Lipase Catalyzed Aminolysis as An Entry to Consecutive Multicomponent Reactions

Inaugural-Dissertation

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presented by

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Research Supervisor: Prof. Dr. Thomas J. J. Müller
Co-examiner: Prof. Dr. Jörg Pietruzska

Date of Examination:

I hereby declare that the work presented here is reflection of my own independent efforts and had been conducted without any unauthorized assistance. Wherever contributions and consultation of other sources are involved, every effort is made to indicate this clearly with due reference to the literature and acknowledgement of collaborative research and discussions, if any. Moreover the dissertation has never been submitted in any form to any other institution.

Düsseldorf, 22.09.2014

(Sidra Hassan)

The present work was conducted during the time period from April 2011 to January 2014, at the Institute of Organic Chemistry and Macromolecular Chemistry, Heinrich Heine University Düsseldorf, under the supervision of Prof. Dr. Thomas J. J. Müller.

Part of this work has already been published or submitted for publication or presented as posters at scientific meetings:

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Poster Presentations

"*One-Pot Chemoenzymatic Synthesis of 1,2,3-Triazoles*"
Third Annual CLIB-GC Retreat 2012, 22-24.02.2012, Bergisch Gladbach, Germany.

"*One-Pot Chemoenzymatic Synthesis of 1,2,3-Triazoles*"
Biotrends: Sustainable Industrial Biocatalysis International Congress, 29-30.11.2012, Dortmund, Germany.

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Oral Presentations

"Development of Chemoenzymatic Amidation-Coupling-Cycloisomerization (ACCI) Sequence in a One-pot Fashion for the Synthesis of Oxazoles"

First Internal(Düsseldorf/Jülich) CLIB-GC Retreat Mülheim, Germany, 30-31.08.2011.

"One-Pot Chemoenzymatic Synthesis of 1,2,3-Triazoles"

Second Internal (Düsseldorf/Jülich) CLIB-GC Retreat Mülheim, Germany, 23-24.08.2012.

"Application of Chemoenzymatic Catalysis in Multicomponent Reactions"

Ecochem Congress: The Sustainable Chemistry and Engineering Event, Basel Switzerland, 19-21.11.2013.

My Thanks to....

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To my mother

for making me certain that it's absolutely normal not to be a normality

...."your longing for Me is My message to you
all your attempts to reach Me
are in reality My attempts to reach you"....

(An excerpt from *Chanting the Name* by Rumi)

"So it was that I began to study not for praise or good opinion of others, but for the independence I thought it could give me. My studies were soon my life. They gave me refuge as well as occupation, for when I was sunk in my books I felt myself not alone, but in company: a company of enlightened souls whose passion was the enlightenment of others. I felt privileged to eavesdrop on the discourse of these great minds, and sometimes resented the intrusion of daily life. I can admit this to you because I know you will understand the temptation of a scholarly existence. Sometimes I think if I had not taken that road, a tumult of emotions might have overwhelmed me. Anger, sorrow and loneliness lay in wait on every side. I had only to stray a step or two from the path and I would be lost to them, and to despair. For what use are the tears of a few gentle souls against the cruelty of so many?"

(An excerpt from *The Einstein Girl* by Philip Sington)

Contents

List of Abbreviations

α	alpha
Å	Ångström
Ac	acyl
ACCI	amidation-coupling-cycloisomerization
ADH	alcohol dehydrogenase
ANAD sequence	**an**hydrides, **a**cetamide and **d**ienophile sequence
Ar	aryl
ATRP	atom transfer radical polymerization
BCL	*Burkholderia cepacia* lipase
[BMIm(BF_4)]	1-butyl-3-methylimidazolium tetrafluoroborate
[BMIm(PF_6)]	1-butyl-3-methylimidazolium hexafluorophosphate
Bn	benzyl
Boc	*t*-butoxycarbonyl
br	broad
BSA	bovine serum albumin
BQ	benzoquinone
Bu	butyl
°C	Degree Centrigrade
CAL	*Candida antarctica* lipase
CAL-A	*Candida antarctica* lipase A
CAL-B	*Candida antarctica* lipase B
CCL	*Candida cylindracea* lipase
CLEA	cross-linked enzyme aggregate
CMP	cytidine monophosphate
3CR	3 component reaction
4CR	4 component reaction
CRL	*Candida rugosa* lipase
CTP	cytidine-5'-phosphate
CuAAC	Cu-catalyzed azide-alkyne cycloaddition
Cy	cyclohexyl
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane

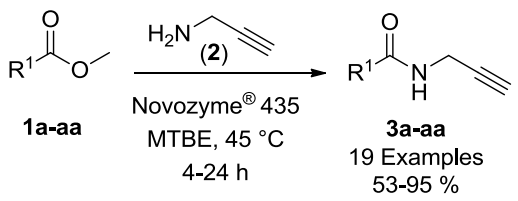
DCM	dichloromethane
dd	doublet of doublets
DEPT	distortionless enhancement by polarization transfer
DFT	density functional theory
DHPMs	dihydropyrimidones
DIPE	diisopropylether
DIPEA	diisopropylethylamine
DKPs	2,5-diketopiperazines
DMF	*N,N*-dimethyl formamide
DMSO	dimethylsulfoxide
ee	enantiomeric excess
EI	electron impact
Enz	enzyme
eq	equivalent
Et	ethyl
EWG	electron withdrawing group
Gal(NAc)	*N*-acetylglactosamine
GC-MS	Gas Chromatography-Mass Spectrometry
Glu	glutamic acid
HheC	halohydrin dehalogenase
h	hour
Hz	Hertz
IPr·HCl	1,3-bis(2,6-diisopropylphenyl)imidazolium chloride
IR	infrared
J	coupling constant
m	multiplet
M	molar
MALDI	matrix assisted laser desorption ionization
MAO-N	monoamine oxidase N
MCP	multicomponent polymerization
MCRs	multicomponent reactions
Me	methyl
min	Minutes
MML	*Mucor miehei* lipase
mol. sieves	molecular sieves
MTBE	methyl-*t*-butyl ether

m/z	mass to charge ratio
N	Normal (solution)
NMR	nuclear magnetic resonance
o	*ortho*
p	*para*
P1	P1 protease
PCL	*Pseudomonas cepacia* lipase
PEG	polyethylene glycol
PestE	thermophilic esterase
PGA	penicillin G amidase
PG	protecting group
Ph	phenyl
PIDA	(diacetoxyiodo)benzene
PIFA	(bis(trifluoroacetoxy)iodo)benzene
PLE	pig liver esterase
PPi	pyrophosphate
PPL	porcine pancreatic lipase
ppm	parts per million
Pr	propyl
PTSA	*p*-toluenesulfonic acid
q	quartet
quat	quaternary
®	registered sign
RAL	*Rhizopus arrhizus* lipase
RT	room temperature
s	singlet
T	temperature
t	triplet
t	tertiary
TBAC	tetrabutylammonium chloride
TBDMS	*t*-butyldimethylsilyl
TEMPO	2,2,6,6-tetramethylpiperidine 1-oxide
TFA	trifluoroacetyl
Tf	triflate
THF	tetrahydrofuran
TMEDA	*N*,*N*,*N*,*N*-tetramethylethylenediamine

TMG	1,1,3,3-tetramethyl guanidine
TMS	trimethylsilyl
v/v	volume to volume ratio
w/w	weight to weight ratio
XANTPHOS	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene

1. Summary

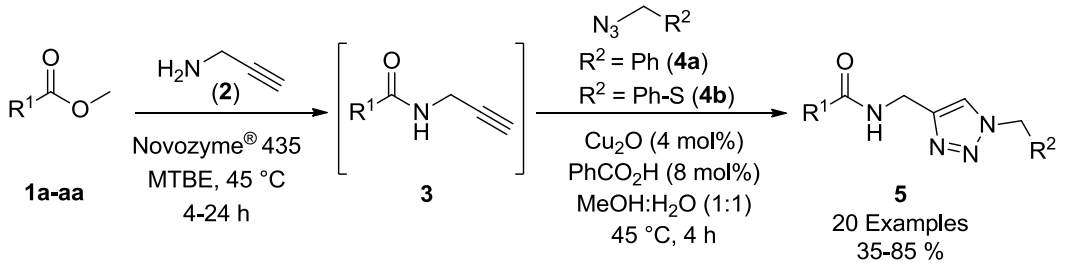
Enzyme catalysis earns its importance owing to its regio-, chemo- and stereoselective mode of action and the application of relatively mild reaction conditions compared to chemical catalysis. Moreover, the mutual incorporation of the concept of enzyme and chemical catalysis involves minimal or no implication of protecting group strategies while designing the synthetic route due to selectivity of a particular enzyme involved. In this study, the focus has been directed towards the application of *Candida antarctica* lipase B (CAL-B) catalyzed aminolysis. Since this class of hydrolases has been established for hydrolysis and esterification/transesterification transformations, therefore, it was considered worthwhile to evaluate the catalytic behaviour of CAL-B towards aminolysis. Lipases, unlike proteases, do not exhibit amidase activity[75] and hence this feature makes them suitable candidates for the synthesis of corresponding propargylamides **3** via aminolysis of methyl ester substrates **1** by propargylamine (**2**). During the early optimization studies, various enzymes, such as *Candida antarctica* lipase B (CAL-B), *Candida antarctica* lipase A (CAL-A), *Candida rugosa* lipase (CRL), porcine pancreatic lipase (PPL), *Pseudomonas cepacia* lipase (PCL) and protease from *Bacillus licheniformis* (Alcalase), have been screened for the desired transformation and various parameters such as solvent selection, nature of ester substrates **1**, operating temperature conditions and the effect of mode of immobilization of enzyme, have been studied. The optimization studies suggested CAL-B (Novozyme® 435) to be the optimum biocatalyst for the desired transformation. A correlation between structural features of ester substrates **1** with that of optimum operating temperature conditions, has been developed that eventually led to the establishment of the control of the regioselectivity of the action of CAL-B for selective site directed aminolysis of diester substrate **1k**. Methyl esters of *N*-protected amino acids (both *D*- and *L*-) have also been employed for screening the suitability of CAL-B catalyzed aminolysis of respective amino acids that in turn instigated the receptivity of CAL-B for such substrates since usually proteases are the enzyme of choice for amide linkage (peptide bond) synthesis. Scheme 1.1, shows the optimized CAL-B catalyzed aminolysis of various ester substrates **1** by propargylamine (**2**).



Scheme 1.1. Optimized CAL-B (Novozyme® 435) catalyzed aminolysis of various ester substrates **1** using propargylamine (**2**).

A variety of ester substrates **1** with different aromatic and heterocyclic side chain substituents, exhibited suitability for aminolysis by CAL-B catalysis for the corresponding propargylamide **3** generation.

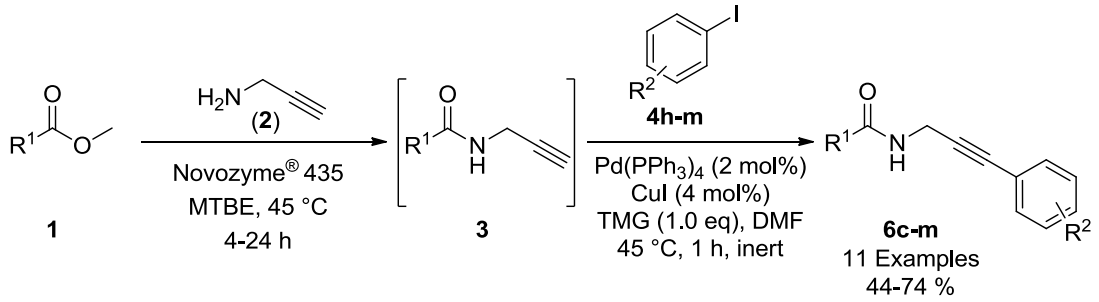
One of the hallmarks of multicomponent syntheses is the attainment of complexity in the target molecules using relatively simple starting materials. Since the benign and selective nature of enzyme catalysis offers the possibility to introduce various sensitive functionalities and hence complexity in a product, therefore, the concept of incorporation of biocatalysis in multicomponent syntheses was considered worth exploring. The terminal alkyne moiety of propargylamides **3** obtained by CAL-B catalysis, acts as a suitable motif for further transformations such as cycloaddition and coupling reactions, hence paving the way to the development of one-pot synthetic schemes. The triazole ring is a bioisostere of amide linkage[70] and amide ligated 1,4-disubstituted 1,2,3-triazoles have been termed as peptidomimetics[70-71] owing to their ability to mimic the properties of peptide linkage. Propargylamides **3** are considered to be "stubborn substrates" towards cycloaddition reactions[174] that explains the limited literature availability pertaining to the syntheses of amide ligated triazole systems **5**. The success of the previously optimized CAL-B catalyzed propargylamide **3** formation, provided the motivation to explore the possibility of the combination of CAL-B catalysis with Cu(I) catalyzed click reaction in a one-pot fashion. The optimization studies for the click reaction of propargylamides **3** using conventional reaction conditions were futile as low yields of the corresponding triazole systems **5** were obtained. However, the application of the concept of ligand promoted click reaction conditions[176a-c] furnished the higher yield of corresponding triazoles **5** under relatively mild reaction conditions using minimum additives (Scheme 1.2).



Scheme 1.2. Chemoenzymatic one-pot synthetic scheme of amide ligated 1,4-disubstituted 1,2,3-triazoles **5**.

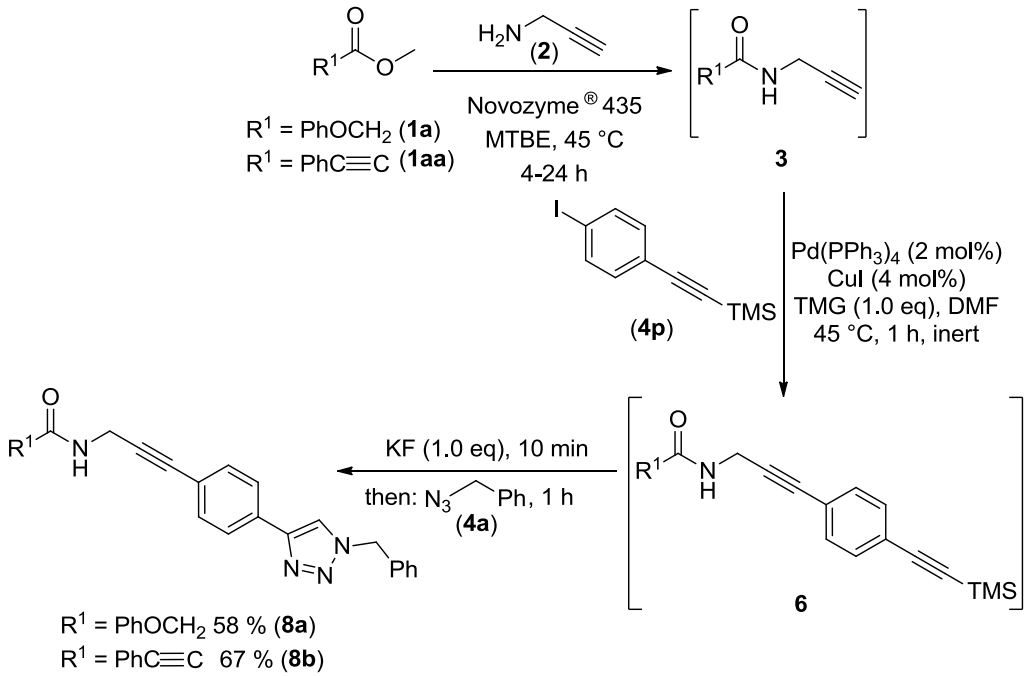
The successful concatenation of biocatalysis (CAL-B) with the chemical catalysis, i.e. Cu(I), further motivated the development of one-pot synthetic strategies involving other metal catalytic systems such as palladium catalyzed Sonogashira coupling reaction of terminal alkyne moiety of propargylamides **3**. Propargylamides **3** have been reported to successfully undergo Sonogashira coupling[144,145] for the synthesis of systems containing a rigid arylated

propargylamide core[161-160] that have established their importance as antagonists against gonadotropin releasing hormone receptor (GnRHR)[160] while propargylated deoxynucleoside derivatives have been pursued for their application in DNA sequencing.[161] One-pot syntheses of such rigid propargylated aryl systems **6** have been successfully developed using a variety of aryl iodides **4h-m** and methyl ester substrates **1** via Pd(0)/Cu(I) catalyzed arylation of propargylamides **3** (Scheme 1.3). Contrary to the previously reported coupling conditions,[144-145] the propargylamides **3** displayed the reactivity towards coupling under Pd(0) catalytic conditions.



Scheme 1.3. Chemoenzymatic one-pot synthetic scheme of arylated propargylamide systems **6**.

During the development of synthetic strategy, the presence of Cu(I) catalysis in the reaction system was further utilized by extending the strategy to triazole ligation on the coupled products **6** (Scheme 1.4).



Scheme 1.4. Chemoenzymatic one-pot synthesis of triazole ligated coupled product **8**.

Thus, the development of chemoenzymatic catalyzed one-pot synthetic strategies has widened the vista of relevance of biocatalysis with metal catalytic systems and further motivates the incorporation of other available chemical catalytic systems with enzyme catalysis for launching novel synthetic strategies.

2. Introduction

2.1. Enzyme Catalysis in Organic Solvents

The use of organic solvents is an inevitable feature in the field of organic synthesis and, therefore, if the incorporation of the concept of biocatalysis into the one-pot synthetic methodological strategies is intended, it is important to be certain of the activity and hence applicability of the enzymes of interest in respective organic medium. Water is a natural medium for enzyme action mainly responsible for maintaining its native active site conformation via noncovalent interactions.[1] In context to their application in the domain of organic syntheses, the studies of catalytic activity of enzymes in pure anhydrous organic solvents have been actively pursued in past decades. The replacement of bulk water with an organic medium has been reasoned since only a few monolayers of water bound to the enzyme's active site, are responsible for maintaining the catalytic activity[1] while the retention of catalytic activity has been rationalized on the basis of acquired structural rigidity in an organic medium keeping the native-like conformation from unfolding.[2] Other potential advantages include a shift in thermodynamic equilibria to favor synthesis over hydrolysis, suppression of water induced side reactions, ease of product recovery and inhibition of microbial contamination.[3]

The pH memory of the ionogenic groups at the active site of the enzyme depends upon the aqueous buffer/environment from where it has been last recovered and remains unchanged in organic media.[1] When in organic solvent, the interaction of the enzyme with the surrounding medium might further activate or deactivate the enzyme depending upon the nature of interactions. These interactions might lead to the disruption of the native conformation by disordering the hydrogen bonding and other noncovalent interactions resulting in instability.[4] Solvent may also interact with diffusible components (substrates or products) of the reaction mixture. This has been shown by the inactivation of enzyme during peroxidase-catalyzed oxidation of phenols in chloroform since it is a quencher of phenoxy radicals. Thereby inhibiting the overall process that requires the initiation by the enzymatic generation of phenoxy radicals.[5] The other mode of interaction depends upon the hydrophilic nature on the organic solvent interacting with essential water bound to the active site of the enzyme. Horseradish peroxidase catalyzed oxidation of *p*-anisidine in various organic solvents showed the reduced activity of the enzyme in highly polar hydrophilic solvents mainly due to their ability to interact with and consequently remove the essential water from the active site of the enzyme while the highest activity was observed in relatively less polar hydrophobic solvents.[5] Various enzymes have been reported to be active in organic media with a certain percentage of water present in it.[6-9]

In continuation to this, porcine pancreatic lipase (PPL) catalyzed transesterification reaction between tributyryl glycerol and various primary and secondary alcohols have been studied in relation to water concentration in the respective organic medium that led to the finding of retention of lipase activity at water concentration as low as 0.015 percent with the half life of 12 h in dry organic medium (decanol) at 100 °C.[10] The thermal stability of PPL was shown to have an inverse relation to the water concentration in the reaction mixture, thus establishing the acquirement of thermal stability and altered substrate selectivity by dry enzyme in dry organic medium. Further, in a comparative study, PPL, yeast and mold lipases have been shown to exhibit catalytic activity towards transesterificatin, esterification, aminolysis, thiotransesterification, and oximolysis in organic media comparable to their activity in water[1] while subtilisin and α-chymotrypsin also displayed catalytic activity for transesterification processes in organic solvents.[2]

2.2. Enhancement of Enzyme Activity via Excipients

The structural rigidity of proteins in non-aqueous environment is responsible for the molecular imprinting phenomenon[11] called "ligand induced enzyme memory" or "bioimprinting".[12] Ligand memory of an enzyme is directly responsible for the activating effect of active site ligands during lyophilization.[13] The structural imprint imposed by the ligand, removed prior to the use of enzyme, on the active site of the enzyme, enables to induce the rigid frozen conformation and readily accepts the substrates of related structural features leading to the consequent enhanced reactivity.[11,13] This approach has been employed for alteration and enhancement of the enzyme selectivity for a given set of structurally related substrates. The precipitation of α-chymotrypsin, which exhibited a high selectivity for *L*-isomer, with *n*-propanol in the presence of *N*-acetyl-*D*-tryptophan, made the enzyme active towards *D*-isomers, an effect absent when no precipitation with *D*-isomer was performed.[14] Another approach for the enhancement of activity of enzyme in organic media involved the addition of excipients or salts to the enzyme before lyophilization that prevented the conformational changes of protein structure in organic media.[15] Salts such as potassium chloride[16] and macrocyclic additives such as crown ethers,[17] cyclodextrin,[18,19] and polymeric additives such as polyethylene glycol (PEG)[20,21] have been shown to augment the enzyme activity in organic medium. Salts are believed to increase the *Mucor javanicus* lipase and subtilisin *Carlsberg* activity by increasing the polarity of the active site thereby stabilizing the transition state.[16] Cyclodextrin amplified the solubility of various substrates via host-guest complex formation[18] while crown ether worked by refolding the protein to a thermodynamically stable catalytically active conformation via macrocyclic interactions.[17] PEG activated the enzyme function by not only conserving the protein structure but also by allowing the dissolution (upto 0.2 mg/ml) of the enzyme in organic media.[20,21]

2.3. Stabilization of Enzymes via Immobilization

In order to increase the stability and operational efficiency of the enzymes, immobilization techniques have been evolving ever since their development. The main idea behind the immobilization of enzymes is to derive the obvious advantages of recycling of catalyst system from the reaction medium and possible anticipated activity related advantages. There is no rule to predict the resultant activity and stability of an enzyme after the immobilization process.[22] It may inhibit or increase the enzyme activity.[23] Immobilized enzymes have been shown to exhibit better thermal stability[24] and enhanced resistance of *Candida rugosa* lipase (CRL) against deactivation induced by aldehyde.[25] Over the years, various methods have been developed such as entrapment within a support (membrane, gel, microcapsule), adsorption by physical interactions and cross linking techniques.[26] Carrier materials such as activated charcoal,[27] alumina,[28] silica,[29,30] celite,[31] cellulose,[32] and synthetic resins,[33] have been employed for the adsorption of enzymes. Adsorption has been adopted as a method of choice when the use of lipophilic organic solvents is involved, where desorption cannot occur.[34] The efficiency of the physical adsorption of the enzyme depends on several factors such as size of the protein to be adsorbed and carrier's specific area and porosity, thereby controlling the rate of diffusion of substrate(s).[22] Another mode of enzyme activation is through "cross-linked enzyme aggregate (CLEA)" formation where the individual molecules (crystals) of an enzyme get cross-linked via covalent bonding either with each other or by using an inert filler such as albumin[34] or bifunctional agents such as glutaraldehyde, diimidates or disulfonylchloride,[35] that could lead to a general enhancement of conformational stability.[36] However, cross linking impeded the enhanced catalytic activity due to the diffusional limitation of substrate molecule(s) to the active site of the enzyme.[34]

2.4. Enzyme Selectivity

One of the pronounced features of enzyme catalysis is the selectivity of their mode of action especially when heterofunctional substrates are involved. In a study, the relative reactivity of hydroxyl and amino group towards acylation was evaluated using aminoalcohols as model substrates without the need of protection of either of the functional groups depending upon the nature of enzyme and acylating agent employed, thus making the chemoselective control of the process possible.[37] In another study, the substrate specificity of the transesterification reaction catalyzed by serine protease, subtilisin Carlsberg, was shown to be strongly dependent upon the physicochemical properties of the solvent[38,39] leading to the derivation of an enzyme-independent thermodynamic model for the prediction of substrate specificity by any enzyme as long as the substrates are inaccessible to the solvent in transition state.[39] Thus the physicochemical control of the solvent could be beneficial for inducing the desired enantioselectivity in an enzyme.

2.5. Chemoenzymatic Multicomponent Reactions

Multicomponent reactions (MCRs) are convergent reactions involving the reaction of three or more starting materials to form the product where all or most of the atoms contribute to the corresponding product formation.[40a] Hence this approach establishes high operational and cost efficiency and atom economy of the overall process with minimum generation of by product(s) leading to high structural diversity.[40b] Depending upon the sequence of addition of reactive components, MCRs can broadly be divided into the following three categories.[40b]

1. Domino MCRs: in which all the components are initially present but intermediates are not isolable.

2. Sequential MCRs: in which the components are introduced into the reaction mixture in sequential manner under constant conditions and intermediates are isolable.

3. Consecutive MCRs: in which reaction conditions are changed in a step wise manner and intermediates are isolable.

Since chemoenzymatic transformations have been holding primordial importance mainly because of their ability to carry out chiral resolution of racemic or meso substrates as a catalytic access to enantiomerically enriched chiral building blocks in organic syntheses,[40c] therefore, the concept of incorporation of biocatalysis with chemical catalyzed processes has been pursued in recent years for enhancing the structural diversity of the desired products. Based upon the work that has been reported in past decade, the approach of incorporation of chemoenzymatic catalysis in MCRs can clearly be divided into the following four categories:

1. MCRs commencing with enantiomerically pure starting materials obtained by enzyme catalyzed resolution of racemic mixtures. (not strictly a one-pot process)

2. Racemic products obtained by MCR strategy that can subsequently be submitted to enzymatic resolution to get the optically pure product of interest. (not a one-pot process)

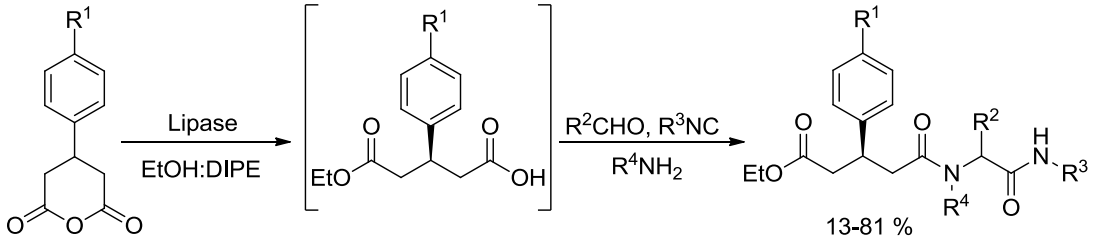
3. Cascade MCRs involving chemo and enzymatic catalysis running side by side. (one-pot process)

4. Enzyme catalyzed MCRs. (one-pot process)

2.5.1. MCRs Utilizing Enzymatic Resolution of Racemic Mixtures

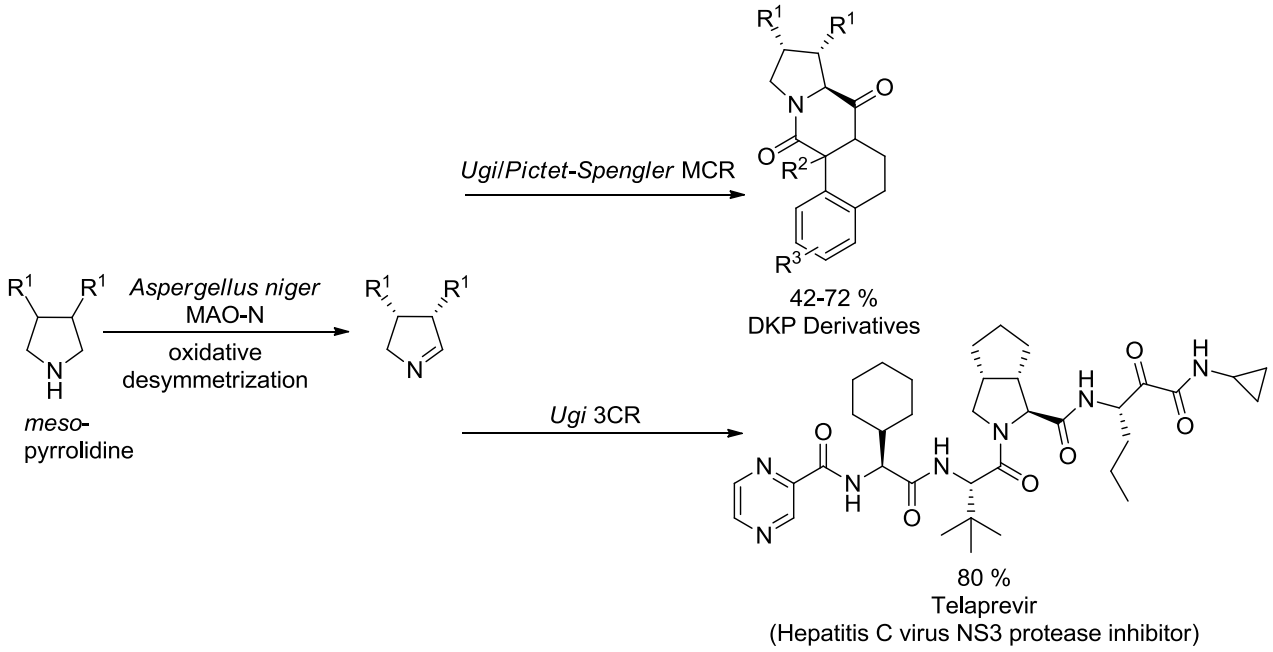
2.5.1.1. Ugi Four-Component Reaction

Integration of enzyme catalyzed desymmetrization with Ugi four-component reaction proceeded in a two step one-pot fashion and the yields were comparable to when conducted in separate step wise manner, however, the one-pot process failed to establish a good diastereoselectivity.[41] With the exceptions of a few starting materials, low overall yields of the products were obtained that were strongly dependent upon the nature of starting materials and the nature of the co solvent for Ugi reaction (Scheme 2.1).



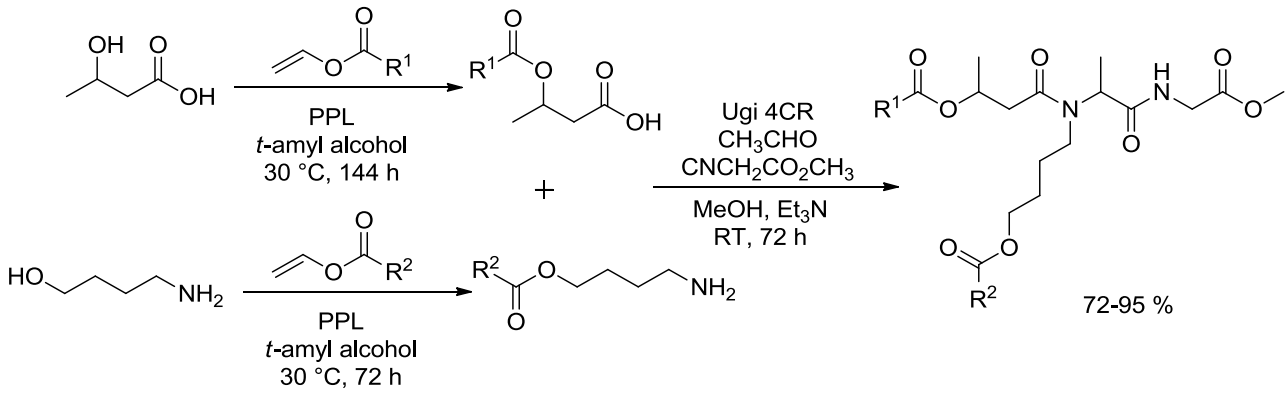
Scheme 2.1. One-pot integration of enzymatic desymmetrization with Ugi reaction.[41]

This feature of enzyme catalyzed desymmetrization has been successfully developed and employed for desymmetrization of *meso*-pyrrolidine catalyzed by monoamine oxidase N (MAO-N) from *Aspergillus niger*.[42] Chirally pure pyrrolidines served as an entry for the synthesis of hepatitis C virus (HCV) NS3 protease inhibitor telaprevir, in combination with Ugi MCR,[43] and further expansion of the study led to the development of a novel Ugi and Pictet-Spengler-type cyclization for the synthesis of polycyclic 2,5-diketopiperazines (DKPs) (Scheme 2.2).[44]



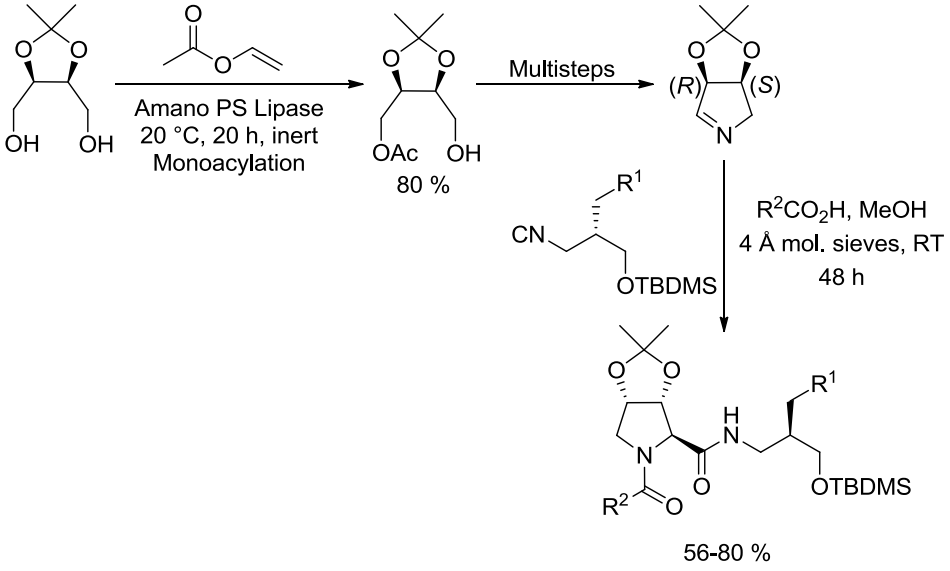
Scheme 2.2. Oxidative desymmetrization of *meso*-pyrrolidine to optically pure diastereomer for subsequent syntheses of DKP derivatives and telaprevir via Ugi-Pictet-Spengler MCR and Ugi MCR sequence respectively.[43,44]

In another approach, a nine member Ugi condensation library has been developed by PPL catalyzed acylation of 3-hydroxybutyrate and 4-amino-1-butanol with a variety of acyl donors, selectively acylating at the hydroxyl moiety of both these precursors for the subsequent Ugi reaction.[45] In this study, the chemoselective nature (selective acylation of hydroxyl moiety) has been exploited for generating the precursors that would subsequently act as two of four components for Ugi reaction but the stereoselectivity was not established (Scheme 2.3).



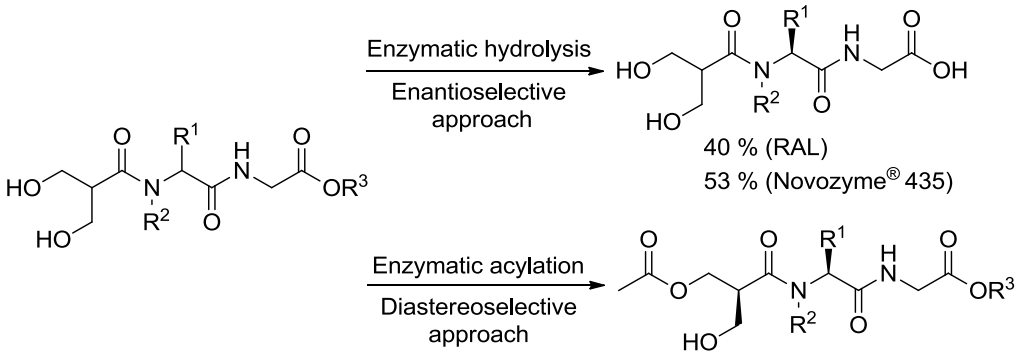
Scheme 2.3. Combination of PPL catalyzed acylation of carboxylic acid and amine precursors for subsequent Ugi four-component reactions in a two step process.[45]

In another study, enantiomerically pure chiral cyclic imine precursors, synthesized by Amano PS lipase catalysis, have been employed for subsequent high diastereoselective Ugi reaction for the synthesis of pharmacologically relevant polyfunctionalized pyrrolidine systems.[46] This approach exploited the enantioselectivity exerted by Amano PS lipase and high diaseteroselectivity of Ugi reaction to achieve a full control of relative and absolute configuration of the final product (Scheme 2.4).



Scheme 2.4. Diastereoselective Ugi reaction using chiral cyclic imine synthesized by Amano PS lipase.[46]

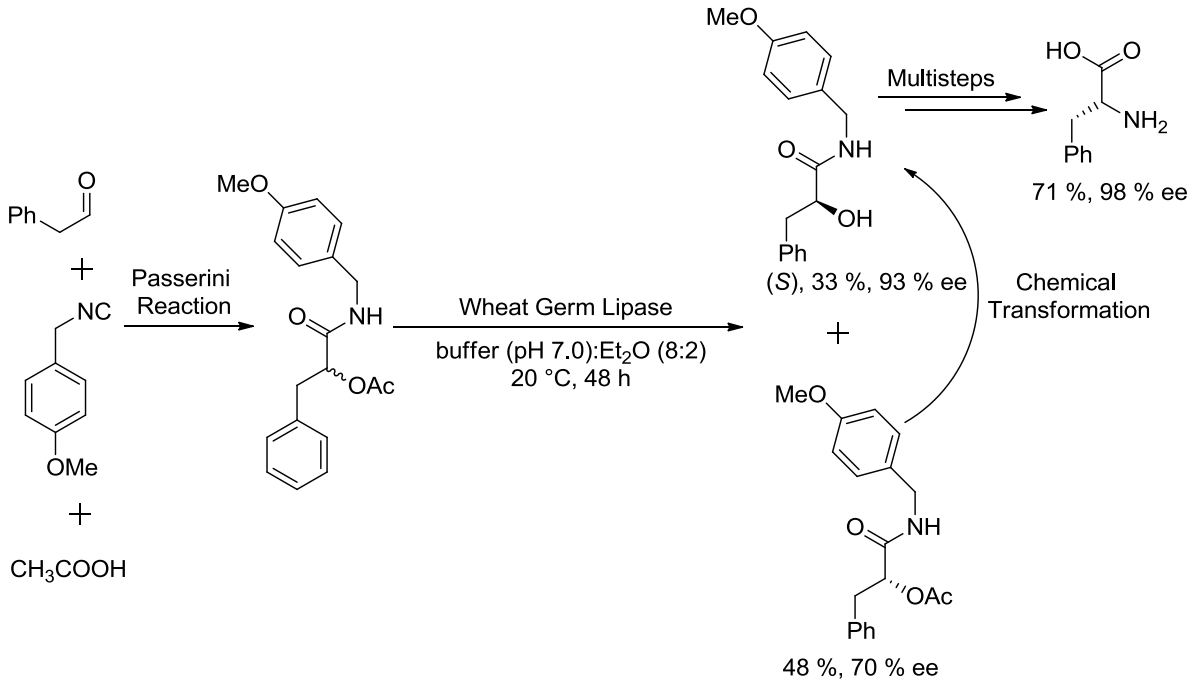
In the previously mentioned approach,[46] the concept of enzyme catalysis was employed for the synthesis of enantiomerically pure precursors for successive Ugi reaction, however this approach has also been envisaged for obtaining enantiomerically pure Ugi product via enzyme mediated kinetic resolution of crude Ugi product for the synthesis of 1,3-diol peptidomimetics.[47] The lipases screened for this study, exhibited poor diastereoselectivity and hence this approach was not pursued, however, the best result of enzymatic hydrolysis (enantioselective approach) was obtained using *Rhizopus arrhizus* lipase (RAL) and *Candida antarctica* lipase B (CAL-B commercially known as Novozyme® 435) with high enantioselectivity (Scheme 2.5).



