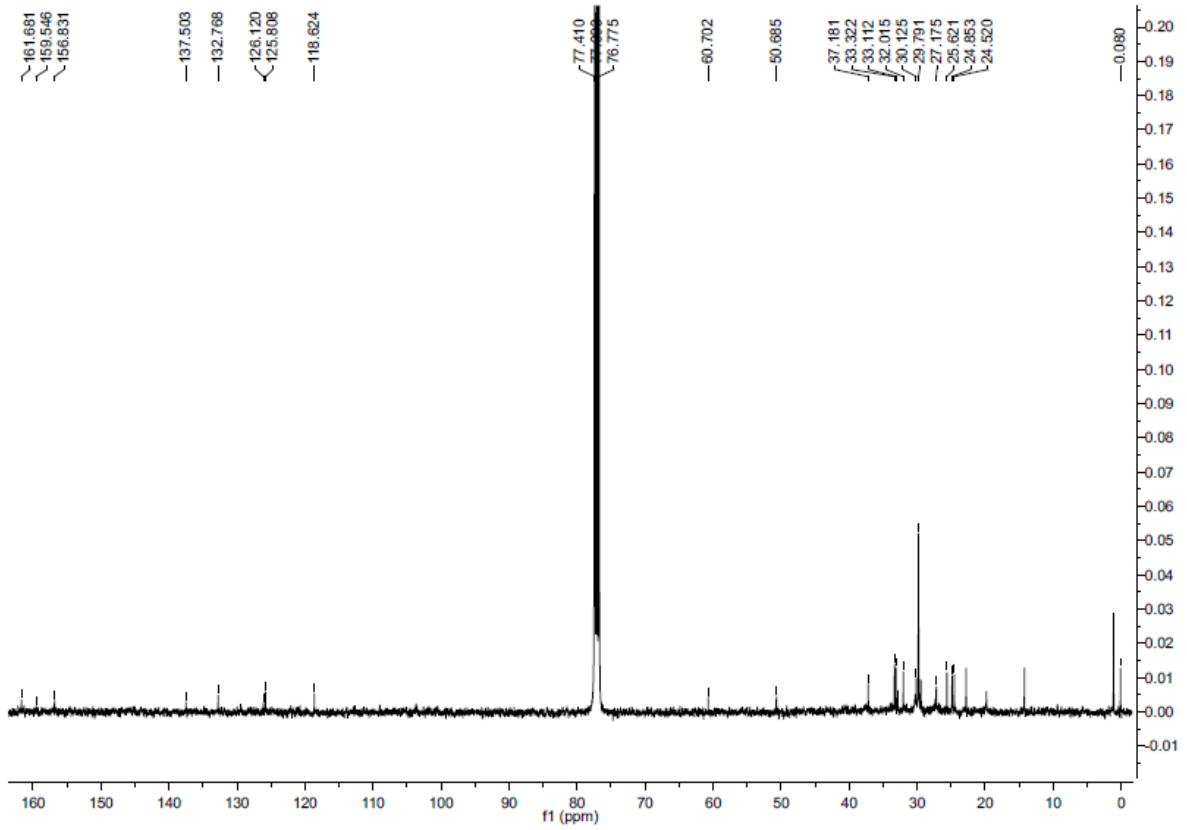
^{1}H and ^{13}C-NMR spectra of 5h



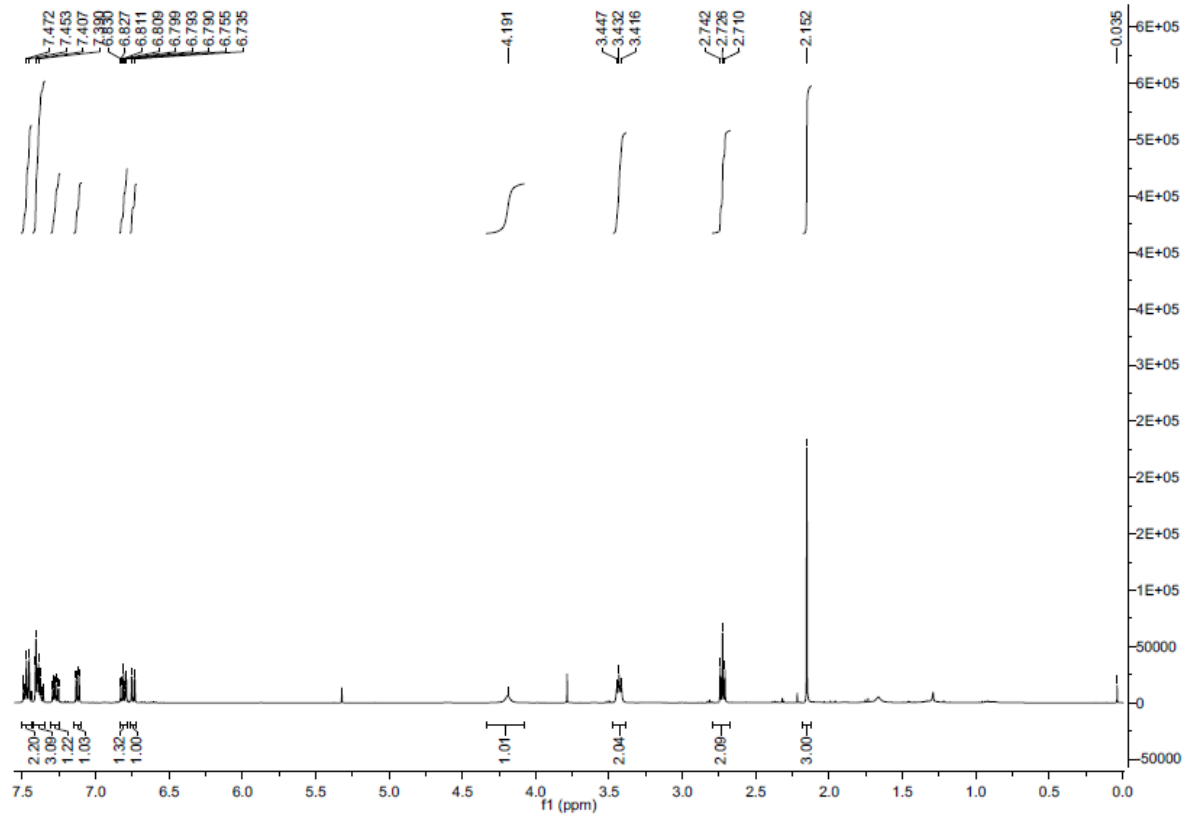


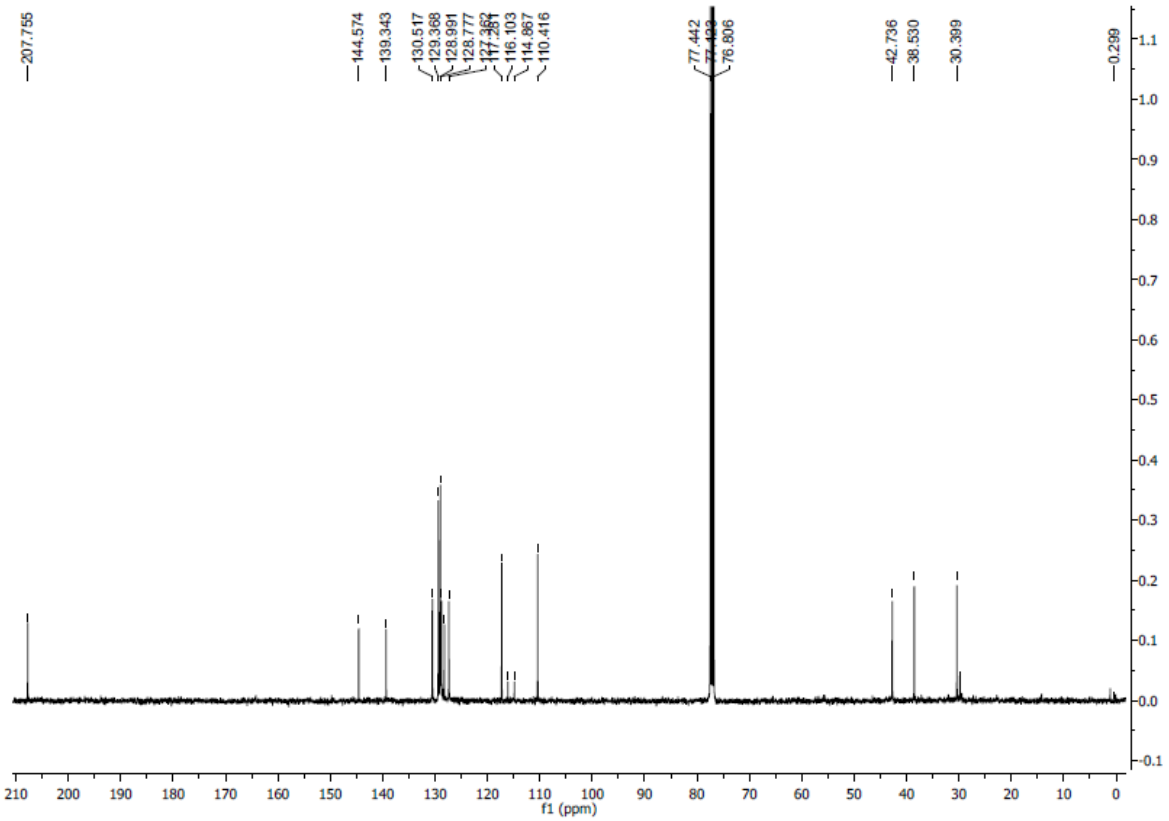
^{1}H and ^{13}C-NMR spectra of 5i