Scheme 2.5. Enzymatic kinetic resolution of crude Ugi product to enantiomerically pure 1,3-diol peptidomimetic.[47]

2.5.1.2. Passerini Multicomponent Reaction

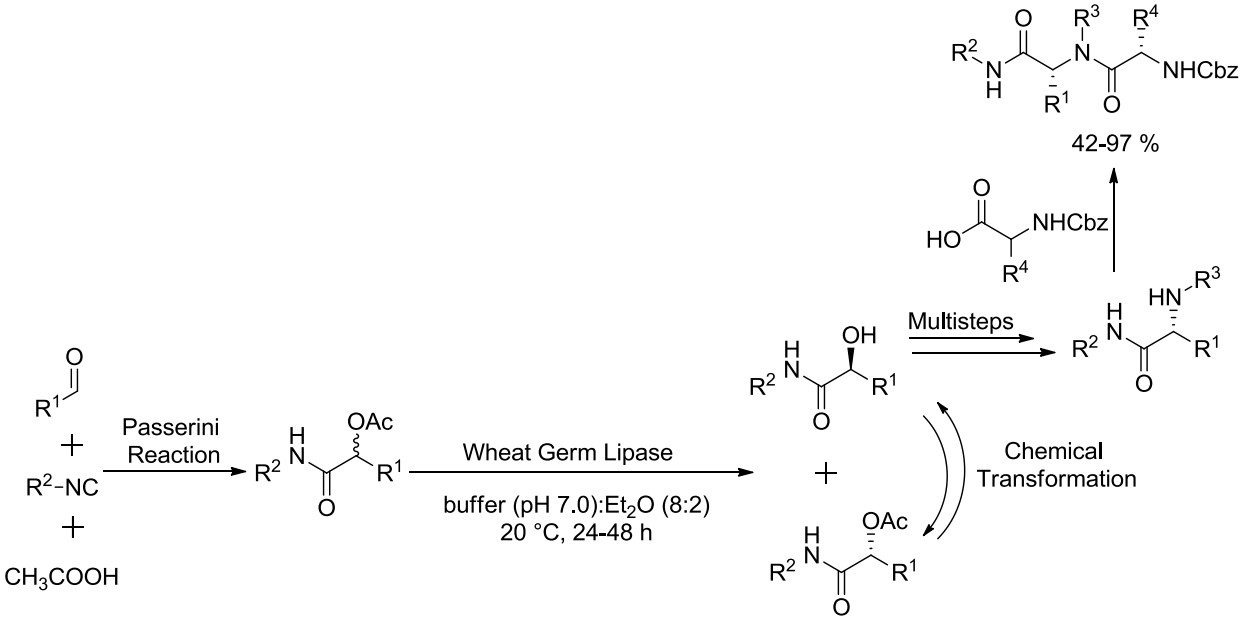
A synthetic route for the synthesis of α-amino acids has been devised via wheat germ lipase catalyzed hydrolysis of α-acetoxyamides, prepared via Passerini reaction, to the corresponding enantiomerically enriched α-hydroxyamides that, in turn, are converted to α-aminoamides. The α-aminoamides so produced were then converted to α-amino acids (Scheme 2.6).[48]



Scheme 2.6. Enzymatic resolution of Passerini product for the synthesis of enantiomerically pure α-amino acid.[48]

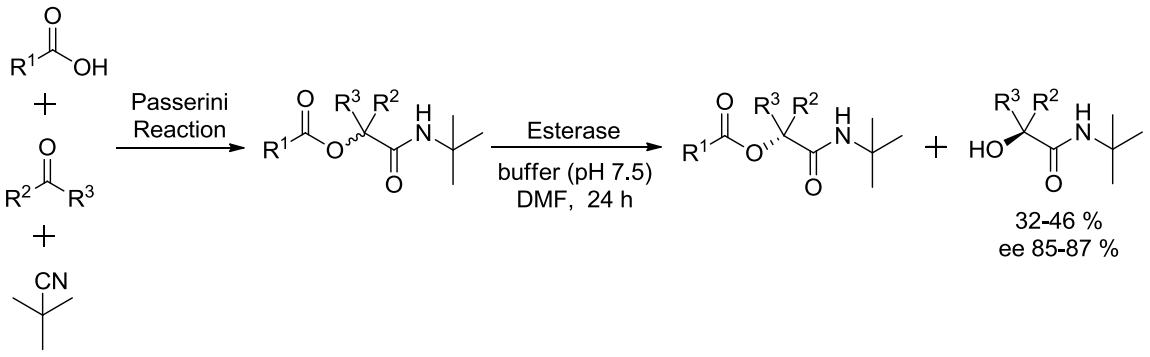
Further investigation revealed the enantioselectivity of the wheat germ lipase to be strongly dependent upon the hydrophobicity of the solvent employed[49] and led to the synthesis of *N*-

methylated amino acids.[50] This method facilitated the introduction of diverse amino acid moieties to the tripeptide scaffold,[50] leading to the synthesis of unnatural peptides[51] that have been difficult to synthesize with high stereocontrol of the chiral centres via Ugi pathway (Scheme 2.7).[52]



Scheme 2.7. Stereocontrolled synthesis of tripeptides by chemoenzymatic Passerini reaction.[51]

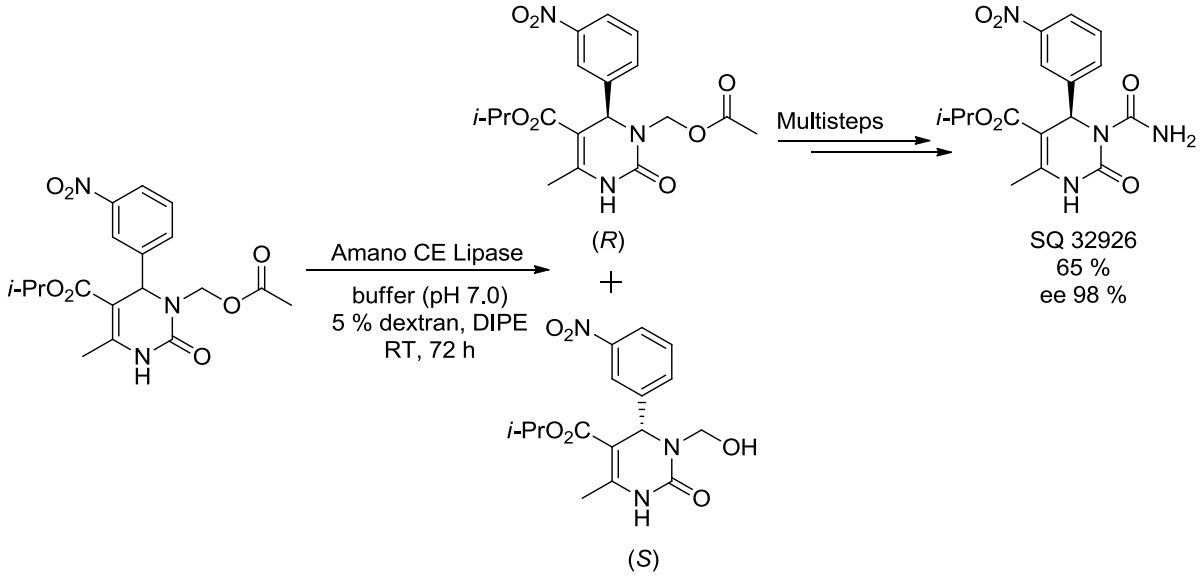
The synthesis of α,α-dialkyl-α-hydroxycarboxylic acid derivatives have been achieved via hydrolase catalyzed kinetic resolution of the racemic mixture obtained by Passerini reaction.[53] Out of 40 screened enzymes, a protease (P1), a thermophilic esterase (PestE), and an esterase of metagenome origin (esterase 8) were the most active and enantioselective (Scheme 2.8).



Scheme 2.8. Synthesis of *N*-(*t*-butyl)-2-hydroxy-2,2-dialkylamides by Passerini MCR and subsequent enzymatic kinetic resolution.[53]

2.5.1.3. Biginelli Multicomponent Reaction

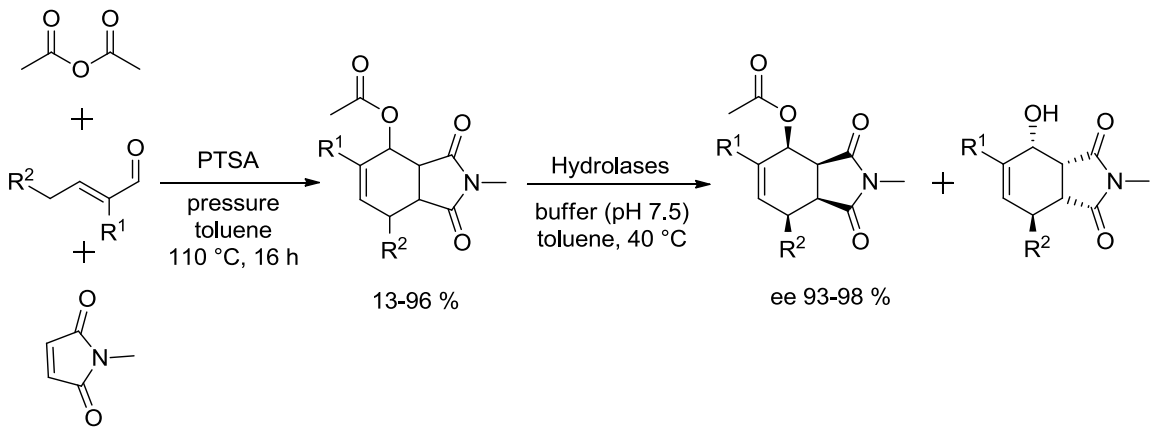
The preparation of enantiomerically pure dihydropyrimidones (DHPMs) obtained by Biginelli reaction, has been achieved by lipase catalyzed resolution of activated DHPM esters but the overall process exhibited lower selectivity when conducted on analytical scale.[54] Activated DHMP derivative with an acetoxymethyl residue at *N*-3 position, underwent selective cleavage by *Thermomyces lanuginosus* lipase (Amano CE lipase) for the synthesis of optically pure enantiomers. Further derivatization of (*R*)-enantiomer furnished antihypertensive agent (*R*)-SQ 32926 (Scheme 2.9).



Scheme 2.9. Lipase catalyzed resolution of DHMPs (Biginelli product) for the subsequent synthesis of antihypertensive agent SQ 32926.[54]

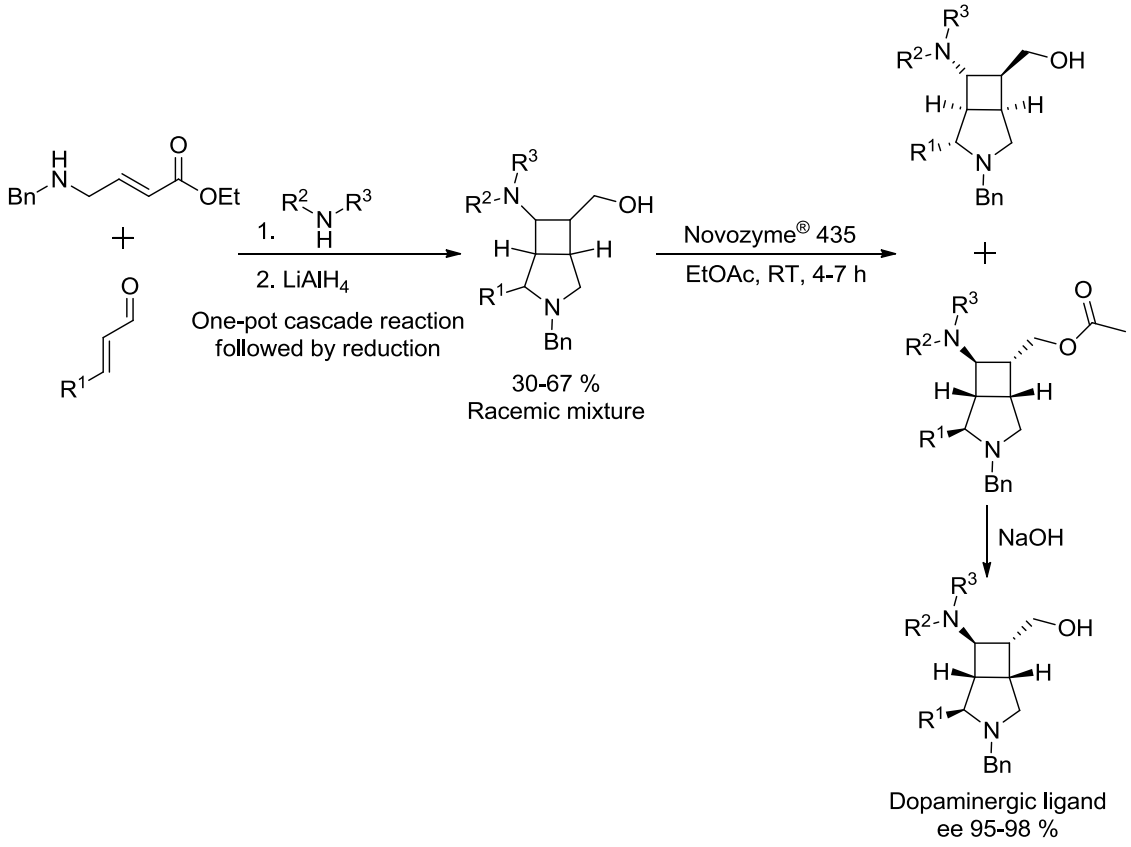
2.5.1.4. Synthesis of Cyclic Systems

4-*N*-Phenylacetylamino derivatives obtained by three-component coupling of anhydrides, phenylacetamide and dienophiles (ANAD sequence) were subjected to kinetic resolution via hydrolysis conducted by Penicillin G amidase (PGA) from *Escherichia coli*.[55] Moreover, in silico predictions based upon the simulation of enzyme-substrate interaction, were found to be in agreement with the observed selectivity of the enzyme.[55] Further study involved the application of a number of different hydrolases such as Chirazyme® E-3, *Burkholderia cepacia* lipase (BCL), CAL-B (Novozyme® 450), PGA-450 (immobilized PGA), and *p*-nitrobenzyl esterase (*p*-NBE), to improve the enantioselectivity of the process (Scheme 2.10).[56]



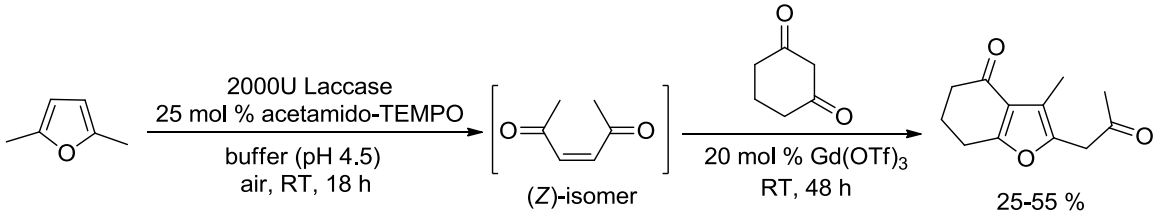
Scheme 2.10. Kinetic resolution of ANAD sequence products mediated by hydrolases.[56]

The kinetic resolution of racemic mixture of the 3-azabicyclo[3.2.0]heptane derivatives, synthesized by a multicomponent cascade reaction (combination of aza-Michael addition, intramolecular Michael addition and Mannich-type reaction), mediated by CAL-B (Novozyme® 450), furnished enantiopure derivatives that exhibited enhanced activity as dopaminergic ligands (Scheme 2.11).[57]



Scheme 2.11. Chemoenzymatic synthesis of dopaminergic ligand 3-aza-bicyclo[3.2.0] heptane.[57]

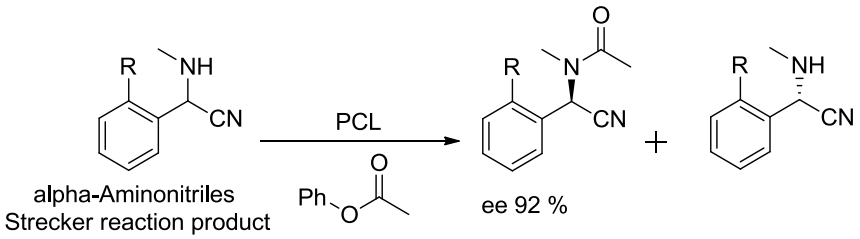
The synthesis of 6,7-dihydrobenzofuran-4(5*H*)-ones has been accomplished by combining laccase-catalyzed formation of (*Z*)-3-hexene-2,5-dione by oxidative cleavage of 2,5-dimethylfuran that subsequently underwent Lewis acid catalyzed domino 1,2-addition/1,4-addition/elimination with 1,3-dicarbonyl compounds in one-pot fashion (Scheme 2.12).[58]



Scheme 2.12. Chemoenzymatic one-pot synthesis of 6,7-dihydrobenzofuran-4(5*H*)-ones.[58]

2.5.1.5. Strecker Reaction

The development of an environmentally benign robust double dynamic asymmetric Strecker multicomponent system coupled with kinetically controlled *Pseudomonas cepacia* lipase (PCL) mediated transacylation facilitated the synthesis of enantiomerically pure acylated-*N*-substituted α-aminonitriles (Scheme 2.13).[59]

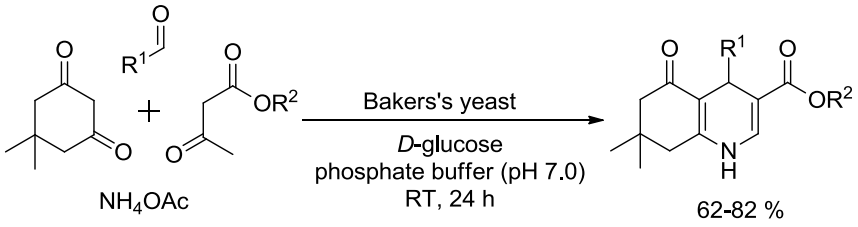


Scheme 2.13. One-pot PCL mediated kinetic resolution of α-aminonitriles obtained by Strecker reaction under thermodynamically controlled double dynamic covalent system.[59]

2.5.2. Enzyme Catalyzed Multicomponent Reactions

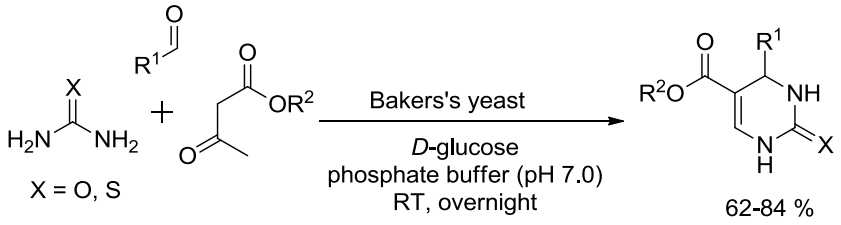
The promiscuity of enzyme action has been a topic of interest for their effective application and eventual design of multicomponent reactions. Thus this led to the development of enzyme catalyzed multicomponent Ugi, Hantzsch, Mannich, Biginelli, and some novel reaction sequences thus showcasing the aptness and capacity of biocatalysis for multicomponent reactions.

Baker's yeast (*Saccharomyces cerevisiae*) has been reported to synthesize dihydropyridyl derivatives via Hantzsch reaction.[60] This aspect of catalysis of Baker's yeast was further explored for the synthesis of aryl substituted polyhydroquinoline derivatives via unsymmetrical Hantzsch reaction (Scheme 2.14).[61]



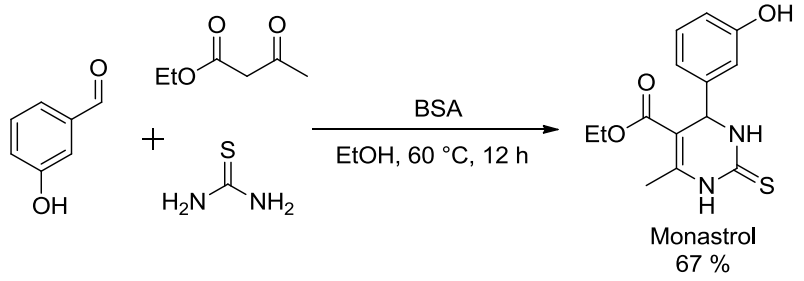
Scheme 2.14. Baker's yeast catalyzed four-component unsymmetrical Hantzsch reaction.[61]

Later, the synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones was conducted via Biginelli reaction catalyzed by Baker's yeast (*Saccharomyces cerevisiae*).[62] The developed methodology represented a set of relatively mild reaction conditions for introducing functional group diversity in the resultant product (Scheme 2.15).



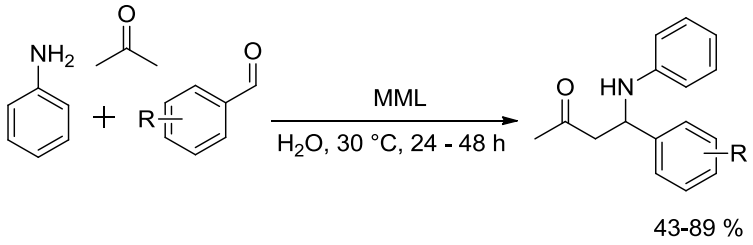
Scheme 2.15. Baker's yeast catalyzed Biginelli reaction for the synthesis of 3,4-dihydropyrimidin-2-(1*H*)-ones.[62]

An inexpensive, environmentally benign waste free methodology was developed for Biginelli reaction using bovine serum albumin (BSA) and the practicality of the methodology was demonstrated by recyclability of the catalyst system (BSA) on gram-scale synthesis of the corresponding bioactive derivative monastrol (Scheme 2.16).[63]



Scheme 2.16. Gram-scale synthesis of monastrol by Biginelli reaction catalyzed by bovine serum albumin (BSA).[63]

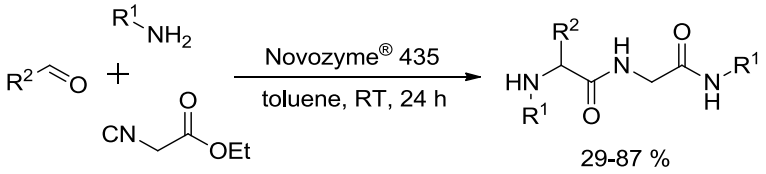
Lipase from *Mucor miehei* (MML) has been effectively employed for Mannich reaction in water for the synthesis of β-amino ketone derivatives (Scheme 2.17).[64]



Scheme 2.17. MML catalyzed Mannich reaction for the synthesis of β-amino ketone derivatives.[64]

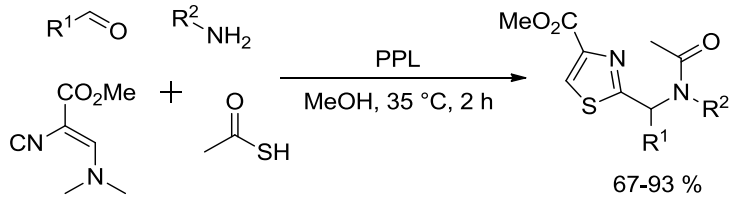
Since one of the reacting species was acetone that also served as co solvent with water but was not considered appropriate for the design of environmentally benign synthesis and also because of the competing reaction of acetone with other ketones to be employed,[65] therefore, further studies in same direction using a variety of substrates, used EtOH:water mixture as an environmentally benign solvent system and *Candida rugosa* lipase (CRL) as the enzyme of choice to achieve a successful conversion to the corresponding structurally diverse β-amino ketone derivatives.[65]

First model reaction for the synthesis of dipeptide via Ugi three-component reaction has further established the suitability of CAL-B (Novozyme® 450) for such multicomponent reactions and expanded the scope of the study by demonstrating its efficacy in aqueous medium. However aqueous medium has not been considered suitable for Ugi reaction due to the susceptibility of the decomposition of isocyanide in the presence of acid (Scheme 2.18).[66]



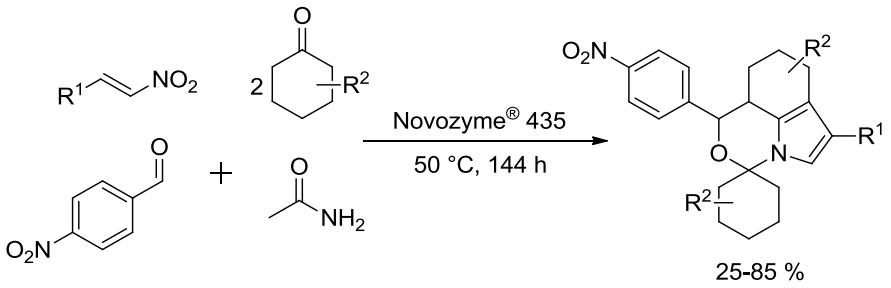
Scheme 2.18. Dipeptide synthesis via *Candida antarctica* lipase B (Novozyme® 435) catalyzed Ugi reaction.[66]

The development of PPL-catalyzed novel one-pot four-component reaction furnished a 2,4-disubstitued thiazole system under milder reaction conditions thus showcasing the suitability of biocatalysis for the synthesis of heterocyclic systems (Scheme 2.19).[67]



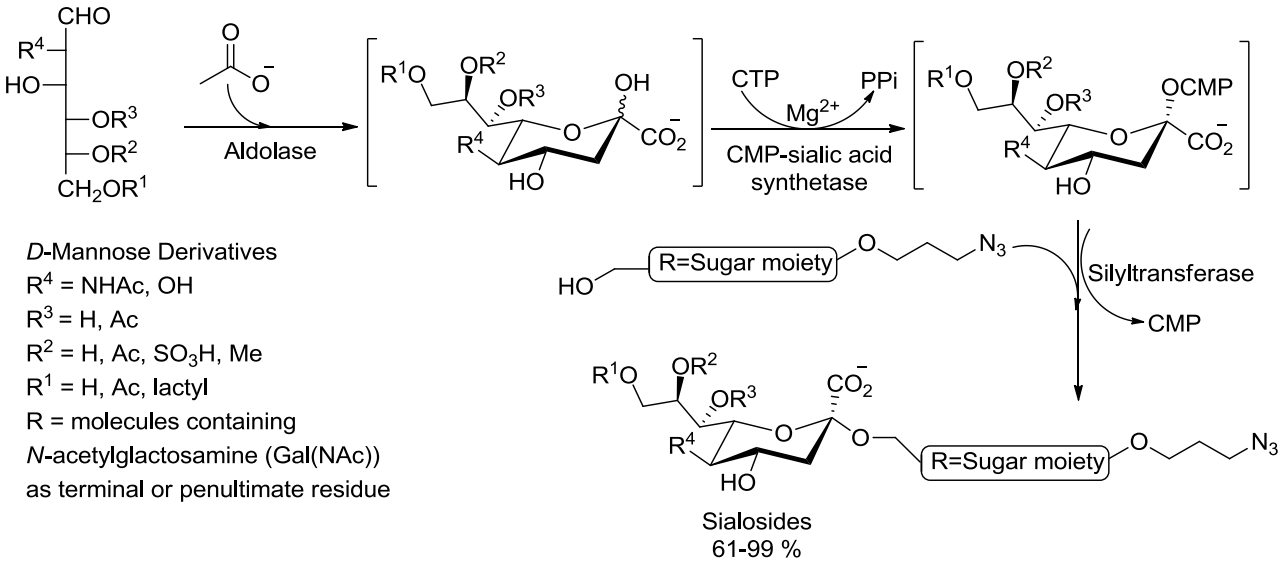
Scheme 2.19. PPL catalyzed novel four-component multicomponent reaction for the synthesis of 2,4-disubstituted thiazole synthesis.[67]

Spirooxazino derivatives have been synthesized by a novel CAL-B (Novozyme® 435) catalyzed multicomponent reaction from readily available starting materials furnishing six new C-C/-N bonds and two ring systems in a single step (Scheme 2.20).[68]



Scheme 2.20. CAL-B (Novozyme® 435) catalyzed novel multicomponent reaction for the synthesis of spirooxazino derivatives.[68]

In context to the physiological and pathological significance of sialic acid derivatives, a number of respective derivatives has been generated via one-pot three enzyme system (Scheme 2.21).[69]

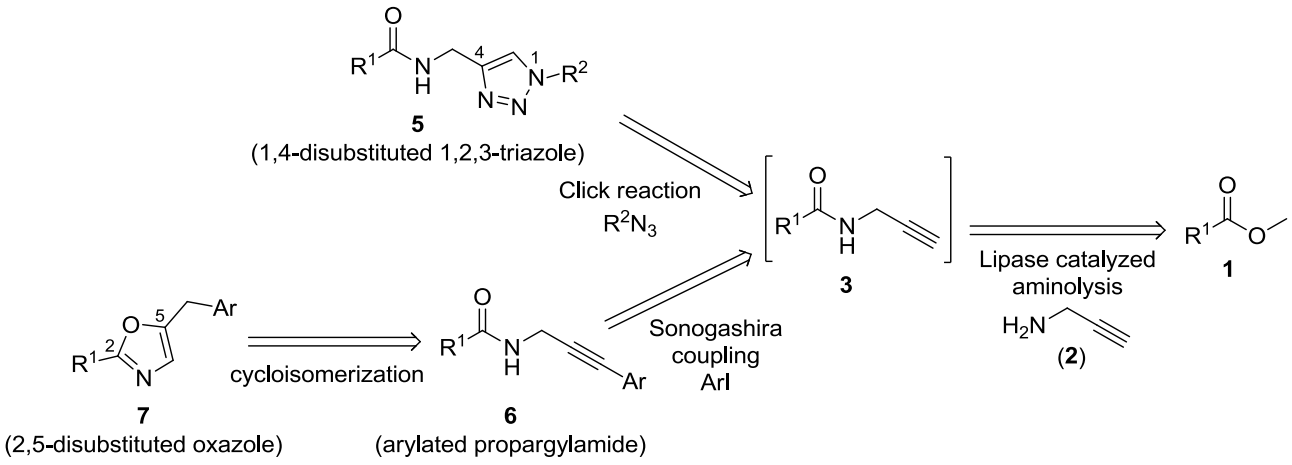


Scheme 2.21. Synthesis of α-2,6-linked sialosides via one-pot three enzyme system.[69]

3. Aim of the Project

In context to the applicability of enzyme catalysis for organic transformations, it was considered worthwhile to incorporate this concept with chemical catalysis for the development of new multicomponent one-pot strategies. The entry to the multicomponent sequence has been designed to be comprised of lipase catalyzed aminolysis of methyl ester substrates **1** for the synthesis of the corresponding propargylamides **3** that, in turn, would act as motif for further transformations to cycloadded (1,4-disubstituted 1,2,3-triazoles **5**), coupled (arylated propargylamides **6**) and cycloisomerized products (oxazoles **7**), owing to the presence of a terminal alkyne moiety. The intended cycloaddition (Cu(I) catalyzed click reaction) would give rise to the corresponding amide ligated 1,4-disubstituted 1,2,3-triazole systems **5**. The triazole nucleus has been established as bioisostere of amide linkage that mimics the atom placement and electronic properties of peptide bond and exhibits enhanced stability due to its resistance towards hydrolytic cleavage[70,71] thus paving the way for amide ligated triazole systems as interesting peptidomimetic motif, to drug development since, unlike amide linkage, triazole nucleus is not susceptible to proteolytic cleavage.[71,72] Moreover, acetylene derived amino acid chains can further be ligated to peptide sequences for labelling or conjugation purposes.[71] Keeping in view the established antitubuline and anti tumor activity of oxazoles,[73] coupling and subsequent cycloisomerization of propargyl-amides **3** has been intended that would give rise to corresponding 2,5-disubstituted oxazoles **7** with possible anticipated biological activities.

The main challenges have been anticipated to be the choice of an appropriate biocatalyst for the desired transformation, solvent selection, synthesis of suitable ester substrates **1** and ultimately, the compatibility, optimization and tunability of biocatalyzed process with the subsequent chemical catalysis (Cu(I) and Pd) for developing viable one-pot processes. A brief outline of the research idea has been sketched as a retrosynthetic analysis in Scheme 3.1.

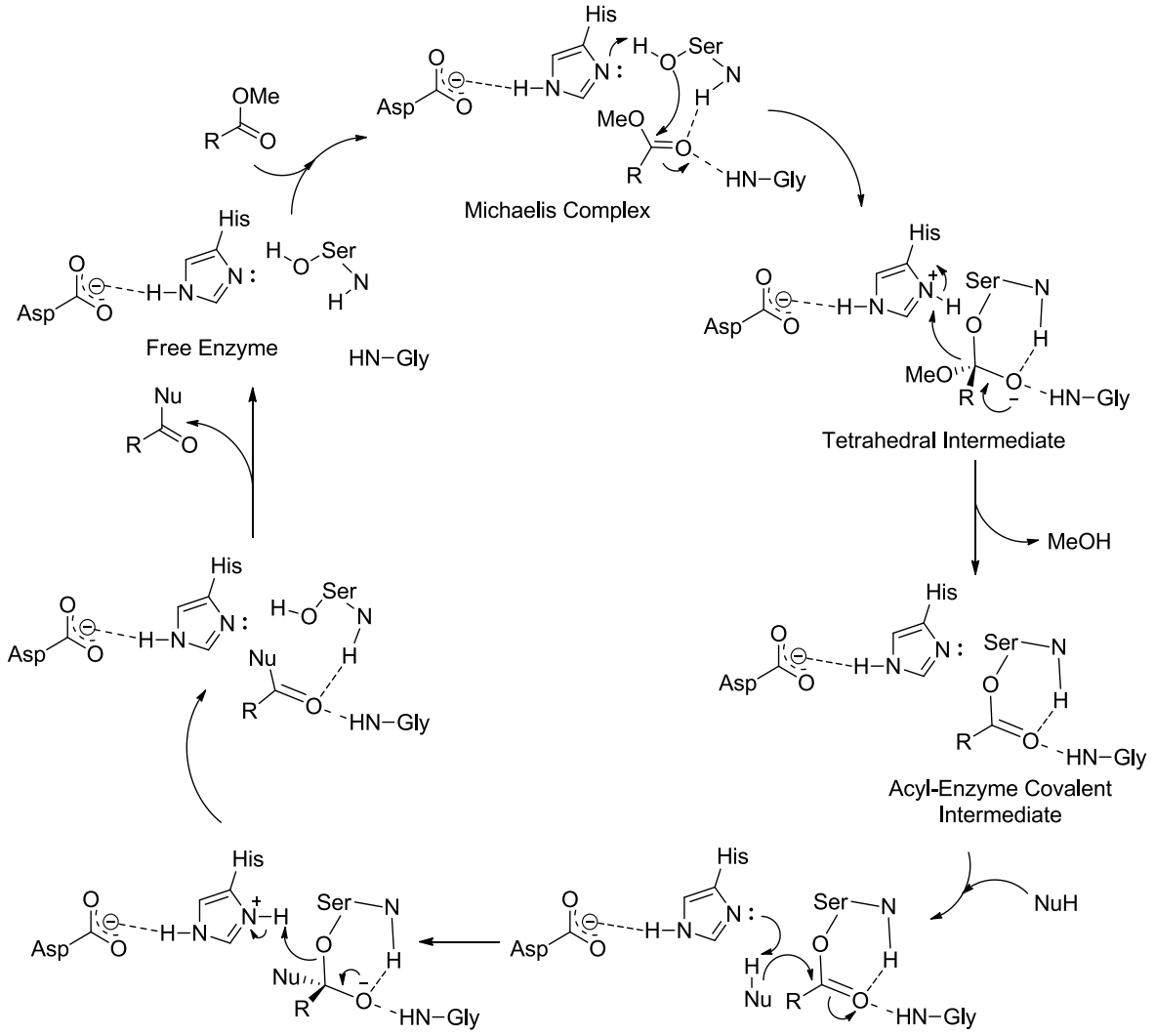


Scheme 3.1. Intended multicomponent one-pot synthetic strategies for the synthesis of amide ligated 1,4-disubstituted 1,2,3-triazole **5** via Cu(I) catalyzed click reaction and the synthesis of coupled product **6** via Sonogashira coupling and its subsequent cycloisomerization to oxazole systems **7** commencing with lipase catalyzed aminolysis of ester substrates **1** by propargylamine (**2**).

4. Literature Review

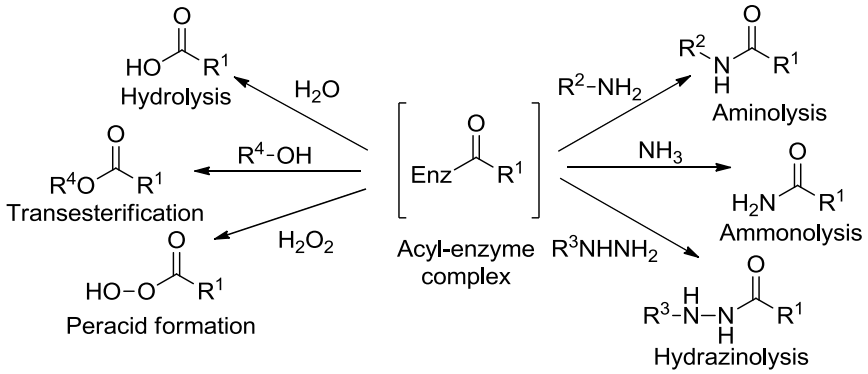
4.1. Mechanistic Aspects of Lipase Catalyzed Aminolysis

Lipases catalyzed aminolysis of ester substrate with a nucleophile, follows "serine hydrolase mechanism".[74] The mechanism involves the activation of an ester substrate by the formation of an acyl-enzyme complex at the active site that makes the acyl moiety of the ester susceptible for the attack by an appropriate nucleophile.[34] The histidine residue, activated by the aspartate side chain,[74] is in proximity to the serine residue at the active site of the enzyme and this arrangement results in its lower pK_a value,[34] making it a suitable nucleophile for the attack at the carbonyl moiety of ester substrate leading to the covalent bonded acyl-enzyme intermediate. Thus this acyl-enzyme intermediate, in the presence of an appropriate nucleophile, regenerates the enzyme and liberates the corresponding product from the active site (Scheme 4.1).



Scheme 4.1. Formation of an acyl-enzyme intermediate at the active site of the enzyme followed by the attack of an appropriate nucleophile for the generation of corresponding product and regeneration of the native conformation of the enzyme.[74,34]

Depending upon the nature of the nucleophile, the acyl-enzyme complex may give rise to the corresponding products (Scheme 4.2).

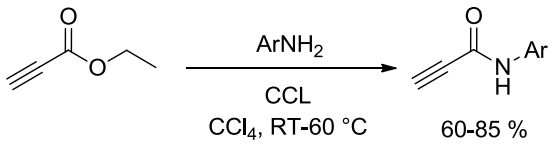


Scheme 4.2. Formation of different products arising from the attack by a nucleophile on the acyl-enzyme complex.[34,74]

Thus this mechanistic feature of serine hydrolases has been actively exploited for organic synthesis in pure organic medium where, under water free conditions, other nucleophiles can equally be compatible for carrying out the respective transformations.[74]

4.2. Development of Lipase Catalyzed Aminolysis

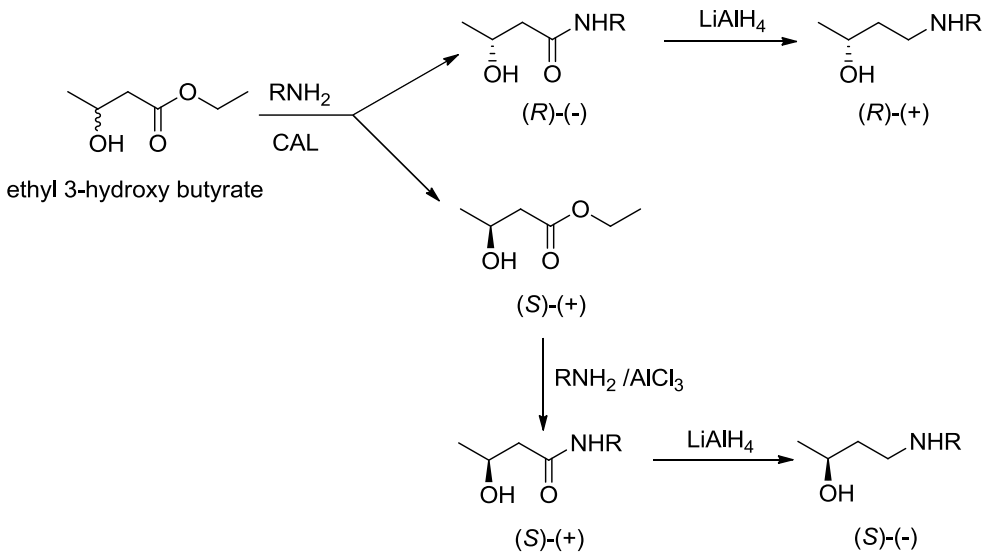
Proteases have been the enzyme of choice for the generation of amide linkage in peptide synthesis however their utility for such transformation has been limited by their amidase activity, preference towards *L*-configuration of amino acids, and stability issue in anhydrous organic solvents.[75] These limitations prompted the exploration of possible application of esterases and lipases for the synthesis of peptides and hence led to the study addressing the potential of porcine pancreatic lipase (PPL), *Candida cylindracea* lipase (CCL), and pig liver esterase (PLE) for peptide synthesis containing usual and unusual amino acids in the sequence.[75] Furthermore, the study of CCL for enantioselective aminolysis of the racemic mixture of ethyl 2-chloropropionate with several aromatic and aliphatic amines for corresponding chiral amide syntheses, established their suitability for the respective transformation even when employed below room temperature.[76] Enantioselective acylation of amino alcohols for corresponding pharmaceutically enantiopure chiral amide synthesis catalyzed by PPL established its efficacy compared to the available relatively expensive chemical method.[77] Propargylamide synthesis has been reported using CCL to catalyze the reaction between ethyl propiolate and aromatic amines[78] since aliphatic amines mainly tend to give the Michael addition product (Scheme 4.3).[79]



Scheme 4.3. CCL catalyzed propargylamides using ethyl propiolate and aromatic amines.[78]

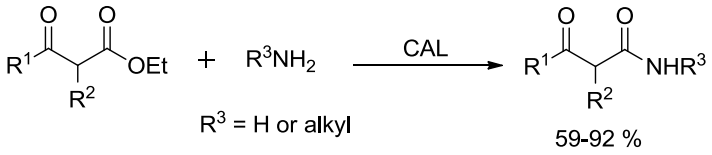
Double aminolysis of racemic ethyl 2-chloropropionate catalyzed by CCL resulted in the formation of the desired (*S*,*S*) diester in high enantiomeric excess compared to Amano P lipase and subtilisin that exhibited poor enantioselectivity.[79] Moreover, the overall transformation to the diester was strongly dependent not only upon the enzyme but also the solvent employed, CCl_4 and diisopropyl ether being the most suitable ones. Further elaboration of the study pertaining to (*S*)-enantioselective monoamidation of racemic ethyl 2-chloropropionate catalyzed by CCL, displayed the crucial role played by the solvent selection, nonpolar solvents (*n*-hexane and CCl_4) being the most suitable ones.[80] This also led to the finding of the reversal of role, enantioselectivity wise, of CCL exhibited (*R*)-enantioselectivity in transesterification reactions.[81] The study of the amidation reaction of racemic ethyl 2-chloropropionate with racemic aliphatic amines showed the preference of CCL for (*S*)-enantiomer of the ester while subtilisin preferred the (*S*)-enantiomer of the amine but no absolute selectivity was observed by the use of either enzymes towards ester and amine concurrently.[82]

RAL have been studied to show high (*R*)-enantioselectivity for the aminolysis of palmitic acid with racemic 2-amino octane compared to CCL, PPL, and *Penicilium cyclopium* lipase.[83] Solvent free aminolysis of ethyl butyrate with various aliphatic and aromatic amines catalyzed by lipase SP 382 (mixture of CAL-A and CAL-B) illustrated the dependence of the reaction strongly upon the structural features of the amines employed.[84] Aliphatic primary amines were more reactive than secondary ones while tertiary amines did not show any receptivity by the enzyme and the aminolysis by aromatic amines produced lower yields of the corresponding amide products. The access to enantiomerically pure 1,3-aminoalcohols was made possible by the enantioselective aminolysis of racemic ethyl 3-hydroxybutyrate effectively catalyzed by CAL compared to PCL, that then underwent subsequent reduction by $LiAlH_4$ with little or no racemization.[85,86] No reactivity was observed when aromatic amines were employed, however, highest enantioselectivity was observed using benzylamine (Scheme 4.4).



Scheme 4.4. CAL catalyzed synthesis of 3-hydroxyamides and their subsequent conversion to enantiomerically pure 1,3-aminoalcohols.[85,86]

PPL exhibited high enantiospecificity when *N*-benzyloxycarbony (Cbz) protected amino alcohols have been used compared to the nonprotected substrates. PPL was more efficient in catalytic activity compared to CCL and CAL.[87] CCL and CAL have been screened for their suitability for chiral amide formation using activated (having an electron withdrawing group at the α-position) and nonactivated ester substrates.[88] The (*S*)-enantioselectivity displayed by CCL in earlier studies,[2,6] has been shown to be independent of the size of the amine while CAL was selective towards the (*R*)-enantiomer with moderate enantiomeric excess of the corresponding products. CAL has been employed as the enzyme of choice for aminolysis and ammonolysis of β-ketoesters for the generation of β-ketoamides (Scheme 4.5).[89]

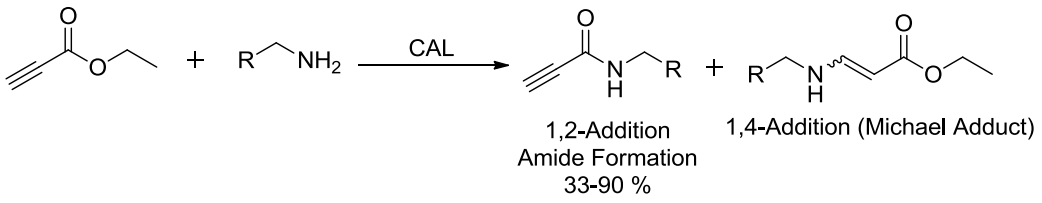


Scheme 4.5. CAL catalyzed aminolysis/ammonolysis of β-ketoesters.[89,90]

With racemic amines, CAL catalyzed the aminolysis of the (*R*)-enantiomer, generating the corresponding β-ketoamide in high enantiomeric excess.[90] Moreover, the ability of CAL-B was explored for a mild and practical enantioselective synthesis of the chiral amides by the ammonolysis of carboxylic acids.[91] This feature of CAL-B catalysis was further explored and effectively employed for their dual role for the esterification of octanoic acid with ethanol followed by ammonolysis to furnish octanamide.[92] A solvent free environmentally benign protocol for the synthesis of chiral amides has been devised using carboxylic acids as acyl

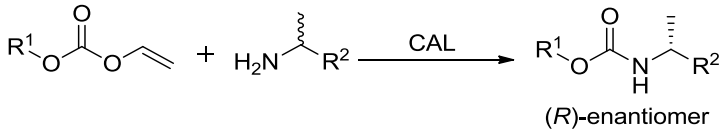
substrate and racemic amines catalyzed by CAL-B that exhibited its (*R*)-enantiopreference for the amines employed.[93]

The synthesis of the corresponding amides by aminolysis of nonactivated esters with β-furyl and β-phenyl groups has been achieved using CAL as the enzyme of choice.[94] Additionally, the aminolysis with the chiral amines established (*R*)-enantioselectivity of CAL with high enantiomeric excess values. The ester substrates with multiple bonds such as propiolic and acrylic esters, have also been converted to the corresponding amides using CAL as enzyme of choice.[95] The choice of solvent was crucial in the suppression of competing Michael 1,4-addition formation. The results of aminolysis with racemic amines was consistent with the previously established (*R*)-enantioselectivity of CAL.[94] The study of suitability of CAL-B for α,β-unsaturated esters led to a selective propiolamide synthesis without the formation of competing Michael adduct formation using immobilized CAL-B.[96] Following the same line of argument, chemoselective control favoring 1,2-addition (amide formation) in a reaction between aliphatic amines and ethyl propiolate was further studied. The optimum conditions for such chemoselectivity involved the use of immobilized CAL-B (Novozyme® 435) in solvents such as toluene, 1,4-dioxane, and MTBE (Scheme 4.6).[97]



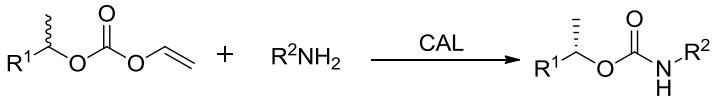
Scheme 4.6. Chemoselective amide formation (1,2-addition) catalyzed by CAL-B using ethyl propiolate and amines.[96]

In order to overcome the difficulties encountered during the chemical procedure of alkoxycarbonylation for chiral carbamate synthesis, the practicality of CAL for such transformation was explored leading to successful generation of corresponding chiral carbamates, with a few exceptions, generally retaining (*R*)-enantioselectivity that strongly depended upon the nature of substrates and solvent employed (Scheme 4.7).[98]



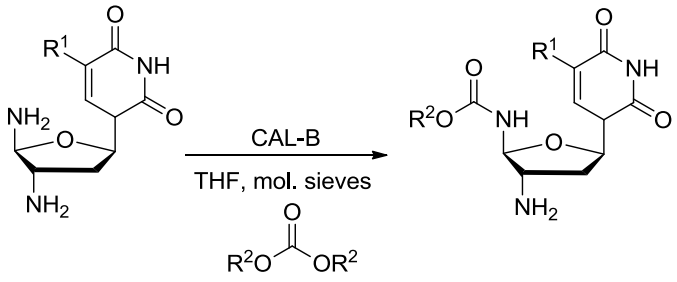
Scheme 4.7. CAL catalyzed alkoxycarbonylation transformation.[98]

The reversal of enantioselectivity was observed when racemic vinyl carbonates were employed, in which case CAL exhibited (*S*)-enantioselectivity for vinyl carbonates (Scheme 4.8).[99]



Scheme 4.8. CAL catalyzed kinetic resolution of vinyl carbonates exhibiting (*S*)-enantioselectivity for carbonates.[99]

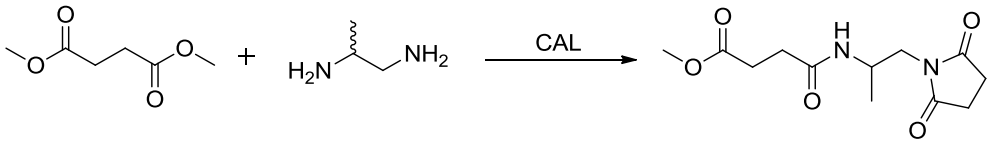
Alkoxycarbonylation of the primary amino group of pyrimidine 3,3-diaminonuceloside has been performed using CAL-B and homocarbonates for the synthesis of corresponding *N*-5-carbamates (Scheme 4.9).[100]



Scheme 4.9. CAL-B catalyzed alkoxycarbonylation of 3,5-diaminonucleoside using homocarbonates. [100]

The results of systematic study of the variables controlling the action of CAL-A and CAL-B towards ammonolysis, demonstrated that both isoforms of lipase from *Candida antarctica*, CAL-A (SP 526) and CAL-B (SP 525), exhibited (*R*)-enantiopreference in the aminolysis of an acyl donor.[101] The enantiomeric purity of the amides obtained, established the higher stereoselectivity of CAL-B compared to CAL-A and immobilization of CAL-B on Lewatit® E (Novozyme® 435) had no effect on (*R*)-enantiopreference.

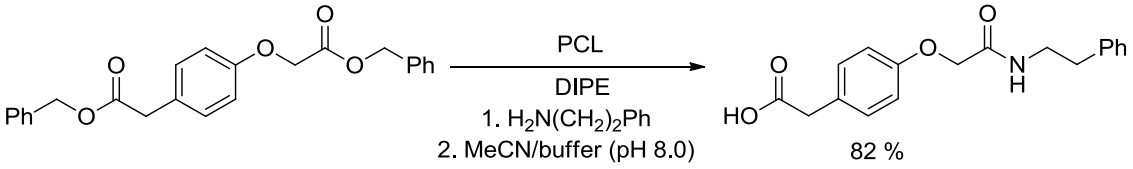
The mono ammonolysis and aminolysis of dimethyl succinate with ammonia and aliphatic amines respectively, have been conducted using CAL-B as the enzyme of choice that also furnished optically active amidoesters when racemic amines were used.[102] Further studies employing diamines with dimethyl succinate and dimethyl glutarate furnished polymeric materials such as *N*-*N*-polymethylenesuccinimides and *N*,*N*-polymethyleneglutarimides catalyzed by CAL.[103] However, in both studies,[102,103] the formation of cyclic imides has been observed along with other desired products (Scheme 4.10).



Scheme 4.10. Cyclic imide formation by CAL via cyclization involving the less hindered nitrogen atom.[103]

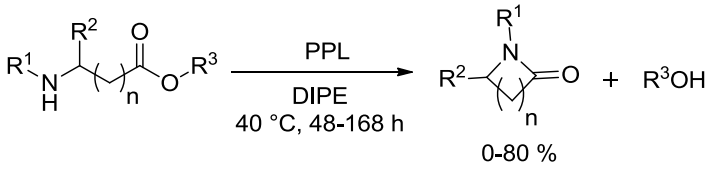
The regioselective action of CAL upon diester substrates was further explored when Cbz-protected glutamic diesters were used.[104] The results of the study showed that Cbz-*L*-Glu diester regioselectively underwent α-amide formation while the *D*-enantiomer furnished γ-amide. CAL exhibited (*R*)-enantioselectivity for the chiral amines used.

Pseudomonas cepacia lipase (Amano P-30) has been shown to catalyze aminolysis of benzyl ester with a variety of different amines.[105] Further, the aminolysis of α-hydroxy benzyl ester with different amines furnished α-hydroxyamides with high stereoselectivity.[106] The enhanced catalytic activity of Amano P-30 lipase towards the aminolysis of esters with oxygenated linkers with both symmetric and unsymmetric diamines[107] showcased the improved adequacy of such substrates that was mainly attributed to the inductive effect of the oxygen atom at the α-position, making the acyl moiety of such substrates more electrophilic and hence susceptible for enzyme attack.[108] However, the enhancement of enzyme action was observed for aminolysis, but opposite effect was observed for hydrolysis when both the processes were carried out in a one-pot fashion (Scheme 4.11).



Scheme 4.11. One-pot aminolysis and hydrolysis catalyzed by PCL showing the selective amide formation at the ester group with oxygenated side linker while hydrolysis occurred at nonactivated ester group.[108]

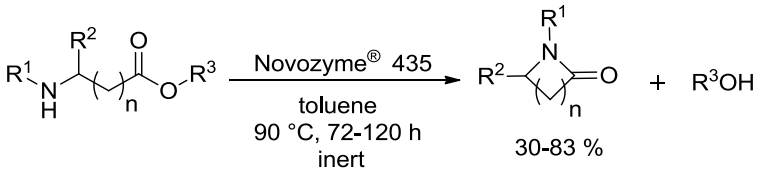
The formation of lactams by intramolecular cyclization of amino esters and macrocyclic bislactams from diesters and diamines, has been reported to be catalyzed by PPL in organic media.[109] Lower yields (40 %) of bislactams were obtained and the process was more selective depending upon the nature of diesters and diamines employed (Scheme 4.12).



Scheme 4.12. PPL catalyzed intramolecular cyclization of amino esters to lactams.[109]

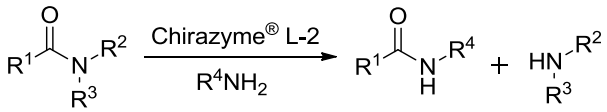
The ability of lipases to catalyze intramolecular cyclization of aminocarboxylic acids to lactams was further investigated and results revealed the suitability of CAL-B (Novozyme® 435) for the synthesis of ε-caprolacton. This methodology led to the synthesis of different sized lactam rings (4-8 membered).[110]

The failure of CAL-B to catalyze the corresponding ring system using mixtures of aminocarboxylic acids, showed the preference of the enzyme for homocyclization (Scheme 4.13).



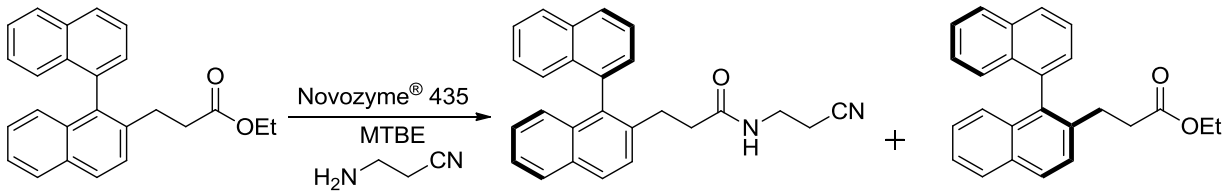
Scheme 4.13. CAL-B catalyzed synthesis of lactams.[110]

CAL-B (Chirazyme® L-2) catalyzed the transamidation of nonactivated amides with amines. The study suggested the crucial role of the size of the side chain of the amide as bulky amide substrates and aromatic amides failed to undergo transamidation while the structural variation of the side chain of primary amines did not substantially improve the process. The incomplete conversion was mainly attributed to the attainment of thermodynamic equilibrium between substrates and the product (Scheme 4.14).[111]



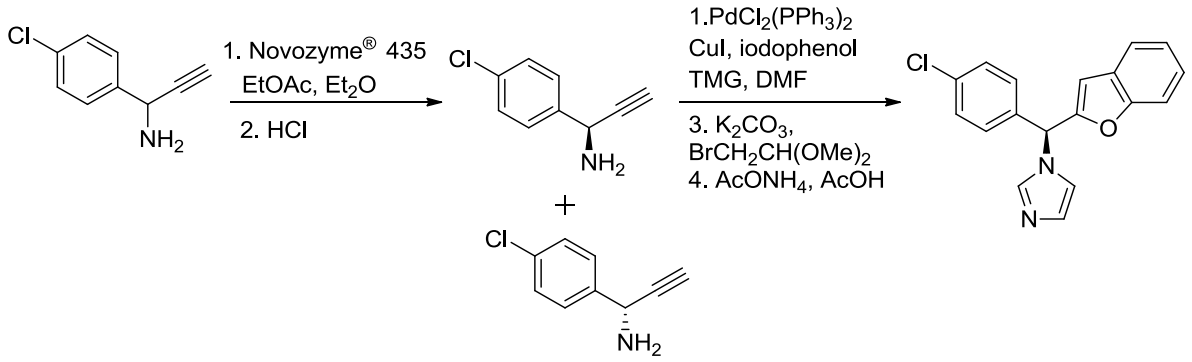
Scheme 4.14. Transamidation catalyzed by CAL-B (Chirazyme® L-2).[111]

Lactams with carboxylic acid/ester substituents such as both (*R*)- and (*S*)-enantiomers of pyroglutamic acids have been transformed to corresponding amide derivatives catalyzed by CAL-B (Novozyme® 435) using ammonia and different primary amines whereas secondary amines failed to furnish any amide product.[112] CAL-B (Novozyme® 435 and Chirazyme® L-2) exhibited the acceptability of bicyclic systems such as binaphthyl ester for their resolution via aminolysis of ethylene spacer between ester functionality and the naphthyl ring (Scheme 4.15).[113]



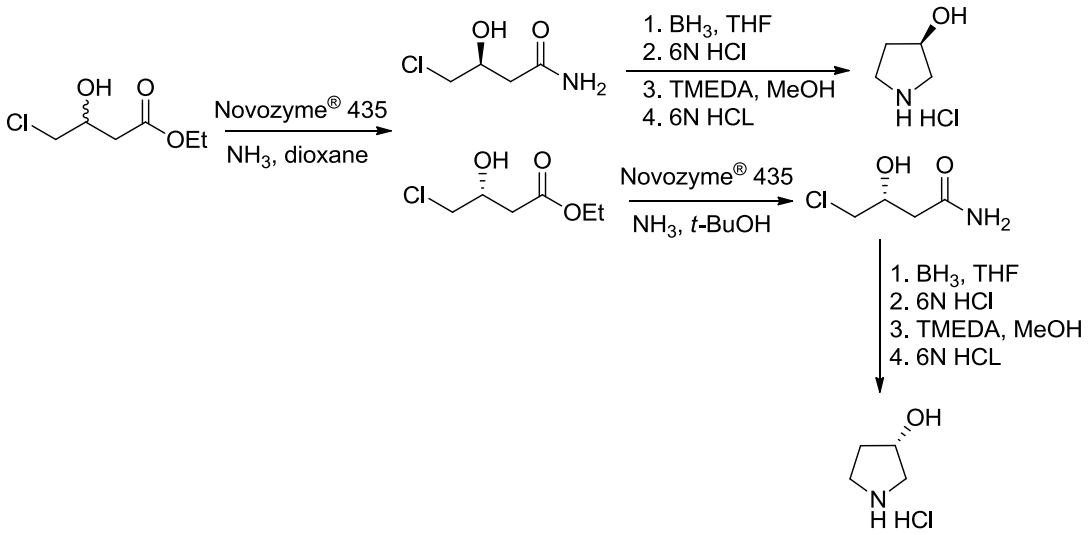
Scheme 4.15. CAL-B (Novozyme® 435) mediated kinetic resolution of binaphthyl system.[113]

The potential of kinetic resolution carried out by lipases has been exploited for the synthesis of enantiomerically pure starting materials for the subsequent chemical transformation to industrially and pharmaceutically interesting compounds. The synthesis of the enantiomerically pure aromatase inhibitor 1-(2-benzofuranyl(4-chlorophenyl)methyl)-1*H*-imidazole has been carried out by CAL-B (Novozyme® 435) catalyzed kinetic resolution of 1-(4-chlorophenyl)-2-propynylamine that served as starting material for the desired transformation (Scheme 4.16).[114]



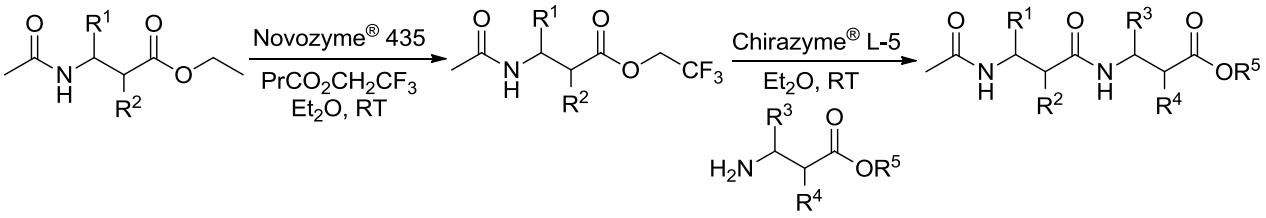
Scheme 4.16. CAL-B catalyzed kinetic resolution of 1-(4-chlorophenyl)-2-propynylamine for subsequent chemical transformation to biologically active imidazole system.[114]

Pyrrolidin-3-ol is a precursor for the synthesis of alkaloids and their derivatives. The syntheses of enantiomerically pure derivatives have been conducted by CAL-B (Novozyme® 435) catalyzed ammonolysis of racemic ethyl 4-chloro-3-hydroxybutanone, that established the crucial role of solvent in controlling the enantioselectivity of the enzyme (Scheme 4.17).[115] Starting with a racemic mixture, selective ammonolysis of the (*S*)-enantiomer took place when 1,4-dioxane was used as solvent, leaving (*R*)-enantiomer as unreacted ester with high ee values. The (*R*)-enantiomer was later transformed to corresponding amide by ammonolysis using the same biocatalyst (CAL-B) in *t*-BuOH.



Scheme 4.17. Solvent dependent chemoenzymatic synthesis of pyrrolidin-3-ol.[115]

A two step, CAL-A (Chirazyme® L-5) and CAL-B (Novozyme® 435), catalyzed procedure has been adopted for the synthesis of pharmaceutically interesting β-dipeptides (Scheme 4.18).[116]

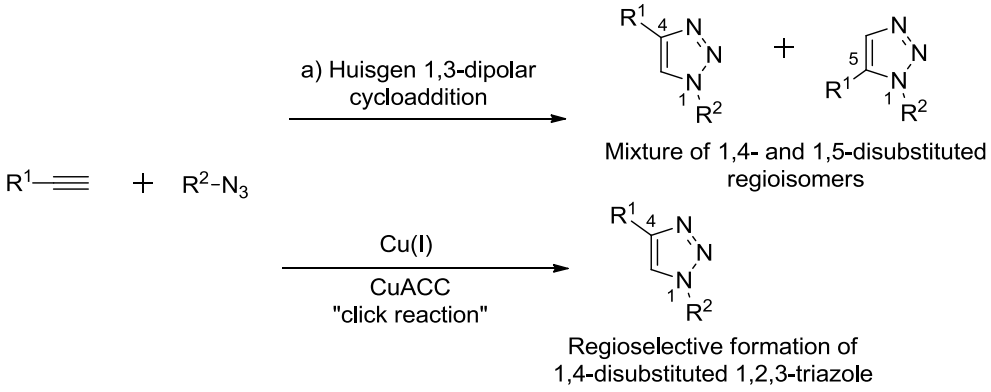


Scheme 4.18. CAL-A and CAL-B catalyzed β-dipeptide synthesis.[116]

CAL-B (Chirazyme® L-2) has been successfully employed for the selective synthesis of the secondary amide surfactant *N*-methyl lauroylethanolamide from methyl laurate and *N*-methylethanol amine. The enzyme remained active upto six catalytic runs, establishing the role of CAL as suitable catalyst for surfactant generation under benign and mild reaction conditions.[117] To maintain the green protocol of the process, in recent years, the suitability of CAL-B towards amide formation has been demonstrated using ionic liquids such as [BMIm(BF_4)][118] and [BMIm(PF_6)],[118,117] for the synthesis of amides with high enantioselectivity[118] with the retention of activity of the enzyme upto four consecutive cycles.[119]

4.3. Development of Synergy of Biocatalysis with Click Multicomponent Synthesis of Triazoles

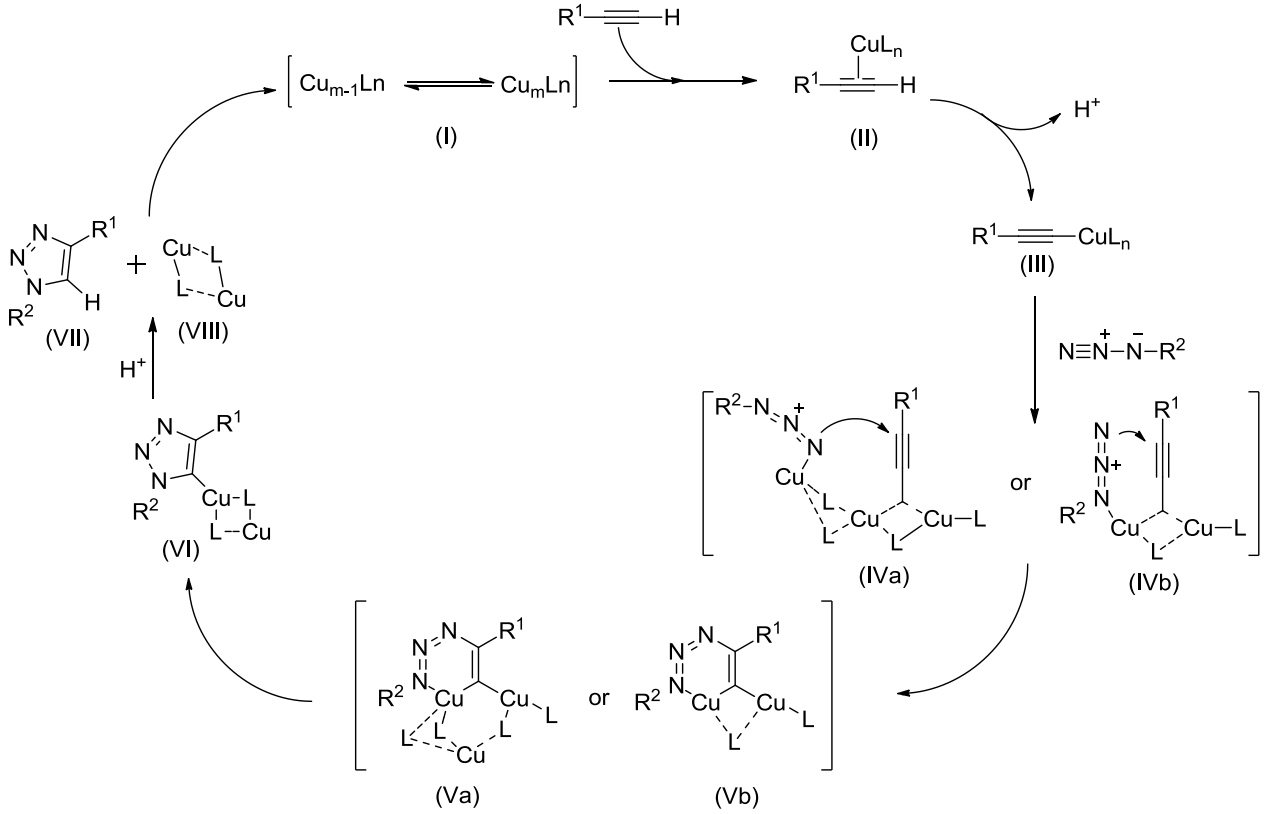
Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC), commonly known as "click reaction", holds its importance mainly in the context of regioselective perfection of thermal Huisgen 1,3-dipolar cycloaddition, giving rise to the selective formation of 1,4-disubstituted 1,2,3-triazole systems (Scheme 4.19).[120]



Scheme 4.19. a) Formation of mixture of products by thermal Huisgen 1,3-dipolar cycloaddition. b) Cu(I) catalyzed regioselective formation of 1,4-disubstituted isomer.

In the uncatalyzed process, the alkyne has been shown to remain a poor dipolarophile.[120] DFT calculations of the copper catalyzed process described the catalysis to be mediated by Cu(I) species and the rate enhancement due to the lowering of activation energy, was reasoned to be due to stepwise process contrary to the thermal uncatalyzed concerted pathway.[121] The reaction pathway commences with the coordination of Cu(I) species to the π-system of alkyne thereby lowering the pK_a value of the acetylenic proton resulting in the exothermic formation of acetylide. The coordination of the azide with Cu(I) acetylide is followed by a rearrangement to a six membered metallocycle (Va, Vb) that further transforms into copper metallated triazole (VI).[122] The copper-triazole complex (VI) then liberates the free triazole (VII) and $L_nCu(I)$ species via protonation or reaction with another electrophile. The kinetic measurements were not in agreement with the mechanism and indicated the involvement of more than two copper atoms coordinated with the terminal carbon atom of acetylene in the transition state.[123] The Cambridge Crystal Database suggested each C-C bond to be coordinated with three (90 % of all Cu(I)-alkyne complexes) or at least two copper atoms and this type of coordination has been established to be energetically favored for acetylide, making the secondary carbon atom more electrophilic.[122] Further DFT calculations suggested the acetylide and azide not to be coordinated to the same copper

atom in the transition state.[70,123,124] Scheme 4.20, shows a summarized depiction of the proposed mechanisms.

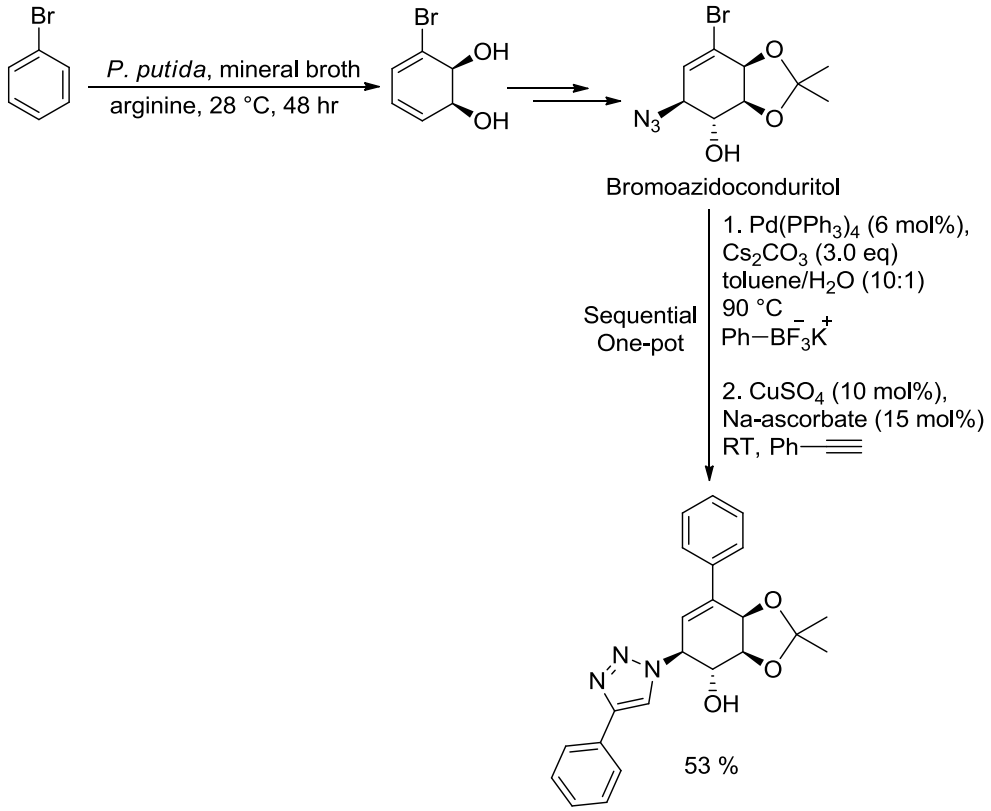


Scheme 4.20. Proposed mechanism for the Cu(I) catalyzed reaction between azides and terminal alkynes.[122]

Triazole ring containing derivatives have been studied to possess anti-HIV,[125] antibacterial,[126] anticancer,[127] antifungal[128] activities and have found an immense importance in the field of material sciences,[129] supramolecular assemblages,[130] and combinatorial drug discovery.[130] Plethora of literature is available pertaining to the role of click chemistry for drug development.[132] However only a limited amount of literature is available corresponding to the application of multicomponent chemoenzymatic synthesis of triazoles via click chemistry in a one-pot fashion.

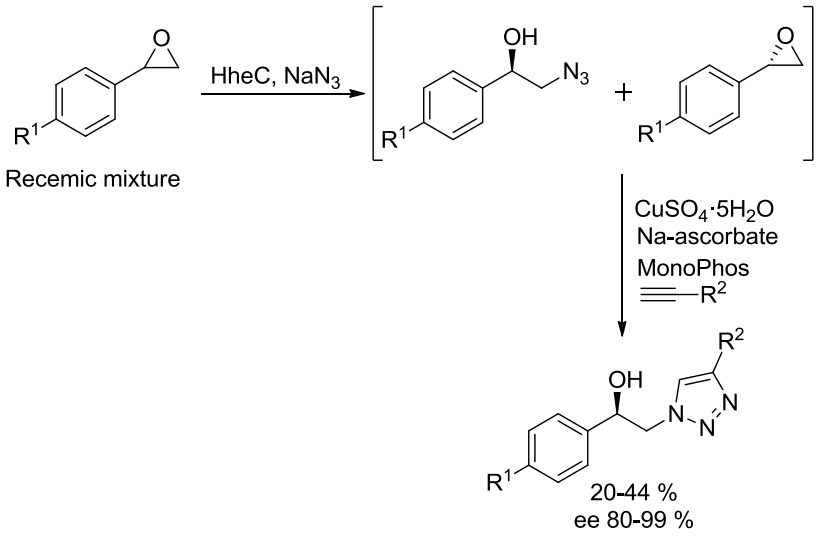
Like other multicomponent reactions, the concept of chemoenzymatic-click sequence has been pursued for the syntheses of enantiomerically pure target compounds via enzyme catalyzed enantioselective preparation of optically pure starting materials. In one of such approaches, enzymatic preparation of bromoazidoconduritol has been reported via whole cell fermentation of bromobenzene with *Pseudomonas putida*. After further protection of diol and derivatization, bromoazidoconduritol was obtained and served as a substrate for

subsequent palladium catalyzed coupling and click transformations, optimized both in tandem and sequential manner (Scheme 4.21).[133]



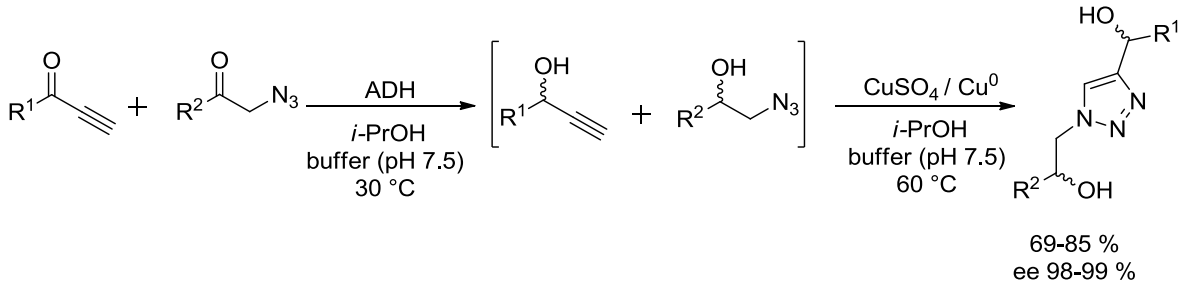
Scheme 4.21. Chemoenzymatic preparation of bromoazidoconduritol for subsequent sequential one-pot coupling-click sequence.[133]

One-pot sequences have been developed that involved the intermediacy of epoxide with subsequent derivatization to azide via ring opening. This mutual compatibility displayed by epoxide ring opening and click reaction in a one-pot process led to the development of a tandem chemoenzymatic-click sequence for the synthesis of enantiomerically pure regioselective chiral hydroxy triazoles. Starting off with racemic epoxide mixtures, it was possible to carry out the enatioselective azidolysis catalyzed by halohydrin dehalogenase (HheC) to give rise to 1,2-azido alcohols. The attack of azide primarily took place at the non-substituted carbon atom of the ring (β-regioselectivity). The azido alcohol so formed further participated in click reaction giving rise to chiral hydroxy triazoles.[134] This methodology accounts for an elegant eco friendly procedure for the synthesis of optically pure chiral derivatives (Scheme 4.22).



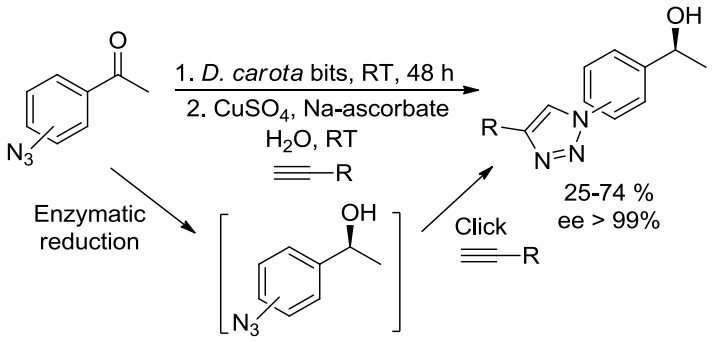
Scheme 4.22. One-pot chemoenzymatic-click sequence for the synthesis of chiral hydroxy triazoles.[134]

The concept of convergent one-pot synthesis involving reduction by alcohol dehydrogenase (ADH) and Cu(I) catalyzed click reaction was successful in synthesizing chiral 1,4-disubstituted 1,2,3-triazole derived diols via intermediacy of chiral alcohols in high yields and excellent enantio- and diastereoselectivities, showing the mutual compatibility of enzymatic and chemical catalysis (Scheme 4.23).[135]



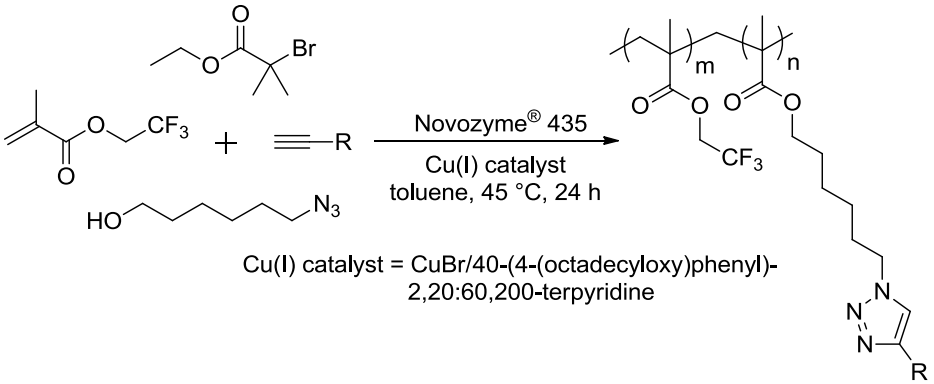
Scheme 4.23. Chemoenzymatic one-pot protocol to synthesize enantioenriched 1,2,3-triazole derived diols.[135]

Systems containing a chiral alcohol moiety may be anticipated as important precursors for the synthesis of chiral auxiliaries. In this context, a sequential two step one-pot procedure was designed taking advantage of the synergy between the enantioselective reduction of azidoketones by *Daucus carota* (carrot root) to azidoalcohols followed by triazole synthesis yielding the corresponding chiral hydroxy disubstituted 1,2,3-triazoles (Scheme 4.24). This chemoenzymatic sequence encompassed the obvious advantages of using cheap and readily available starting materials in water as a green solvent.[136]



Scheme 4.24. One-pot synthesis of chiral 1,2,3-triazoles from azidoacetophenones.[136]

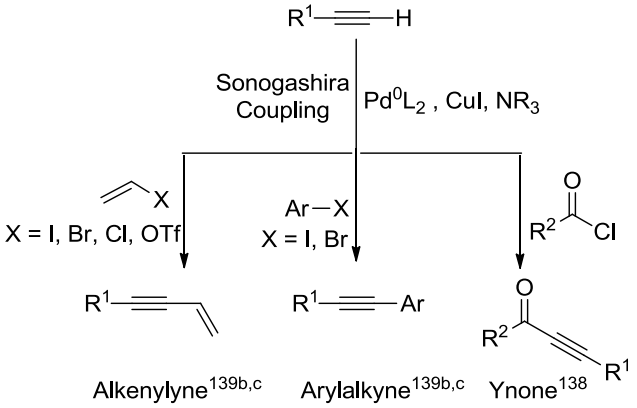
A simultaneous chemoenzymatic one-pot sequence has been developed by combining click reaction and CAL-B (Novozyme® 435) catalyzed transesterification, coupled with an atom transfer radical polymerization (ATRP) giving rise to multicomponent polymerization (MCP) for a tricomponent polymer system (Scheme 4.25).[137]



Scheme 4.25. Click–chemoenzymatic–ATRP system in one-pot to prepare designable functional polymers.[137]

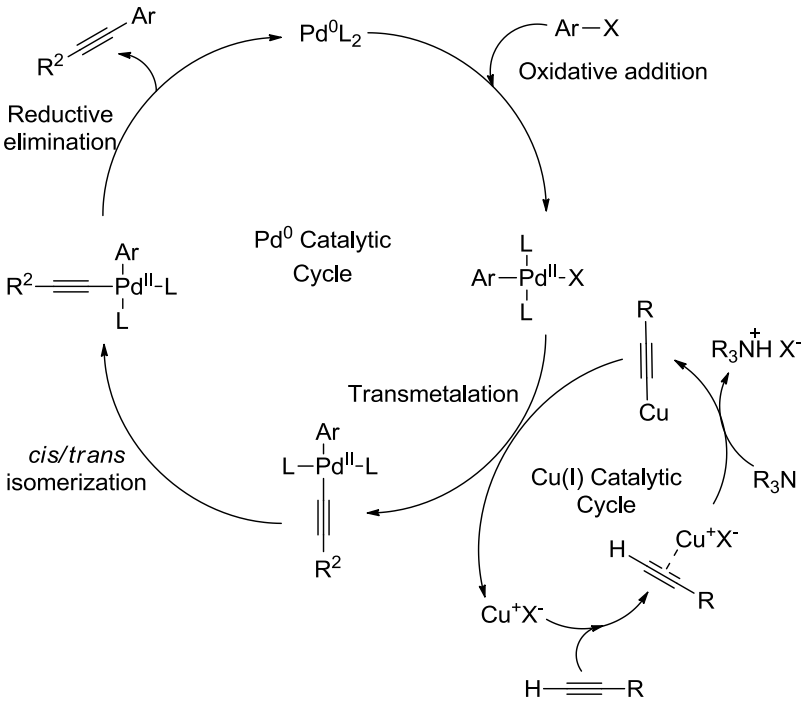
4.4. Synergy of Biocatalysis with Palladium Catalyzed Coupling Reactions

The Sonogashira coupling reaction has developed its efficacy as one of the most widely employed C(sp^2)-C(sp) coupling methods between aryl, alkenyl, acyl halides or triflates and terminal alkynes for the synthesis of corresponding arylalkynes, alkenylynes and ynones[138] respectively, catalyzed by Pd(0)/Pd(II) phosphane complexes and Cu(I) as co catalyst in the presence of an appropriate base (Scheme 4.26).[139] The relative reactivities of sp^2 species for Sonogashira coupling reaction follow the order: vinyl iodide > vinyl triflate > vinyl bromide > vinyl chloride > aryl iodide > aryl triflate > aryl bromide >> aryl chloride. This methodology has been immensely employed in the fields of dyes, sensors, electronics, polymers, natural product chemistry and heterocycle synthesis.[139b,c]



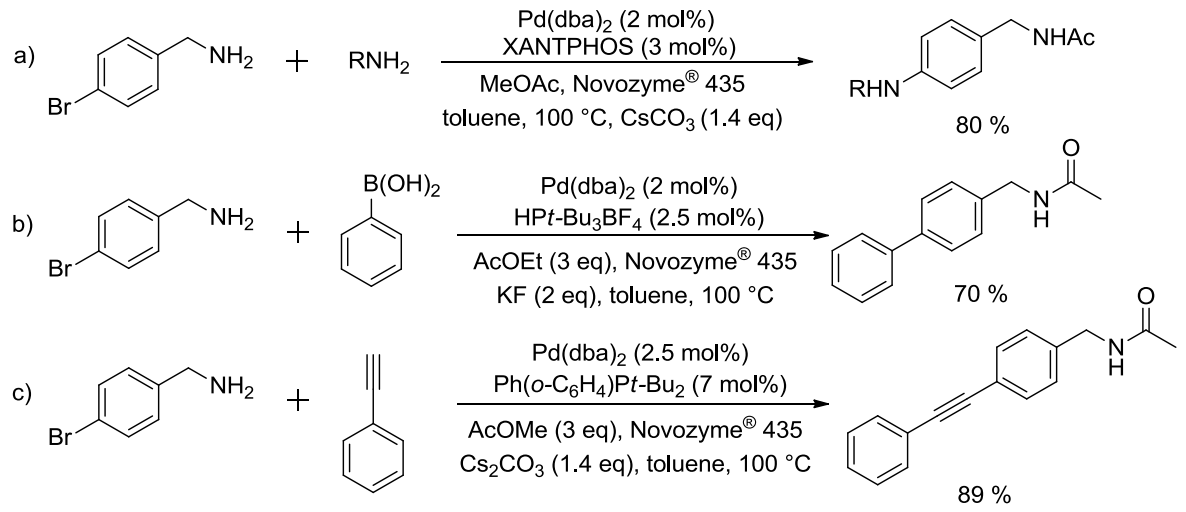
Scheme 4.26. An overview of the attainment of product diversity utilizing Sonogashira coupling.

Despite of its extensive application in the field of organic synthesis, the exact mechanism of the Sonogashira coupling reaction remained an issue of debate mainly due to the involvement of two independent metal catalytic cycles (Pd(0) and Cu(I)) and the difficulty to isolate the organometallic intermediates from homogeneous mixture to confirm several mechanistic aspects.[139c] However, a concise description of the proposed mechanism is depicted in Scheme 4.27. The mechanism is comprised of two catalytic cycles. The palladium catalyzed cycle commences with active $Pd(0)L_2$ species that can be formed from Pd(0) catalyst such as $Pd(PPh_3)_4$ or Pd(II) ($PdX_2(L_n)_2$ such as $PdCl_2(PPh_3)_2$) species via coordination with terminal alkyne that then regenerates the active $Pd(0)L_2$ species after reductive elimination.[140] Since in present work Sonogashira coupling of aryl halides **4h-4p** with propargylamides **3** has been performed, therefore, for this purpose the consideration of energy barrier of oxidative addition, which is a rate limiting step of the process, has been considered important that follows the order ArI < ArBr < ArCl.[141] After transmetalation with copper acetylide, generated in Cu(I) catalyzed cycle, the resulting adduct undergoes *cis*/*trans* isomerization and then reductive elimination, generating active $Pd(0)L_2$ species again (Scheme 4.27). The nature of Cu(I) catalytic cycle is not well developed mainly due to possibility of copper-ligand interaction(s) and inter transfer from one metal to other in tandem palladium/copper coupling cycle.[142]



Scheme 4.27. Proposed mechanistic involvement of two separate metal catalytic cycles (Pd^0 and Cu(I)) for Sonogashira coupling.

Due to the synthetic magnanimity of palladium catalyzed cross couplings, the concept of incorporation of Sonogashira coupling with lipase catalyzed transformations can very well be apprehended and anticipated to further increase the synthetic potential of the overall chemoenzymatic process when conducted in tandem or consecutive manner. This synergistic behaviour has been reported by carrying out lipase mediated aminolysis with palladium catalyzed couplings (Buchwald-Hartwig, Suzuki, Sonogashira) simultaneously on the same substrate leading to the development of a lipase-Pd cascade reaction sequence.[143] The efficiency of the developed cascade reactions strongly depended upon the nature of Pd catalyst, alcohol side product and optimization of the cascade process as a whole contrary to the generally followed approach of stepwise optimization strategies (Scheme 4.28).