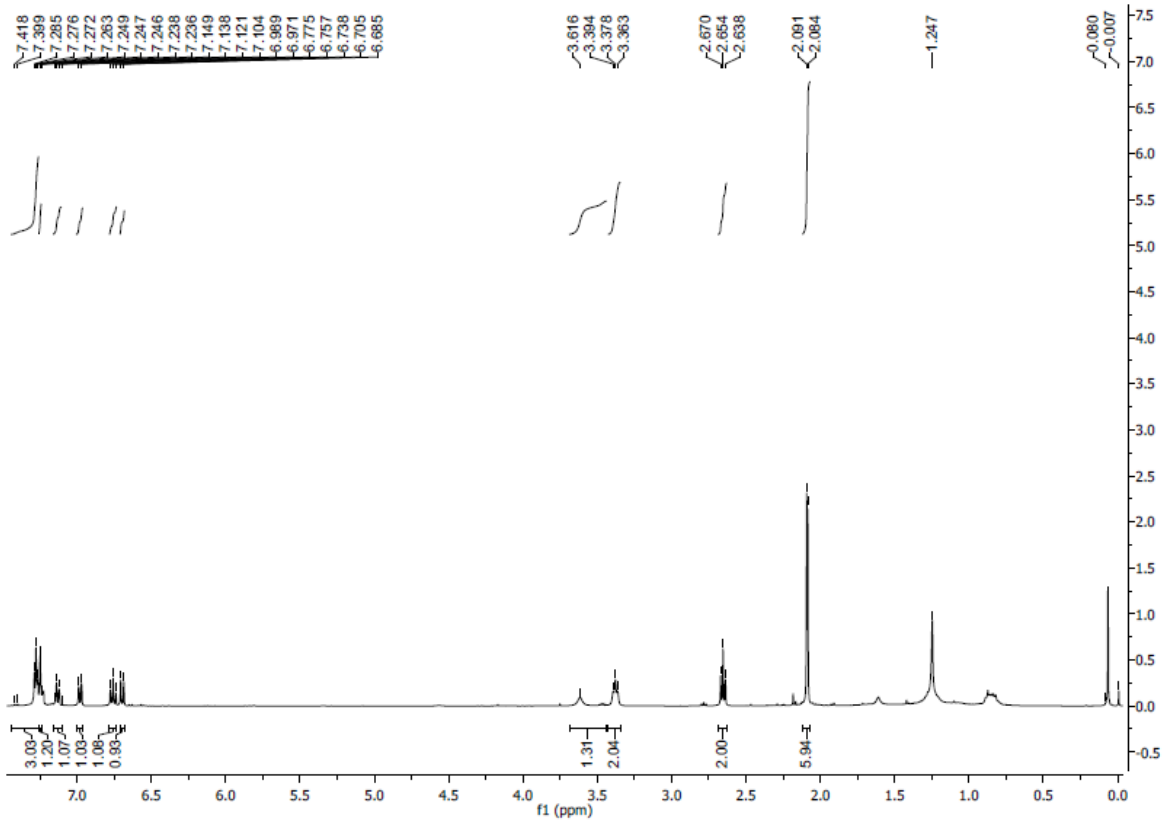


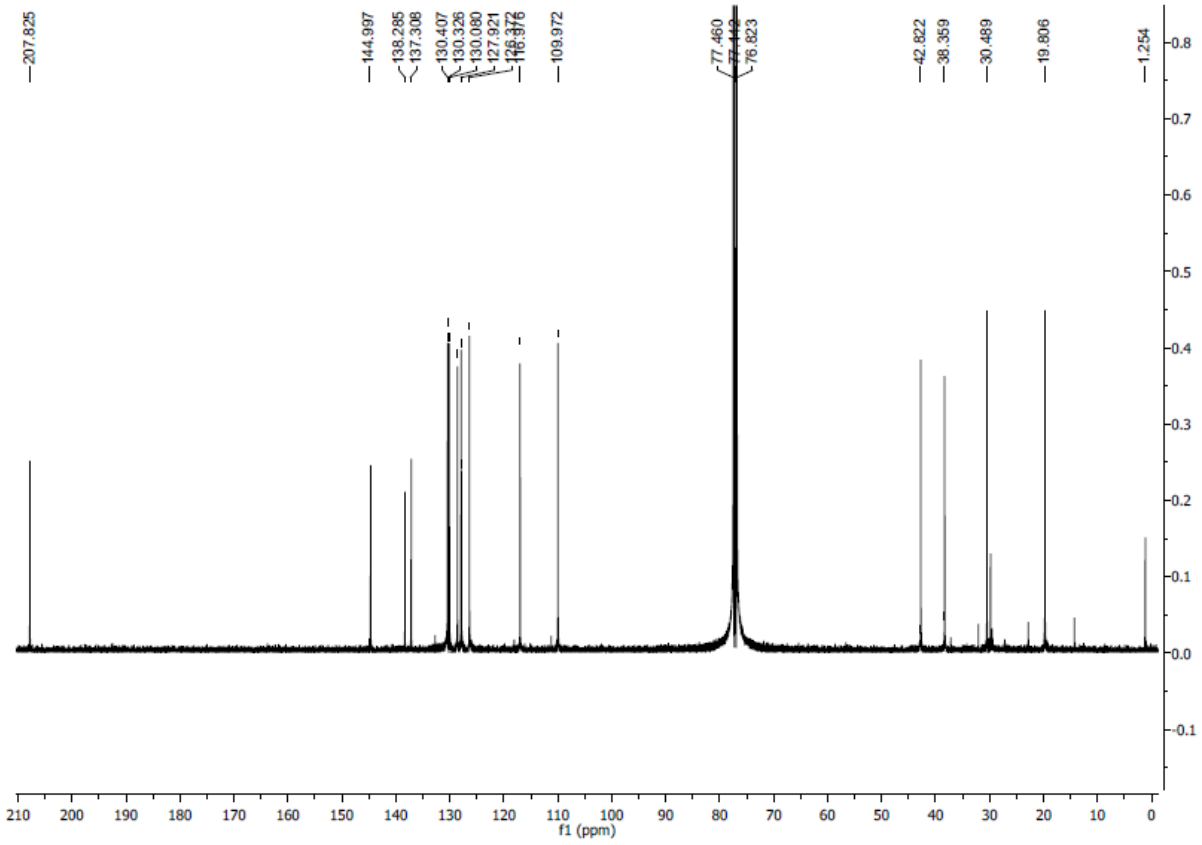
^{1}H and ^{13}C-NMR spectra of 6a



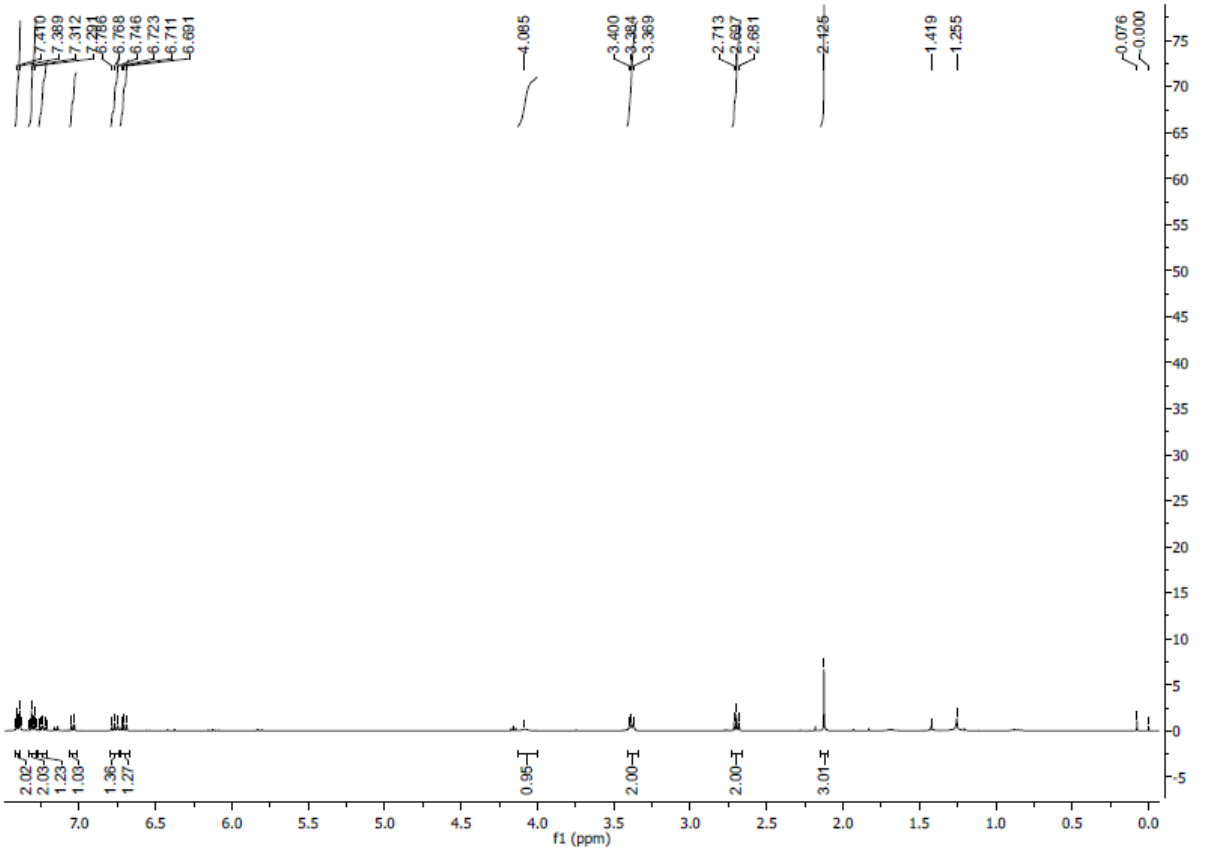


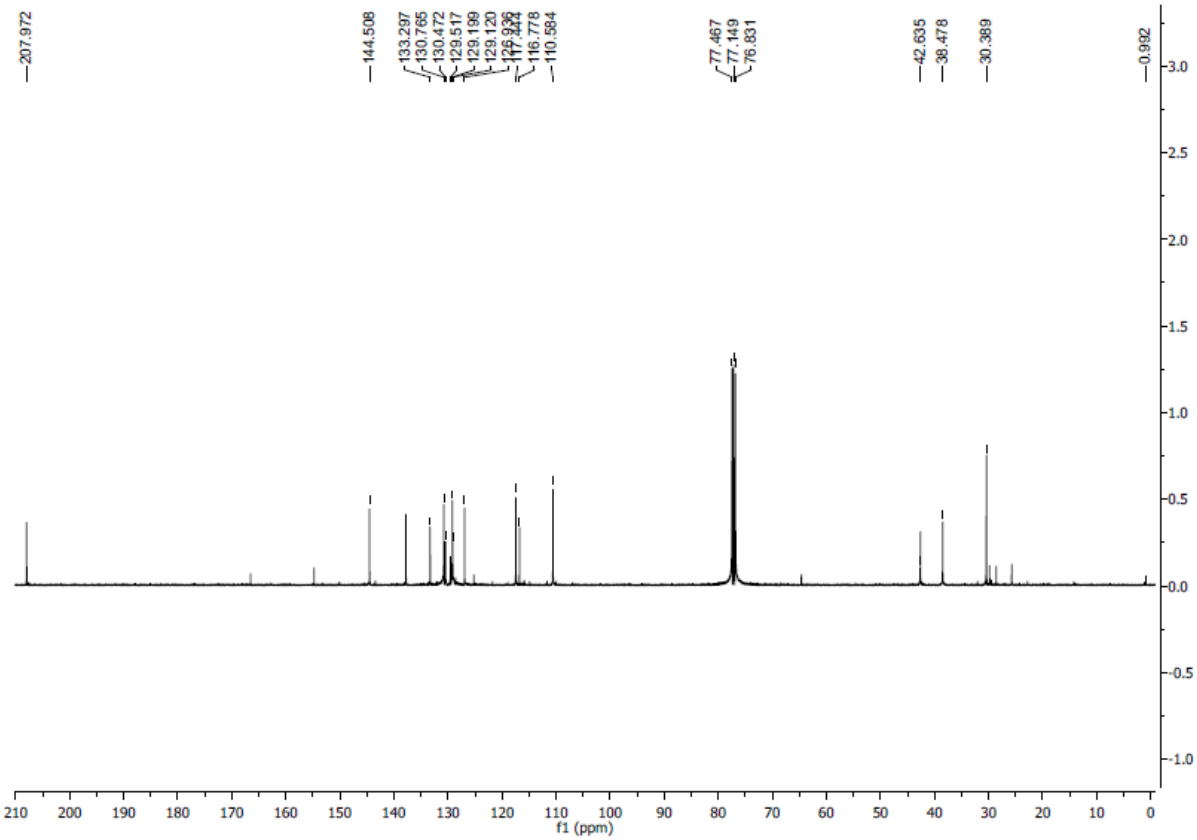
^{1}H and ^{13}C-NMR spectra of 6b