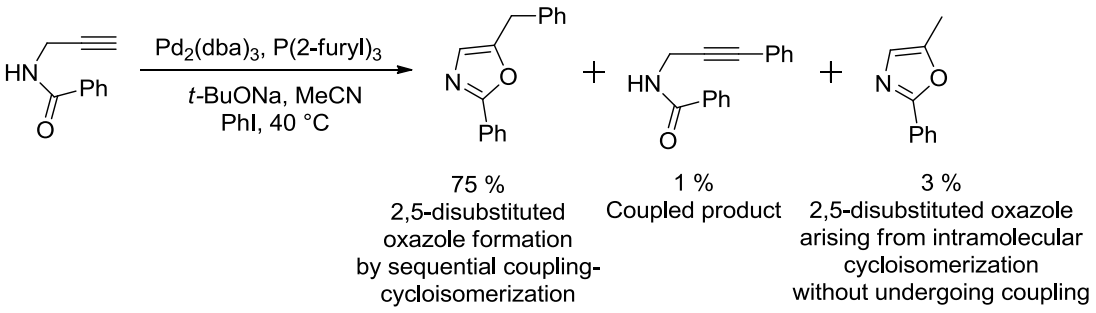


Scheme 4.28. Study of the synergistic effect of lipase-palladium cascade reactions: a) Buchwald-Hartwig Coupling. b) Suzuki Coupling. c) Sonogashira Coupling.[143]

4.5. Heterocyclization of Propargylamides

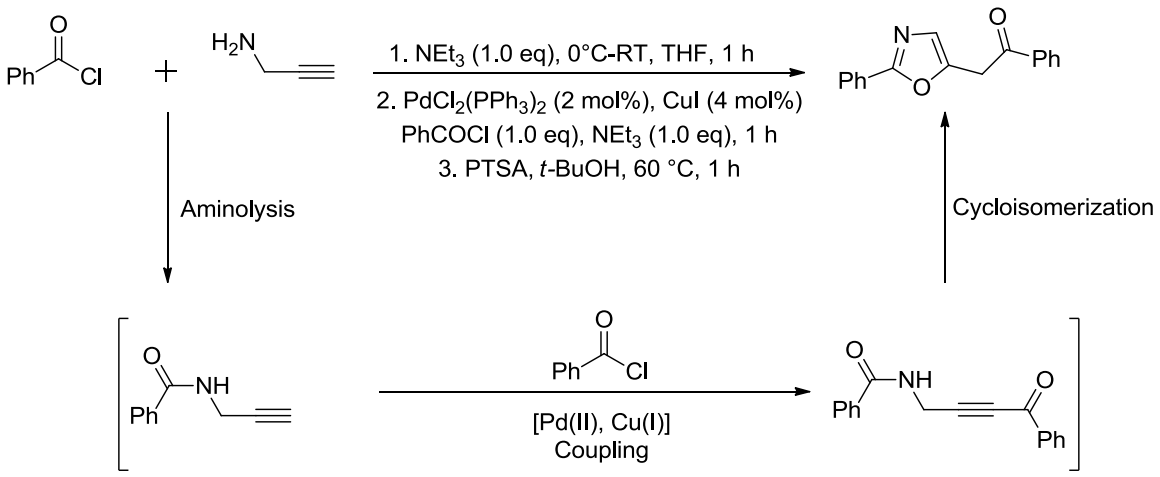
4.5.1. Oxazole Syntheses

Synthesis of the biologically active 2,5-disubstituted oxazole structure has been achieved via sequential coupling and cycloisomerization of *N*-propargylamides.[144] Formation of the oxazole system was reasoned to take place following the coupling step. The subtlety and control of reaction conditions were crucial. The coupling step was promoted by the implementation of electron withdrawing phosphane ligands while cycloisomerization was induced by the choice of an appropriate base and base to alkyne ratio, since excess of base could also promote the intramolecular cycloisomerization of the propargylamide without undergoing any coupling reaction (Scheme 4.29).



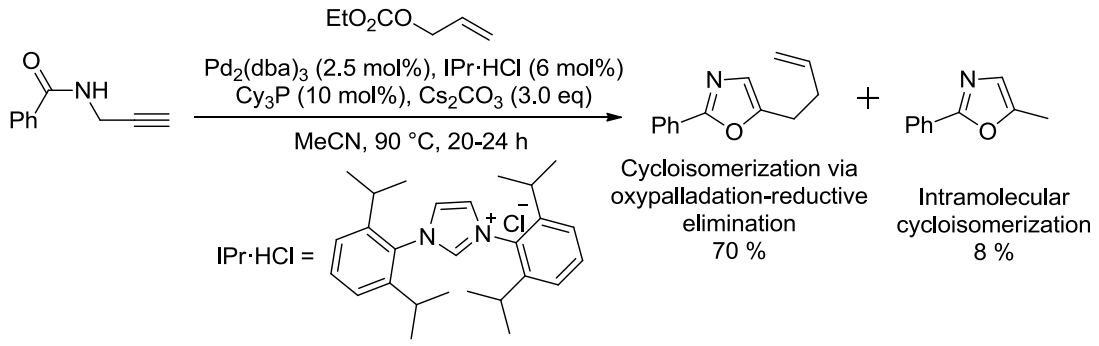
Scheme 4.29. Preparation of 2,5-disubstituted oxazole system by sequential coupling-cycloisomerization sequence.[144]

The propensity of propargylamides to undergo cycloisomerization to the oxazole ring was further exploited and led to the development of a consecutive three-component one-pot oxazole synthesis via an amidation-coupling-cycloisomerization (ACCI) sequence.[145] The cycloisomerization of acylated propargylamide was mediated by PTSA unlike the previously reported method in which silica gel was used as an inducer for this transformation (Scheme 4.30).[146]



Scheme 4.30. A consecutive three-component one-pot synthesis of 2,5-disubstituted oxazoles.[145]

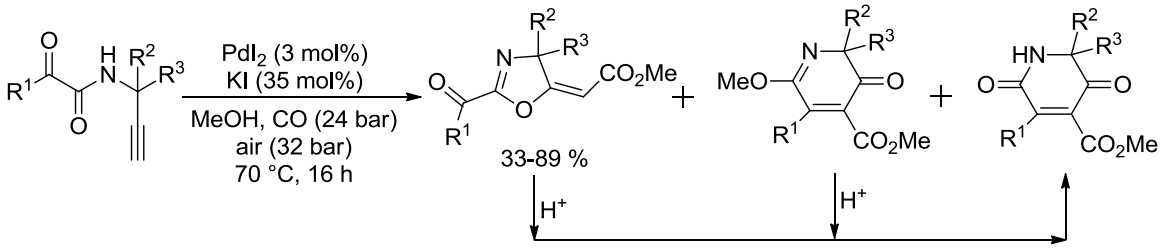
In another approach, the synthesis of the oxazole derivatives possessing unsaturated side chains through a palladium catalyzed cycloisomerization-allylation reaction of propargylamide derivatives with allyl carbonates using tricyclohexylphosphane (Cy_3P) and 1,3-bis(2,6-diisopropylphenyl)imidazole-2-ylidene (IPr·HCl) ligands has been established (Scheme 4.31).[147] The mechanistic steps have been rationalized to be following the cyclization of propargylamides through the oxypalladation caused by a π-allyl palladium complex, generated by Pd(0) and allyl ethyl carbonate, followed by reductive elimination[147] unlike previously studied mechanisms where the coupling step was followed by the acid[145]/base[144] cycloisomerization step.



Scheme 4.31. Palladium catalyzed cycloisomerization-allylation of propargylamide with allyl carbonate for 2,5-disubstituted oxazole synthesis.[147]

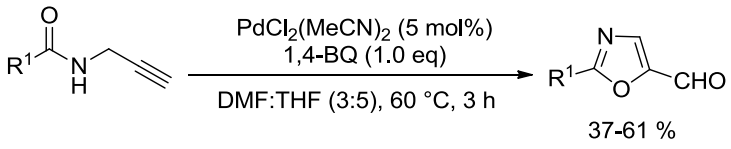
4.5.2. Intramolecular Cycloisomerization of Terminal Propargylamides

The capability of palladium to induce cycloisomerization was employed for heterocyclization-alkoxycarbonylation to synthesize (*E*)-5-(alkoxycarbonyl)methylene-3-oxazolines (Scheme 4.32).[148] The process of heterocyclization has been shown to be strongly dependent upon the structural features of propargylamides and the nature of the solvent. Moreover, the oxazolines so formed can further undergo rearrangement to six-membered ring systems under acidic conditions.



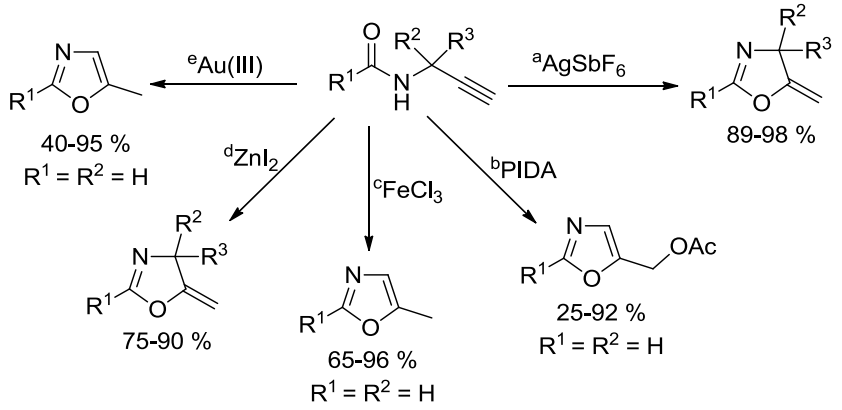
Scheme 4.32. Pd-catalyzed oxidative cyclization-alkoxycarbonylation of propargylamides.[148]

The concept of Pd(II) catalyzed oxidative cycloisomerization gave rise to the syntheses of 2-substituted-5-oxazolecarbaldehydes (Scheme 4.33).[149] However, the mechanistic role of palladium was not clear, it was deduced to undergo complexation with triple bond, making it susceptible to nucleophilic attack (oxygen atom of amide moiety) giving rise to 5-*exo*-*dig* cyclization to the corresponding oxazole structure. The presence of oxidant promoted the regeneration of active Pd(II) species in catalytic cycle and dehydrogenation of 4,5-dihydrooxazole-5-carbaldehyde to oxazole.



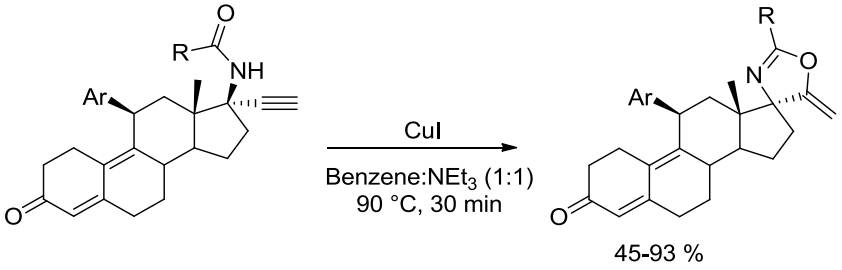
Scheme 4.33. Intramolecular Pd(II)-oxidative cyclization of propargylamides to 2-substituted- 5-oxazolecarbaldehyde.[149]

Over the years many metal catalytic systems such as silver,[150] gold (Au(III)[151-153] and Au(I)),[154-155] copper,[156] Lewis acid[146,157] and non-metal catalyst such as hypervalent iodine oxidants (PIDA)[158] have emerged for cycloisomerization of terminal propargylamides under relatively milder and robust reaction conditions (Scheme 4.34).



Scheme 4.34. Different metal and non-metal catalytic systems for cycloisomerization of terminal propargylamides (a,[150] b,[158] c,[157] d,[157] e,[151-153]).

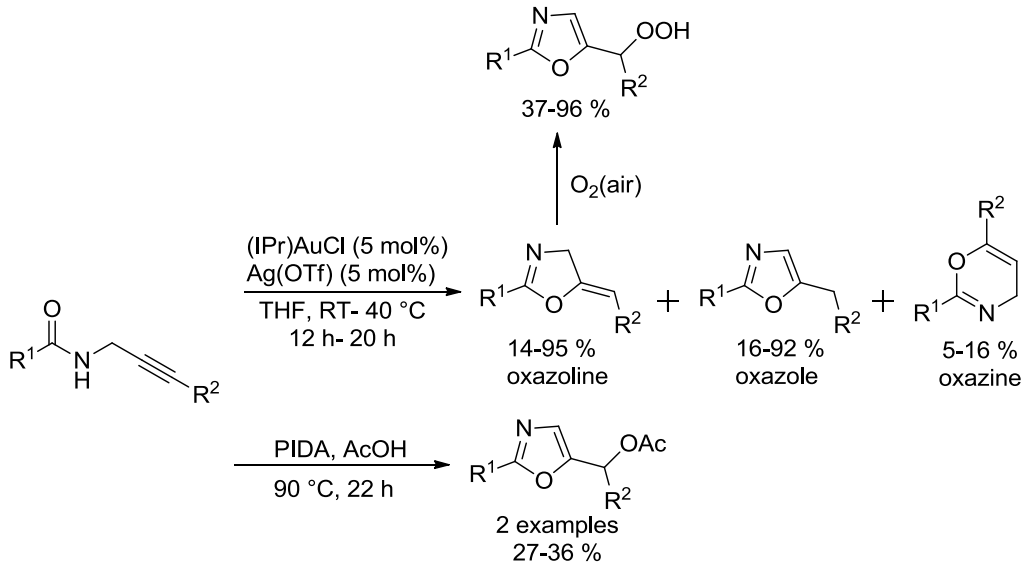
Scheme 4.35, depicts the utility of Cu(I) for the syntheses of a variety of novel 11*β*-aryl-17,17-spiro[(4'*H*,5'-methylene)oxazol]-substituted steroids in moderate to good yields via cyclization of acylaminoacetylenes.[156]



Scheme 4.35. Cu(I) catalyzed cycloisomerization of steroidal acylaminoacetylenes to dihydrooxazole.[156]

4.5.3. Intramolecular Cycloisomerization of Non-terminal Propargylamides

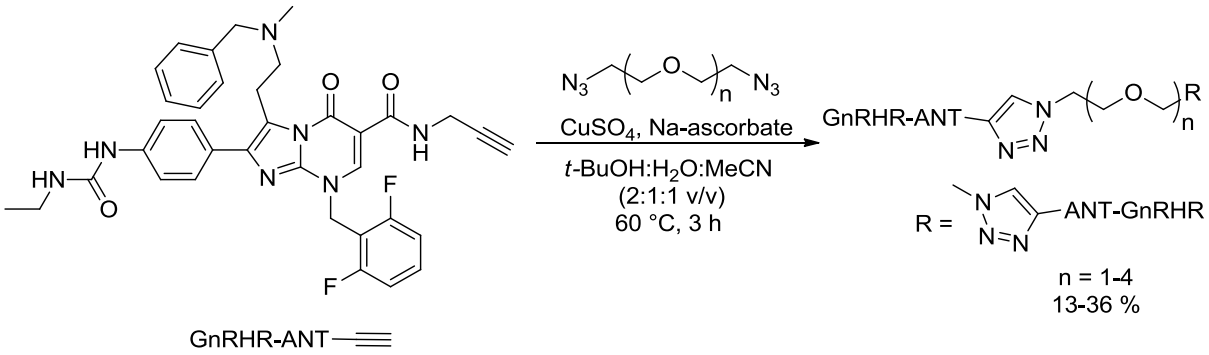
PIDA mediated cycloisomerization of non-terminal propargylamides was successful in furnishing the corresponding oxazole but lower yields of the products were obtained (Scheme 4.36).[158] Under Au(III) reaction conditions, non-terminal propargylamides have been shown to remain unresponsive to the desired transformation therefore a Au(I) catalyst was developed that gave rise to the corresponding oxazolines (via 5-*exo*-*dig* cyclization) and oxazines (via 6-*endo*-*dig* cyclization) using gold-carbene catalyst system.[154] The selectivity between the two products was strongly dependent upon the nature of the catalyst system and the alkyl side chain length of the propargylamides. No generality of the scope could be concluded as mixture of products was obtained using various substituted propargylamide substrates. However, oxazoline derivatives were further converted to peroxide oxazole derivatives under oxidizing conditions (atmospheric oxygen) (Scheme 4.36).[155]



Scheme 4.36. PIDA[157] and Au(I)[154-155] mediated cycloisomerization of non-terminal propargylamides.

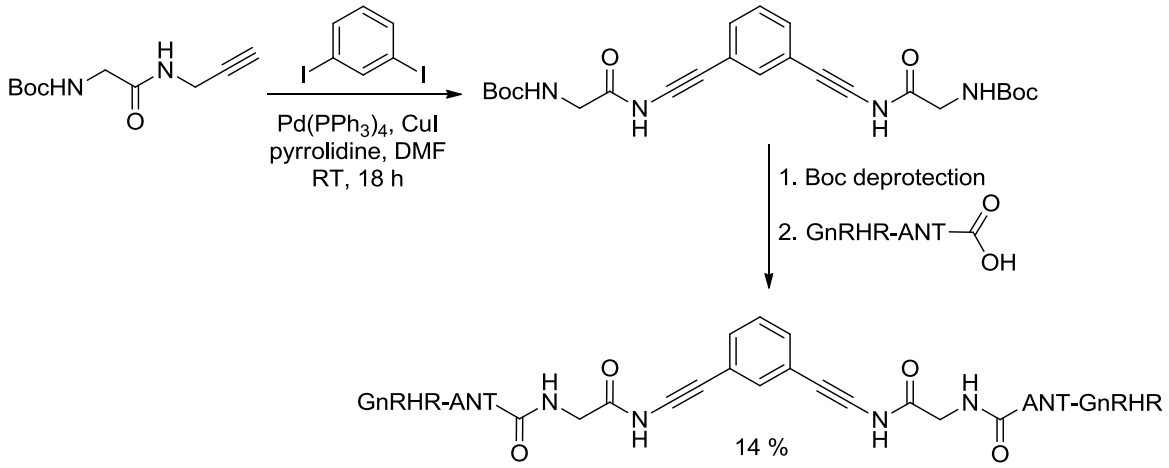
4.5.4. Coupling and Cycloaddition Reactions of Propargylamides

Syntheses of modified homodimeric "Gonadotropin Releasing Hormone Receptor (GnRHR)" antagonist dimers with rigid functionalities such as bistriazole with hydrophilic polyethylene glycol (PEG) spacer[159] (Scheme 4.37) and a propargylated aryl system replacing the PEG spacer,[160] have been accessed via cycloaddition and coupling reaction, respectively.



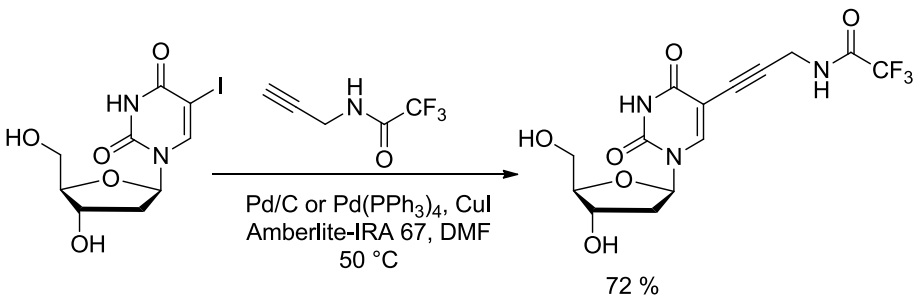
Scheme 4.37. Synthesis of homo-bistriazole GnRHR ligands via click reaction of propargylated receptor with bisazide ethyleneglycol.[159]

Continuation of this study led to the replacement of the bistriazole system with a more rigid hydrophobic propargylated aryl system that was synthesized by the coupling between terminal alkyne moiety of propargyl protected amino acid derivatives with the respective iodoaryl systems (Scheme 4.38).[160]



Scheme 4.38. Synthesis of homodimeric GnRHR antagonists having a rigid bis propargylated benzene core.[160]

While in another study, deoxynucleoside derivatives have been synthesized by coupling of propargylated trifluoroacetyl systems with iodo substituted pyramidinones, for their biological evaluation (Scheme 4.39).[161]



Scheme 4.39. Synthesis of propargylated trifluoroacetyl derivative of pyramidinone.[161]

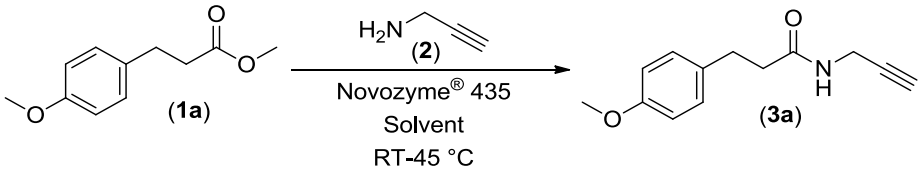
5. Results and Discussion

5.1. Optimization of Enzyme Catalyzed Aminolysis

5.1.1. Solvent Selection

The solvent selection for the enzyme catalyzed reaction of interest is not straightforward and there are always exceptions to the general rules. However log *P* values (logarithm of the partition coefficient of a given solvent between 1-octanol and water)[34] have been well established as one of the most reliable criteria for solvent selection for an enzyme catalyzed process.[2,10,162,163] Solvents possessing intermediate polarity, that are non-substrate to enzyme, have been suggested to be the most suitable ones since the highly polar solvents may render the enzyme inactive by removing the essential water layer off the active site of the enzyme.[164] The solvent selection also crucially depends upon the substrates as the selection becomes difficult if the substrates exhibit dissimilar solubility characteristics.[164]

In the present study, different solvents have been screened based on their log *P* values, for the intended aminolysis and optimization study was conducted using most commonly used lipase, CAL-B (Novozyme® 435) (Scheme 5.1). The screened organic solvents with their respective log *P* values are mentioned in Table 5.1.



Scheme 5.1. Model reaction for Novozyme® 435 catalyzed aminolysis.

Table 5.1. log *P* Values of different organic solvents screened for optimization of Novozyme® 435 catalyzed aminolysis (Scheme 5.1).

Entry	Solvents	log *P*	*t* [h] until product 3a detection by GC-MS
1	*n*-hexane[34]	3.5	2.5
2	cyclohexane[34]	3.2	2.5
3	toluene[34]	2.5	-
4	MTBE[165]	1.49	0.5
5	DIPE[34]	1.9	0.5
6	DCM[166]	2.0	-
7	THF[34]	0.49	-

Entry	Solvents	log *P*	*t* [h] until product 3a detection by GC-MS
8	1,4-dioxane[34]	-1.1	-
9	*t*-BuOH[34]	0.80	-
10	MeOH[34]	-0.76	-
11	acetonitrile[34]	-0.33	-
12	acetone[34]	-0.23	-
13	1,2-dimethoxyethane	-	-

The results can be rationalized on the basis of the polarity of the solvents used. In highly non polar solvents such as *n*-hexane and cyclohexane, the product formation was observed after 2.5 h of reaction time and the product **3a** so formed, began to crystallize out in the reaction vial. This observation has been attributed to the poor solubility of the product **3a** in these solvents. The fastest progress of the reaction was observed when ether solvents such as MTBE and DIPE were used but when the moderately polar to highly polar solvents were employed (Table 5.1, entries 7-12), no product formation was observed. Therefore, a subtle balance between the polarity of the solvent employed and its hydrophilicity, has been considered important to maintain. The solvent to be used has to be polar enough to solubilize the polar substrates but not hydrophilic to the extent rendering the enzyme inactive. So, for the present study, the use of nonpolar solvents such as *n*-hexane and cyclohexane was not appropriate because these solvents do not offer a wide spectrum of solubility options for the different types of ester substrates **1** to be used. Since the ether solvents were the most efficient ones, therefore, MTBE was chosen as solvent of choice.

5.1.2. Screening of Enzymes

According to the literature pertaining to biocatalysis, lipases have been the enzymes of choice for esterification and transesterification reaction but their utility as a biocatalyst for aminolysis is rather limited.[74] In the past few decades the studies attributed towards applicability of CAL-B for aminolysis and ammonolysis, have credited CAL-B for such transformations.[85-94,101-104,111-117] Not only CAL-B but also the utility of PCL has been established in the past years for aminolysis reactions.[105-107] CAL-A has been shown to be active in aminolysis of esters but not in the esterification reactions.[101] According to conducted literature survey, no reference was available stating the aminolysis of an ester substrate using propargylamine (**2**). Various lipases such as PPL, CRL, PCL (immobilized on immobead® 150), CAL-A (immobilized on immobead® 150), CAL-B (two types of commercially available immobilized CAL-B have been used : a) immobilized on immobead®

150 and, b) immobilized on acrylic resin that is commercially known as Novozyme® 435) and protease from *Bacillus licheniformis* (Alcalase CLEA®) were used for the model aminolysis reaction (Scheme 5.1). The results of enzyme screening have been shown in Table 5.2.

Table 5.2. Screening of different enzymes for aminolysis of ester **1a** for the synthesis of propargylamide **3a** shown in Scheme 5.1.

Entry	Enzyme	Mode of Immobilization	*t* [h]	**3a** Yield (%)
1	CAL-B	Immobilized on immobead® 150	24	35
2	CAL-B	Immobilized on immobead® 150	48	67
3	CAL-B	Immobilized on acrylic resin (Novozyme® 435)	24	68
4	CAL-A	Immobilized on immobead® 150	24	-
5	CRL	-	24	-
6	PPL	-	24	-
7	PCL	-	24	-
8	Alcalase	CLEA®	24	-

According to the optimization studies mentioned in Table 5.2, only CAL-B (Novozyme® 435) was able to catalyze the corresponding aminolysis by propargylamine (**2**) (Table 5.2, entries 1-3) while the rest of the enzymes failed to catalyze this reaction. CAL-B (Novozyme® 435) exhibited similar results in half the time (Table 5.2, entry 3) that was taken by CAL-B immobilized on immobead® 150 (Table 5.2, entry 2) therefore Novozyme® 435 was chosen for the catalysis of aminolysis of the ester **1a**. This observation can be rationalized by taking into account the mode of immobilization of CAL-B on solid support. In case of Novozyme® 435, CAL-B is immobilized on hydrophilic anionic resin, Lewatit® E, through ionic binding[101] while in case of immobead® 150, the enzyme is immobilized on cross linked co-polymer of methacrylate (carrying oxirane groups) through covalent binding. Some loss of activity has always been reported in case when the enzyme is bound by covalent binding and consequently the residual activity of the enzyme is not believed to exceed 60-80 % while such an extent of loss of activity has not been observed when the mode of binding is ionic.[34] This explains the longer time requirement of CAL-B immobilized on immobead® 150 for furnishing the yield of the corresponding product **3a**, that was produced by Novozyme® 435

in half the time. Effect of the mol ratio of ester **1** to propargylamine (**2**) and the amount of enzyme used, on the product **3a** yield was determined. The results are shown in Table 5.3.

Table 5.3. Effect of mol ratio of ester **1a** and propargylamine (**2**) on isolated yield of the product **3a** at 45 °C and 24 h of reaction time (Scheme 5.1).

Entry	**Amount of CAL-B** (Novozyme® 435) (% w/w of ester substrate **1**)	**Mol Ratio** Ester **1a**:Propargylamine (**2**)	**3a Yield** (%)
1	10 %	1:1	13
2	10 %	1.2:1	14
3	20 %	1:1	14
4	20 %	1.2:1	16
5	30 %	1.2:1	39
6	40 %	1.2:1	44
7	50 %	1:1	60
8	50 %	1.2:1	68
9	50 %	1:1.2	56
10	50 %	2:1	67
11	50 %	1:2	53
12	100 %	1.2:1	62
13	100 %	2:1	59
14	100 %	1:2	51

The data mentioned in Table 5.3, showed that the highest yield of the product **3a** was obtained using a slight excess of ester **1a** with Novozyme® 435 in a 50% w/w ratio to ester **1a** (Table 5.3, entry 8). Therefore, these reaction conditions were chosen for further optimization of Novozyme® 435 catalyzed aminolysis of esters **1** by propargylamine (**2**).

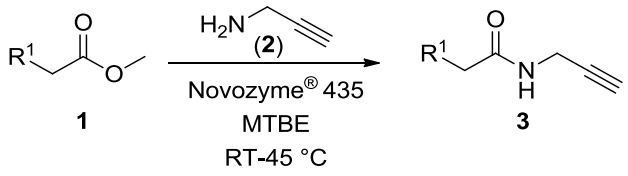
5.1.3. Substrate (Acyl Donor) Selection

Studies pertaining to structural features of CAL-B established that, compared to other lipases, it possesses rather limited available space in the active site pocket. This feature is believed to be responsible for its enhanced substrate specificity.[167] X-ray crystallographic studies revealed the active site to be composed of a relatively more spacious acyl channel than the alcohol channel[168] and based on this, CAL-B is expected to exhibit comparatively a broad specificity for acyl donors.[169]

A wide range of acyl donors (ester substrates) have been successful in establishing their suitability for CAL-B catalyzed transesterification reaction.[167] This observation prompted the screening of different ester substrates for the synthesis of structurally diverse propargylamides **3** through aminolysis that, in turn, would bring the structural variety to the corresponding products intended to be obtained by one-pot processes.

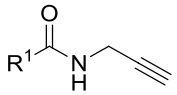
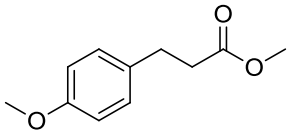
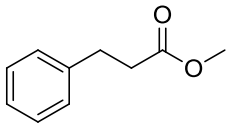
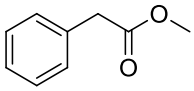
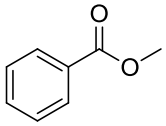
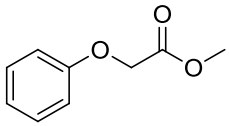
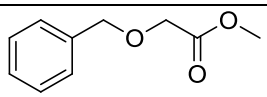
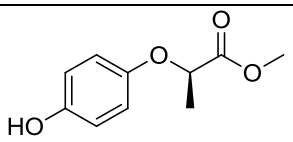
5.1.3.1. Substrates with Heteroatom Side Chain Linkers

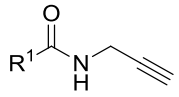
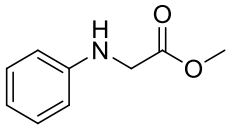
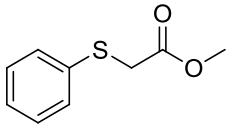
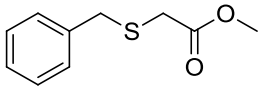
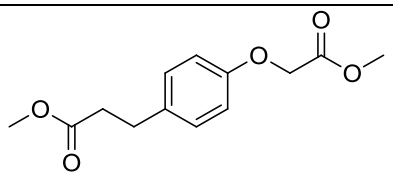
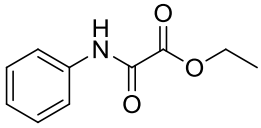
There exists a correlation between structural features of ester substrates **1** and the operating temperature dependency of CAL-B (Novozyme® 435) catalysis. The Novozyme® 435 catalyzed aminolysis of ester substrate **1a** employed in the model reaction (Scheme 5.1) could produce the corresponding amide product **3a** only when the temperature of 40-45 °C was maintained and yield did not exceed 68 % despite of longer reaction time and increased catalyst loading (Table 5.3, entries 12 and 13). So, the next step was to search for the ester substrates **1** compatible with Novozyme® 435. Enhanced reactivity of ester substrates with the oxygenated side chain linkers in PCL catalyzed aminolysis has been reported in literature.[105-107] In the present work not only the effect of presence of oxygen but also the other heteroatoms, such as sulphur and nitrogen, in the side chain of the ester has been studied and an enhanced acceptability of such ester substrates has been observed. Table 5.4, shows the operating temperature dependency of Novozyme® 435 catalyzed aminolysis on the nature of ester substrates **1** used.



Scheme 5.2. Novozyme® 435 catalyzed aminolysis using different ester substrates **1**.

Table 5.4. Effect of structural features of the ester substrates **1** on the operating temperature and time taken by Novozyme® 435 catalyzed aminolysis (Scheme 5.2).

Entry	Ester Substrates 1	*T* [°C]	*t* [h]	 **3** **Yield (%)**
1	 **1a**	RT	24	-
		45	24	68
2	 **1b**	RT	24	-
		45	24	62
3	 **1c**	RT	24	-
		45	24	73
4	 **1d**	RT-45	24	-
5	 **1e**	RT	6	86
6	 **1f**	RT	6	86
7	 **1g**	RT	4	95

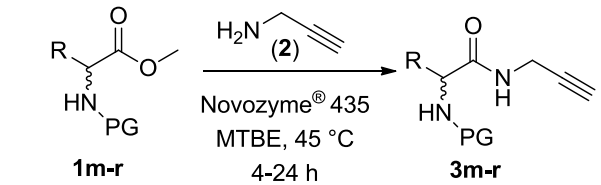
Entry	Ester Substrates 1	*T* [°C]	*t* [h]	 **3** **Yield (%)**
8	 **1h**	RT	8	74
9	 **1i**	RT	8	76
10	 **1j**	RT	8	82
11	 **1k**	RT	4	80
12	 **1l**	RT-45	-	-

The results, shown in Table 5.4, indicate a clear demarcation between various types of esters **1** used. The activated ones are those containing hetero atom linkers in their side chain (Table 5.4, entries 5-11) and consequently exhibited higher acceptability by the enzyme resulting high product **3** yield in shorter period of time at room temperature. While the rest produced satisfactory yields of the respective products **3**, relative to activated esters Table 5.4, entries 5-11), over a prolonged reaction time as well as operating temperature of 45 °C. The high acceptability of the activated ester substrates **1e-k** can be rationalized based on the inductive effect of the hetero atom side chain linkers in their structure. The presence of the hetero atom in the side chain renders the carbonyl moiety of the ester more electrophilic, hence making it more susceptible for the enzyme attack. While such a possibility does not exist in the case of esters without hetero atom in their side chains and

hence results in relatively a low acceptability of these substrates by the enzyme. Site selective preference of Novozyme® 435 of such sort was further exploited when diester **1k** (Table 5.4, entry 11) was used and the reaction selectively took place at the ester moiety with oxygenated side chain linker. However the reaction did not proceed beyond the mono amidation product mainly due to the insolubility of the product **3k** in MTBE. Methylbenzoate (**1d**), (Table 5.4, entry 4) could not produce the corresponding "aminolyzed" product but ester substrate **1c** (Table 5.4, entry 4) furnished the respective amide product, though the substrates **1c** and **1d** differ only by the presence of a methylene group between aromatic ring and ester linkage thus showcasing the dependence of selectivity of enzyme upon subtle structural differences.

5.1.3.2. *N*-Protected Amino Acids as Ester Substrates

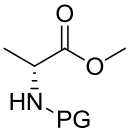
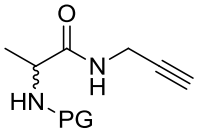
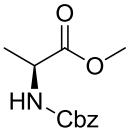
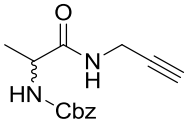
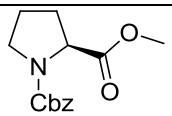
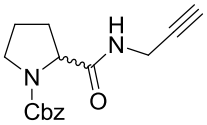
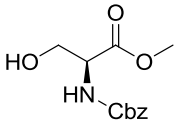
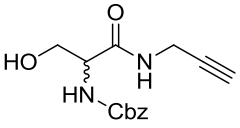
Since Novozyme® 435 is able to successfully catalyze the intended aminolysis reaction (Scheme 5.1), therefore it was considered interesting to assess its receptive ability for methyl esters of *N*-protected amino acids for aminolysis by propargylamine (**2**). Lipases, unlike proteases, do not possess amidase activity[75] and therefore it is always interesting to employ lipases as the catalyst of choice for peptide synthesis. PPL is considered to be the only class of lipases that is active towards peptide synthesis while CRL catalyzed reactions involving the use of *N*-protected amino esters as electrophiles and free amino acids as nucleophiles.[170] Subtilisin has been studied to catalyze the incorporation of *D*-amino acids (via acylation) into the peptide sequence.[171] Synthesis of β-dipeptides has been reported using CAL-A for catalyzing the acylation of β-amino esters.[114] Since Novozyme® 435 has not previously been employed for acylation reactions involving amino acids, therefore, in present study methyl esters of different *N*-protected amino acids **1m-r**, were used as ester substrates and the effect of different protecting groups towards the reactivity of amino acids was determined. The results revealed that CAL-A and CRL could not catalyze the intended reaction (Scheme 5.3) but *Bacillus licheniformis* protease (Alcalase CLEA®) could produce the desired respective, **3n** and **3o**, product over prolonged reaction time (24-48 h) in lower yield (15-20 %).



Scheme 5.3. Novozyme® 435 catalyzed aminolysis of methyl ester of *N*-protected amino acids **1m-r**.

Results (Table 5.5) suggested that the catalytic activity of Novozyme® 435 was highly dependent upon the nature of the protecting group employed. Different *N*-protected *D*- and *L*-amino esters were used and the results showed that amino esters protected with a Boc group were not suitable as no product formation was observed. This can be attributed to the steric bulk of the Boc group making it unbefitting for Novozyme® 435. While *N*-protected *D*-alanine methyl esters, **1n** and **1o** (Table 5.5 entry 1)**,** exhibited more flexible behaviour compared to its counterpart *N*-protected *L*-alanine methyl ester **1p**, as *D*-alanine methyl ester derivatives, protected by TFA **1n** and Cbz **1o**, were active towards aminolysis but *L*-alanine methyl ester could only be converted to the corresponding amide when protected by Cbz group (Table 5.5, entry 2), in shorter time period. This observation also suggested that selectivity can be achieved in instances involving both *D*- and *L*-amino acids owing to the differences of behaviour these *N*-protected amino methyl esters exhibited towards Novozyme® 435 catalyzed aminolysis when protected by different protecting groups.

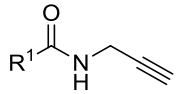
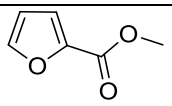
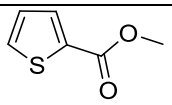
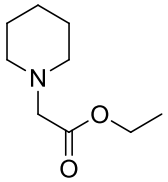
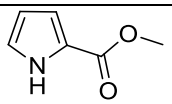
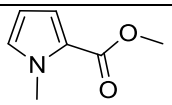
Table 5.5. Effect of the nature of different protecting groups on the receptive behaviour of Novozyme® 435 towards different methyl esters of *N*-protected amino acids.

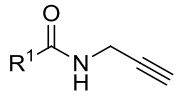
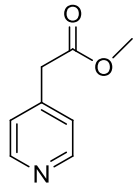
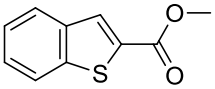
Entry	Ester Substrate 1	*t* [h]	Propargylamide 3
1	 **1m** : PG = Boc **1n** : PG = TFA **1o** : PG = Cbz	4	 **3m** (no product formation) **3n** (62 %) **3o** (82 %)
2	 **1p**	4	 **3p** (80 %)
3	 **1q**	24	 **3q** (68 %)
4	 **1r**	24	 **3r** (53 %)

5.1.3.3. Ester Substrates with Heterocyclic Systems

Ester substrates with heterocyclic systems, have also been screened for their susceptibility to aminolysis catalyzed by Novozyme® 435. The results are shown in Table 5.6. The ester substrates with furan, thiophene and *N*-substituted piperidine systems (Table 5.6, entries 1-3) were successfully converted to the corresponding products **3** while pyrrole, both unsubstituted **1v** and *N*-methyl substituted **1w** (Table 5.6, entries 4 and 5), pyridine **1x**, (Table 5.6, entry 6) and benzothiophene system **1y** (Table 5.6, entry 7), failed to furnish the corresponding amide products.

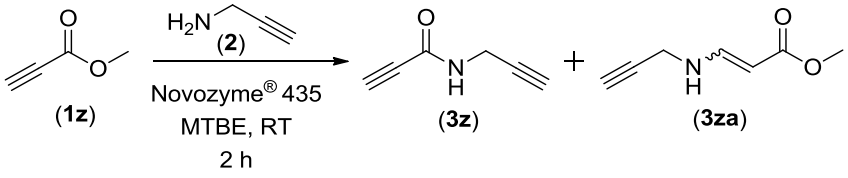
Table 5.6. Ester substrates **1** with heterocyclic systems for Novozyme® 435 catalyzed aminolysis.

Entry	Ester Substrates 1	*T* [°C]	*t* [h]	 **3** Yield (%)
1	 **1s**	RT	24	-
		45	24	71
2	 **1t**	RT	24	-
		45	24	77
3	 **1u**	RT	24	-
		45	24	70
4	 **1v**	RT	24	-
		45	24	-
5	 **1w**	RT	24	-
		45	24	-

Entry	Ester Substrates 1	*T* [°C]	*t* [h]	 **3** **Yield (%)**
6	 **1x**	RT	24	-
		45	24	-
7	 **1y**	RT	24	-
		45	24	-

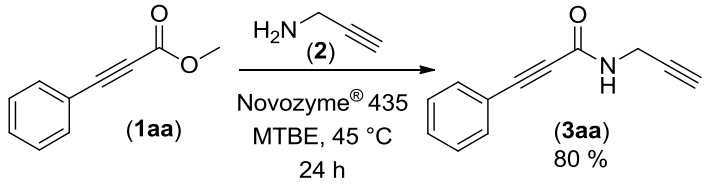
5.1.3.4. Methyl propiolates as Acyl Donor

Ester substrate with multiple bonds in their side chain **1z** and **1aa** were screened for their suitability for aminolysis. Novozyme® 435 catalyzed aminolysis of methyl propiolate (**1z**) with propargylamine (**2**) not only gave rise to the corresponding desired amide product **3z** but also the formation of the Michael adduct **3za** was observed in GC-MS analysis, an experimental observation contrary to the previously reported selective amidation (1,2-addition) by CAL-B in MTBE[96,97] (Scheme 5.4).



Scheme 5.4. Reaction outcome of aminolysis of methyl propiolate (**1z**) by propargylamine (**2**).

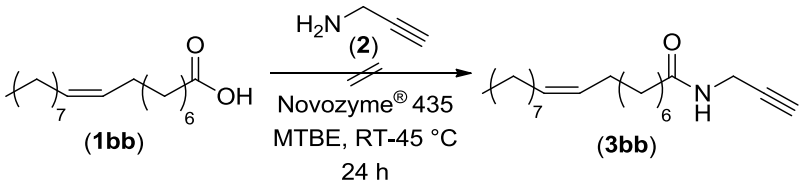
Since the aminolysis of methyl propiolate (**1z**) resulted in the formation of both amide (1,2 addition) and undesired Michael (1,4-addition) adduct product, therefore, in order to prevent the formation of the Michael product, a substituted propiolate, such as methyl phenylpropiolate (**1aa**), was employed and as expected, no Michael adduct formation was observed and corresponding amide **3aa** was the sole product of Novozyme® 435 catalyzed aminolysis (Scheme 5.5).



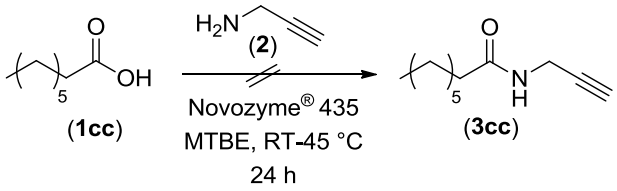
Scheme 5.5. Aminolysis of methyl phenylpropiolate (**1aa**) by propargylamine (**2**).

5.1.3.5. Long Chain Fatty acids

Since long chain fatty acids are natural substrates for lipases, therefore, Novozyme® 435 catalyzed aminolysis by propargylamine (**2**) of long esters and acids was screened. For this purpose oleic acid (**1bb**) and octanoic acid (**1cc**) were screened. The reaction was allowed to run at room temperature and when no product formation was observed, the temperature was increased to 45 °C. The reaction was monitored via both TLC and GC-MS techniques that showed no product formation in the reaction mixture (Schemes 5.6 and 5.7).



Scheme 5.6. Aminolysis of oleic acid (**1bb**) with propargylamine (**2**) catalyzed by Novozyme® 435.

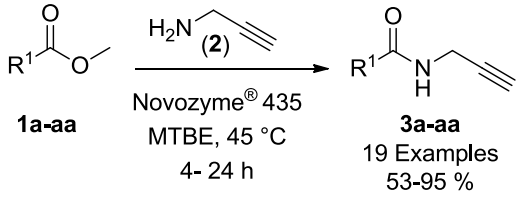


Scheme 5.7. Aminolysis of octanoic acid (**1cc**) with propargylamine (**2**) catalyzed by Novozyme® 435.

Thus the screening of various possible ester substrates **1** for the intended lipase catalyzed aminolysis revealed the suitability and compatibility of Novozyme® 435 with a number of possible ester substrates **1** under relatively mild reaction conditions.

5.2. Optimized CAL-B (Novozyme® 435) Catalyzed Aminolysis

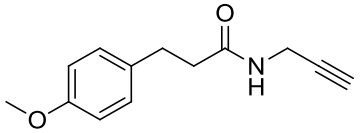
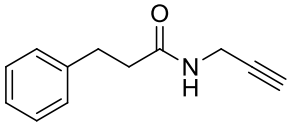
With the exception of activated esters, **1e-k**, the rest required the reaction temperature of 45 °C for furnishing the corresponding propargylamides **3** in satisfactory yields. Therefore the optimum temperature of Novozyme® 435 catalyzed aminolysis was chosen to be 45 °C. Depending upon the type of ester substrates **1** employed, the reaction was completed between 4 to 24 h of reaction time (Scheme 5.8).

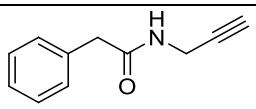
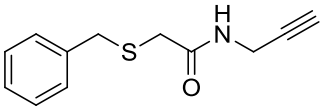
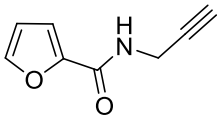
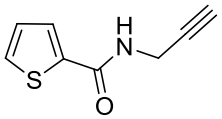


Scheme 5.8. Optimized Novozyme® 435 aminolysis of various ester substrates **1** by propargylamine (**2**).

In order to develop the efficacy of the optimized Novozyme® 435 catalyzed aminolysis, a comparison was made between the yields of the products **3** obtained by base (triethylamine) catalyzed (synthesized and literature reported values) and Novozyme® 435 catalyzed aminolysis as shown in Table 5.7. The comparison revealed that comparable yields of the corresponding propargylamides **3** could be obtained by Novozyme® 435 catalyzed protocol under milder reaction conditions.

Table 5.7. Comparison of yields of propargylamides **3** prepared by base (NEt_3) catalyzed and Novozyme® 435 catalyzed aminolysis.

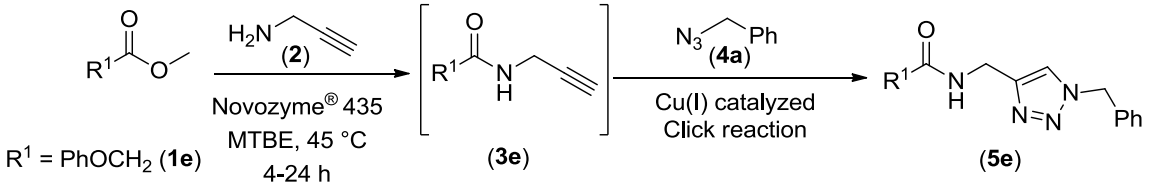
Entry	Propargylamides 3	Base (NEt_3) catalyzed aminolysis Yield (%)	Novozyme® 435 catalyzed aminolysis Yield (%)
1	 **3a**	73[a]	68[b]
2	 **3b**	75[a]	62[b]

Entry	Propargylamides 3	Base (NEt_3) catalyzed aminolysis Yield (%)	Novozyme® 435 catalyzed aminolysis Yield (%)
3	 **3c**	92[c]	73[b]
4	 **3j**	84[d]	82[e]
5	 **3s**	85[f]	71[b]
6	 **3t**	65[g]	77[b]

Reaction conditions: [a]NEt_3 (1.0 eq), DCM, 0-20 °C, 4 h, [b]24 h, [c]literature reported value,[172] [d]literature reported value,[173] [e]4 h, [f]literature reported value,[151] [g]literature reported value.[149]

5.3. Optimization Studies for Chemoenzymatic One-pot Synthesis of 1,4-Disubstituted 1,2,3-Triazoles 5

Classical reaction conditions for the Cu(I) catalyzed click reaction involves the application of *t*-BuOH:H_2O (1:1) as solvent of choice and the reaction usually takes place at room temperature. The application of such sort of trivial reaction conditions was not possible in the present case as most of the propargylamides **3**, were not soluble in *t*-BuOH. Therefore, other reported reaction conditions involving the use of solvents other than *t*-BuOH for click reaction were employed. Propargylamides **3** have been established as substrates that are known for their low reactivity towards Huisgen 1,3-dipolar cycloaddition with azides,[174] but have been reported to exhibit high reactivity in Cu(I) catalyzed azide-alkyne cycloaddition (CuACC) and produced 1,2,3-triazoles in quantitative yields when DCM was used as solvent.[175] Table 5.8, summarizes the outcome of the optimization study of one-pot click reaction using different solvents, copper catalyst species and ligands.



Scheme 5.9. Chemoenzymatic one-pot synthetic scheme of 1,4-disubstituted 1,2,3-triazole **5e**.

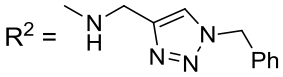
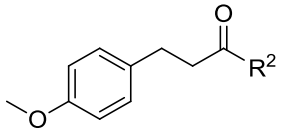
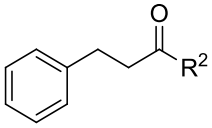
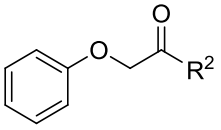
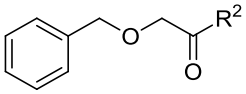
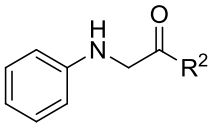
Of all the reaction conditions employed, only the ones involving the use of base and Cu(I) stabilizing ligand, could produce the corresponding 1,2,3-triazole **5e** in satisfactory yield (Table 5.8, entries 9-11). The possible interaction(s) of the enzyme with the respective metal catalytic species leading to its consequent reduction in concentration in the same reaction mixture, has been a topic that is not being addressed vividly. But the success of ligand promoted click reaction in a one-pot methodology, pointed towards the diminishing concentration of Cu(I) species in the reaction mixture when conducted under ligand free conditions. Recently, a relatively new concept for CuACC has been introduced involving the application of Cu_2O as direct Cu(I) source in the presence of carboxylic acids as bidentate Cu(I) stabilizing ligands[176a-c] and the incorporation of this concept has been successful in a one-pot fashion (Table 5.8, entry 11).

Table. 5.8. Optimization studies of chemoenzymatic one-pot click reaction (Scheme 5.9).

Entry	Cu Source	Reducing agent (Na-ascorbate)	Base	Ligand	Solvent	*T* [°C]	*t* [h]	5e Yield (%)
1	$CuSO_4 \cdot 5H_2O$ (5 mol%)	20 mol%	-	-	THF:H_2O (2:1)	RT	48	35
2	$CuSO_4 \cdot 5H_2O$ (5 mol%)	20 mol%	-	-	EtOH:H_2O (1:1)	RT	24	52
3	$CuSO_4 \cdot 5H_2O$ (4 mol%)	20 mol%	Na_2CO_3 (20 mol %)	-	EtOH:H_2O (1:1)	RT	24	42
4	$CuSO_4 \cdot 5H_2O$ (4 mol%)	20 mol%	Na_2CO_3 (20 mol %)	-	EtOH:H_2O (2:1)	RT	24	38
5	$Cu(OAc)_2 \cdot H_2O$ (6 mol%)[177]	6 mol%	-	-	MeCN: H_2O (1:1)	RT	42	72
			Na_2CO_3 (20 mol %)	*L*-Proline (20 mol%)		RT	24	61
			Na_2CO_3 (20 mol %)	*L*-Proline (20 mol%)		45	19	85
6	$CuSO_4 \cdot 5H_2O$ (5 mol%)[175]	15 mol%	-	-	DCM: H_2O (1:1)	RT	48	60
						45	24	63
7	CuI (10 mol%)	-	DIPEA (1.0 eq)	Ethylene diamine (1.0 eq)	Dry THF under argon	RT	24	61
8	$CuSO_4 \cdot 5H_2O$ (5 mol%)[178]	20 mol%	Na_2CO_3 (20 mol %)	*L*-Proline (20 mol%)	DMSO:H_2O (9:1)	45	16	53
9	$CuSO_4 \cdot 5H_2O$ (5 mol%)	20 mol%	Na_2CO_3 (20 mol %)	*L*-Proline (20 mol%)	MeOH: H_2O (1:1)	RT	24	61
						45	19	85
10	$CuSO_4 \cdot 5H_2O$ (5 mol%)	20 mol%	Na_2CO_3 (20 mol %)	*L*-Proline (20 mol%)	DCM: H_2O (1:1)	45	19	82
11	Cu_2O (4 mol%)[176]	-	-	$PhCO_2H$ (8 mol %)	H_2O:MeOH	RT	8	61
						45	4	83

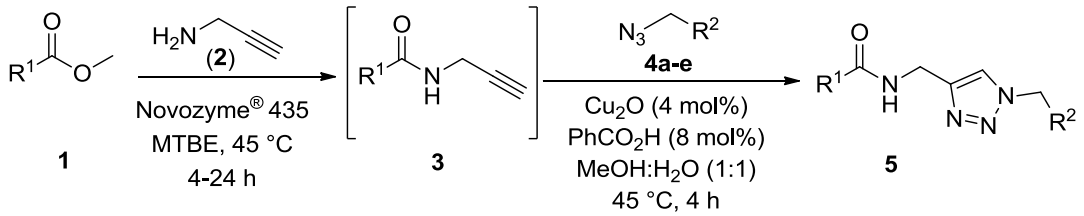
Considering the outcome of the one-pot triazole synthesis under different reaction conditions, entries 9 and 10 in Table 5.8, were two of the three high yielding sets of reaction conditions for triazole **5e** synthesis but these reaction conditions were disadvantageous as these involved the usage of additives and prolonged reaction time compared to carboxylic acid promoted CuACC sequence (Table 5.8, entry 11). Table 5.9, shows the comparison of the isolated yields of the products obtained by using both the optimized reaction conditions.

Table 5.9. Yield of the 1,2,3-triazoles **5** obtained by chemoenzymatic one-pot synthesis.

Entry	1,2,3-Triazoles R^2 = 	Yield (%)	
		Catalyst System A	Catalyst System B
1	 **5a**	71	73
2	 **5b**	61	63
3	 **5e**	83	85
4	 **5f**	70	72
5	 **5h**	74	51

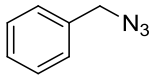
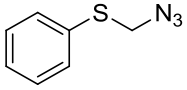
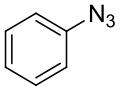
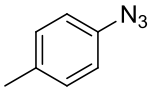
Catalyst System A: Cu_2O (4 mol%), $PhCO_2H$ (8 mol%), 45 °C, 4 h, MeOH:H_2O (1:1). Catalyst System B: $CuSO_4 \cdot 5H_2O$ (5 mol%), Na-ascorbate (20 mol%), *L*-Proline (20 mol%), Na_2CO_3 (20 mol%) 45 °C, 18-20 h, MeOH:H_2O (1:1). MeOH as co solvent was used only when propargylamide **3** was insoluble in MTBE.

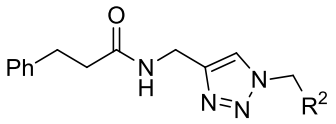
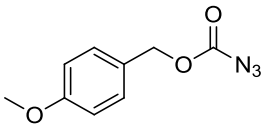
Since, more or less, the same yield of the respective 1,2,3-triazole products **5** was obtained using the two sets of reaction conditions mentioned in Table 5.9, therefore, the reaction conditions involving the use of less additives and a shorter period of reaction time i.e. Cu_2O as direct Cu(I) source in the presence of benzoic acid as bidentate ligand, was chosen for the one-pot sequence. Various commercially available azides **4a-e** were employed, but only azides **4a** and **4b** could successfully participate and generate the corresponding triazoles **5** (Scheme 5.10).



Scheme 5.10. Screening of azides **4a-e** for the synthesis of corresponding triazole systems **5** via chemoenzymatic one-pot procedure.

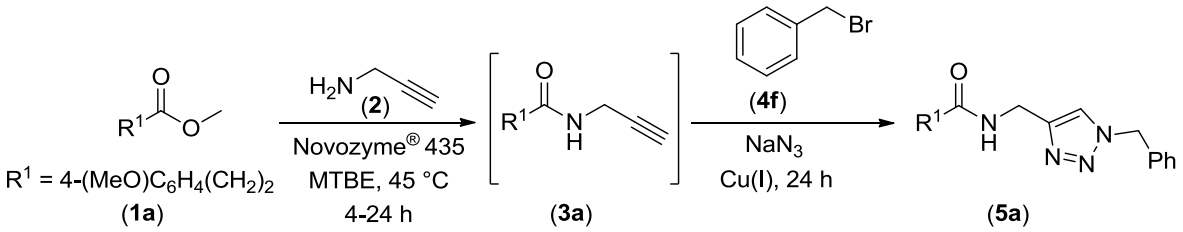
Table 5.10. Azides **4a-e** used in chemoenzymatic one-pot sequence for the synthesis of corresponding triazoles **5**.

Entry	Azides	Yield (%) **5**
1	 **4a**	**5b** (61 %)
2	 **4b**	**5ff** (63 %)
3	 **4c**	-
4	 **4d**	-

Entry	Azides	Yield (%)  **5**
5	 **4e**	-

5.4. Synthesis of Triazoles 5 via *In situ* Azide Generation

In order to circumvent the use of organic azides, considering their perilous nature, the concept of the synthesis of 1,2,3-triazole system **5** via in situ generation of benzyl azide (**4a**) has also been carried out but no satisfactory corresponding product **5a** yield was obtained. The optimization studies were conducted in a one-pot fashion and also utilizing isolated propargylamide **3a** but no adequate outcome was obtained (Scheme 5.11).



Scheme 5.11. Synthesis of 1,2,3-triazole **5a** via *in situ* generation of benzyl azide (**4a**).

Table 5.11. Chemoenzymatic one-pot procedure via in situ benzyl azide (**4a**) generation.

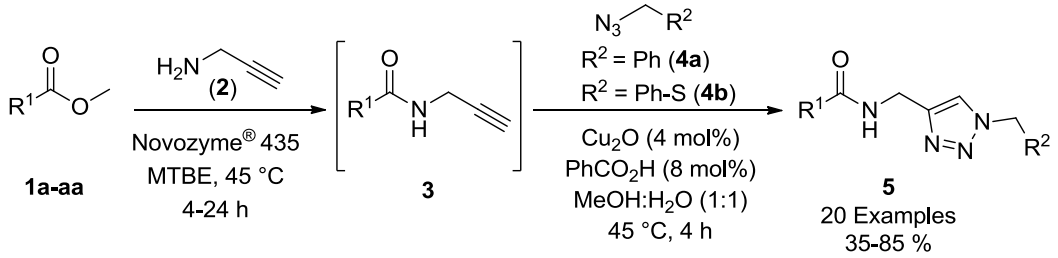
Entry	Catalyst System	*T* [°C]	Solvent	5a Yield (%)
1	Na-ascorbate (20 mol%) *L*-Proline (20 mol%) Na_2CO_3(20 mol%) $CuSO_4 \cdot 5H_2O$ (5 mol%)	45-55	DCM	36
2	Na-ascorbate (20 mol%) *L*-Proline (20 mol%) Na_2CO_3(20 mol%) $CuSO_4 \cdot 5H_2O$ (5 mol%)	45-55	MeOH	44

Entry	Catalyst System	*T* [°C]	Solvent	5a Yield (%)
3	Cu_2O (4 mol%) Benzoic acid (8 mol%)	45-55	MeOH	43
4	[a]Cu_2O (4 mol%) Benzoic acid (8 mol%)	45-55	MeOH	45[a]

[a]Reaction was commenced using isolated propargylamide **3a**.

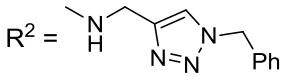
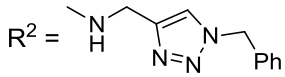
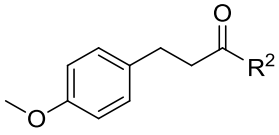
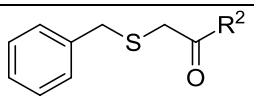
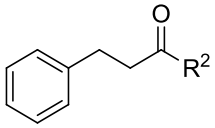
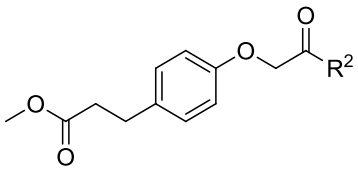
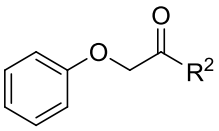
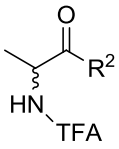
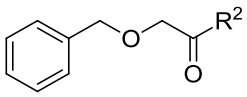
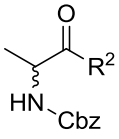
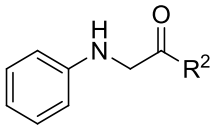
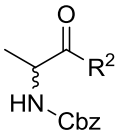
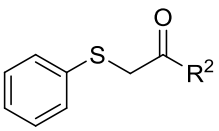
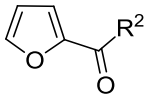
5.5. Optimized Chemoenzymatic One-pot Synthesis of Amide Ligated 1,4-Disubstituted 1,2,3-Triazoles 5

The screening of different copper sources as possible Cu(I) catalytic species, various solvents, bases and ligand stabilizing species, led to the optimized chemoenzymatic one-pot synthesis of amide ligated 1,4-disubstituted 1,2,3-triazoles **5** (Scheme 5.12). The optimized one-pot methodology exhibited a fair level of compatibility with a wide variety of acyl donors **1** except for the ester substrates with functionalities that might act as Cu(I) stabilizing motifs (**1g**, **1q**, **1r**, **1u**) that in turn failed to produce the corresponding triazole products.



Scheme 5.12. Optimized chemoenzymatic one-pot synthesis of 1,4-disubstituted 1,2,3-triazoles **5**.

Table 5.12. Triazoles **5** obtained by chemoenzymatic one-pot sequence using benzyl azide (**4a**).

Entry	Product 5 (R^2 = )	Yield (%)	Entry	Product 5 (R^2 = )	Yield (%)
1	 **5a**	71	7	 **5j**	66
2	 **5b**	61	8	 **5k**	51
3	 **5e**	83	9	 **5n**	59
4	 **5f**	70	10	 **5o** (using substrate **1o**)	70
5	 **5h**	85	11	 **5p** (using substrate **1p**)	73
6	 **5i**	74	12	 **5s**	68

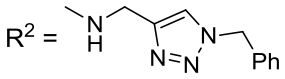
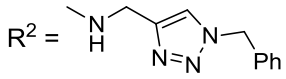
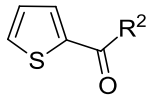
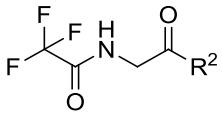
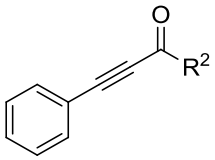
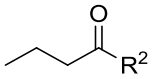
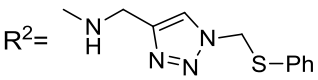
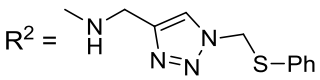
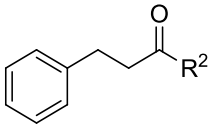
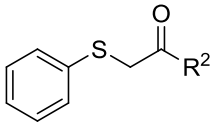
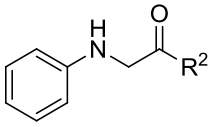
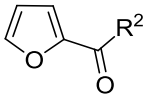
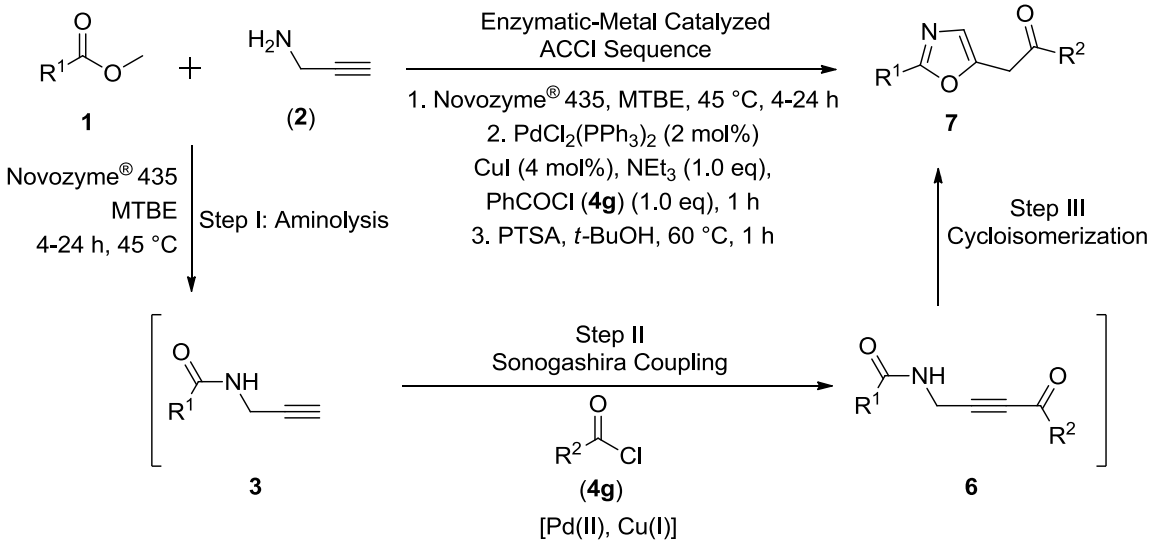
Entry	Product 5 (R^2 = )	Yield (%)	Entry	Product 5 (R^2 = )	Yield (%)
13	 **5t**	35	15	 **5dd**	40
14	 **5aa**	71	16	 **5ee**	61

Table 5.13. Triazoles **5** obtained by chemoenzymatic one-pot sequence using azidomethyl phenyl sulphide (**4b**).

Entry	Product 5 (R^2= )	Yield (%)	Entry	Product 5 (R^2 = )	Yield (%)
17	 **5ff**	63	19	 **5hh**	59
18	 **5gg**	78	20	 **5ii**	85

5.6. Intended Modification of Amidation-Coupling-Cycloisomerization (ACCI) Sequence

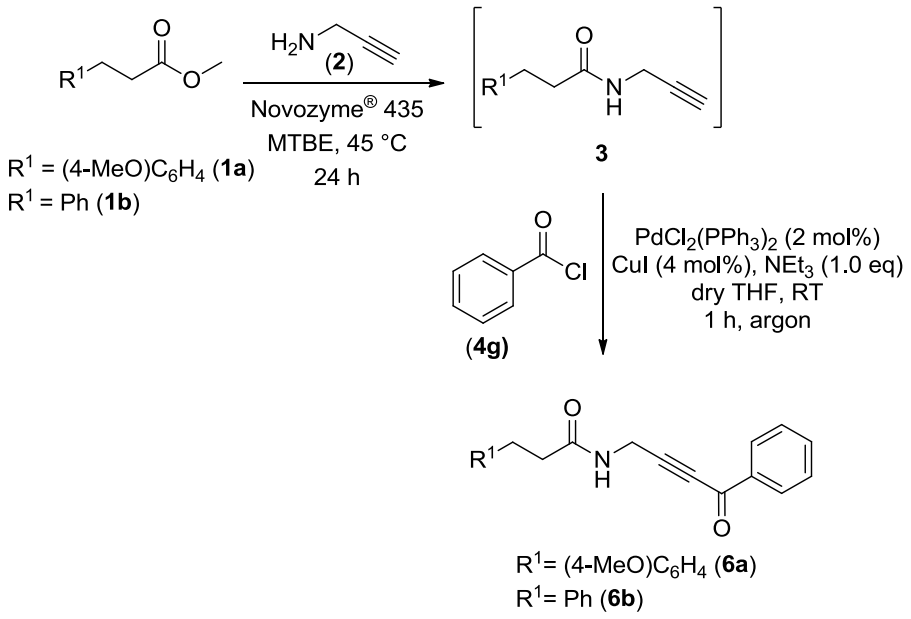
Propargylamides **3** have been used as an entry to multicomponent one-pot synthesis of oxazoles **7** via amidation-coupling-cycloisomerization (ACCI) sequence.[145] In this context, a modification of this sequence has been carried out by replacing base (NEt_3) catalyzed aminolysis of benzoyl chloride (**4g**) (Scheme 5.13) with Novozyme® 435 catalyzed aminolysis. Base catalyzed aminolysis does not allow the liberty to introduce functional group diversity in the corresponding product due to the possibility of their sensitivity towards the base. However base could be replaced by such catalytic system that would allow the introduction of base labile groups and present a relatively mild approach, therefore it was considered worthwhile to incorporate the concept of Novozyme® 435 catalyzed aminolysis to the designed synthesis of oxazoles **7** (Scheme 5.13).



Scheme 5.13. Intended chemoenzymatic one-pot synthesis of 2,5-disubstituted oxazoles **7** via amidation-coupling-cycloisomerization (ACCI) sequence.

5.6.1. Optimization Studies of Sonogashira Coupling Reaction

Optimization studies were conducted by carrying out one-pot synthesis using the reaction conditions for coupling reported previously (Scheme 5.14).[145]



Scheme 5.14. First attempt to chemoenzymatic one-pot synthesis of coupled products **6a** and **6b**.

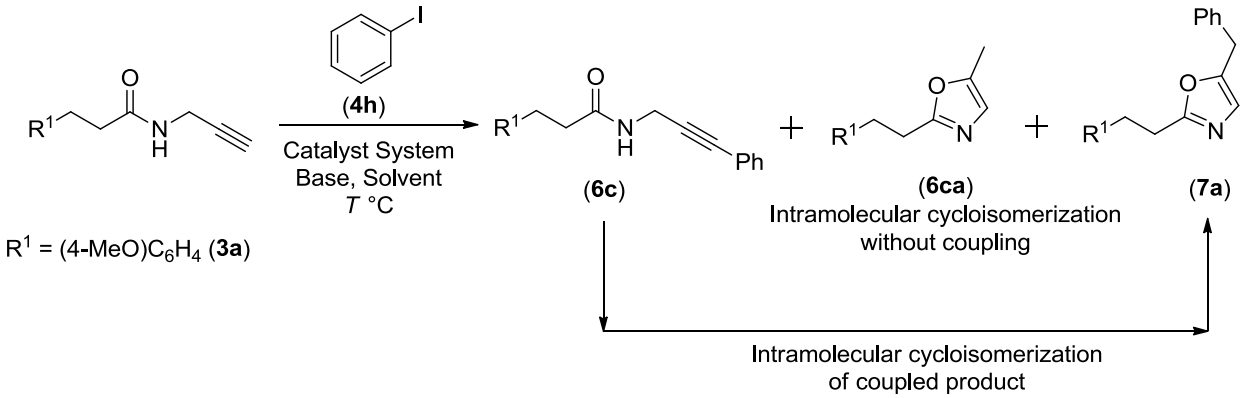
The expected coupled products **6a** and **6b** could not be obtained and the GC-MS spectrum of the reaction mixture revealed the presence of unreacted starting material **1** while almost complete consumption of benzoyl chloride (**4g**) but no corresponding product **6** formation was observed in the spectrum. Moreover there was a prominent peak of m/z = 136 in GC-MS spectrum corresponding to the molecular mass of methyl benzoate. The formation of methyl benzoate can be rationalized since methanol has been formed as the by product of the Novozyme® 435 catalyzed aminolysis, so it immediately reacted with benzoyl chloride (**4g**) forming methyl benzoate which resulted in the low concentration of benzoyl chloride in the reaction mixture to be able to couple with propargylamide **3**. So in order to overcome this, reaction was repeated using 5 Å molecular sieves for quenching the methanol formed during aminolysis. The GC-MS profile of the reaction mixture showed the presence of unreacted species, **3a**, **3b** or **4g**, but no product **6** formation was observed. In another attempt, the sequence was performed in stepwise manner by reacting propargylamide (**3a** and **3b**, isolated separately) with benzoyl chloride (**4g**) using standard conditions for Sonogashira coupling[145] but no product formation, **6a** or **6b,** was observed. This indicated the inability of the chemical catalytic system (Pd(II)/Cu(I)) in catalyzing the desired reaction. Therefore the coupling step, using isolated propargylamide **3,** was optimized by employing different catalytic systems. The results of initial optimization studies are shown in Table 5.14.

Table 5.14. Optimization studies of coupling reaction, shown in Scheme 5.14, using standard conditions for Sonogashira coupling reaction.[145]

Entry	Solvent	*T* [°C]	Other Additives	*t* [h]	Yield (%)
1	THF	RT-50	-	1	-
2	THF	RT-50	5 Å molecular sieves	20	-
3	1,4-dioxane	RT-70	5 Å molecular sieves	20	-

Reaction conditions for coupling step: $PdCl_2(PPh_3)_2$ (2 mol%), CuI (4 mol%), NEt_3 (1.0 eq), benzoyl chloride (**4g**, 1.0 eq).

Since this strategy possessed the inherent problem of the presence of methanol in the reaction mixture, therefore the attention was diverted towards using starting materials that are not reactive towards methanol and would successfully get engaged into the coupling reaction. So for this purpose, iodobenzene (**4h**) was used as a coupling partner as shown in Scheme 5.15, and the results of further optimization studies, only focusing on Sonogashira coupling, are shown in Table 5.15.



Scheme 5.15. Optimization studies of Sonogashira coupling reaction using iodobenzene (**4h**) with the possibility of the formation of cycloisomerization to oxazole systems **6ca** or **7a**.

Table 5.15. Results of optimization studies of Sonogashira reaction shown in Scheme 5.15.

Entry	Catalyst System	Ligand	Solvent	*T* [°C]	Base/Additive	Yield (%)
1	[a]Pd(II), Cu(I)	-	THF	RT-50	NEt_3 (1.0 eq)	-
2	[a]Pd(II), Cu(I)	-	1,4-dioxane	RT-70	NEt_3 (1.0 eq)	13
3	[a]Pd(II), Cu(I)	-	1,4-dioxane	RT-70	Pyrrolidine (1.0 eq)	25
4	[a]Pd(II), Cu(I)	-	1,4-dioxane	RT-70	Pyrrolidine (1.5 eq)	33
5	[b]Pd(0), Cu(I)	-	DMF	45	Pyrrolidine:DMF (1:4 v/v)	75
6	[c]Pd(0)	P(2-furyl)$_3$	MeCN	45	NaO*t*-Bu (2.0 eq)	-
7	[d]Pd(0)	IPr·HCl	MeCN	45	NaO*t*-Bu (2.0 eq)	-
8	[e]Pd(II)	IPr·HCl	MeCN	45	K_2CO_3/TBAC	-
9	[f]Pd(0), Cu(I)	-	DMF	RT	NEt_3 (1.0 eq)	53
10	[g]Pd(0), Cu(I)	-	DMF	45	DIPEA (1.5 eq)	67
11	[h]Pd(0), Cu(I)	-	DMF	45	Pyrrolidine (1.0 eq)	56
12	[i]Pd(0), Cu(I)	-	DMF	45	Pyrrolidine (1.5 eq)	85
13	[j]Pd(0), Cu(I)	-	DMF	45	DABCO (1.0 eq)	57
14	[k]Pd(0), Cu(I)	-	DMF	45	DBU (1.0 eq)	69
15	[l]Pd(0), Cu(I)	-	DMF	45	TMG (1.0 eq)	83

Reaction Conditions: [a]$PdCl_2(PPh_3)_2$ (2 mol%), CuI (4 mol%), 24 h,[145] [b]$Pd(PPh_3)_4$ (5 mol%), CuI (1 mol%), 6 h,[160] [c]$Pd_2(dba)_3$ (2.5 mol%), P(2-furyl)$_3$ (10 mol%), 24 h,[144] [d]$Pd_2(dba)_3$ (2.5 mol%), IPr·HCl (10 mol%), 24 h,[147] [e]$Pd(OAc)_2$ (5 mol%), IPr·HCl (10 mol%), K_2CO_3 (2.0 eq), TBAC (2.0 eq), 24 h, [f]$Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), 8-24 h, [g]$Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), 6 h, [h]$Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), 6-8 h, [i]$Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), 4 h, [j]$Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), 6-8 h, [k]$Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), 6-8 h, [l]$Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%), 1-2 h.

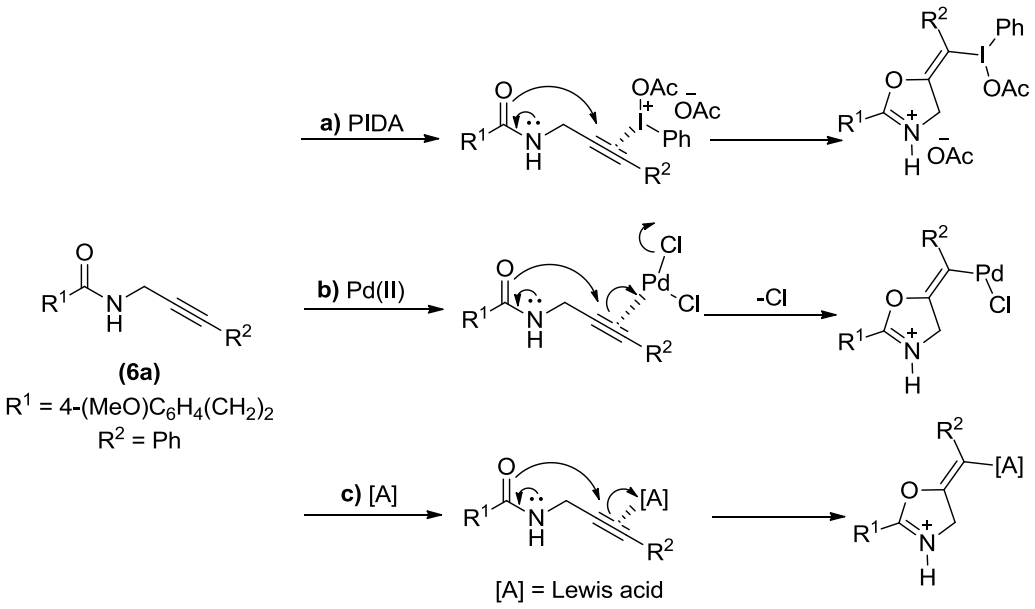
Electron deficient ligands are considered favorable for promoting the coupling reaction while strongly coordinating ligand (electron rich) are considered to retard the coordination of catalytic palladium species with alkyne for σ-alkenylpalladium complex formation (Scheme 5.16b), hence promoting the intramolecular cycloisomerization to **6ca**[144] but the implication of such reaction condition (Table 5.15, entry 6) remained futile in furnishing the corresponding coupled product **6c** and no formation of the intramolecular cycloisomerized product **6ca** was observed. The use of Pd(II) catalyst in conjunction with carbene ligands, has been established as an efficient approach for Sonogashira coupling of unactivated systems[179] and has also been shown to mediate the cyclization to heterocyclic systems.[147,180] But these reaction conditions were not successful for the coupling reaction either (Table 5.15, entries 7 and 8). The outcome of optimization studies showed $Pd(PPh_3)_4$/CuI (Table 5.15, entry 15) to

be the active and efficient catalyst system for the coupling of propargylamide **3a** with iodobenzene (**4h**). Tetramethylguanidine (TMG) was the only base, of all the screened bases, that was successful in furnishing the desired product **6a** in good yield by the usage of 1.0 equivalent and under this set of reaction conditions, no cycloisomerized products, **6ca** or **7a**, were formed. Therefore, these reaction conditions (Table 5.15, entry 15) were chosen for the one-pot synthesis of corresponding coupling product **6c**.

5.6.2. Cycloisomerization of Propargylamides 3

5.6.2.1. Lewis/Protic Acids mediated Cycloisomerization

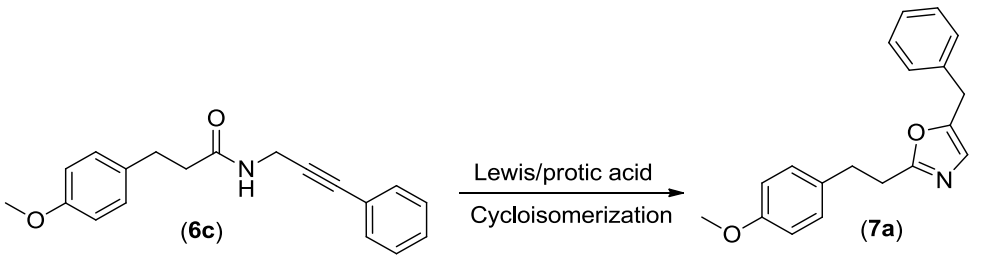
The third and final step in the sequence shown in Scheme 5.13, is the cycloisomerization of coupled product **6c** to the corresponding 2,5-disubstituted oxazole **7a**. Scheme 5.16, represents an overview of the proposed mechanisms of cycloisomerization of propargylamides **3** to corresponding oxazole system **7** via complexation of inducers (PIDA, Pd(II), Lewis acid) with alkyne moiety of propargylamide **6a**.



Scheme 5.16. Proposed mechanisms for a) activation of the alkyne moiety by hypervalent iodine species (PIDA)[158] b) σ-alkenylpalladium complex formation[149] c) complexation with Lewis acid.[151-153]

Propargylamides **3** have been studied to undergo cycloisomerization induced by PTSA at elevated temperature.[145,181] However this set of reaction conditions could not produce the desired oxazole product **7a** (Table 5.16, entry 1). Various reaction conditions involving the use of Lewis acids and protic acids, were employed but all of them failed to induce the

cycloisomerization to give the desired product **7a** (Scheme 5.17). The optimization studies for cycloisomerization reaction are mentioned in Table 5.16.



Scheme 5.17. Lewis/protic acid induces cycloisomerization to 2,5-disubstituted oxazole **7a**.

Table 5.16. Optimization study for the cycloisomerization reaction shown in Scheme 5.17.

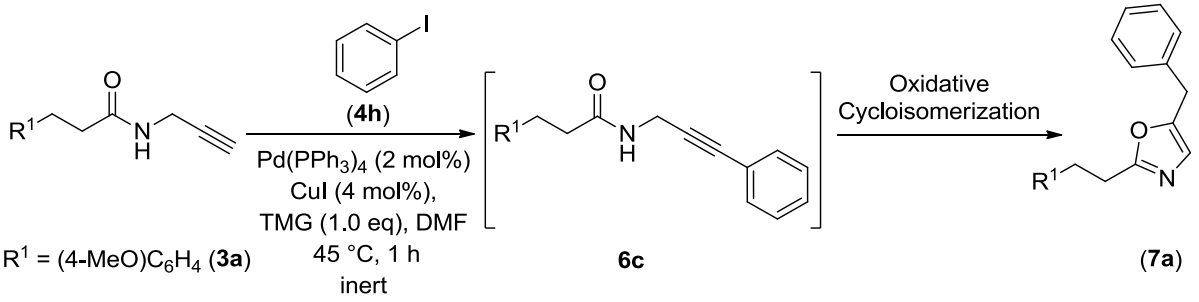
Entry	Lewis/Protic Acid	Solvent	*T* [°C]	*t* [h]	Yield (%)
1	[a]PTSA	THF:*t*-BuOH (1:1)	60	24	-
2	[b]Methanesulfonic acid	DCM	RT-45	24	-
3	[c]Trifluoromethanesulfonic acid	DCM	45	24	-
4	[d]$ZnCl_2$	DCM	RT-45	24	-
5	[e]$Zn(OTf)_2$	DCM	RT-45	24	-
6	[f]$FeCl_3$	DCM	45	24	-
7	[f]$FeCl_3$	DCE	60	24	-
8	[g]$AlCl_3$	DCM	45	24	-
9	[h]SiO_2	DCM	RT	24	-
10	[i]$Ag(OTf)_2$	DCM	RT-45	24	-
11	[j]$AuCl_3$	DCM	45	24	-
12	[j]$AuCl_3$	MeCN	45	24	-

Reaction Conditions for 1.0 eq of isolated **6c**: [a]PTSA (1.0 eq),[145] [b]Methanesulfonic acid (1.0 eq), [c]trifluoromethanesulfonic acid (1.0 eq), [d]0.1M solution of $ZnCl_2$ in diethylether,[157] [e]$Zn(OTf)_2$ (10 mol%), [f]$FeCl_3$ (0.5 eq),[157] [g]$AlCl_3$ (10 mol%), [h]SiO_2 (300 % w/w),[146] [i]$Ag(OTf)_2$ (10 mol%), [j]$AuCl_3$ (10 mol%).[151-152]

5.6.2.2. Oxidative Cycloisomerization

Propargylamides with terminal nonsubstituted alkyne moiety **3** have been reported to undergo cycloisomerization under oxidative conditions using hypervalent iodine reagents such as PIDA[158] or Pd(II) species with oxidant such as 1,4-benzoquinone.[149] Since the use of Lewis and protic acid could not catalyze the desired transformation (Table 5.16), therefore, the concept of oxidative cycloisomerization using PIDA and Pd(II) species in the presence of an oxidizing reagent, was employed but this approach also failed to carry out the required

transformation (Scheme 5.18). Table 5.17, summarizes the reaction conditions that were employed using oxidative cycloisomerization concept.



Scheme 5.18. Oxidative-cycloisomerization for oxazole **7a** synthesis.

Table 5.17. Optimization studies for oxidative cycloisomerization reaction shown in Scheme 5.18.

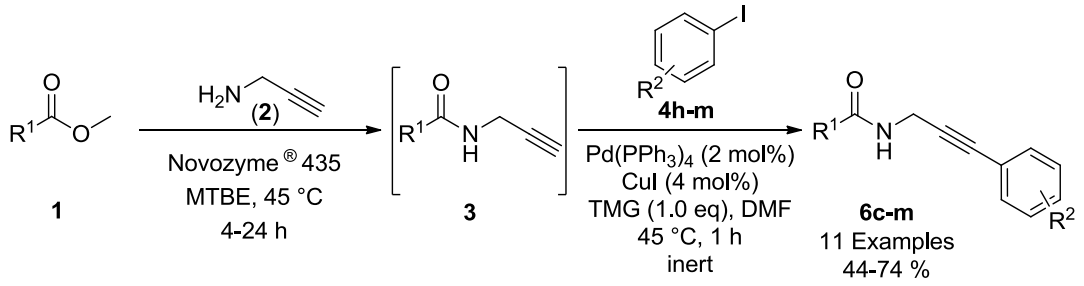
Entry	Additives for Oxidative Cycloisomerization	*T* [°C]	7a Yield (%)
1	[a]1,4-Benzoquinone	RT-80	-
2	[b]$Pd(MeCN)_2Cl_2$, 1,4-BQ	RT-80	-
3	[c]$PdCl_2$, 1N HCl, TBAC	RT	-
4	[d]PIDA, AcOH	90	-

Reaction Conditions: [a]1,4-BQ (1.0 eq), 24 h, [b]$PdCl_2(MeCN)_2$ (5 mol%), 1,4-BQ (1.0 eq), 24 h,[149] [c]$PdCl_2$ (5 mol %) TBAC (1.0 eq), 24 h, [d]PIDA (1.5 eq), AcOH (5.0 eq), 24 h.[158]

Based on the observations pertaining to cycloisomerization studies, it could be concluded that arylation of propargylamide **3a** furnished a rigid propargylated aryl system **6c**[160] that is not prone to undergo intended cycloisomerization via acid/base catalysis or σ-alkenyl-palladium complex formation by Pd(II) species or by the activation of alkyne moiety by hypervalent iodine (PIDA) (Scheme 5.16).