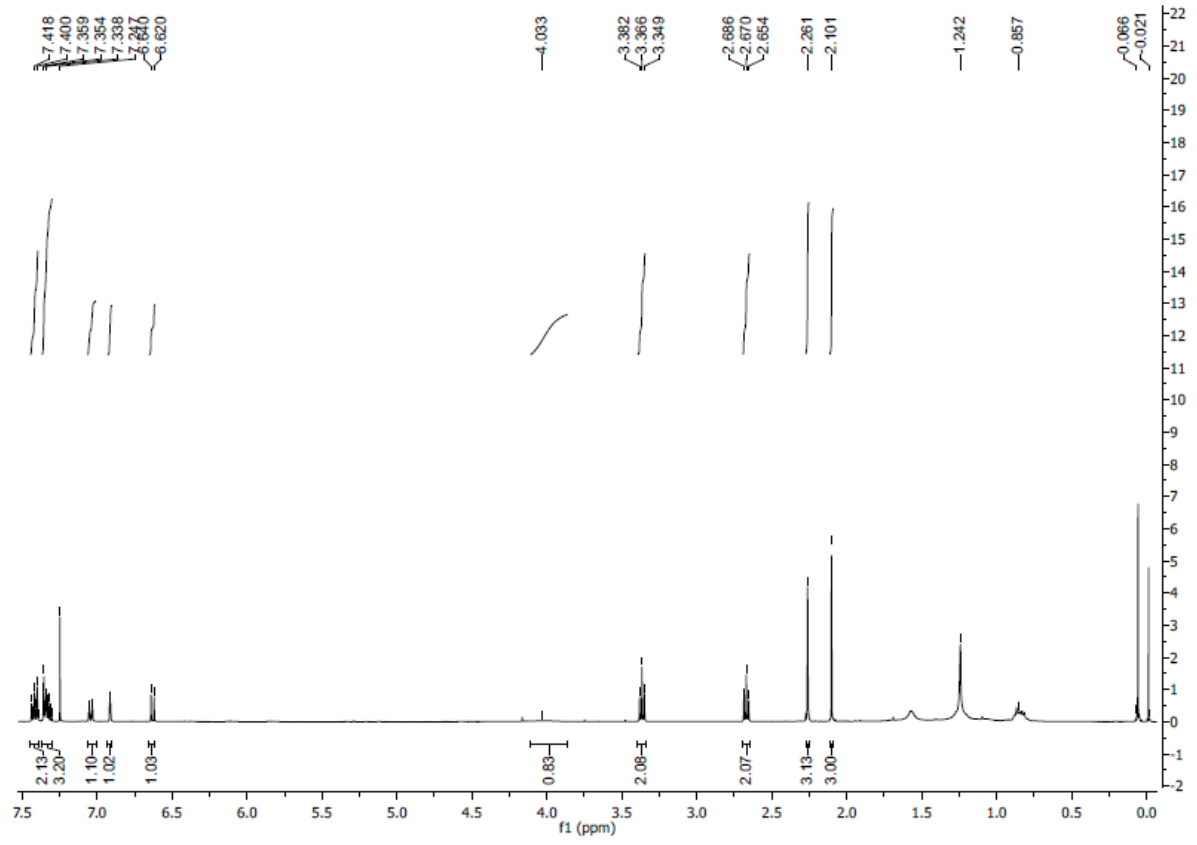


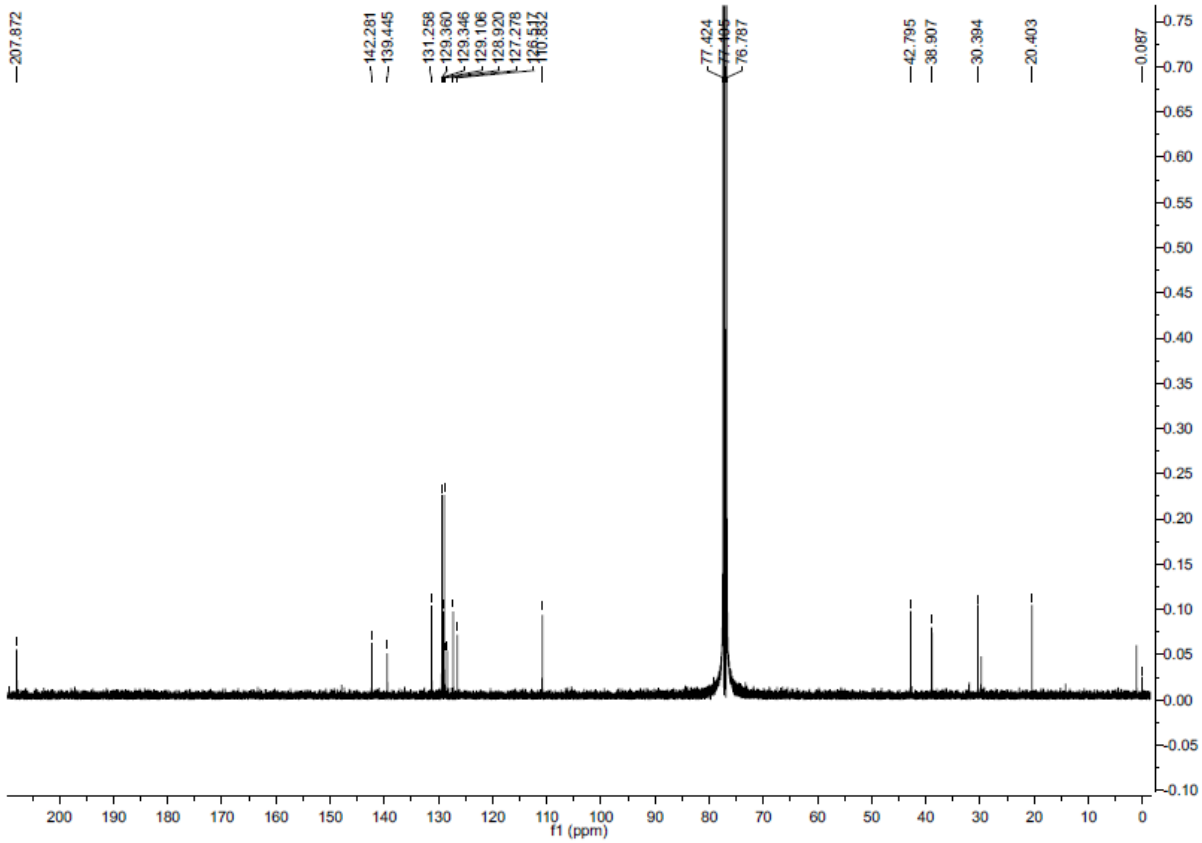
^{1}H and ^{13}C-NMR spectra of 6c



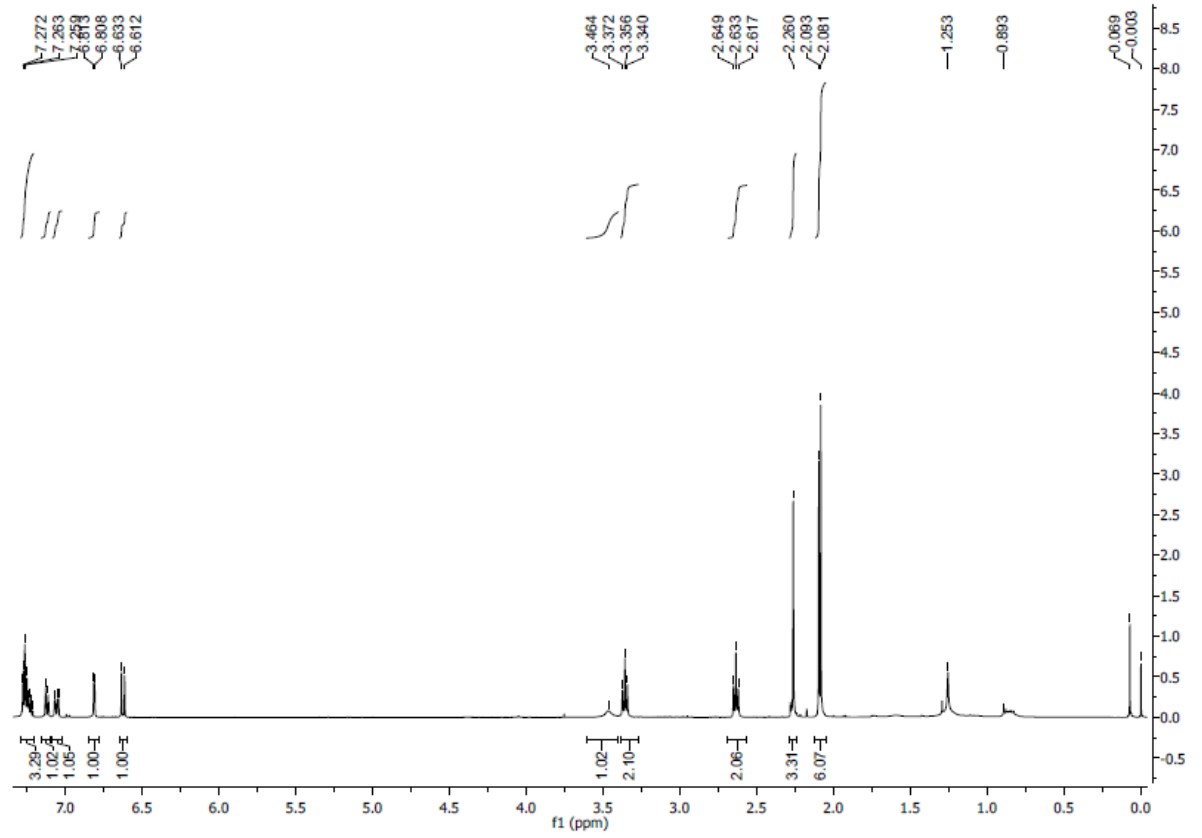


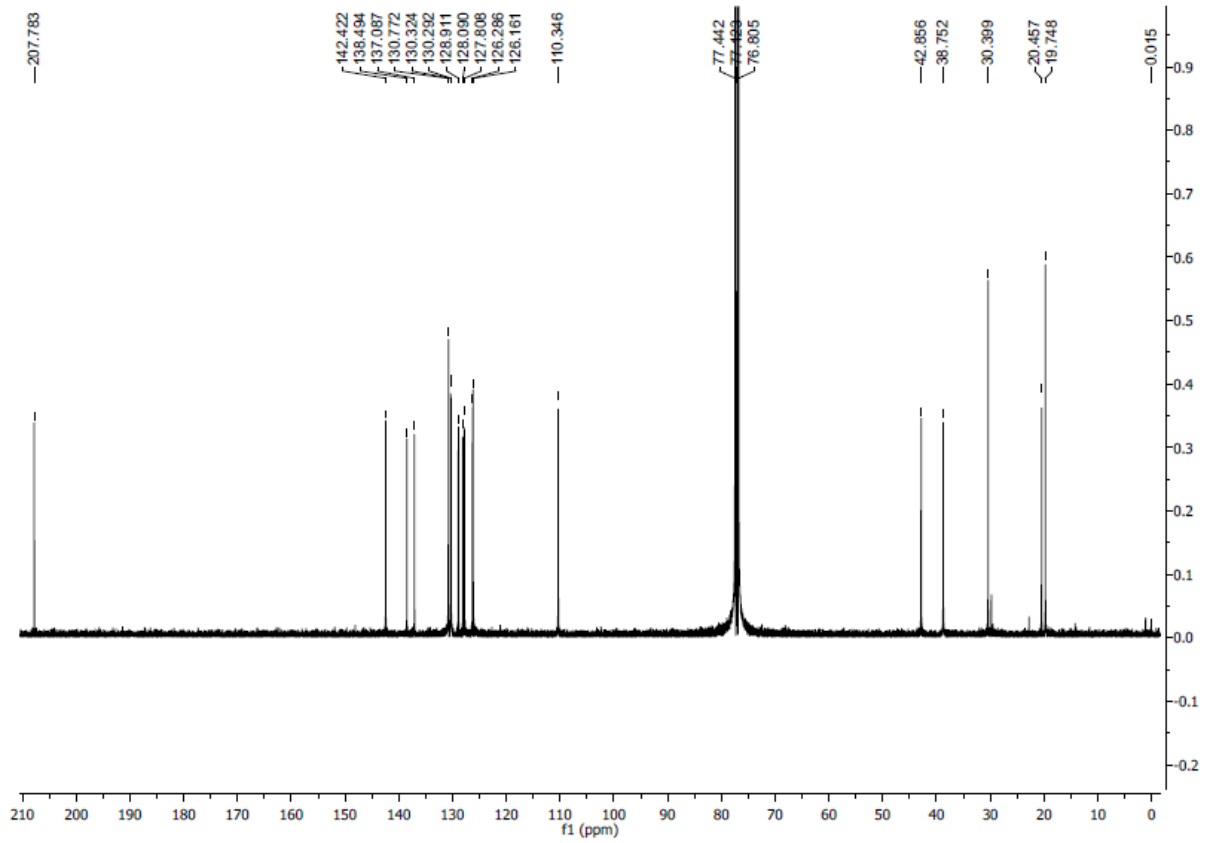