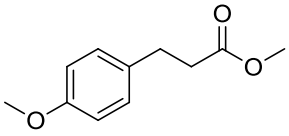
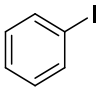
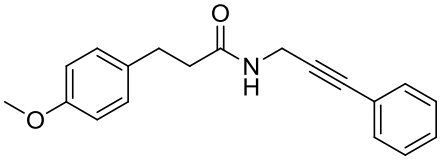
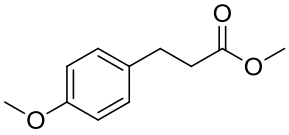
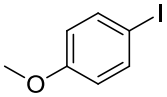
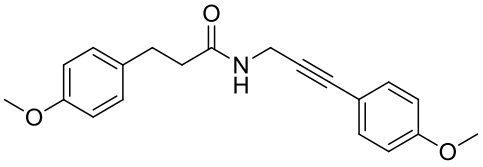
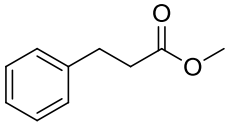
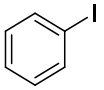
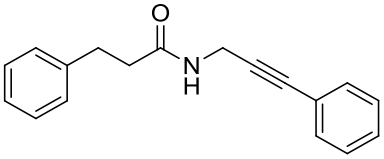
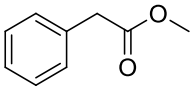
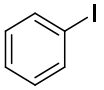
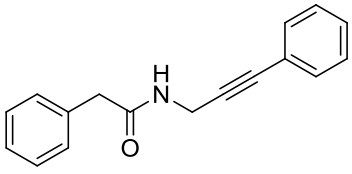
5.7. Optimized Chemoenzymatic One-pot Amidation-Sonogashira Coupling Sequence

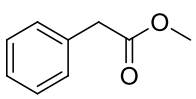
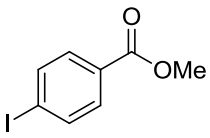
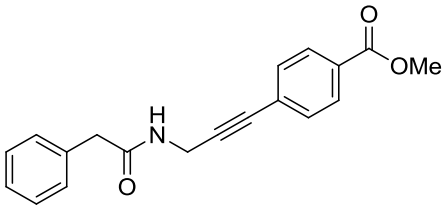
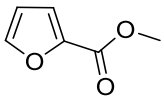
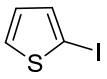
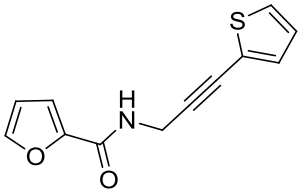
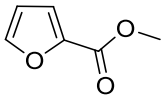
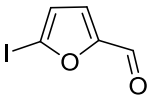
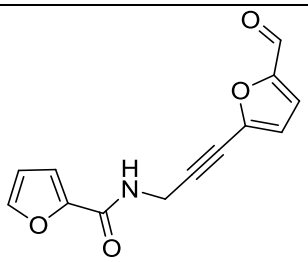
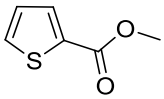
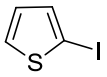
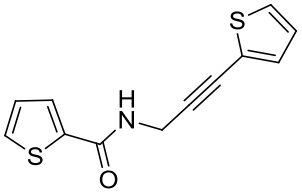
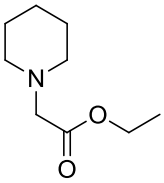
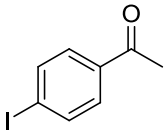
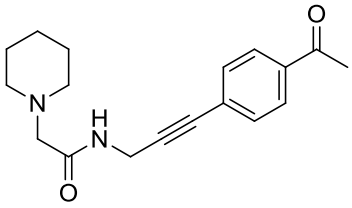
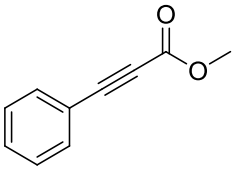
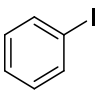
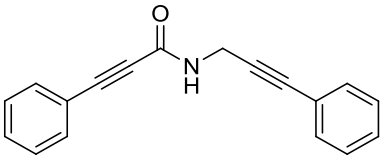
Cycloisomerization could not be induced to generate the desired product by employing the already reported reaction conditions.[149-158] Therefore, the sequence was closed after the coupling step and eleven coupled products **6** were synthesized using the optimized one-pot amidation-coupling sequence (Scheme 5.19). However, diiodoaryl systems (Table 5.18, entries 12 and 13) and iodopyridine (Table 5.18, entry 14) failed to furnish the corresponding coupled products **6n** and **6o**.

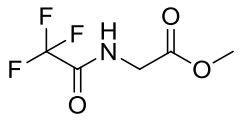
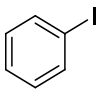
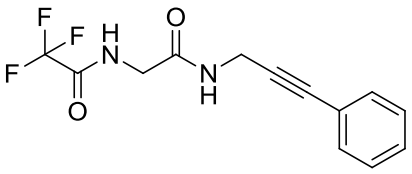
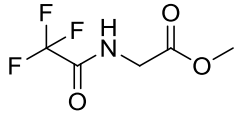
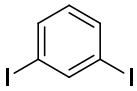
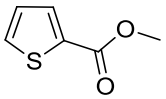
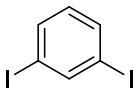
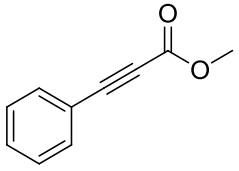
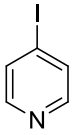


Scheme 5.19. Optimized chemoenzymatic one-pot amidation-coupling sequence.

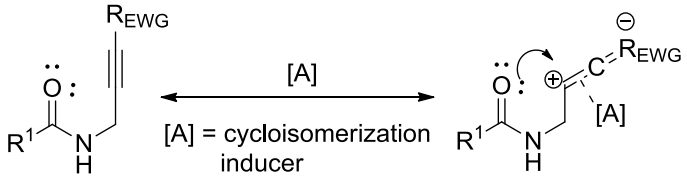
Table 5.18. Arylated propargylamides **6** obtained via amidation-coupling sequence (Scheme 5.19).

Entry	Ester Used 1	Iodoaryl 4h-o	Product 6 Yield (%)
1	 **1a**	 **4h**	 **6c** (58 %)
2	 **1a**	 **4i**	 **6d** (62 %)
3	 **1b**	 **4h**	 **6e** (54 %)
4	 **1c**	 **4h**	 **6f** (53 %)

Entry	Ester Used 1	Iodoaryl 4h-o	Product 6 Yield (%)
5	 **1c**	 **4j**	 **6g** (74 %)
6	 **1s**	 **4k**	 **6h** (71 %)
7	 **1s**	 **4l**	 **6i** (62 %)
8	 **1t**	 **4k**	 **6j** (44%)
9	 **1u**	 **4m**	 **6k** (64 %)
10	 **1aa**	 **4h**	 **6l** (51 %)

Entry	Ester Used 1	Iodoaryl 4h-o	Product 6 Yield (%)
11	 **1dd**	 **4h**	 **6m** (71 %)
12	 **1dd**	 **4n**	-
13	 **1t**	 **4n**	-
14	 **1aa**	 **4o**	-

Presence of electron withdrawing groups facilitate cycloisomerization due to their inductive effect[154] as shown in Scheme 5.20.

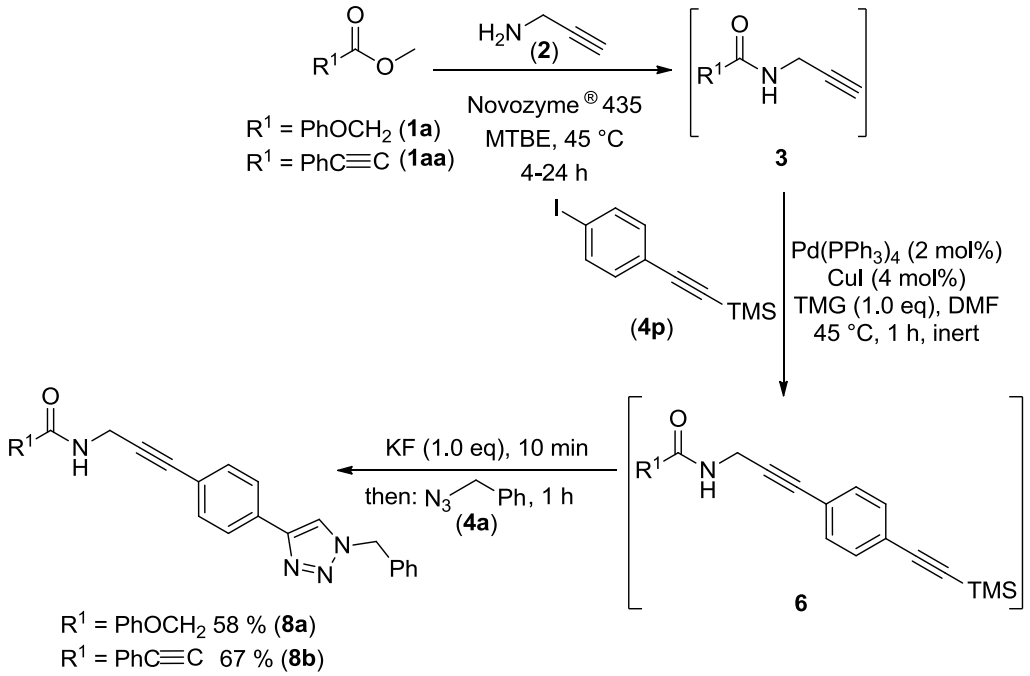


Scheme 5.20. Ease of cycloisomerization due to the inductive effect of electron withdrawing group.[154]

But no cycloisomerized product formation was observed when iodoaryl systems with electron withdrawing groups, **4j** and **4m**, were used for coupling reaction (Table 5.18, entries 5 and 9). That could be rationalized on the basis of structural rigidity of arylated

propargylamides **6** that are too rigid to acquire the proper structural conformational changes required for cycloisomerization.

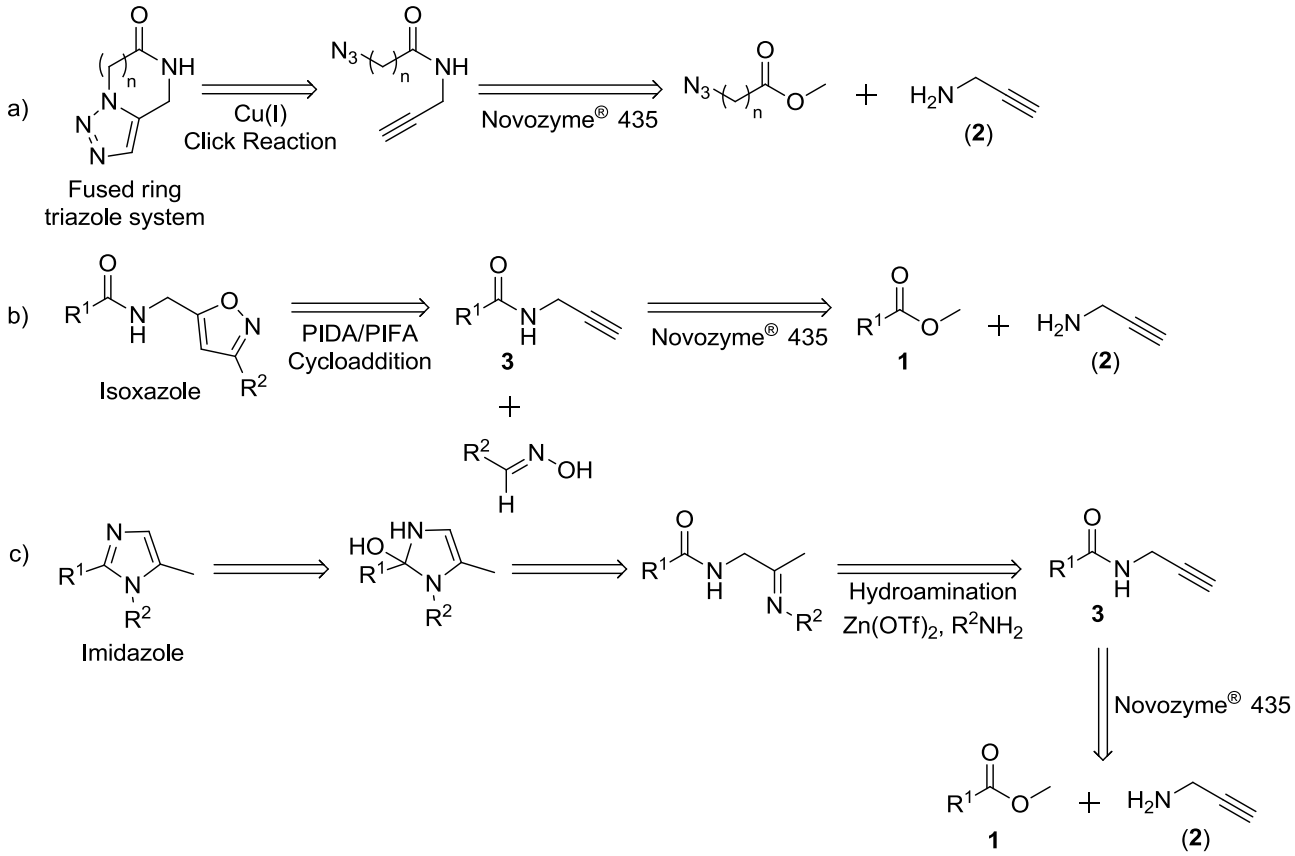
During the development of synthetic strategy, the presence of Cu(I) catalysis in the reaction mixture, was further utilized by extending the strategy to triazole ligation on the coupled product **6** hence extending the methodology by the incorporation of click reaction as the terminal step of the one-pot procedure (Scheme 5.20).



Scheme 5.20. Synthesis of triazole ligated coupled product **8** via chemoenzymatic one-pot amidation-coupling-clicks sequence.

6.0. Outlook

The successful integration of the concept of lipase catalyzed aminolysis with subsequent metal catalyzed cycloaddition and coupling reactions in a consecutive one-pot manner, validates the synergy of biocatalysis with chemical catalyzed processes for the development of novel multicomponent methodologies. In this study, propargylamine (**2**) has been utilized as a suitable motif for the generation of corresponding amide functionality with terminal alkyne group for further modifications in one-pot fashion. Some of the other possibilities utilizing alkyne moiety of propargylamide **3** for the syntheses of heterocyclic systems, have been depicted in Scheme 6.1, below.



Scheme 6.1. Retrosynthetic analyses for the syntheses of a) fused ring triazoles, b) Isoxazoles, c) Imidazoles, suggesting the integration of Novozyme® 435 catalysis for the development of novel one-pot strategies.

One possibility might be the generation of fused ring triazole systems via click reaction following the lipase catalyzed aminolysis (Scheme 6.1a). The optimized lipase catalyzed aminolysis for propargylamide **3** generation can further be tuned to other catalyst systems for the syntheses of heterocyclic systems. In this context, hypervalent iodine species (PIDA,

PIFA) have been actively employed for the synthesis of isoxazole systems[181] and this methodology can be assimilated with lipase catalyzed aminolysis for the development of metal free one-pot isoxazole syntheses (Scheme 6.1b). Synthesis of imidazoles have been reported by hydroamination of propargylamides in the presence of zinc catalyst system ($Zn(OTf)_2$).[182] Since the synergistic behaviour of lipases with zinc catalyst systems has not yet been explored, therefore, this synthetic void also provides the opportunity to study the integration of lipase catalysis with zinc catalyzed hydroamination of alkyne moiety of propargylamides **3** leading to imidazole system (Scheme 6.1c).

7.0. Experimental

7.1. General Considerations

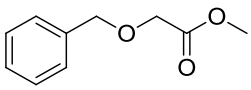
All enzyme catalyzed and coupling reactions were performed in flame dried Schlenk tubes. The enzyme catalyzed reactions were carried out under normal atmospheric conditions in an incubating shaker IKA® KS 4000i, whereas the Sonogashira coupling reactions were executed under argon atmosphere. MTBE was dried over 3 Å molecular sieves prior to use. The reaction progress was monitored qualitatively by thin layer chromatography using silica gel layered aluminium foil (60 F254 Merck, Darmstadt). The detection was made by irradiating under UV light of wavelength 254 and 366 nm or stained with $KMnO_4$, ninhydrin and molybdate solution (saturated ethanolic solution of molybdatophosphoric acid). Crude mixtures were absorbed on Celite® 545 (0.02-0.20 mm, Carl Roth GmbH Co.KG) for column chromatography through flash technique. All the chemicals that have not been synthesized, were purchased from Sigma Aldrich, Alfa Aesar and ACROS and were used as received without any further purification. The ^{1}H-NMR and ^{13}C-NMR spectra were measured on the device Avance DRX 500 , AV or AV III 600 III 300 Bruker. Chemical shifts are given in ppm (δ) and were referenced to the internal solvent signal: d_6-acetone (^{1}H δ 2.05 ^{13}C δ 29.9), $CDCl_3$ (^{1}H δ 7.26 , ^{13}C δ 77.2), d_6-DMSO (^{1}H δ 2.50 ^{13}C δ 39.5). Multiplicities are stated as: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), dq (doublet of quartet), m (multiplet), br m (broad multiplet). Coupling constants (*J)* are given in Hz. The assignment of the primary (CH_3), secondary (CH_2), tertiary (CH) and quaternary carbon nuclei (C_{quat}), was made using DEPT-135 spectra. The mass spectrometric investigations were carried out in the Department of Mass Spectrometry of Inorganic and Organic Chemistry of the University of Düsseldorf. The IR spectra were recorded with a Bruker Vector 22 FT-IR or Shimadzu IRAffinity-1. The intensities of the IR bands were abbreviated as w (weak), m (medium), s (strong). Melting points (uncorrected) were measured using Reichert Thermovar (PeakTeck®). Combustion analyses were measured on a Perkin Elmer Series II Analyser 2400 in the Institute for Pharmaceutical and Medicinal Chemistry Heinrich-Heine University, Düsseldorf.

7.2. Synthesis of Starting Materials

Ester starting materials have been synthesized according to the respective methods reported in literature.

Methyl 2-(benzyloxy) acetate (**1f**),[183] methyl 2-(phenylamino)acetate (**1h**),[184] methyl 2-(phenylthio)acetate (**1i**),[185] methyl 2-(benzylthio)acetate (**1j**),[185] methyl 3-(4-(2-methoxy-2-oxoethoxy)phenyl)propanoate (**1k**),[186] (*R*)-methyl 2-((*t*-butoxycarbonyl)amino)propanoate (**1m**),[187] (*R*)-methyl 2-(2,2,2-trifluoroacetamido)propanoate (**1n**),[188] (*R*)-methyl 2-((benzyloxy) carbonyl)amino)propanoate (**1o**),[189] (*S*)-methyl 2-((benzyloxy)carbonyl)amino) propanoate (**1p**),[189] methyl 2-(2,2,2-trifluoroacetamido)acetate (**1dd**).[188]

7.2.1. Methyl 2-(benzyloxy)acetate (1f)[183]

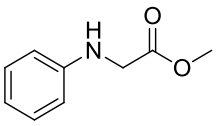


$C_{10}H_{12}O_3$
180.20 g/mol

A solution of 2-(benzyloxy) acetic acid (2.0 g, 12.00 mmol) in MeOH (40.0 mL), was cooled to 4 °C in an ice bath. Thionyl chloride (1.7 g, 14.00 mmol) was added drop wise maintaining the temperature below 8 °C. The resulting solution was stirred for 30 minutes at 4 °C and for 2.5 h at room temperature. The solvent was evaporated under reduced pressure and the resulting residue was dissolved in ethylacetate (20.0 mL) and washed with saturated aqueous $NaHCO_3$ solution (20.0 mL). The aqueous layer was extracted with additional portion of ethylacetate (2 x 10.0 mL). The combined organic layers were washed with brine (20.0 mL), dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to get 1.62 g (75 %) of title compound as colorless clear liquid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.76 (s, 3 H), 4.11 (s, 2 H), 4.64 (s, 2 H), 7.27-7.38 (m, 5 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 51.2 (CH_3), 61.1 (CH_2), 73.37 (CH_2), 128.0 (CH), 128.1 (CH), 128.5 (CH), 137.0 (C_{quat}), 170.8 (C_{quat}). **EI-MS:** *m/z* (%) = 180 (M^+, 1.3), 121 ($C_8H_9O^+$, 4.9), 105 ($C_4H_9O_3^+$, 3.6), 107 ($C_7H_7O^+$, 79.9), 91 ($C_7H_7^+$, 100), 89 ($C_3H_5O_3^+$, 5.4). Anal. calcd. for $C_{10}H_{12}O_3$ (180.2): C 66.65, H 6.71. Found C 66.51, H 6.80.

7.2.2. Methyl 2-(phenylamino)acetate (1h)[184]

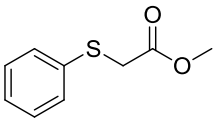


$C_9H_{11}N_{O2}$
165.19 g/mol

In a 50 mL round bottom flask containing acetone (10.0 mL), were added aniline (1.03 g, 11.10 mmol), and potassium carbonate (2.3 g, 16.70 mmol). The reaction mixture was allowed to stir at 60 °C for 1 h after which methyl bromoacetate (1.85 g, 12.10 mmol), was added drop wise to the suspension and the mixture was stirred for 20 h at 60 °C. After 20 h, the reaction mixture was filtered and diluted with ethylacetate (20.0 mL) for subsequent washing with water (10.0 mL) and brine (10.0 mL). The organic layer was dried with anhydrous Na_2SO_4 followed by silica gel column chromatography using *n*-hexane:ethylacetate (4:1) as eluent to get 1.23 g (67 %) of title compound as greyish brown solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.79 (s, 3 H), 3.93 (s, 2 H), 4.29 (br s, 1 H), 6.62 (d, ^3J = 8.5 Hz, 2 H), 6.76 (m, 1 H), 7.20 (m, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 45.8 (CH_2), 52.4 (CH_3), 113.1 (CH), 118.4 (CH), 129.5 (CH), 147.1 (C_{quat}), 171.8 (C_{quat}). **EI-MS:** *m/z* (%) = 165 (M^+, 16.7), 106 ($C_8H_{10}N^+$, 100), 77 ($C_6H_5^+$, 30.3). Anal. calcd. for $C_9H_{11}O_2N$ (165.2): C 65.44 N 8.48, H 6.71. Found C 65.63 N 8.30 H 6.52.

7.2.3. Methyl 2-(phenylthio)acetate (1i)[185]



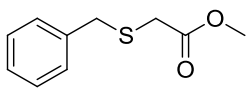
$C_9H_{10}O_2S$
182.24 g/mol

In a 50 mL round bottom flask, a mixture of thiophenol (1.11 g, 10.00 mmol), triethylamine (1.01 g, 10.0 mmol), methyl bromoacetate (1.53 g, 10.00 mmol), and benzene (13.0 mL) was stirred at room temperature for 4 h after which the reaction mixture was filtered and diluted with ethylacetate (20.0 mL) followed by subsequent washing with water (10.0 mL) and brine (10.0 mL). The organic layer was dried with anhydrous Na_2SO_4 followed by silica gel column

chromatography (*n*-hexane:ethylacetate 10:1) to get 1.39 g (96 %) of title compound as colorless liquid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.65 (s, 2 H), 3.71 (s, 2 H), 7.23 (t, ^3J = 7.5 Hz, 1 H), 7.30 (t, ^3J = 7.5, 2 H), 7.41 (d, ^3J = 7.3 Hz, 2 H).**^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 52.6 (CH_3), 36.6 (CH_2), 127.1 (CH), 129.2 (CH), 130.0 (CH), 135.0 (C_{quat}), 170.2 (C_{quat}). **EI-MS:** *m/z* (%) = 182 (M^+, 32.9), 123 ($C_7H_7S^+$, 100), 109 ($C_6H_5S^+$, 17.8), 77 ($C_6H_5^+$, 30.5). Anal. calcd. for $C_9H_{10}O_2S$ (182.2): C 59.32 H 5.53. Found C 59.26 H 5.35.

7.2.4. Methyl 2-(benzylthio)acetate (1j)[185]

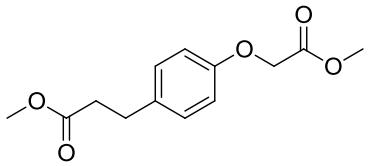


$C_{10}H_{12}O_2S$
196.27 g/mol

Benzyl mercaptan (0.62 g, 5.00 mmol), triethylamine (0.51 g, 5.00 mmol) and methyl bromoacetate (0.76 g, 5.00 mmol) in benzene (7.5 mL), were stirred at room temperature for 4 h in a 50 mL round bottom flask. After the aqueous work up, same as mentioned for **1h** and **1i**, and drying with anhydrous Na_2SO_4, the product was purified by silica gel chromatography (*n*-hexane:ethylacetate (10:1)) to get 0.77 g (78 %) of title compound as colorless liquid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.06 (s, 2 H), 3.69 (s, 3 H), 3.90 (s, 2 H), 7.2 -7.31 (m, 5 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ 32.2 (CH_2), 36.5 (CH_2), 52.5 (CH_3), 127.4 (CH), 128.7 (CH), 129.3 (CH), 137.3 (C_{quat}), 171.0 (C_{quat}).

7.2.5. Methyl 3-(4-(2-methoxy-2-oxoethoxy)phenyl)propanoate (1k)[186]



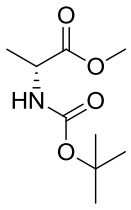
$C_{13}H_{16}O_5$
252.26 g/mol

To a mixture of methyl 3-(4-hydroxyphenyl)propionate (2.70 g, 15.00 mmol) and K_2CO_3 (8.09 g, 57.00 mmol), was added methyl bromoacetate (4.58 g, 30.00 mmol) and the resulting mixture was allowed to stir at 70 °C for 30 h. After 30 h of reaction time, the reaction mixture was filtered and acetone was removed under vacuum. The residue was redissolved in water

and extracted with chloroform (3 x 20.0 mL). The combined organic layers were dried with anhydrous Na_2SO_4 and solvent was removed under vacuum to get crude solid that was subjected to vacuum distillation with the gradual increase of temperature to get the 1.44 g (38 %) of pure product (bp. 210-213 °C) as low melting colorless solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.59 (t, ^3J = 7.4 Hz, 2 H), 2.89 (t, ^3J = 7.7 Hz, 2 H), 3.66 (s, 1 H), 3.80 (s, 3 H), 4.60 (s, 2 H), 6.83 (d, ^3J = 8.6 Hz, 2 H) , 7.11 (d, ^3J = 8.4 Hz, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 30.6 (CH_2), 36.4 (CH_2), 52.2 (CH_3), 52.8 (CH_3), 65.8 (CH_2), 115.3 (CH), 129.9 (CH), 134.4 (C_{quat}), 156.9 (C_{quat}), 170.0 (C_{quat}), 173.9 (C_{quat}). **GC-MS:** *m/z* (%) = 252 (M^+, 21.1), 179 ($C_{10}H_{11}O_3^+$, 100), 193 ($C_{11}H_{13}O_3^+$, 6.3), 221 ([M - CH_3O]$^+$, 3.3), 91 ($C_7H_7^+$, 37.6), 77 ($C_6H_5^+$, 30.8), 59 ($C_2H_3O_2^+$, 21.4). Anal. calcd for $C_{13}H_{16}O_5$ (252.3): C 61.90 H 6.39. Found C 61.96 H 6.38.

7.2.6. (*R*)-Methyl 2-((*t*-butoxycarbonyl)amino)propanoate (1m)[187]

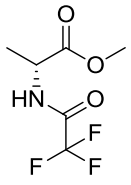


$C_9H_{17}NO_4$
203.24 g/mol

To a suspension of *D*-alanine (0.89 g, 10.00 mmol) in ice cooled methanol (17.0 mL) in a 50 mL round bottom flask, thionyl chloride (1.69 g, 14.20 mmol) was drop wise added maintaining the temperature between 0-8 °C. After the complete drop wise addition of thionyl chloride, the reaction mixture was allowed to stir for 20 min at 0 °C and then at room temperature overnight. After overnight stirring, the reaction mixture was heated to 80 °C for 3 h and solvent was removed under reduced pressure to get viscous light yellow colored residue that was then redissolved in saturated aqueous $NaHCO_3$ solution (24.0 mL) followed by drop wise addition of 1,4-dioxane (25.0 mL) solution of Boc-anhydride (3.00 g, 13.70 mmol). The reaction mixture was allowed to run for 12 h at room temperature after which it was filtered and extracted with ethylacetate (3 x 15.0 mL). The combined organic layers were dried with anhydrous Na_2SO_4. The evaporation of organic solvent under reduced pressure followed by drying under vacuum, gave 1.48 g (72 %) of title compound as colorless viscous liquid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.39 (d, ^3J = 7.2 Hz, 3 H), 1.45 (s, 9 H), 3.75 (s, 3 H), 4.33 (m, 1 H), 5.05 (br s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 18.4 (CH_3), 28.0 (CH_3), 48.9 (CH), 52.0 (CH_3), 79.6 (C_{quat}), 154.8 (C_{quat}), 173.6 (C_{quat}). **GC-MS:** *m/z* (%) = 203 (M^+, 0.2), 188 ($C_8H_{14}NO_4^+$, 188.0), 144 ($C_7H_{14}NO_2^+$, 18.1), 130 ($C_5H_8NO_3^+$, 3.7), 116 ($C_5H_{10}NO_2^+$, 2.3), 102 ($C_4H_8NO_2^+$, 12.7), 88 ($C_4H_8O_2^+$, 23.1), 70 (5.3), 57 ($C_4H_9^+$, 100).

7.2.7. (*R*)-Methyl 2-(2,2,2-trifluoroacetamido)propanoate (1n)[188]

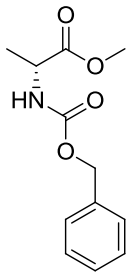


$C_6H_8F_3NO_3$
199.13 g/mol

To a suspension of *D*-alanine (0.89 g, 10.00 mmol) in ice cooled methanol (17.0 mL) in a 50 mL round bottom flask, thionyl chloride (1.69 g, 14.20 mmol) was drop wise added maintaining the temperature between 0-8 °C. After the complete drop wise addition of thionyl chloride, the reaction mixture was allowed to stir for 20 min at 0 °C and then at room temperature overnight. Colorless liquid was obtained after evaporating the solvent under reduced pressure. To this colorless liquid, was added DCM (20.0 mL) in an ice bath followed by slow addition of triethylamine (3.03 g, 30.00 mmol) maintaining the temperature below 5 °C. After the complete addition of triethylamine, trifluoroacetic anhydride (2.31 g, 11.00 mmol) was added drop wise in the reaction mixture maintaining the temperature below 5 °C. The reaction mixture was allowed to run for 30 min on ice bath and for 5 h at room temperature after which water (15.0 mL) was added into it for subsequent extraction with ethylacetate (3 x 15.0 mL) and washing with 2N HCl solution. The combined organic layers were dried with anhydrous Na_2SO_4. Purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)) furnished 1.25 g (63 %) of the title compound as viscous brown liquid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51 (d, ^3J = 7.2 Hz, 3 H), 3.81 (s, 3 H), 4.61 (m, 1 H), 6.99 (br s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 17.6 (CH_3), 48.3 (CH_3), 52.8 (CH), 115.3 (d, ^1J = 287.6 Hz, C_{quat}), 156.6 (d, ^2J = 37.9 Hz, C_{quat}), 171.7 (C_{quat}). **GC-MS:** *m/z* (%) = 199 (M^+, 0.9), 184 ($C_5H_5F_3NO_3^+$, 1.1), 167 (0.9), 140 (100), 129 (2.4), 102 ($C_4H_8NO_2^+$, 8.4), 113 (3.4), 92 (10.7), 70 (23.3), 69 (CF_3^+, 48.7), 59 ($C_2H_3O_2^+$, 13.9).

7.2.8. (*R*)-Methyl 2-((benzyloxy)carbonyl)amino)propanoate (1o)[189]

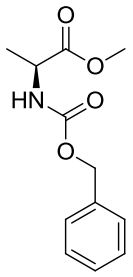


$C_{12}H_{15}NO_4$
237.25 g/mol

To a suspension of *D*-alanine (0.89 g, 10.00 mmol) in ice cooled methanol (17.0 mL) in a 50 mL round bottom flask, thionyl chloride (1.69 g, 14.20 mmol) was drop wise added maintaining the temperature between 0-8 °C. After the complete drop wise addition of thionyl chloride, the reaction mixture was allowed to stir for 20 minutes at 0 °C and then at room temperature overnight after which it was heated to 80 °C for 3 h. Removal of the solvent under reduced pressure gave the respective methyl ester as colorless liquid that was then dissolved in saturated aqueous $NaHCO_3$ solution (20.0 mL) followed by the addition of benzyl chloroformate (1.70 g, 10.00 mmol) under vigorous stirring overnight. The reaction mixture was extracted with diethylether (3 x 15.0 mL) and washed with 2N HCl (20.0 mL) solution. The combined organic layers were dried with anhydrous Na_2SO_4. Purification by silica gel column chromatography (*n*-hexane:ethylacetate (3:1)) furnished 1.76 g (74 %) of the title compound as colorless waxy solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.41 (d, ^3J = 7.2 Hz, 3 H), 3.74 (s, 3 H), 4.39 (m, 1 H), 5.11 (s, 2 H), 5.34 (br s, 1 H), 7.31-7.36 (m, 5 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 18.4 (CH_3), 43.3 (CH_3), 52.2 (CH), 66.7 (CH_2), 127.8 (CH), 127.9 (CH), 128.2 (CH), 136.0 (C_{quat}), 155.3 (C_{quat}), 173.2 (C_{quat}). **GC-MS:** *m/z* (%) = 237 (M^+, 2.6), 178 ($C_{10}H_{12}NO_2^+$, 12.6), 179 (1.3), 135 ($C_8H_6O_2^+$, 1.6), 134 (15.3), 108 ($C_7H_8O^+$, 36.5), 109 ($C_7H_9O^+$, 2.7), 91 ($C_7H_7^+$, 100), 92 (7.5), 102 ($C_4H_8NO_2^+$, 1.7), 59 ($C_2H_3O_2^+$, 1.4), 89 ($C_4H_9O_2^+$, 3.7), 70 (1.8), 79 (3.3), 65 ($C_5H_5^+$, 5.8), 59 (1.4), 51 (1.4), 44 (2.4).

7.2.9. (*S*)-Methyl 2-((benzyloxy)carbonyl)amino)propanoate (1p)[189]

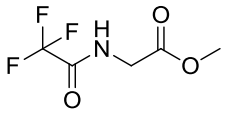


$C_{12}H_{15}NO_4$
237.25 g/mol

L-alanine methyl ester (1.40 g, 10.00 mmol) was dissolved in aqueous saturated $NaHCO_3$ solution (20.0 mL) in a 50 mL round bottom flask. Ethylacetate (20.0 mL) was added to the reaction mixture followed by the addition of benzyl chloroformate (1.70 g, 10.00 mmol) and reaction was allowed to run overnight. After the overnight stirring, the organic layer was separated and aqueous layer was extracted with ethylacetate (3 x 10.0 mL). The combined organic layers were washed with 2N HCl solution (15.0 mL) and dried with anhydrous Na_2SO_4. Evaporation of the solvent yielded 1.96 g (83 %) of the title compound as colorless solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.75 (s, 3 H), 4.39 (br m, 1 H), 5.11 (s, 2 H), 5.31 (br s, 1 H), 7.29-7.42 (br m, 5 H).**^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 18.8 (CH_3), 49.7 (CH_3), 52.6 (CH), 67.1 (CH_2), 128.2 (CH), 128.3 (CH), 128.7 (CH), 136.4 (C_{quat}), 155.7 (C_{quat}). **GC-MS:** *m/z* (%) = 237 (M^+, 1.1), 178 ($C_{10}H_{12}NO_2^+$, 2.3), 148 ($C_5H_{10}NO_4^+$, 1.7), 134 (5.7), 108 ($C_7H_8O^+$, 21.5), 109 ($C_7H_9O^+$, 1.7), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 7.3), 65 ($C_5H_5^+$, 5.9).

7.2.10. Methyl 2-(2,2,2-trifluoroacetamido)acetate (1dd)[188]



To a mixture of glycine methyl ester hydrochloride (1.26 g, 10.00 mmol) in DCM (20.0 mL), was added triethylamine (3.04 g, 30.00 mmol) at 0 °C followed by addition of trifluoroacetic anhydride (2.31 g, 11.00 mmol) maintaining the temperature between 0-8 °C. After the complete addition of trifluoroacetic anhydride, the reaction mixture was allowed to stir at room temperature for 5 h after which the reaction mixture was treated with water (20.0 mL) and extracted with ethylacetate (3 x 15.0 mL). The combined organic layers were washed with 2N HCl solution (20.0 mL) and dried with anhydrous Na_2SO_4. The solvent was removed

under reduced pressure and 1.12 g (61 %) of the title compound was obtained as colorless liquid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (3:1)).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 3.76 (s, 3 H), 4.07 (d, ^{3}J = 5.1, 2 H), 6.83 (br s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 39.6 (CH_2), 48.7 (CH_3), 117.6 (d, ^{1}J = 286.8 Hz, C_{quat}), 156.9 (d, ^{2}J = 37.5 Hz, C_{quat}), 169.8 (C_{quat}). **GC-MS:** *m/z* (%) = 185 (M^+, 0.6), 154 ([M - CH_3O]$^+$, 3.2), 126 ($C_3H_3F_3NO^+$, 100), 106 (4.89), 88 ([M - C_2F_3O]$^+$, 13.9), 69 (CF_3^+, 48.6), 59 ($C_2H_3O_2^+$, 15.7).

7.3. General Procedure for CAL-B (Novozyme® 435) Catalyzed Aminolysis

To a solution of 55 mg (1.00 mmol) of propargylamine (**2**), in dry MTBE (2.0 mL) in a screwcap Schlenk vessel, were added 1.20 mmol of the methyl ester **1** (experimental details mentioned in Table 7.1) and Novozyme® 435 (50 % w/w of corresponding ester substrate **1** used) and the reaction was shaken in an incubating shaker at 45 °C for 4 or 24 h (depending upon the nature of ester **1** used). After the given reaction time (Table 7.1), the enzyme beads were filtered off and then the filtered reaction mixture was subjected to silica gel column chromatography using *n*-hexane:ethylacetate as eluent to obtain the pure product **3**.

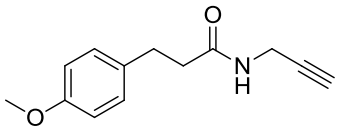
Table 7.1. Experimental details for *Candida antarctica* lipase B (Novozyme® 435) catalyzed aminolysis by propargylamine (**2**, 55.0 mg,1.00 mmol) at 45 °C.

Entry	Ester Substrate 1	*t* [h]	Propargylamide 3
1	233 mg (1.20 mmol) of **1a**	24	148 mg (68 %) of **3a**
2	197 mg (1.20 mmol) of **1b**	24	116 mg (62 %) of **3b**
3	180 mg (1.20 mmol) of **1c**	24	126 mg (73 %) of **3c**
4	199 mg (1.20 mmol) of **1e**	4	165 mg (87%) of **3e**
5	216 mg (1.20 mmol) of **1f**	4	175 mg (86 %) of **3f**
6	235 mg (1.20 mmol) of **1g**	4	208 mg (95 %) of **3g**
7	198 mg (1.20 mmol) of **1h**	4	152 mg (81 %) of **3h**
8	219 mg (1.20 mmol) of **1i**	4	158 mg (77 %) of **3i**
9	236 mg (1.20 mmol) of **1j**	4	180 mg (82 %) of **3j**
10	303 mg (1.20 mmol) of **1k**	4	220 mg (80%) of **3k**
11	239 mg (1.20 mmol) of **1n**	4	138 mg (62 %) of **3n**
12	285 mg (1.20 mmol) of **1o**	4	213 mg (82 %) of **3o**
13	285 mg (1.20 mmol) of **1p**	4	208 mg (80 %) of **3p**
14	316 mg (1.20 mmol) of **1q**	24	195 mg (68 %) of **3q**
15	304 mg (1.20 mmol) of **1r**	24	146 mg (53 %) of **3r**

Entry	Ester Substrate 1	*t* [h]	Propargylamide 3
16	151 mg (1.20 mmol) of **1s**	24	106 mg (71 %) of **3s**
17	171 mg (1.20 mmol) of **1t**	24	127 mg (77 %) of **3t**
18	205 mg (1.20 mmol) of **1u**	4	126 mg (70 %) of **3u**
19	192 mg (1.20 mmol) of **1aa**	24	147 mg (80 %) of **3aa**

7.4. Analytical Data of Propargylamides 3

7.4.1. 3-(4-Methoxyphenyl)-*N*-prop-2-yn-1-yl)propanamide (3a)

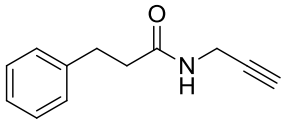


$C_{13}H_{15}NO_2$
217.26 g/mol

148 mg (0.68 mmol, 68 %) of **3a** as colorless crystalline substance was obtained after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 88 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.21 (t, ^4J = 2.6 Hz, 1 H), 2.46 (t, ^3J = 7.7 Hz, 2 H), 2.90 (t, ^3J = 7.7 Hz, 2 H), 3.77 (s, 3 H), 4.01 (dd, ^3J = 5.2 Hz, ^4J = 2.5 Hz, 2 H), 5.70 (br s, 1 H), 6.82 (d, ^3J = 8.6 Hz, 2 H), 7.10 (d, ^3J = 8.6 Hz, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.3 (CH_2), 30.8 (CH_2), 38.6 (CH_2), 55.4 (CH_3), 71.7 (CH), 79.6 (C_{quat}), 114.1 (CH), 129.4 (CH), 132.8 (C_{quat}), 158.2 (C_{quat}), 172.0 (C_{quat}). **EI-MS:** *m/z* (%) = 217 (M^+, 5.8), 121 ([M - $C_5H_6NO]^+$, 100), 134 (12.3), 180 ($C_{10}H_{14}NO_2^+$, 21.8), 77 ($C_6H_5^+$, 7.6), 91 ($C_7H_7^+$, 9.3), 65 ($C_5H_5^+$, 3.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3068 (w), 2962 (w), 2922 (w), 2860 (w), 2835 (w), 2673 (w), 1634 (s), 1612 (w), 1544 (m), 1512 (s), 1456 (w), 1421 (w), 1375 (w), 1340 (w), 1301 (w), 1296 (w), 1240 (s), 1228 (s), 1178 (m), 1111 (w), 1087 (w), 1028 (s), 923 (w), 887 (w), 821 (w), 802 (m), 771 (w), 721 (s), 688 (s), 615 (w). Anal. calcd. for $C_{13}H_{15}NO_2$ (217.3): C 71.87, H 6.96, N 6.45. Found: C 71.87, H 6.94, N 6.24.

7.4.2. 3-Phenyl-*N*-(prop-2-yn-1-yl)propanamide (3b)

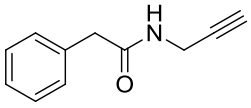


$C_{12}H_{13}NO$
187.24 g/mol

116 mg (0.62 mmol, 62 %) of **3b** as colorless crystalline substance was obtained after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 69 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.20 (t, ^4J = 2.5 Hz, 1 H), 2.50 (t, ^3J = 7.6 Hz, 2 H), 2.97 (t, ^3J = 7.6 Hz, 2 H), 4.01 (dd, ^3J = 5.3 Hz, ^4J = 2.6 Hz, 2 H), 5.74 (br s, 1 H), 7.18-7.21 (m, 3 H), 7.27-7.29 (m, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.3 (CH_2), 31.6 (CH_2), 38.2 (CH_2), 71.7 (CH), 79.6 (C_{quat}), 126.4 (CH), 128.4 (CH), 128.7 (CH), 140.8 (C_{quat}), 171.9 (C_{quat}). **EI-MS:** *m/z* (%) = 188 ([M + H]$^+$, 25.1), 187 (M$^+$, 97.4), 186 ([M - H]$^+$, 18.6), 170 (8.3), 169 (32.0), 158 (5.2), 146 (8.1), 144 (13.8), 143 (18.9), 141 (16.6), 131 ($C_9H_7O^+$, 12.9), 129 ($C_9H_5O^+$, 33.0), 128 ($C_9H_4O^+$, 30.0), 117 (4.4), 116 (3.3), 115 (5.5), 110 ([M - C_6H_5]$^+$, 9.3), 105 ($C_8H_9^+$, 59.3), 104 ($C_8H_8^+$, 38.3), 96 ([M - C_7H_7]$^+$, 76.0), 91 ($C_7H_7^+$, 100), 82 (4.6), 65 ($C_5H_5^+$, 15.5), 55 ($C_3H_5N^+$, 16.0), 51 (C_3HN^+, 12.4), 39 ($C_3H_3^+$, 25.8). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3055 (w), 2988 (w), 2951 (w), 2901 (w), 2886 (w), 2835 (w), 2581 (w), 1732(s), 1599 (w), 1585 (w), 1558 (w), 1437 (m), 1368 (m), 1317 (w), 1261 (w), 1223 (m), 1217 (m), 1179 (m), 1138 (m), 1076 (w), 1057 (w), 1028 (w), 984 (w), 957 (w), 916 (w), 870 (w), 839 (w), 820 (w), 754 (m), 741 (s), 694 (s), 602 (w). Anal. calcd. for $C_{12}H_{13}NO$ (187.2): C 76.98, H 7.00, N 7.48. Found: C 76.89, H 7.01, N 7.49.

7.4.3. 2-Phenyl-*N*-(prop-2-yn-1-yl)acetamide (3c)

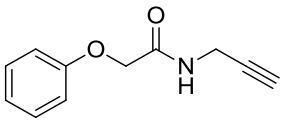


$C_{11}H_{11}NO$
173.21 g/mol

126 mg (0.73 mmol, 73 %) of **3c** as colorless crystalline substance was obtained after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 71 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.18 (t, ^4J = 2.3 Hz, 1 H), 3.59 (s, 2 H), 4.01 (dd, ^3J = 5.3, ^4J = 2.6, 2 H), 5.67 (br s, 1 H), 7.25 (m, 1 H), 7.27-7.39 (m, 4 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.4 (CH_2), 43.6 (CH_2), 71.7 (CH), 79.5 (C_{quat}), 127.6 (CH), 129.2 (CH), 129.6 (CH), 134.5 (C_{quat}), 170.7 (C_{quat}). **EI-MS:** *m/z* (%) = 174 ($[M + H]^+$, 3.6), 173 (M^+, 16.7), 172 ($[M - H]^+$, 1.1), 119 [$C_8H_7O^+$, 1.8), 96 ($C_5H_6NO^+$, 3.2), 91 ($C_7H_7^+$, 100), 82 ($C_4H_4NO^+$, 10.4), 77 ($C_6H_5^+$, 1.3), 65 ($C_5H_5^+$, 16.3), 43 ($C_2H_3O^+$, 3.1), 39 ($C_3H_3^+$, 22.1). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3034 (w), 2972 (w), 2922 (w), 2866 (w), 2804 (w), 1664 (w), 1627 (m), 1539 (m), 1490 (m), 1448 (m), 1429 (w), 1394 (w), 1363 (w), 1330 (w), 1315 (w), 1292 (w), 1263 (w), 1199 (w), 1155 (w), 1103 (w), 1082 (w), 1070 (w), 1028 (w), 1002 (w), 920 (w), 906 (w), 800 (w), 769 (w), 731 (w), 690 (s), 677 (s), 650 (m), 607 (m). Anal. calcd. for $C_{11}H_{11}NO$ (173.2): C 76.28, H 6.40, N 8.09. Found C 76.20, H 6.25, N 7.81.

7.4.4. 2-Phenoxy-*N*-(prop-2-yn-1-yl) acetamide (3e)



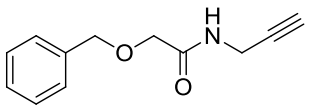
$C_{11}H_{11}NO_2$
189.21 g/mol

165 mg (0.87 mmol, 87%) of **3e** as colorless crystalline substance was obtained after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 102 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.26 (t, ^4J = 2.6 Hz, 1 H), 4.15 (dd, ^3J = 5.5 Hz, ^4J = 2.6 Hz, 2 H), 4.51 (s, 2 H), 6.84 (br s, 1 H), 6.92 (dd, ^3J = 8.6 Hz, ^4J = 0.8 Hz, 2 H), 7.03 (tt, ^3J = 7.4 Hz, ^4J = 0.8 Hz, 1 H), 7.32 (dd, ^3J = 8.7 Hz, 7.4 Hz, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 28.8 (CH_2), 67.4 (CH_2), 72.0 (CH), 79.1 (C_{quat}), 114.8 (CH), 122.4 (CH), 129.9 (CH), 157.2 (C_{quat}), 168.1 (C_{quat}). **EI-MS:** *m/z* (%) = 190 ($[M + H]^+$, 13.8), 189 (M^+, 100), 108

([M - C_4H_3NO]$^+$, 53.9), 107 ($C_7H_7O^+$, 24.4), 96 ([M - C_6H_5O]$^+$, 26.1), 77 ($C_6H_5^+$, 54.6), 55 ($C_3H_5N^+$, 8.8). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3039 (w), 2914 (w), 1658 (m), 1598 (w), 1527 (m), 1494 (m), 1442 (m), 1413 (w), 1363 (w), 1350 (w), 1284 (w), 1170 (s), 1080 (w), 1062 (m), 1028 (w), 840 (w), 750 (s), 675 (m), 648 (m), 609 (m). Anal. calcd. for $C_{11}H_{11}NO_2$ (189.2): C 69.83, H 5.89, N 7.40. Found: C 69.86, H 5.69, N 7.36.

7.4.5. 2-(Benzyloxy)-*N*-(prop-2-yn-1-yl)acetamide (3f)

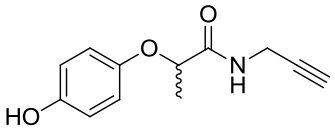


$C_{12}H_{13}NO_2$
203.24 g/mol

175 mg (0.86 mmol, 86 %) of **3f** was obtained as colorless liquid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.24 (t, ^4J = 2.6 Hz, 1 H), 4.01 (s, 2 H), 4.08 (dd, ^3J = 5.6 Hz, ^4J = 2.6 Hz, 2 H), 4.58 (s, 2 H), 6.77 (br s, 1 H), 7.31-7.42 (m, 5 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 28.6 (CH_2), 69.5 (CH_2), 71.8 (CH), 73.8 (CH_2), 79.4 (C_{quat}), 128.2 (CH), 128.5 (CH), 128.8 (CH), 136.8 (C_{quat}), 169.4 (C_{quat}). **EI-MS:** *m/z* (%) = 91 ($C_7H_7^+$, 100), 96 ([M - C_7H_7O]$^+$, 62.3), 77 ($C_6H_5^+$, 8.2), 55 ($C_3H_5N^+$, 18.0). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3032 (w), 2912 (w), 2862 (w), 2121 (w), 1880 (w), 1813 (w), 1662 (s), 1519 (s), 1454 (w), 1442 (w), 1421 (w), 1340 (w), 1278 (w), 1259 (w), 1207 (w), 1099 (s), 1026 (w), 1002 (w), 966 (w), 925 (w), 912 (w), 850 (w), 819 (w), 738 (m), 698 (s), 667 (m), 650 (m). Anal. calcd. for $C_{12}H_{13}NO_2$ (203.2): C 70.92, H 6.47, N 6.89. Found: C 70.97, H 6.47, N 6.95.

7.4.6. 2-(4-Hydroxyphenoxy)-*N*-(prop-2-yn-1-yl)propanamide (3g)



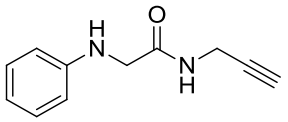
$C_{12}H_{13}NO_3$
219.24 g/mol

208 mg (0.95 mmol, 95 %) of **3g** was obtained as white crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 112 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 1.32 (d, ^3J = 6.6 Hz, 3 H), 3.06 (t, ^4J = 2.5 Hz, 1 H), 3.85 (m, 2 H), 4.50 (q, ^3J = 6.6 Hz, 1 H), 6.61-6.65 (m, 2 H), 6.71-6.74 (m, 2 H), 8.46 (t, ^3J = 5.6 Hz, 1 H), 8.98 (s, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 18.6 (CH_3), 27.8

(CH_2), 72.7 (CH), 74.6 (CH), 81.1 (C_{quat}), 115.7 (CH), 116.8 (CH), 149.9 (C_{quat}), 151.8 (C_{quat}), 171.4 (C_{quat}). **EI-MS:** *m/z* (%) = 220 ($[M + H]^+$, 9.2), 219 (M^+, 65.6), 137 ($[M - C_4H_4NO]^+$, 88.6), 110 ($[M - C_6H_5O_2]^+$, 100), 109 ($C_6H_5O_2^+$, 11.5), 111 ($C_6H_7O_2^+$, 8.6), 93 ($C_6H_5O^+$, 8.1), 82 ($C_4H_4NO^+$, 19.2), 81 ($C_4H_3NO^+$, 22.8), 65 ($C_5H_5^+$, 14.8), 56 ($C_3H_6N^+$, 5.1), 55 ($C_3H_5N^+$, 15.9), 39 ($C_3H_3^+$, 21.3). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3296 (w) 3259 (m), 2976 (w), 2966 (w), 2931 (w), 2900 (w), 2819 (w), 2744 (w), 2692 (w), 2609 (w), 2567 (w), 2505 (w), 2476 (w), 2434 (w), 2125 (w), 1988 (w), 1651 (s), 1604 (w), 1556 (m), 1508 (s), 1463 (w), 1440 (w), 1425 (w), 1373 (w), 1344 (w), 1303 (w), 1220 (s), 1207 (s), 1176 (w), 1141 (m), 1118 (w), 1093 (m), 1058 (m), 1047 (w), 1008 (w), 941 (w), 916 (w), 902 (w), 850 (w), 823 (m), 802 (w), 783 (w), 759. (s), 711 (w), 688 (m), 640 (w). Anal. calcd. for $C_{12}H_{13}NO_3$ (219.2): C 65.74, H 5.98, N 6.39. Found C 65.58, H 6.06, N 6.48.

7.4.7. 2-(Phenylamino)-*N*-(prop-2-yn-1-yl)acetamide (3h)

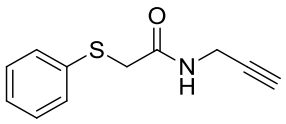


$C_{11}H_{12}N_2O$
188.23 g/mol

152 mg (0.81 mmol, 81 %) of **3h** was obtained as brownish yellow viscous liquid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.25 (t, ^4J = 2.6 Hz, 1 H), 3.88 (d, ^3J = 5.4 Hz, 2 H), 4.13 (dd, ^3J = 5.6 Hz, ^4J = 2.6 Hz, 2 H), 4.37 (br s, 1 H), 6.69 (d, ^3J = 7.6 Hz, 2 H), 6.90 (t, ^3J = 7.4 Hz, 1 H), 7.01 (br s, 1 H), 7.27-7.32 (m, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.0 (CH_2), 49.0 (CH_2), 71.7 (CH), 79.4 (C_{quat}), 113.4 (CH), 119.4 (CH), 129.6 (CH), 147.2 (C_{quat}), 170.6 (C_{quat}). **EI-MS:** *m/z* (%) = 189 ($[M + H]^+$, 2.5), 188 (M^+ , 19.9), 106 ($C_7H_8N^+$, 100), 104 (3.0), 93 (1.1), 77 ($C_6H_5^+$, 12.1), 51 (4.2), 39 ($C_3H_3^+$, 2.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3356 (w), 3263 (m), 3024 (w), 1643 (s), 1602 (m), 1514 (s), 1496 (m), 1458 (w), 1435 (w), 1423.47 (w), 1354 (w), 1338 (w), 1319 (m), 1261 (w), 1240 (w), 1226 (w), 1182 (w), 1155 (w), 1109 (w), 1078 (w), 1022 (w), 941 (w), 881 (w), 756 (s), 698 (m), 677 (m), 659 (m), 630. (m), 617 (w). Anal. calcd. for $C_{11}H_{12}N_2O$ (188.2): C 70.19, H 6.43, N 14.88. Found: C 70.01, H 6.70, N 14.75.

7.4.8. 2-(Phenylthio)-*N*-(prop-2-yn-1-yl)acetamide (3i)

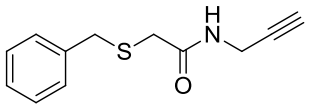


$C_{11}H_{11}NOS$
205.28

158 mg (0.77 mmol, 77 %) of **3i** was obtained as colorless crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 63 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.20 (t, ^4J = 2.6 Hz, 1 H), 3.64 (s, 2 H), 4.04 (dd, ^3J = 5.4 Hz, ^4J = 2.5 Hz, 2 H), 6.99 (br s, 1 H), 7.19-7.25 (m, 1 H), 7.28-7.31(m, 4 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.6 (CH_2), 37.6 (CH_2), 71.9 (CH), 79.0 (C_{quat}), 127.0 (CH), 128.5 (CH), 129.5 (CH), 134.5 (C_{quat}), 167.8 (C_{quat}). **EI-MS:** *m/z* (%) = 206 ([M + H]$^+$, 7.6), 205 (M$^+$, 48.4), 204 (2.2), 172 (6.5), 162 (3.3), 148 (2.7), 123 ($C_7H_7S^+$, 48.3), 124 ($C_7H_8S^+$, 48.7), 125 (6.2), 109 ($C_6H_5S^+$, 15.3), 110 (7.0), 111 (1.3), 96 ([M - C_6H_5S]$^+$, 100), 83 (1.1), 91 (17.0), 79 (5.8), 78 (12.8), 77 ($C_6H_5^+$, 12.7), 65 ($C_5H_5^+$, 7.8), 45 (27.5), 39 ($C_3H_3^+$, 18.2). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3062 (w), 2958 (w), 2358 (w), 1649 (s), 1624 (w), 1583 (w), 1544 (m), 1508 (w), 1479 (m), 1452 (w), 1436 (w), 1404 (w), 1354 (w), 1332 (w), 1309 (w), 1234 (w), 1215 (m), 1186 (w), 1165 (w), 1114 (w), 1089 (w), 1072 (w), 1022 (m), 937 (w), 887 (w), 783 (w), 736 (s), 646 (m), 615 (w). Anal. calcd. for $C_{11}H_{11}NOS$ (205.3): C 64.36, H 5.40, N 6.82. Found: C 64.11, H 5.54, N 6.83.

7.4.9. 2-(Benzylthio)-*N*-(prop-2-yn-1-yl)acetamide (3j)



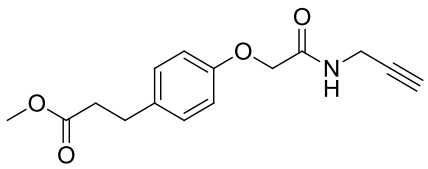
$C_{12}H_{13}NOS$
219.30 g/mol

180 mg (0.82 mmol, 82 %) of **3j** was obtained as colorless crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 57 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.25 (t, ^4J = 2.6 Hz, 1 H), 3.14 (s, 2 H), 3.73 (s, 2 H), 3.96 (dd, ^3J = 5.4 Hz, ^4J = 2.6 Hz, 2 H), 6.83 (br s, 1 H), 7.27-7.36 (m, 5 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.5 (CH_2), 35.2 (CH_2), 37.2 (CH_2), 71.9 (CH), 79.3 (C_{quat}), 127.6 (CH), 128.9 (CH), 129.1 (CH), 137.0 (C_{quat}), 168.3 (C_{quat}). **EI-MS:** *m/z* (%) = 220 ([M + H]$^+$, 2.6), 219 (M$^+$, 11.1), 123 ($C_7H_7S^+$, 12.9), 121 ($C_7H_5S^+$, 2.9), 97 ($C_5H_7NO^+$, 100), 96 ($C_5H_6NO^+$, 52.2), 98 ($C_5H_8NO^+$, 7.5), 91 ($C_7H_7^+$, 69.7), 89 (2.8), 77 ($C_6H_5^+$, 2.6), 65 ($C_5H_5^+$, 11.6), 39

($C_3H_3^+$, 10.8). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3032 (w), 2908 (w), 1637 (s), 1533 (m), 1494 (w), 1452 (w), 1440 (w), 1411 (w), 1373 (w), 1350 (w), 1300 (w), 1251 (w), 1228 (w), 1199 (w), 1153 (w), 1411 (w), 1373 (w), 1350 (w), 1300 (w), 1251 (w), 1228 (w), 1199 (w), 1153 (w), 1068 (w), 1014 (w), 891 (w), 771 (w), 702 (s), 684 (m), 665 (w), 638 (s). Anal. calcd. for $C_{12}H_{13}NOS$ (219.3): C 65.72, H 5.97, N 6.39. Found: C 65.89, H 6.11, N 6.30.

7.4.10. Methyl 3-(4-(2-oxo-2-(prop-2-yn-1-ylamino)ethoxy)phenyl)propanoate (3k)

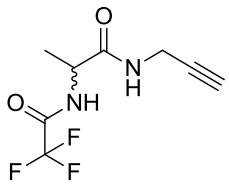


$C_{15}H_{17}NO_4$
275.30 g/mol

220 mg (0.80 mmol, 80 %) of **3k** was obtained as colorless crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (3:1)).

Mp. 89 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.26 (t, ^4J = 2.5 Hz, 1 H), 2.59 (t, ^3J = 7.6 Hz, 2 H), 2.90 (t, ^3J = 7.8 Hz, 2 H), 3.66 (s, 3 H), 4.14 (dd, ^3J = 2.5, 5.5 Hz, 2 H), 4.48 (s, 2 H), 6.81 (br s, 1 H), 6.84 (d, ^3J = 8.6 Hz, 2 H), 7.15 (d, ^3J = 8.5 Hz, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 28.8 (CH_2), 30.1 (CH_2), 35.9 (CH_2), 51.7 (CH_3), 67.5 (CH_2), 72.0 (CH), 79.1 (C_{quat}), 114.9 (CH), 129.7 (CH), 134.5 (C_{quat}), 155.7 (C_{quat}), 168.1 (C_{quat}), 173.4 (C_{quat}). **EI-MS:** *m/z* (%) = 276 ([M + H]$^+$, 6.4), 275 (M^+, 34.6), 215 (6.6), 202 ([M - $C_3H_5O_2$]$^+$, 38.3), 133 (10.7), 137 (59.3), 138 (5.3), 193 (6.6), 179 ([M - C_5H_6NO]$^+$, 100), 163 (5.3), 121 ($C_8H_9O^+$, 14.6), 107 ($C_7H_7O^+$, 33.8), 105 (5.9), 103 (8.5), 96 ($C_5H_6NO^+$, 20.7), 90 (6.0), 77 ($C_6H_5^+$, 8.0), 55 ($C_3H_5N^+$, 7.9), 39 ($C_3H_3^+$, 12.03). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 2997 (w), 2953 (w), 2935 (w), 2918 (w), 2866 (w), 1720 (m), 1658. (s), 1624 (w), 1608 (w), 1587 (w), 1570 (w), 1529 (m), 1508 (s), 1448 (m), 1438 (m), 1417 (w), 1340 (m), 1300 (m), 1288 (m), 1232 (s), 1174 (m), 1141 (m), 1107 (m), 1093 (w), 1066 (m), 1024 (m), 993 (m), 925 (w), 875 (w), 856 (m), 840 (m), 821 (m), 808 (m), 731 (m), 698 (m), 657 (m). Anal. calcd. for $C_{15}H_{17}NO_4$ (275.3): C 65.44, H 5.09, N 6.22. Found: C 65.37, H 5.06, N 6.32.

7.4.11. (*R*)-*N*-(Prop-2-yn-1-yl)-2-(2,2,2-trifluoroacetimido)propanamide (3n)

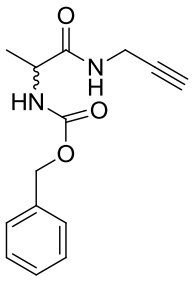


$C_8H_9F_3N_2O_2$
222.16 g/mol

138 mg (0.62 mmol, 62 %) of **3n** was obtained as colorless solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (3:1)).

Mp. 99 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.48 (d, ^3J = 6.9 Hz, 3 H), 2.26 (t, ^4J = 2.6 Hz, 1 H), 4.06 (dd, ^3J = 5.3 Hz, ^4J = 2.6 Hz, 2 H), 4.56 (dq, ^3J = 7.1 Hz, 7.1 Hz, 1 H), 6.46 (br s, 1 H), 7.41 (d, ^3J = 5.4 Hz, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 18.6 (CH_3), 29.7 (CH_2), 49.3 (CH), 72.4 (CH), 78.5 (C_{quat}), 115.7 (d, ^1J = 287.2 Hz, C_{quat}), 157.1 (d, ^2J = 37.9 Hz, C_{quat}), 170.4 (C_{quat}). **EI-MS:** *m/z* (%) = 223 ($[M + H]^+$, 0.4), 222 (M^+, 0.5), 141 ($[M - C_4H_3NO]^+$, 100), 140 ($[M - C_4H_4NO]^+$, 31.5), 72 (12.3), 69 (8.9), 39 ($C_3H_3^+$, 12.2). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3298 (w), 3093 (w), 1701 (m), 1660 (m), 1548 (m), 1452 (w), 1421 (w), 1381 (w), 1361 (w), 1344 (w), 1305 (w), 1265 (w), 1246 (w), 1182 (s), 1157 (s), 1062 (w), 1047 (w), 1006 (w), 929 (w), 894 (w), 840 (w), 786 (w), 607 (w), 702 (w), 659 (s). Anal. calcd. for $C_8H_9F_3N_2O_2$ (222.2): C 43.25, H 4.08, N 12.61. Found: C 43.36, H 4.18, N 12.56.

7.4.12. (*R*)-Benzyl (1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl)carbamate (3o)

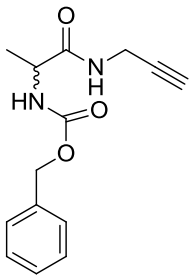


$C_{14}H_{16}N_2O_3$
260.29 g/mol

213 mg (0.82 mmol, 82 %) of **3o** was obtained as colorless solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 123 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 1.34 (d, ^3J = 7.1 Hz, 3 H), 2.65 (t, ^4J = 2.6 Hz, 1 H), 3.99 (dd, ^3J = 5.6 Hz, ^4J = 2.6 Hz, 2 H), 4.22 (m, 1 H), 5.06 (d, ^4J = 3.2 Hz, 2 H), 6.52 (br s, 1 H), 7.27-7.38 (br m, 5 H), 7.62 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 18.1 (CH_3), 28.0 (CH_2), 49.9 (CH), 65.4 (CH_2), 73.0 (CH), 81.1 (C_{quat}), 127.8 (CH), 127.8 (CH), 128.3 (CH), 137.0 (C_{quat}), 155.7 (C_{quat}), 172.3 (C_{quat}). **EI-MS:** *m/z* (%) = 260 (M^+, 1.0), 178 ([M - C_4H_4NO]$^+$, 33.6), 153 (1.9), 134 ([M - $C_6H_{10}N_2O$]$^+$, 27.2), 91 ($C_7H_7^+$, 100), 107 (3.2), 82 (3.0), 77 ($C_6H_5^+$, 2.2), 65 ($C_5H_5^+$, 5.9), 39 ($C_3H_3^+$, 6.8). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 2980 (w), 2935 (w), 1681 (s), 1643 (s), 1529 (s), 1498 (w), 1498 (w), 1448 (w), 1386 (w), 1367 (w), 1328 (w), 1261 (m), 1232 (s), 1172 (w), 1111 (w), 1074 (w), 1058 (w), 1045 (m), 1029 (w), 1006 (w), 954 (w), 916 (w), 840 (w), 777 (w), 756 (w), 736 (w), 698 (m), 680 (m), 651 (m), 636 (m). Anal. calcd. for $C_{14}H_{16}N_2O_3$ (260.3): C 64.60, H 6.20, N 10.76 Found: C 64.31, H 6.41, N 10.55.

7.4.13. (*S*)-Benzyl (1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl)carbamate (3p)



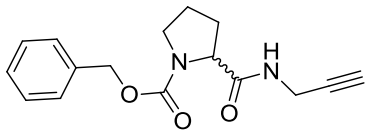
$C_{14}H_{16}N_2O_3$
260.29 g/mol

208 mg (0.80 mmol, 80 %) of **3p** was obtained as colorless solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 123 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 1.33 (d, ^3J = 7.1 Hz, 3 H), 2.64 (t, ^4J = 2.6 Hz, 1 H), 3.99 (dd, ^3J = 5.6, ^4J = 2.6 Hz, 2 H), 4.21 (m, 1 H), 5.07 (d, ^4J = 3.1 Hz, 2 H), 6.50 (br s, 1 H), 7.27-7.38 (m, 5 H), 7.60 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-acetone): δ = 18.7 (CH_3), 29.0 (CH_2), 51.3 (CH), 66.8 (CH_2), 72.0 (CH), 81.2 (C_{quat}), 128.6 (CH), 129.2 (CH), 138.2 (C_{quat}), 156.7 (C_{quat}), 172.8 (C_{quat}). **EI-MS:** *m/z* (%) = 261 ([M + H]$^+$, 0.8), 260 (M^+, 1.3), 178 ([M - C_4H_4NO]$^+$, 35.9), 153 ([M - C_7H_7O]$^+$, 2.6), 146 (4.1), 134 ($C_8H_6O_2^+$, 28.9), 107 (3.0), 91 ($C_7H_7^+$, 100), 88 (8.2), 82 (2.9), 77 ($C_6H_5^+$, 2.1), 65 ($C_5H_5^+$, 6.9), 39 ($C_3H_3^+$, 7.4). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3037 (w), 2976 (w), 2929 (w), 2781 (w), 2771 (w), 1681 (s), 1643 (s), 1529 (s), 1498 (w), 1448 (w), 1415 (w), 1366 (w), 1367 (w), 1328 (w), 1259 (s), 1232 (s), 1172 (w), 1111 (w), 1074 (w), 1056 (m), 1045 (m), 1030 (w), 1008 (w), 956 (w), 916 (w), 840 (w), 777

(w), 756 (w), 736 (w), 698 (s), 678 (s), 651 (s), 636 (s), 621 (w). Anal. calcd. for $C_{14}H1_6N_2O_3$ (260.3): C 64.40, H 6.20, N, 10.76. Found: C 64.40, H 6.02, N 10.63.

7.4.14. (*S*)-Benzyl 2-(prop-2-yn-1-ylcarbamoyl)pyrrolidine-1-carboxylate (3q)

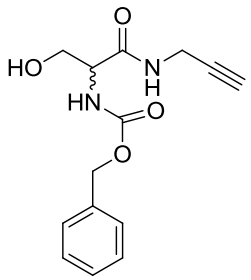


$C_{16}H_{18}N_2O_3$
286.33 g/mol

195 mg (0.68 mmol, 68 %) of **3q** was obtained as colorless crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (1:1)).

Mp. 96 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 1.90-1.94 (m, 2 H), 1.98-2.01 (m, 1 H), 2.12-2.22 (m, 1 H), 2.64 (t, ^4J = 2.6 Hz, 1 H), 3.46-3.54 (m, 2 H), 3.98 (dd, ^3J = 5.5 Hz, ^4J = 2.3 Hz, 2 H), 4.28 (dd, ^3J = 8.3 Hz, ^4J = 3.3 Hz, 1 H), 5.02-5.18 (m, 2 H), 7.34-7.39 (m, 5H), 7.63 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-acetone): δ = 24.1 (CH_2), 28.9 (CH_2), 32.0 (CH_2), 47.6 (CH_2), 61.5 (CH), 67.0 (CH_2), 71.9 (CH), 81.4 (C_{quat}), 128.3 (CH), 128.5 (CH), 129.2 (CH), 138.2 (C_{quat}), 155.9 (C_{quat}), 172.8 (C_{quat}). **EI-MS:** *m/z* (%) = 286 (M^+, 1.8), 204 ([M - $C_4H_4NO]^+$, 40.1), 178 (3.2), 161 (3.8), 160 (37.0), 151 ([M - $C_8H_7O_2]^+$, 3.1), 134 (3.6), 111 (1.2), 105 (2.9), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 2.2), 65 ($C_5H_5^+$, 5.8), 57 (2.6), 39 ($C_3H_3^+$, 5.1), 41 (3.5). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3062 (w), 2970 (w), 2947 (w), 2929 (w), 2872 (w), 2773 (w), 2459 (w), 2125 (w), 1747 (w), 1707 (s), 1651 (s), 1606.70 (w), 1541 (w), 1496 (w), 1485 (w), 1458 (w), 1458 (w), 1446 (w), 1415 (s), 1357 (s), 1309 (w), 1278 (w), 1236 (w), 1205 (w), 1186 (w), 1170 (w), 1126 (m), 1091 (w), 1076 (w), 1028 (w), 1014 (w), 987 (w), 968 (w), 925 (w), 900 (w), 887 (w), 802 (w), 769 (w), 752 (w), 729 (s), 694 (m), 680 (m), 621 (w), 605 (w). Anal. calcd. for $C_{16}H_{18}N_2O_3$ (286.3): C 67.12, H 6.34, N 9.78. Found: C 67.00, H 6.15, N 9.62.

7.4.15. Benzyl (3-hydroxy-1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl)carbamate (3r)

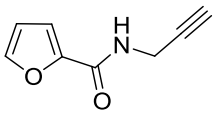


$C_{14}H_{16}N_2O_4$
276.29 g/mol

146 mg (0.53 mmol, 53 %) of **3r** was obtained as colorless crystalline solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate 1:1, product obtainment with *n*-hexane:ethylacetate (1:4)).

Mp. 128 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 2.64 (t, ^4J = 2.6 Hz, 1 H), 3.74-3.88 (m, 2 H), 4.00 (dd, ^3J = 5.5 Hz, ^4J = 2.5 Hz, 2 H), 4.16-4.27 (m, 2 H), 5.09 (d, ^4J = 1.4 Hz, 2 H), 6.39 (br s, 1 H), 7.28-7.435 (m, 5 H), 7.70 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 28.1 (CH_2), 57.1 (CH), 61.7 (CH_2), 65.5 (CH_2), 73.0 (CH), 81.1 (C_{quat}), 127.8 (CH), 127.8 (CH), 128.4 (CH), 137.0 (C_{quat}), 155.9 (C_{quat}), 169.9 (C_{quat}). **EI-MS:** *m/z* (%) = 276 (M^+, 0.7), 258 ([M - H_2O]$^+$, 2.6), 194 ([M - C_4H_4NO]$^+$, 24.5), 177 (2.1), 169 ([M - C_7H_7O]$^+$, 2.9), 150 ([M - $C_6H_8NO_2$]$^+$, 20.9), 151 ($C_8H_9NO_2^+$, 2.3), 91 ($C_7H_7^+$, 100). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3336 (w), 3273 (m), 3265 (w), 3057 (w), 2980 (w), 2970 (w), 2931 (w), 2860 (w), 2808 (w), 1701 (s), 1670 (s), 1544 (s), 1492 (w), 1458 (m), 1419 (w), 1392 (w), 1328 (w), 1284 (m), 1265 (w), 1244 (m), 1234 (m), 1211 (m), 1118 (w), 1093 (m), 1070 (m), 1058 (m), 1039 (m), 1012 (m), 987 (w), 939 (w), 925 (w), 825 (w), 785 (w), 750 (s), 663 (s), 615 (w). Anal. calcd. for $C_{14}H_{16}N_2O_4$ (276.3): C 60.86, H 5.84, N 10.14. Found: C 60.75, H 5.98, N 10.01.

7.4.16. *N*-(Prop-2-yn-1-yl)furan-2-carboxamide (3s)

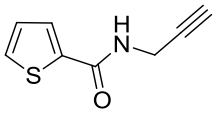


$C_8H_7NO_2$
149.15 g/mol

106 mg (0.71 mmol, 71 %) of **3s** was obtained as colorless crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 81 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 2.27 (t, ^4J = 2.6 Hz, 1 H), 4.22 (dd, ^3J = 2.6 Hz, 5.5 Hz, 2 H), 6.49 (dd, ^3J = 1.8 Hz, 3.5 Hz, 1 H), 6.58 (br s, 1 H), 7.14 (d, ^3J = 3.5 Hz, 1 H), 7.44 (d, ^3J = 1.5 Hz, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 28.9 (CH_2), 71.9 (CH), 79.4 (C_{quat}), 112.3 (CH), 114.9 (CH), 144.3 (CH), 147.5 (C_{quat}), 158.0 (C_{quat}). **EI-MS:** *m/z* (%) = 150 ($[M + H]^+$, 4.4), 149 (M^+, 21.9), 148 (2.3), 121 ($[M - C_2H_4]^+$, 21.2), 106 (5.8), 95 ($[M - C_3H_4N]^+$, 100), 93 ($C_5HO_2^+$, 40.8), 78 (7.1), 67 ($[M - C_4H_4NO]^+$, 10.8), 52 (5.4), 39 ($C_3H_3^+$, 31.7). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3263 (w), 3180 (w), 1653 (m), 1637 (s), 1589 (m), 1571 (w), 1525 (m), 1473 (m), 1436 (w), 1415 (m), 1381 (w), 1348 (w), 1300 (m), 1265 (w), 1255 (w), 1230 (w), 1186 (m), 1147 (w), 1082 (w), 1018 (m), 923 (w), 908 (w), 885 (w), 835 (w), 786 (w), 756 (s), 721 (m), 671 (m), 636 (s), 613 (s). Anal. calcd. for $C_8H_7NO_2$ (149.1): C 64.43, H 9.39, N 4.73. Found: C 64.21, H 9.36, N 4.59.

7.4.17. *N*-(Prop-2-yn-1-yl)thiophene-2-carboxamide (3t)

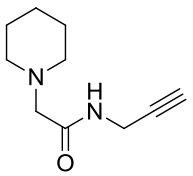


C_8H_7NOS
165.21 g/mol

127 mg (0.77 mmol, 77 %) of **3t** was obtained as colorless crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 116 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.27 (t, ^4J = 2.6 Hz, 1 H), 4.22 (dd, ^3J = 5.3 Hz, ^4J = 2.6 Hz, 2 H), 6.41 (br s, 1 H), 7.07 (dd, ^3J = 5.0 Hz, 3.8 Hz, 1 H), 7.49 (dd, ^3J = 4.9 Hz, ^4J = 1.1 Hz, 1 H), 7.56 (dd, ^3J = 3.7 Hz, ^4J = 1.2 Hz, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.8 (CH_2), 72.0 (CH), 79.5 (C_{quat}), 127.8 (CH), 128.7 (CH), 130.5 (CH), 138.2 (C_{quat}), 161.7 (C_{quat}). **EI-MS:** *m/z* (%) = 166 ($[M + H]^+$, 2.0), 165 (M^+, 15.7), 164 (7.3), 122 (9.5), 138 (3.0), 137 (8.2), 136 (54.8), 132 (5.3), 121 (5.1), 111 ($[M - C_3H_4N]^+$, 100), 109 (4.5), 83 ($C_4H_3S^+$, 10.0), 39 ($C_3H_3^+$, 22.5). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3266 (w), 1625 (m), 1546 (s), 1514 (w), 1413 (w), 1357 (w), 1344 (w), 1305 (m), 1263 (w), 1246 (w), 1145 (w), 1078 (w), 1033. (w), 962 (w), 910 (w), 846 (w), 788 (w), 752 (w), 711 (m), 663 (m), 632 (s). Anal. calcd. for C_8H_7NOS (165.2): C 58.16, H 4.27, N 8.48. Found: C 58.20, H 4.13, N 8.37.

7.4.18. 2-(Piperidine-1-yl)-*N*-(prop-2-yn-1-yl)acetamide (3u)

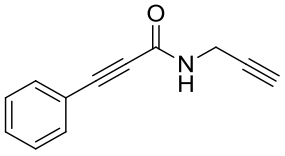


$C_{10}H_{16}N_2O$
180.25 g/mol

126 mg (0.70 mmol, 70 %) of **3u** was obtained as brown solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (2:1)).

Mp. 62 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 1.43 (m, 2 H), 1.58 (m, 4 H), 2.21 (t , ^4J = 2.6 Hz, 1 H), 2.44 (t, ^3J = 5.4 Hz, 4 H), 2.95 (s, 2 H), 4.06 (dd, ^3J = 5.6 Hz, ^4J = 2.6 Hz, 2 H), 7.43 (br s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 23.8 (CH_2), 26.3 (CH_2), 28.7 (CH_2), 55.1 (CH_2), 62.3 (CH_2), 71.3 (CH), 79.9 (C_{quat}), 170.8 (C_{quat}). **EI-MS:** *m/z* (%) = 180 (M^+, 0.2), 98 ([M - $C_4H_4NO]^+$, 100), 84 ([M - $C_5H_6NO]^+$, 6.4), 69 ($C_5H_9^+$, 5.3), 55 ($C_3H_5N^+$, 16.2). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3336 (w), 2935 (m), 2912 (w), 2852 (w), 2810 (w), 2758 (w), 2117 (w), 1654 (s), 1622 (w), 1514 (s), 1506 (w), 1492 (w), 1454 (w), 1415 (m), 1384 (w), 1367 (w), 1350 (w), 1332 (m), 1298 (w), 1273 (m), 1257 (m), 1161 (w), 1130 (m), 1109 (m), 1085 (m), 1039 (m), 1020 (w), 1001 (m), 979 (m), 960 (w), 931 (w), 866 (w), 852 (w), 792 (w), 731 (s), 702 (s), 671 (m), 611 (m). Anal. calcd. for $C_{10}H_{16}N_2O$ (180.2): C 66.63, H 8.95, N 15.54. Found: C 66.44, H 8.73, N 15.38.

7.4.19. 3-Phenyl-*N*-(prop-2-yn-1-yl)propiolamide (3aa)



$C_{12}H_9NO$
183.21 g/mol

147 mg (0.80 mmol, 80 %) of **3aa** was obtained as colorless crystalline solid after purification by silica gel column chromatography (*n*-hexane:ethylacetate (3:1)).

Mp. 62 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.29 (t, ^4J = 2.6 Hz, 1 H), 4.14 (dd, ^3J = 2.6 Hz, ^4J = 5.4 Hz, 2 H), 6.11 (br s, 1 H), 7.33-7.46 (m, 3 H), 7.52-7.60 (m, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.7 (CH_2), 72.4 (CH), 78.6 (C_{quat}), 82.5 (C_{quat}), 85.8 (C_{quat}), 120.1 (C_{quat}), 128.7 (CH), 130.4 (CH), 132.7 (CH), 153.1 (C_{quat}). **EI-MS:** *m/z* (%) = 184 ([M + H]$^+$, 4.0), 183 (M^+,

17.5), 182 ([M - H]$^+$, 13.3), 154 (37.5), 155 (12.2), 141 (3.8), 140 (25.9), 129 ([M - C_3H_4N]$^+$, 100), 130 (9.7), 139 (16.4), 114 (2.2), 126 (31.1), 101 ($C_8H_5^+$, 11.1), 102 ($C_8H_6^+$, 13.8), 74 (5.2), 77 ($C_6H_5^+$, 4.8), 75 ($C_6H_3^+$, 16.0), 63 (2.3), 52 (2.2), 51 (6.8), 39 ($C_3H_3^+$, 3.1). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3047 (w), 2667 (w), 2501 (w), 2216 (w), 2088 (w), 1749 (m), 1697 (m), 1683 (m), 1624 (m), 1595 (w), 1541 (s), 1521 (m), 1489 (w), 1338 (w), 1303 (m), 1288 (m), 1219 (w), 925 (w), 756 (w), 684 (m), 657 (m), 636 (m), 609 (w). Anal. calcd. for $C_{12}H_9NO$ (183.2): C 78.67, H 4.95, N 7.65. Found: C 78.54, H 5.12, N 7.59.

7.5. Procedure for Chemoenzymatic One-pot Synthesis of Amide ligated 1,4-Disubstituted 1,2,3-Triazoles 5

To a 2.0 mL MTBE solution of 55 mg (1.00 mmol) of propargylamine (**2**), in a screwcap Schlenk vessel, were added 1.20 mmol of the ester **1** (experimental details mentioned in Table 7.2) and Novozyme® 435 (50 % w/w of corresponding ester substrate **1** used) and the reaction was shaken in an incubating shaker at 45 °C for 4-24 h (depending upon the nature of ester **1** used). After the completion of aminolysis, 1.50 mmol of the azide **4a-b**, 6.0 mg (0.04 mmol) of Cu_2O, 9.7 mg (0.08 mmol) of benzoic acid, and 1.0 mL of water, were added to the reaction vessel and the reaction mixture was shaken at 45 °C for 4 h. It is important to mention that methanol as co solvent was added only if the amide product **3** of enzymatic step was insoluble in MTBE and crystallized out of the reaction mixture during the course of the reaction. The reaction mixture containing colorless precipitated triazole product **5** was dissolved in ethylacetate (10.0 mL) to filter the enzyme beads. To this diluted filtered reaction mixture was added brine (5.0 mL) followed by extraction with ethylacetate (3 x 10.0 mL). The combined organic layers were dried with anhydrous Na_2SO_4 and the crude product was purified by flash chromatography on silica gel using *n*-hexane/ethylacetate as eluent, to get the analytically pure triazoles **5**.

Table 7.2. Experimental details of chemoenzymatic one-pot synthesis of 1,4-disubstituted 1,2,3-triazoles **5** using 1.50 mmol (200 mg) of benzylazide (**4a**).

Entry	Ester Substrate 1	Triazole 5
1	233 mg (1.20 mmol) of **1a**	249 mg (71 %) of **5a**[a]
2	197 mg (1.20 mmol) of **1b**	195 mg (61 %) of **5b**[a]
3	199 mg (1.20 mmol) of **1e**	268 mg (83 %) of **5e**[b]
4	216 mg (1.20 mmol) of **1f**	235 mg (70 %) of **5f**[b]
5	198 mg (1.20 mmol) of **1h**	273 mg (85 %) of **5h**[b]
6	219 mg (1.20 mmol) of **1i**	250 mg (74 %) of **5i**[b]
7	236 mg (1.20 mmol) of **1j**	233 mg (66 %) of **5j**[b]
8	303 mg (1.20 mmol) of **1k**	208 mg (51 %) of **5k**[b]
9	239 mg (1.20 mmol) of **1n**	210 mg (59 %) of **5n**[b]
10	285 mg (1.20 mmol) of **1o**	275 mg (70 %) of **5o**[b]
11	285 mg (1.20 mmol) of **1p**	287 mg (73 %) of **5p**[b]
12	151 mg (1.20 mmol) of **1s**	192 mg (68 %) of **5s**[a]
13	171 mg (1.20 mmol) of **1t**	104 mg (35 %) of **5t**[a]
14	192 mg (1.20 mmol) of **1aa**	225 mg (71 %) of **5aa**[a]
15	204 mg (1.20 mmol) of **1dd**	103 mg (40 %) of **5dd**[b]
16	222 mg (1.20 mmol) of **1ee**	208 mg (61 %) of **5ee**[a]

[a]Reaction time of 24 h for Novozyme® 435 catalyzed aminolysis. [b]Reaction time of 4 h for Novozyme® 435 catalyzed aminolysis.