^{1}H and ^{13}C-NMR spectra of 6d



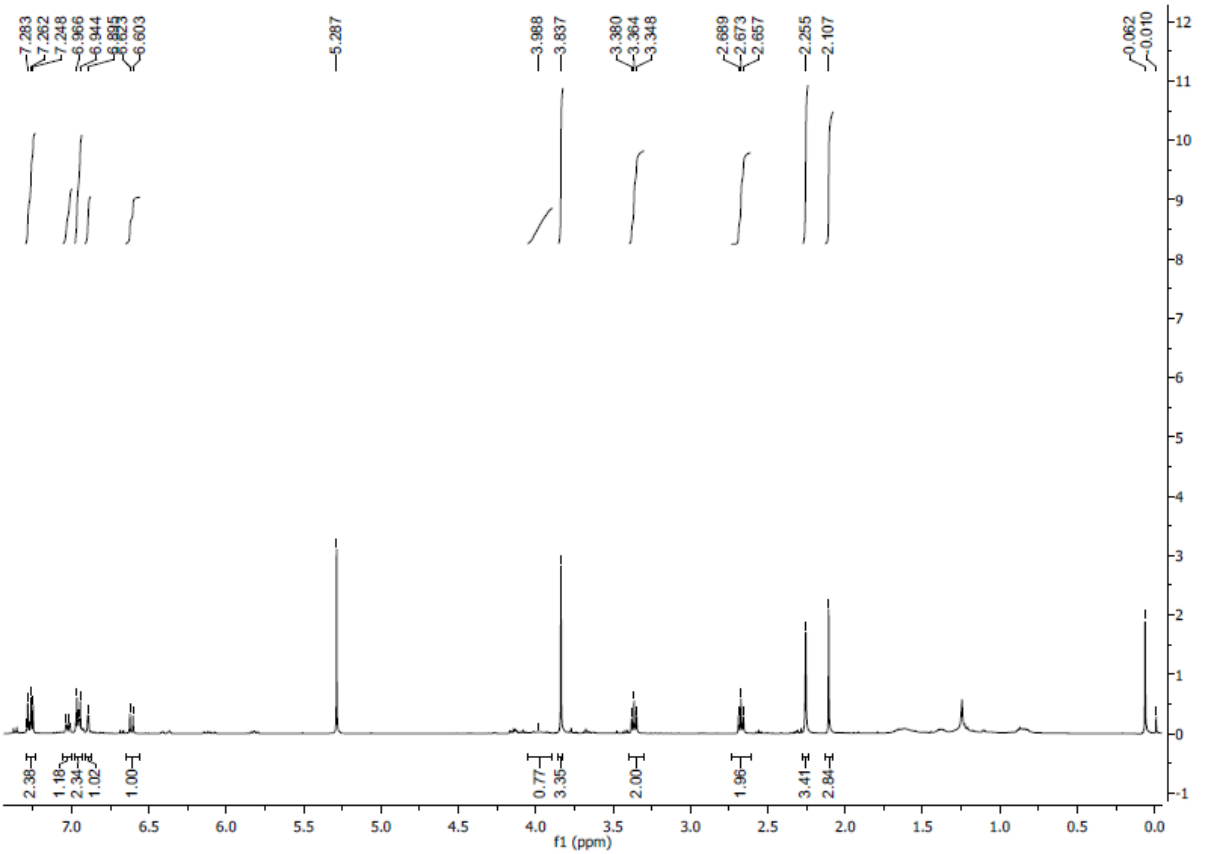


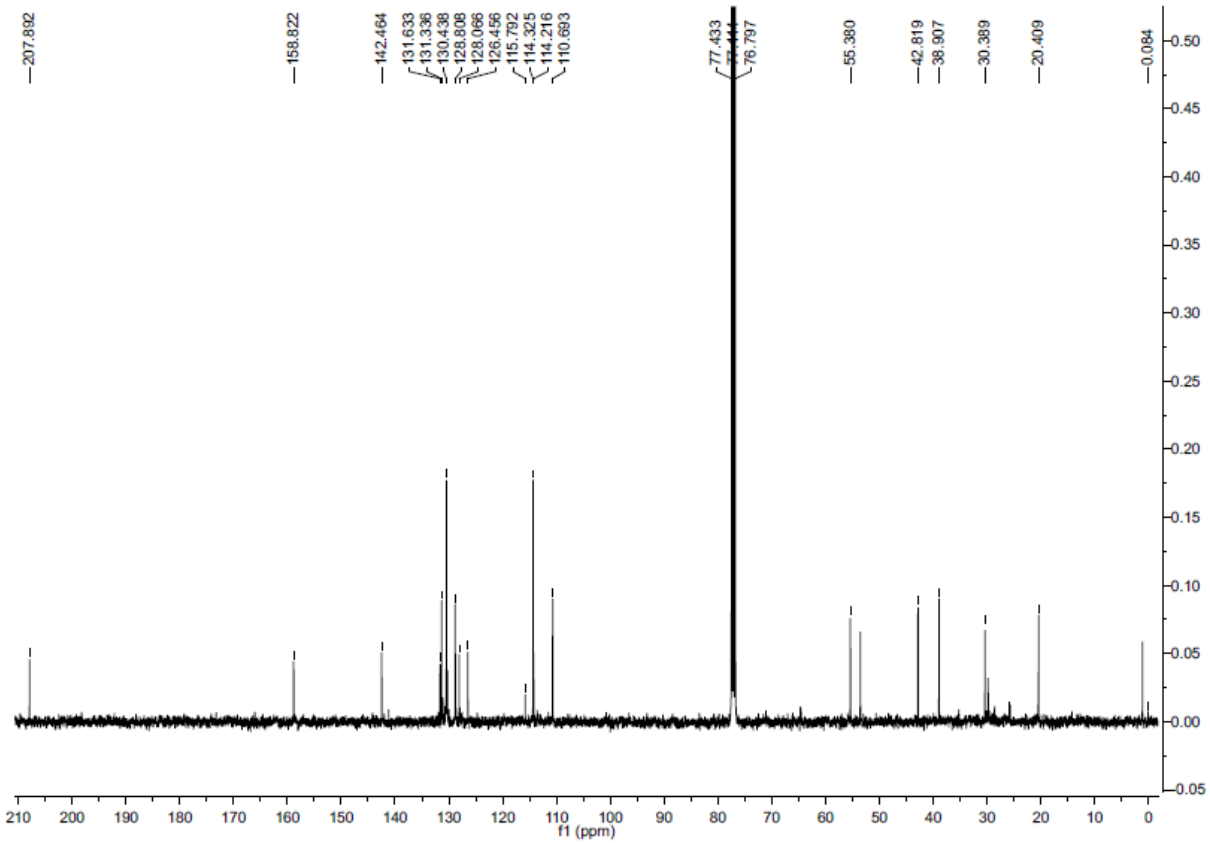
^{1}H and ^{13}C-NMR spectra of 6e



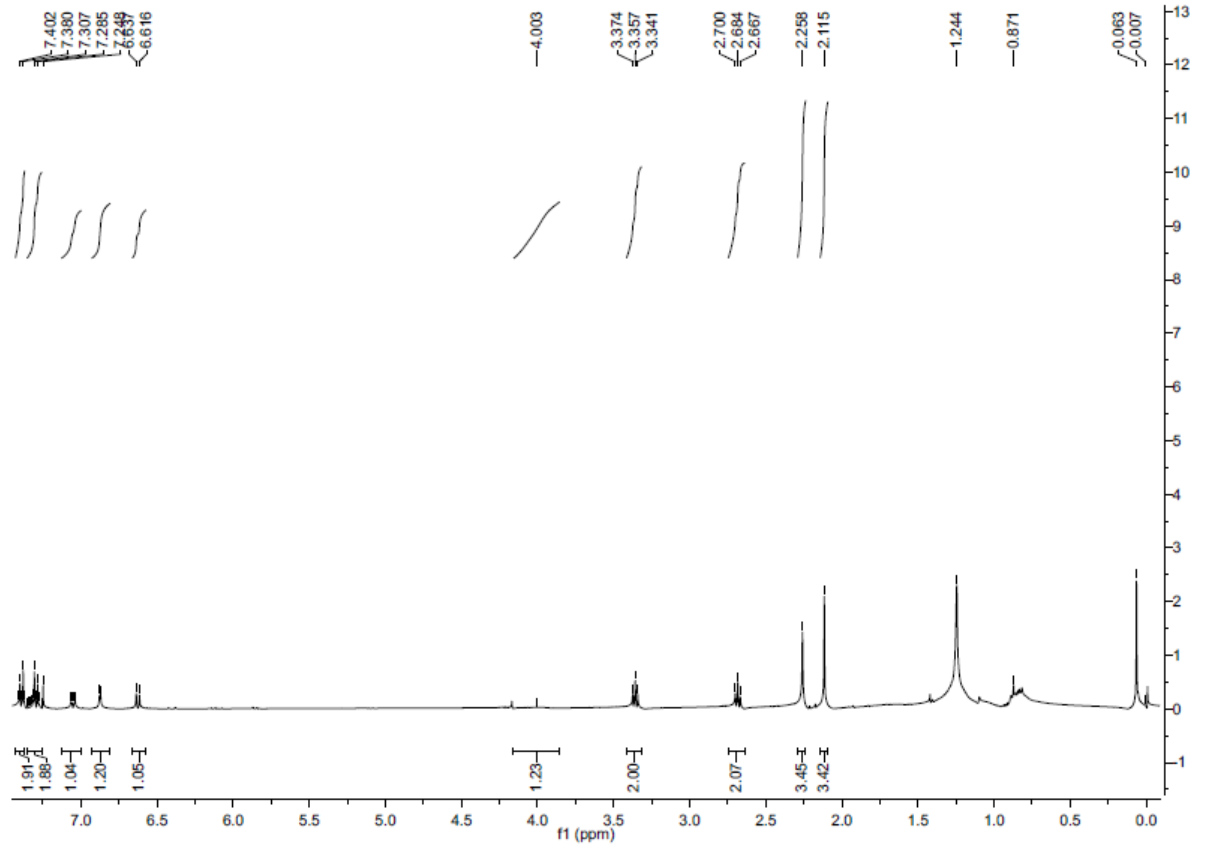


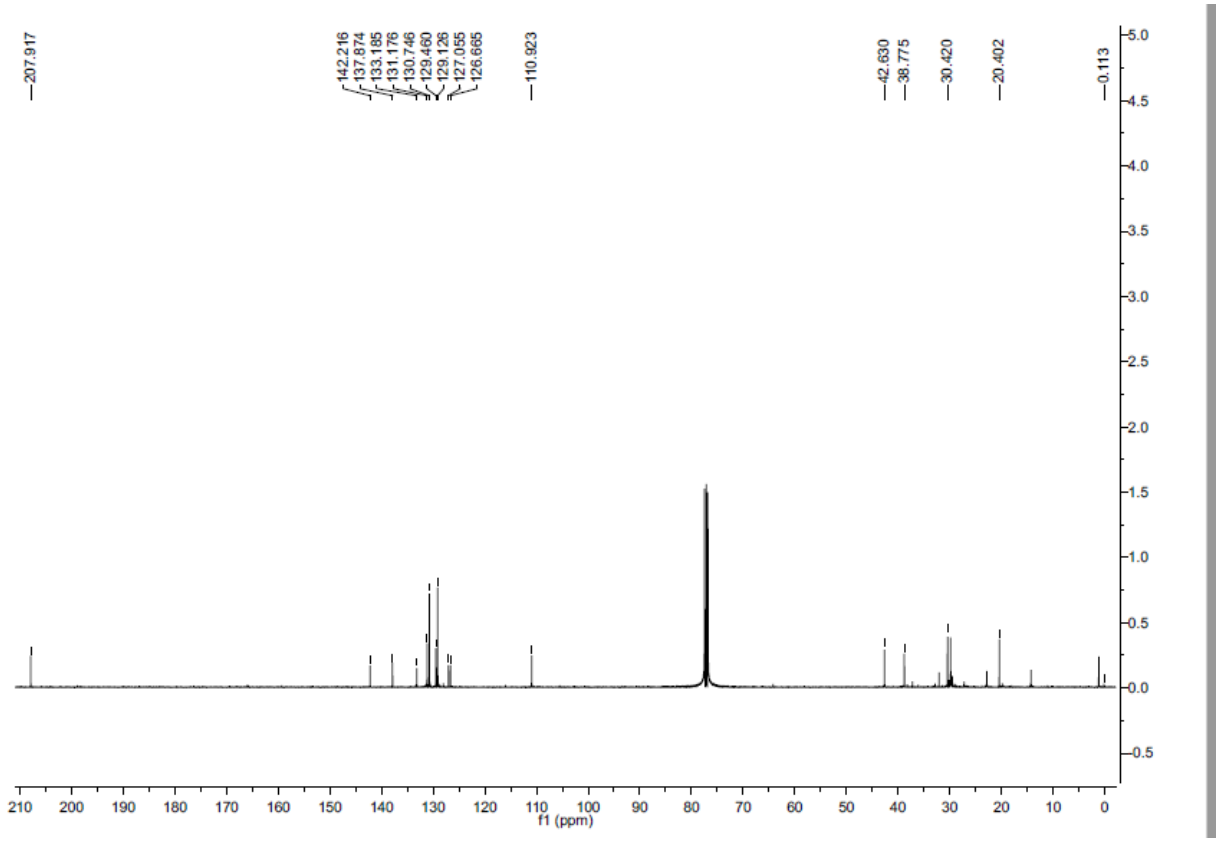
^{1}H and ^{13}C-NMR spectra of 6f



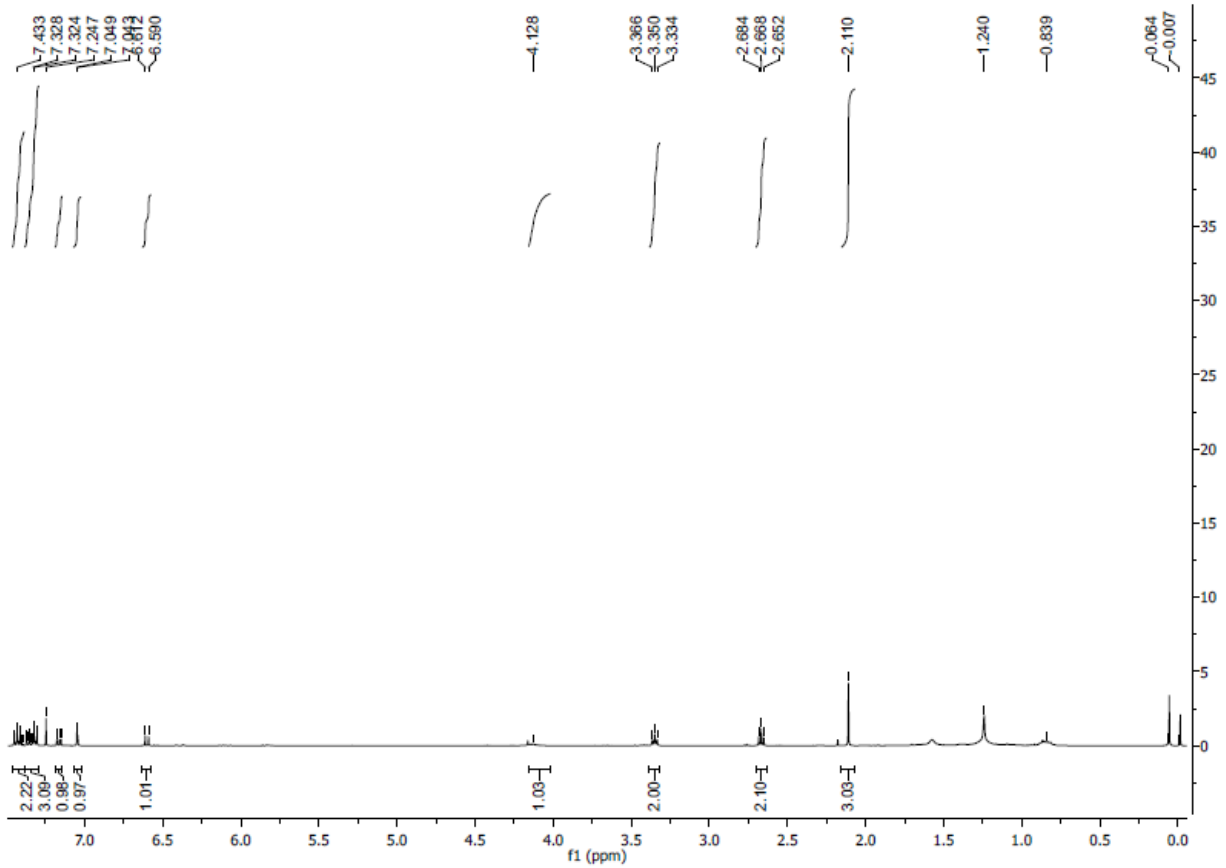


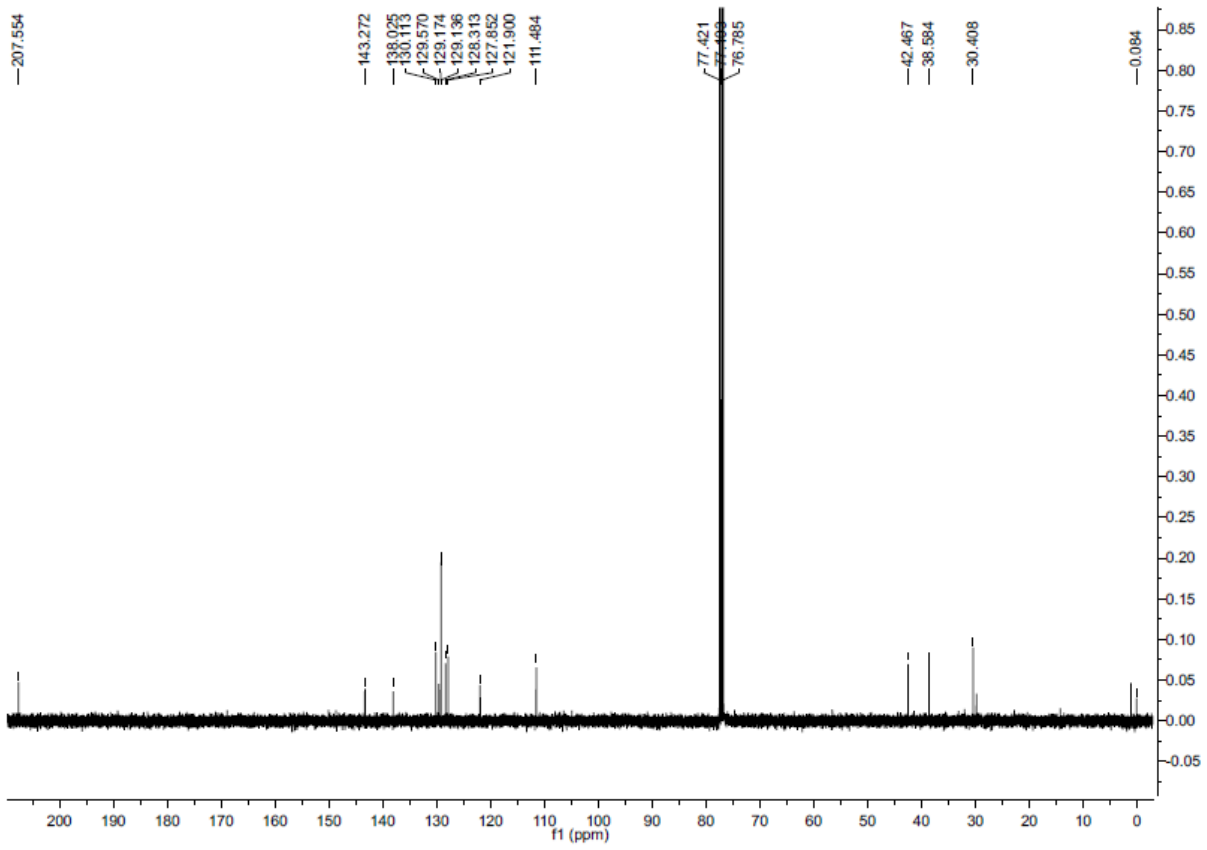
^{1}H and ^{13}C-NMR spectra of 6g





^{1}H and ^{13}C-NMR spectra of 6h




207.554
143.272
138.025
130.113
129.570
129.174
129.136
128.313
127.852
121.900
111.484
77.421
77.103
76.785
42.467
38.584
30.408
0.084
f1 (ppm)

PUBLICATIONS

1. Khan, I.; Singh, J.; Khan, I.; **Dutt, S.**; Khan, S.; Tyagi, V. *Arkivoc.* **2019**, *5*, 279-291.

2. Kamboj, P.; **Dutt, S.**; Chakroborty, S.; Tyagi, V.; *Tetrahedron Lett.***2019**, *60*, 151162.

3. **Dutt, S.**; Goel, V.; Garg, N.; Choudhury, D.; Mallick, D.; Tyagi, V. *Adv. Synth. Catal.* **2020**, *362*, 858-866.

4. **Dutt, S.**; Tyagi, V.; *Tetrahedron Lett.* **2021**, *87*, 153527.

5. **Dutt, S.**; Tyagi, V.; *Biocatal. Biotransformation.* **2023**, 1-11 **.**