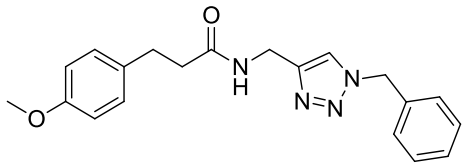
Table 7.3. Experimental details of chemoenzymatic one-pot synthesis of 1,4-disubstituted 1,2,3-triazoles **5** using 1.50 mmol (248 mg) of azidomethyl phenyl sulphide (**4b**).

Entry	Ester Substrate 1	Triazole 5
1	197 mg (1.20 mmol) of **1b**	222 mg (63 %) of **5ff**[a]
2	198 mg (1.20 mmol) of **1h**	276 mg (78 %) of **5gg**[b]
3	219 mg (1.20 mmol) of **1i**	218 mg (59 %) of **5hh**[b]
4	151 mg (1.20 mmol) of **1s**	267 mg (85 %) of **5ii**[a]

[a]Reaction time of 24 h for Novozyme® 435 catalyzed aminolysis. [b]Reaction time of 4 h for Novozyme® 435 catalyzed aminolysis.

7.6. Analytical Data of Amide Ligated 1,4-Disubstituted 1,2,3-Triazoles (5)

7.6.1. *N*-((1-Benzyl-1*H*- 1,2,3-triazol-4-yl)methyl)-3-(4-methoxyphenyl)propanamide (5a)

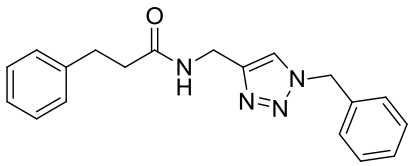


$C_{20}H_{22}N_4O_2$
350.41 g/mol

249 mg (0.71 mmol, 71 %) of **5a** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:20)).

Mp. 128 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 2.35 (t, ^3J = 8.0 Hz, 2 H), 2.74 (t, ^3J = 8.0 Hz, 2 H), 3.70 (s, 3 H), 4.26 (d, ^3J = 5.6 Hz, 2 H), 5.55 (s, 2 H), 6.80 (d, ^3J = 8.6 Hz, 2 H), 7.09 (d, ^3J = 8.6 Hz, 2 H), 7.30-7.39 (m, 5 H), 7.83 (s, 1 H), 8.30 (t, ^3J = 5.5 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 30.1 (CH_2), 34.1 (CH_2), 37.1 (CH_2), 52.7 (CH_3), 54.9 (CH_2), 113.7 (CH), 122.8 (CH), 127.9 (CH), 128.1 (CH), 128.7 (CH), 129.1 (CH), 133.1 (C_{quat}), 136.1 (C_{quat}), 145.3 (C_{quat}), 157.5 (C_{quat}), 171.3 (C_{quat}). **EI-MS:** *m/z* (%) = 351 ($[M + H]^+$, 22.8), 350 (M^+, 92.9), 189 ($C_{10}H_{13}N_4^+$, 10.7), 187 ($C_{10}H_{11}N_4^+$, 21.7), 173 ($C_{10}H_{11}N_3^+$, 45.2), 159 (5.2), 144 (10.7), 135 (17.3), 134 ($C_9H_{10}O^+$, 100), 121 ($C_8H_9O^+$, 63.4), 97 (8.5), 91 ($C_7H_7^+$, 77.8), 86 (4.0), 77 ($C_6H_5^+$, 7.5), 69 (4.1), 65 ($C_5H_5^+$, 8.1). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3010 (w), 2960 (w), 2880 (w), 1639 (m), 1612 (w), 1548 (m), 1512 (m), 1492 (w), 1438 (w), 1425 (w), 1367 (w), 1288 (w), 1249 (m), 1220 (m), 1174 (w), 1107 (w), 1074 (w), 1055 (m), 1029 (m), 1002 (w), 817 (m), 788 (w), 761 (w), 719 (s), 694 (m), 669 (w). Anal. calcd. for $C_{20}H_{22}N_4O_2$ (350.4): C 68.55, H 6.33, N 15.99. Found: C 68.49, H 6.14, N 15.87.

7.6.2. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-phenylpropanamide (5b)

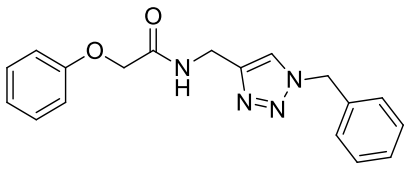


$C_{19}H_{20}N_4O$
320.39 g/mol

195 mg (0.61 mmol, 61 %) of **5b** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1: 20)).

Mp. 121 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 2.39 (t, ^3J = 7.7 Hz, 2 H), 2.81 (t, ^3J = 7.7 Hz, 2 H), 4.27 (d, ^3J = 5.6 Hz, 2 H), 5.55 (s, 2 H), 7.12-7.40 (br m, 10 H), 7.80 (s, 1 H), 8.32 (t, ^3J = 5.3 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 31.0 (CH_2), 34.1 (CH_2), 36.8 (CH_2), 52.7 (CH_2), 122.8 (CH), 125.9 (CH), 127.9 (CH), 128.1 (CH), 128.2 (CH), 128.2 (CH), 128.7 (CH), 136.1 (C_{quat}), 141.3 (C_{quat}), 145.0 (C_{quat}), 171.2 (C_{quat}). **EI-MS:** *m/z* (%) = 322 ([M + 2H]$^+$, 1.3), 321 ([M + H]$^+$, 13.1), 320 (M^+, 59.0), 211 (3.4), 187 ([M - C_9H_9O]$^+$, 26.0), 173 ($C_{10}H_{11}N_3^+$, 36.7), 159 ($C_9H_9N_3^+$, 9.2), 144 (13.8), 143 (21.7), 132 ($C_7H_6N_3^+$, 4.0), 133 (2.3), 105 ($C_8H_9^+$, 14.7), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 4.8), 65 ($C_5H_5^+$, 8.9), 39 ($C_3H_3^+$, 2.3). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3030 (w), 2929 (w), 2912 (w), 1637 (m), 1604 (w), 1546 (m), 1492 (w), 1454 (w), 1438 (w), 1381 (w), 1365 (w), 1336 (w), 1321 (w), 1301 (w), 1290 (w), 1259 (w), 1002 (w), 1220 (w), 1174 (w), 1163 (w), 1126 (w), 1076 (w), 1055 (w), 1028 (w), 860 (w), 842 (w), 827 (w), 785 (w), 761 (w), 717 (s), 694 (w), 669 (w), 651 (w), 621 (w). Anal. calcd. for $C_{19}H_{20}N_4O$ (320.4) : C 71.23, H 6.29, N 17.49. Found: C 71.20, H 6.36, N 17.47.

7.6.3. *N*-((1-Benzyl-1*H*-1,2,3-triazole-4-yl)methyl)-2-phenoxyacetamide (5e)

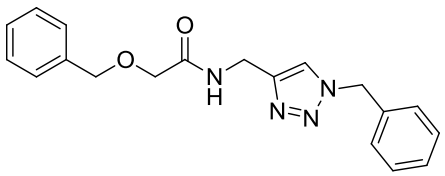


$C_{18}H_{18}N_4O_2$
322.36 g/mol

268 mg (0.83 mmol, 83 %) of **5e** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:10)).

Mp. 129 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.44 (d, ^3J = 6.0 Hz, 2 H), 4.55 (s, 2 H), 5.45 (s, 2 H), 6.84 (d, ^3J = 8.7 Hz, 2 H), 6.96 (t, ^3J = 7.4 Hz, 1 H), 7.20 (br s, 1 H), 7.21-7.25 (m, 4 H), 7.32-7.36 (m, 3 H), 7.39 (s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 34.5 (CH_2), 54.4 (CH_2), 67.3 (CH_2), 114.7 (CH), 122.2 (CH), 128.3 (CH), 129.0 (CH), 129.3 (CH), 129.9 (CH), 134.5 (C_{quat}), 144.8 (C_{quat}), 157.2 (C_{quat}), 168.4 (C_{quat}). **EI-MS:** *m/z* (%) = 322 (M^+, 4.4), 231 ([M - C_7H_7]$^+$, 1.6), 229 ([M - C_6H_5O]$^+$, 5.9), 215 ([M - C_7H_7O]$^+$, 6.0), 174 (9.2), 173 ($C_{10}H_{11}N_3^+$, 72.8), 187 (6.6), 107 (8.7), 91 ($C_7H_7^+$, 100). **IR (ATR**) $\tilde{\nu}$ [cm^{-1}] = 2939 (w), 2908 (w), 2852 (w), 1656 (m), 1600 (w), 1539 (w), 1492 (w), 1438 (w), 1427 (w), 1291 (w), 1029 (w), 1226 (m), 1176 (w), 1130 (w), 1083 (w), 1053 (m), 855 (w), 833 (w), 798 (w), 748 (s), 719 (m), 692 (s), 669 (w). Anal. calcd. for $C_{18}H_{18}N_4O_2$ (322.4): C 67.07, H 5.63, N 17.38. Found: C 66.93, H 5.68, N 17.30.

7.6.4. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(benzyloxy)acetamide (5f)

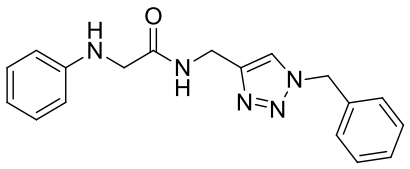


$C_{19}H_{20}N_4O_2$
336.39 g/mol

235 mg (0.70 mmol, 70 %) of **5f** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:10)).

Mp. 100 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.96 (s, 2 H), 4.52 (s, 2 H), 4.54 (s, 2 H), 5.49 (s, 2 H), 7.14 (br s, 1 H), 7.27-7.39 (m, 10 H), 7.43 (s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 34.4 (CH_2), 54.3 (CH_2), 69.5 (CH_2), 73.7 (CH_2), 122.2 (CH), 128.1 (CH), 128.3 (CH), 128.4 (CH), 128.7 (CH), 128.9 (CH), 129.3 (CH), 134.6 (C_{quat}), 136.8 (C_{quat}), 145.0 (C_{quat}), 169.7 (C_{quat}). **EI-MS:** *m/z* (%) = 336 (M^+, 0.1), 245 (0.2), 230 ($C_{12}H_{14}N_4O^+$, 13.6), 187 (5.0), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 2.6), 65 ($C_5H_5^+$, 16.0). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3062 (w), 2999 (w), 2953 (w), 1654 (m), 1517 (w), 1494 (w), 1452 (w), 1373 (w), 1344 (w), 1311 (w), 1242 (w), 1226 (w), 1207 (w), 1132 (w), 1116 (m), 1074 (w), 1058 (m), 1029 (w), 1010 (w), 958 (w), 889 (w), 827 (w), 798 (w), 777 (w), 732 (m), 723 (s), 694 (s), 673 (w), 644 (w), 615 (w). Anal. calcd. for $C_{19}H_{20}N_4O_2$ (336.4): C 67.84, H 5.99, N 16.66. Found: C 68.04, H 6.14, N 16.78.

7.6.5. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(phenylamino)acetamide (5h)

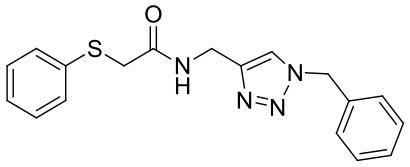


$C_{18}H_{19}N_5O$
321.38 g/mol

273 mg (0.85 mmol, 85 %) of **5h** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:20)).

Mp. 116 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.78 (br s, 2 H), 4.31 (br s, 1 H), 4.50 (d, ^3J = 5.4 Hz, 2 H), 5.44 (s, 2 H), 6.54 (d, ^3J = 7.3 Hz, 2 H), 6.77 (t, ^3J = 6.7 Hz, 1 H), 7.14 (m, 2 H), 7.24 (m, 2 H), 7.36 (br m, 5 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 34.8 (CH_2), 48.8 (CH_2), 54.3 (CH_2), 113.3 (CH), 119.2 (CH), 122.1 (CH), 128.3 (CH), 128.9 (CH), 129.3 (CH), 129.5 (CH), 134.6 (C_{quat}), 145.2 (C_{quat}), 147.1 (C_{quat}), 170.9 (C_{quat}). **EI-MS:** *m/z* (%) = 323 ($[M + 2H]^+$, 1.5), 322 ($[M + H]^+$, 11.7), 321 (M^+, 52.1), 215 ($[M - C_7H_8N]^+$, 3.2), 189 ($C_{10}H_{13}N_4^+$, 94.2), 173 ($C_{10}H_{11}N_3^+$, 67.0), 159 ($C_9H_9N_3^+$, 4.5), 144 ($C_8H_6N_3^+$, 29.5), 106 ($C_7H_8N^+$, 100), 91 ($C_7H_7^+$, 95.0), 77 ($C_6H_5^+$, 21.7), 65 ($C_5H_5^+$, 8.2), 39 ($C_3H_3^+$, 3.1). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3334 (w), 3032 (w), 2879 (w), 2831 (w), 1633 (s), 1602 (m), 1516 (m), 1494 (m), 1456 (w), 1429 (w), 1363 (w), 1313 (m), 1274 (w), 1261 (w), 1242 (w), 1228 (w), 1207 (w), 1180 (w), 1134 (w), 1118 (w), 1105 (w), 1074 (w), 1053 (m), 1026 (w), 991 (w), 858 (w), 796 (w), 767 (w), 748 (m), 725 (s), 686 (s), 663 (w), 638 (w), 617 (w). Anal. calcd. for $C_{18}H_{19}N_5O$ (321.4): C 67.27, H 5.96, N 21.79. Found: C 67.00, H 5.89, N 21.72.

7.6.6. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(phenylthio)acetamide (5i)

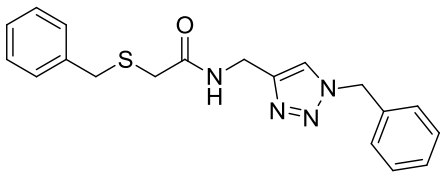


$C_{18}H_{18}N_4OS$
338.43 g/mol

250 mg (0.74 mmol, 74 %) of **5i** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:10)).

Mp. 112 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.62 (s, 2 H), 4.48 (d, ^3J = 5.9 Hz, 2 H), 5.43 (s, 2 H), 7.13-7.24 (m, 8 H), 7.33-7.38 (m, 4 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 35.4 (CH_2), 37.5 (CH_2), 54.3 (CH_2), 122.0 (CH), 126.8 (CH), 128.2 (CH), 128.5 (CH), 128.9 (CH), 129.3 (CH), 129.3 (CH), 134.6 (C_{quat}), 144.9 (C_{quat}), 168.1 (C_{quat}). **EI-MS:** *m/z* (%) = 339 ($[M + H]^+$, 4.3), 338 (M^+, 16.0), 320 (2.2), 305 (2.0), 229 (1.3), 215 (5.8), 189 ($C_{10}H_{13}N_4^+$, 86.7), 187 (5.5), 172 (11.2), 159 (2.2), 150 (4.3), 144 ($C_8H_6N_3^+$, 31.7), 123 (12.5), 109 (4.6), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 6.2), 65 ($C_5H_5^+$, 10.9), 39 ($C_3H_3^+$, 3.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3059 (w), 2985 (w), 2951 (w), 2908 (w), 1651 (s), 1622 (w), 1516 (m), 1479 (w), 1454 (w), 1438 (w), 1427 (w), 1404 (w), 1372 (w), 1300 (w), 1244 (w), 1199 (w), 1184 (w), 1168 (w), 1128 (w), 1091 (w), 1074 (w), 1055 (m), 1024 (w), 1002 (w), 896 (w), 871 (w), 788 (w), 769 (w), 734 (s), 719 (s), 688 (s), 661 (w), 613 (w). Anal. calcd. for $C_{18}H_{18}N_4OS$ (338.4): C 63.88, H 5.36, N 16.56. Found: C 63.67, H 5.20, N 16.44.

7.6.7. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(benzylthio)acetamide (5j)

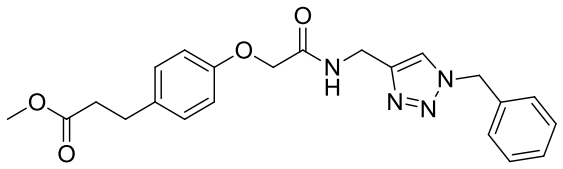


$C_{19}H_{20}N_4OS$
352.45 g/mol

233 mg (0.66 mmol, 66 %) of **5j** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:10)).

Mp. 107 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.10 (s , 2 H), 3.65 (s , 2 H), 4.41 (d , ^3J = 5.8 Hz, 2 H), 5.49 (s, 2 H), 7.17-7.24 (m, 6 H), 7.27-7.40 (m, 5 H), 7.42 (s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 35.2 (CH_2), 35.3 (CH_2), 37.1 (CH_2), 54.3 (CH_2), 122.2 (CH), 127.5 (CH), 128.2 (CH), 128.79 (CH), 129.0 (CH), 129.1 (CH), 129.3 (CH), 134.6 (C_{quat}), 137.1 (C_{quat}), 145.0 (C_{quat}), 168.8 (C_{quat}). **EI-MS:** *m/z* (%) = 353 ($[M + H]^+$, 0.4), 352 (M^+, 0.4), 261 ($[M - C_7H_7]^+$, 2.2), 230 ($C_{12}H_{14}N_4O^+$, 98.1), 212 (1.2), 187 ($[M - C_9H_9OS]^+$, 18.6), 172 ($[M - C_9H_{10}NOS]^+$, 15.1), 159 ($C_9H_9N_3^+$, 2.0), 144 ($C_8H_6N_3^+$, 9.4), 139 ($C_8H_{11}S^+$, 7.3), 115 (2.1), 111 (9.8), 97 ($C_4H_5N_2O^+$, 8.6), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 1.7), 65 ($C_5H_5^+$, 9.3), 39 ($C_3H_3^+$, 2.4). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3034 (w), 2964 (w), 1668 (s), 1635 (w), 1558 (w), 1541 (w), 1494 (w), 1446 (w), 1408 (w), 1325 (w), 1303 (w), 1222 (m), 1213 (w), 1165 (w), 1149 (w), 1132 (w), 1056 (w), 1026 (w), 869 (w), 792 (w), 775 (w), 746 (w), 719 (m), 696 (s), 663 (w). Anal. calcd. for $C_{19}H_{20}N_4OS$ (352.5): C 64.75, H 5.72, N 15.90. Found C 64.85, H 5.70, N 16.15.

7.6.8. Methyl-3-(4-(2-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-2-oxoethoxy) phenyl)propanoate (5k)

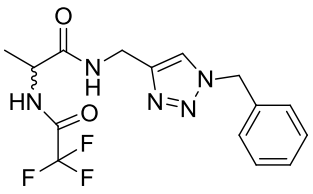


$C_{22}H_{24}N_4O_4$
408.45 g/mol

208 mg (0.51 mmol, 51 %) of **5k** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1: 20)).

Mp. 106 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 2.57 (t, ^3J = 7.4 Hz, 2 H), 2.78 (t, ^3J = 7.5 Hz, 2 H), 3.57 (s, 3 H), 4.37 (d, ^3J = 5.9 Hz, 2 H), 4.46 (s, 2 H), 5.56 (s, 2 H), 6.84 (d, ^3J = 8.7 Hz, 2 H), 7.12 (d, ^3J = 8.6 Hz, 2 H), 7.29-7.32 (m, 2 H), 7.34-7.40 (m, 3 H), 7.93 (s, 1 H), 8.59 (t, ^3J = 5.8 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 29.4 (CH_2), 34.0 (CH_2), 35.1 (CH_2), 51.3 (CH_3), 52.7 (CH_2), 66.9 (CH_2), 114.6 (CH_2), 122.9 (CH), 128.0 (CH), 128.1 (CH), 128.7 (CH), 129.2 (CH), 133.1 (C_{quat}), 136.1 (C_{quat}), 145.1 (C_{quat}), 156.1 (C_{quat}), 167.8 (C_{quat}), 172.7 (C_{quat}). **EI-MS:** *m/z* (%) = 409 ($[M + H]^+$, 4.3), 408 (M^+, 16.7), 377 (6.8), 349 (1.8), 230 ($[M - C_{10}H_{10}O_3]^+$, 45.2), 229 (9.6), 187 (9.0), 173 ($C_{10}H_{11}N_3^+$, 40.1), 144 ($C_8H_6N_3^+$, 11.4), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 21.7), 65 ($C_5H_5^+$, 8.2), 39 ($C_3H_3^+$, 2.1). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3053 (w), 2954 (w), 2933 (w), 1730 (s), 1666 (s), 1612 (w), 1514 (s), 1494 (w), 1456 (w), 1435 (w), 1423 (w), 1375 (w), 1363 (w), 1332 (w), 1280 (w), 1244 (m), 1215 (w), 1203 (w), 1168 (m), 1118 (w), 1076 (w), 1053 (s), 1026 (w), 985 (w), 866 (w), 835 (w), 823 (s), 796 (w), 767 (w), 719 (s), 705 (s), 690 (w), 665 (w). Anal. calcd. for $C_{22}H_{24}N_4O_4$ (408.5): C 64.69, H 5.92, N 13.72. Found: C 64.82, H 6.11, N 13.59.

7.6.9. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(2,2,2-trifluoroacetamido)propanamide (5n)

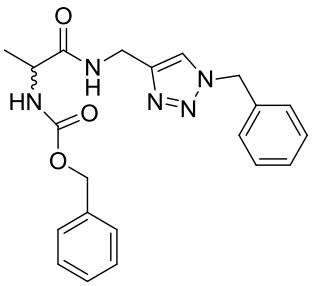


$C_{15}H_{16}F_3N_5O_2$
355.32 g/mol

210 mg (0.59 mmol, 59 %) of **5n** was obtained as colorless solid after purification by column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:20)).

Mp. 163 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 1.41 (d, ^3J = 7.1 Hz , 3 H), 4.44 (d, ^3J = 5.7 Hz, 2 H), 4.48-4.55 (m, 1 H), 5.59 (s, 2 H), 7.28-7.44 (m, 5 H), 7.82 (s, 1 H), 7.87 (br s, 1 H), 8.44 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-acetone): δ = 18.1 (CH_3), 35.7 (CH_2), 50.2 (CH), 54.1 (CH_2), 117.0 (d, ^1J = 287.4 Hz, C_{quat}), 123.2 (CH), 128.9 (CH), 129.1 (CH), 129.7 (CH), 137.1 (C_{quat}), 146.0 (C_{quat}), 157.1 (d, ^2J = 36.83 Hz, C_{quat}), 171.4 (C_{quat}). **EI-MS:** *m/z* (%) = 356 ([M + H]$^+$, 3.1) , 355 (M^+, 15.8), 215 ([M - $C_4H_5F_3NO$]$^+$, 9.0), 187 ($C_{10}H_{11}N_4^+$, 11.0), 173 ($C_{10}H_{11}N_3^+$, 8.8), 149 (4.8), 143 ($C_8H_5N_3^+$, 32.0), 97 ($C_2F_3O^+$, 9.7), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 6.9), 69 (CF_3^+, 69.2). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3078 (w), 3005 (w), 2951 (w), 2929 (w), 2879 (w), 1705 (m), 1656 (m), 1543 (m), 1496 (w), 1458 (w), 1348 (w), 1323 (w), 1213 (m), 1182 (s), 1155 (s), 1132 (w), 1107 (w), 1074 (w), 1053 (w), 1029 (w), 977 (w), 837 (w), 792 (w), 752 (w), 729 (m), 719 (m), 694 (m), 669 (w), 651 (w), 628 (w). Anal. calcd. for $C_{15}H_{16}F_3N_5O_2$ (353.3): C 50.70, H 4.54, N 19.71 Found C 50.88, H 4.80, N 19.52.

7.6.10. Benzyl 1-{[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amino}-1-oxopropan-2-yl carbamate (5o)

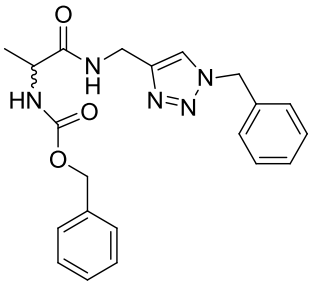


$C_{21}H_{23}N_5O_3$
393.44 g/mol

275 mg (0.70 mmol, 70 %) of **5o** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1: 20)).

Mp. 166 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 1.19 (d, ^3J = 7.2 Hz, 3 H), 4.02 (m, 1 H), 4.30 (d, ^3J = 5.6 Hz, 2 H), 5.00 (d, ^4J = 6.4, 2 H), 5.56 (s, 2 H), 7.29-7.39 (m, 10 H), 7.44 (d, ^3J = 7.4 Hz, 1 H), 7.91 (s, 1 H), 8.36 (t, ^3J = 5.5 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 18.1 (CH_3), 34.4 (CH_2), 50.1 (CH), 52.7 (CH_2), 65.4 (CH_2), 122.8 (CH), 127.7 (CH), 127.8 (CH), 127.9 (CH), 128.1 (CH), 128.3 (CH), 128.7 (CH), 136.1 (C_{quat}), 137.0 (C_{quat}), 145.3 (C_{quat}), 155.7 (C_{quat}), 172.5 (C_{quat}). **EI-MS:** *m/z* (%): 393 (M^+, 2.9), 350 (1.8), 286 (1.5), 244 (2.6), 216 (6.4), 221 (1.5), 215 (7.9), 189 (1.7), 188 (6.0), 174 ($C_{10}H_{12}N_3^+$, 10.0), 173 ($C_{10}H_{11}N_3^+$, 77.5), 143 (10.5), 144 (7.4), 117 (3.1), 107 (4.6), 108 (6.0), 91 ($C_7H_7^+$, 100), 79 (5.8), 65 ($C_5H_5^+$, 7.0). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3062 (w), 2968 (w), 2954 (w), 1685 (m), 1639 (s), 1602 (w), 1587 (w), 1537 (m), 1496 (w), 1454 (w), 1454 (w), 1435 (w), 1392 (w), 1332 (w), 1305 (w), 1257 (m), 1232 (w), 1232 (w), 1220 (w), 1120 (w), 1078 (w), 1047 (m), 1029 (w), 848 (w), 779 (w), 758 (w), 717 (w), 698 (m), 671 (m), 646 (w). Anal. calcd. for $C_{21}H_{23}N_5O_3$ (393.4): C 64.11, H 5.89, N 17.80. Found: C 64.08, H 5.98, N 17.77.

7.6.11. Benzyl-1-{[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amino}-1-oxopropan-2-yl carbamate (5p)

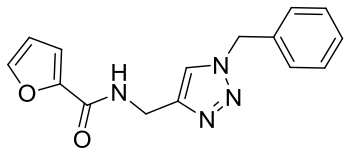


$C_{21}H_{23}N_5O_3$
393.44 g/mol

287 mg (0.73 mmol, 73 %) of **5p** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1: 20)).

Mp. 166 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 1.19 (d, ^3J = 7.1 Hz, 3 H), 4.00 (m, 1 H), 4.30 (d, ^3J = 5.6 Hz, 2 H), 5.00 (d, ^4J = 6.4 Hz, 2 H), 5.56 (s, 2 H), 7.29-7.39 (m, 10 H), 7.44 (d, ^3J = 7.4 Hz, 1 H), 7.91 (s, 1 H), 8.37 (t, ^3J = 5.5 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 18.1 (CH_3), 34.4 (CH_2), 50.1 (CH), 52.8 (CH_2), 65.4 (CH_2), 122.8 (CH), 127.7 (CH), 127.8 (CH), 128.0 (CH), 128.1 (CH), 128.4 (CH), 128.7 (CH), 136.1 (C_{quat}), 137.0 (C_{quat}), 145.3 (C_{quat}), 155.7 (C_{quat}), 172.6 (C_{quat}). **EI-MS:** *m/z* (%) = 394 ($[M + H]^+$, 1.9), 393 (M^+, 5.3), 350 (3.7), 286 (2.3), 215 (8.7), 188 (6.5), 189 (1.8), 173 ($C_{10}H_{11}N_3^+$, 87.2), 174 (10.5), 159 (2.5), 143 (10.4), 144 (8.6), 130 (2.5), 108 (5.2), 91 ($C_7H_7^+$, 100), 79 (4.9), 65 ($C_5H_5^+$, 6.9). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3013 (w), 2913 (w), 2946 (w), 2906 (w), 2879 (w), 2853 (w), 2826 (w), 1685 (s), 1639 (s), 1616 (w), 1602 (w), 1587 (w), 1537 (s), 1496 (w), 1454 (w), 1435 (w), 1392 (w), 1332 (w), 1305 (w), 1257 (m), 1232 (m), 1220 (m), 1120 (w), 1078 (w), 1047 (w), 1028 (w), 848 (w), 821 (w), 779 (w), 758 (w), 734 (m), 717 (m), 698 (s), 671 (m), 646 (w), 619 (w). Anal. calcd. for $C_{21}H_{23}N_5O_3$ (393.4): C 64.11, H 5.89, N 17.80. Found: C 63.90, H 5.86, N 17.66.

7.6.12. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)furan-2-carboxamide (5s)

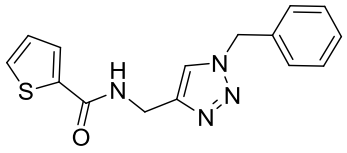


$C_{15}H_{14}N_4O_2$
282.30 g/mol

192 mg (0.68 mmol, 68 %) of **5s** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1: 20)).

Mp. 94 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.65 (d, ^3J = 5.9 Hz, 2 H), 5.49 (s, 2 H), 6.46 (m, 1 H), 7.04 (br s, 1 H), 7.07 (dd, ^3J = 3.5 Hz, ^4J = 0.8 Hz, 1 H), 7.24 (m, 1 H), 7.28-7.38 (m, 4 H), 7.41 (m, 1 H), 7.51 (s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 34.7 (CH_2), 54.4 (CH_2), 112.2 (CH), 114.5 (CH), 122.4 (CH), 128.3 (CH), 128.9 (CH), 129.3 (CH), 134.5 (C_{quat}), 144.2 (CH), 145.0 (C_{quat}), 147.7 (C_{quat}), 158.5 (C_{quat}). **EI-MS:** *m/z* (%) = 283 ($[M + H]^+$, 7.4), 282 (M^+, 36.2), 254 ($[M - N_2]^+$, 5.0), 191 ($[M - C_7H_7]^+$, 11.7), 187 ($[M - C_5H_3O_2]^+$, 13.6), 159 (4.2), 144 (11.0), 95 ($C_5H_3O_2^+$, 48.7), 91 ($C_7H_7^+$, 100), 77 ($C_6H_5^+$, 2.6), 65 ($C_5H_5^+$, 12.0), 39 ($C_3H_3^+$, 10.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3037 (w), 2958 (w), 2924 (w), 2856 (w), 1639 (m), 1591 (m), 1575 (w), 1546 (w), 1525 (w), 1496 (w), 1469 (w), 1456 (w), 1423 (w), 1375 (w), 1328 (w), 1298 (w), 1284 (w), 1207 (w), 1192 (m), 1143 (w), 1111 (w), 1076 (w), 1041 (w), 1018 (w), 987 (w), 912 (w), 883 (w), 835 (w), 823 (w), 796 (w), 771 (w), 752 (s), 727 (w), 109 (s), 696 (s), 667 (w), 653 (w), 605 (m). Anal. calcd. for $C_{15}H_{14}N_4O_2$ (282.3): C 63.82, H 5.00, N 19.85. Found: C 63.56, H 4.93, N 19.79.

7.6.13. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)thiophene-2-carboxamide (5t)

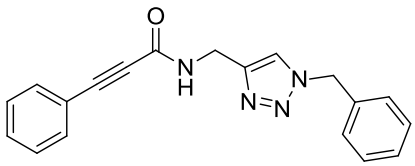


$C_{15}H_{14}N_4OS$
298.36 g/mol

104 mg (0.35 mmol, 35 %) of **5t** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1: 20)).

Mp. 123 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.63 (d, ^3J = 5.8 Hz, 2 H), 5.48 (s, 2 H), 7.02 (dd, ^3J = 3.8 Hz, 4.9 Hz, 1 H), 7.25 (br s, 1 H), 7.27-7.39 (m, 5 H), 7.43 (dd, ^3J = 5.0 Hz, ^4J = 1.1 Hz, 1 H), 7.55 (dd, ^3J = 3.7 Hz, ^4J = 1.1 Hz, 1 H), 7.59 (s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 35.7 (CH_2), 54.8 (CH_2), 123.3 (CH), 128.2 (CH), 128.7 (CH), 128.8 (CH), 129.4 (CH), 129.7 (CH), 130.7 (CH), 134.9 (C_{quat}), 139.2 (C_{quat}), 145.6 (C_{quat}), 162.6 (C_{quat}). **EI-MS:** *m/z* (%) = 300 ([M + 2H]$^+$, 2.6), 299 ([M + H]$^+$, 7.5), 298 (M$^+$, 38.6), 280 (3.1), 269 ([M - N_2 - H]$^+$, 1.8), 207 ([M - C_7H_7]$^+$, 3.7), 187 ([M - C_5H_3OS]$^+$, 25.8), 179 (9.9), 173 ($C_{10}H_{11}N_3^+$, 2.5), 159 ($C_9H_9N_3^+$, 8.2), 151 (1.9), 143 ($C_8H_5N_3^+$, 39.0), 130 (8.2), 111 ($C_5H_3OS^+$, 59.3), 104 (3.1), 91 ($C_7H_7^+$, 100), 83 ($C_4H_3S^+$, 5.5), 77 ($C_6H_5^+$, 2.4), 65 ($C_5H_5^+$, 12.7), 39 ($C_3H_3^+$, 11.8). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3080 (w), 3064 (w), 2927 (w), 2860 (w), 1620 (s), 1556 (s), 1514 (w), 1494 (w), 1456 (w), 1417 (m), 1377 (w), 1354 (m), 1327 (w), 1200 (s), 1263 (w), 1240 (m), 1228 (w), 1207 (w), 1145 (w), 1134 (w), 1111 (w), 1076 (w), 1047 (m), 1029 (w), 1018 (w), 952 (w), 860 (w), 819 (w), 794 (w), 752 (w), 725.23 (s), 709 (s), 694 (s), 665 (m), 653 (m), 619 (w). Anal. calcd. for $C_{15}H_{14}N_4OS$ (298.4): C 60.38, H 4.73, N 18.78. Found C 60.32, H 4.64, N 18.60.

7.6.14. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-3-phenylpropiolamide (5aa)

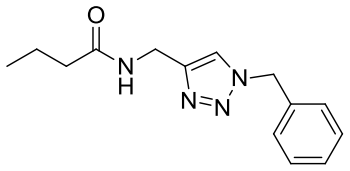


$C_{19}H_{16}N_4O$
316.36 g/mol

225 mg (0.71 mmol, 71 %) of **5aa** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (2:1), product obtainment with *n*-hexane:ethylacetate (1:3)).

Mp. 137 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.51 (d, ^3J = 5.8 Hz, 2 H), 5.42 (s, 2 H), 6.91 (br s, 1 H), 7.16-7.25 (m, 3 H), 7.28-7.35 (m, 5 H), 7.42-7.43 (m, 2 H), 7.45 (s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 35.4 (CH_2), 54.4 (CH_2), 82.8 (C_{quat}), 85.4 (C_{quat}), 120.2 (C_{quat}), 122.5 (CH), 128.3 (CH), 128.6 (CH), 129.0 (CH), 129.3 (CH), 130.2 (CH), 132.7 (CH), 134.5 (C_{quat}), 144.4 (C_{quat}), 153.5 (C_{quat}). **EI-MS:** *m/z* (%) = 317 ($[M + H]^+$, 4.5), 316 (M^+, 18.5), 287 (6.8), 273 (4.2), 259 (3.7), 244 (4.2), 197 (11.5), 187 ($[M - C_9H_5O]^+$, 16.8), 169 (3.8), 161 (4.4), 129 ($C_9H_5O^+$, 58.3), 101 (4.8), 91 ($C_7H_7^+$, 100), 75 (6.2), 65 ($C_5H_5^+$, 8.5), 39 ($C_3H_3^+$, 2.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3223 (w), 3143 (w), 3051 (w), 3034 (w), 2939 (w), 2862 (w), 2511 (w), 2229 (w), 1950 (w), 1730 (w), 1637 (s), 1552 (w), 1537 (w), 1489 (w), 1454 (w), 1159 (w), 1051 (w), 1421 (w), 1338 (w), 1300 (m), 1280 (m), 1238 (w), 1219 (s), 1122 (w), 1024 (w), 995 (w), 912 (w), 873 (w), 821 (w), 794 (w), 752 (s), 719 (s), 698 (s), 686 (s), 673 (m), 655 (m). Anal. calcd. for $C_{19}H_{16}N_4O$ (316.4): C 72.13, H 5.10, N 17.71. Found: C 72.22, H 5.20, N 17.61.

7.6.15. *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)butyramide (5dd)

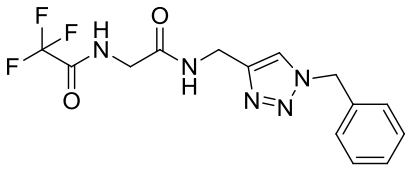


$C_{14}H_{18}N_4O$
258.32 g/mol

103 mg (0.40 mmol, 40 %) of **5dd** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:10)).

Mp. 126 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 0.89 (t, ^3J = 7.4 Hz, 3 H), 1.57-1.69 (m, 2 H), 2.15 (t, ^3J = 7.4 Hz, 2 H), 4.46 (d, ^3J = 5.7 Hz, 2 H), 5.48 (s, 2 H), 6.27 (br s, 1 H), 7.24 (m, 1 H), 7.27 (m, 1 H), 7.35-7.40 (m, 3 H), 7.45 (s, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 13.8 (CH_3), 19.1 (CH_2), 35.0 (CH_2), 38.5 (CH_2), 54.4 (CH_2), 122.2 (CH), 128.2 (CH), 129.0 (CH), 129.3 (CH), 134.6 (C_{quat}), 145.3 (C_{quat}), 173.2 (C_{quat}). **EI-MS:** *m/z* (%) = 259 ($[M + H]^+$, 6.8), 258 (M^+, 34.5), 240 (3.1), 229 ($[M - C_2H_5]^+$, 1.7), 215 ($[M - C_3H_7]^+$, 1.2), 187 ($[M - C_4H_7O]^+$, 30.0), 173 ($C_{10}H_{11}N_3^+$, 2.2), 167 ($[M - C_7H_7]^+$, 2.9), 159 ($C_9H_9N_3^+$, 13.9), 143 ($C_8H_5N_3^+$, 51.7), 139 (12.4), 130 (4.8), 115 (3.7), 104 (2.5), 97 (16.9), 91 ($C_7H_7^+$, 100), 83 (3.8), 71 ($C_4H_7O^+$, 11.1), 65 ($C_5H_5^+$, 11.4), 43 ($C_3H_7^+$, 19.2). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3078 (w), 3035 (w), 2962 (w), 2931 (w), 2873 (w), 2827 (w), 2779 (w), 1631 (s), 1608 (w), 1544 (m), 1494 (w), 1454 (w), 1436 (w), 1404 (w), 1367 (w), 1342 (w), 1334 (w), 1276 (w), 1222 (w), 1209 (w), 1165 (w), 1130 (w), 1109 (w), 1056 (w), 1041 (w), 1028 (w), 1001 (w), 858 (w), 829 (w), 808 (w), 781 (w), 742 (w), 717 (w), 692 (m), 667 (w). Anal. calcd. for $C_{14}H_{18}N_4O$ (258.3): C 65.09, H 7.02, N 21.69. Found C 65.34, H 7.01, N 21.76.

7.6.16. *N*-(2-(((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)-2-oxoethyl)-2,2,2-trifluoro acetamide (5ee)

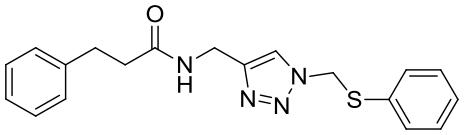


$C_{14}H_{14}F_3N_5O_2$
341.29 g/mol

208 mg (0.61 mmol, 61 %) of **5ee** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:20)).

Mp. 177 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 3.81 (d, ^3J = 4.3 Hz , 2 H), 4.32 (d, ^3J = 5.6 Hz, 2 H), 5.57 (s, 2 H), 7.31-7.41 (m, 5H), 7.98 (s, 1H), 8.57 (t, ^3J = 5.4 Hz, 1H), 9.63 (br s, 1H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 34.3 (CH_2), 41.9 (CH_2), 52.8 (CH_2), 115.9 (d, ^1J = 287.9 Hz, C_{quat}), 123.1 (CH), 128.0 (CH), 128.14 (CH), 128.8 (CH), 136.1 (C_{quat}), 144.8 (C_{quat}), 156.6 (d, ^2J = 36.4 Hz, C_{quat}), 166.96 (C_{quat}). **EI-MS:** *m/z* (%) = 343 ([M + 2H]$^+$, 3.2), 342 ([M + H]$^+$, 16.3), 341 (M$^+$, 25.8), 299 ([M - 2HF - 2H]$^+$, 8.5), 187 ([M - $C_4H_3F_3NO_2$]$^+$, 11.9), 172 ([M - $C_4H_4F_3N_2O_2$]$^+$, 1.9), 159 ($C_9H_9N_3^+$, 2.5), 144 ($C_8H_6N_3^+$, 12.1), 130 (3.8), 126 ($C_3H_3F_3NO^+$, 9.0), 104 (2.7), 97 ($C_2F_3O^+$, 9.0), 91 ($C_7H_7^+$, 100), 65 ($C_5H_5^+$, 8.7), 43 ($C_3H_7^+$, 2.7), 39 ($C_3H_3^+$, 2.9). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3068 (w), 2947 (w), 2924 (w), 2906 (w), 2854 (w), 1701 (s), 1658 (s), 1558 (m), 1544 (m), 1494 (w), 1456 (w), 1427 (w), 1382 (w), 1359 (w), 1327 (w), 1282 (w), 1242 (w), 1213 (m), 1184 (s), 1161 (s), 1132 (w), 1089 (w), 1076 (w), 1058 (w), 692 (m), 1028 (w), 1004 (w), 844 (w), 763 (w), 742 (w), 723 (m), 713 (m), 659 (w). Anal. calcd. for $C_{14}H_{14}F_3N_5O_2$ (341.3): C 49.27, H 4.13, N 20.52. Found C 49.42, H 4.35, N 20.33.

7.6.17. 3-Phenyl-*N*-((1-((phenylthio)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)propanamide (5ff)

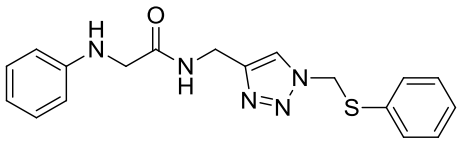


$C_{19}H_{20}N_4OS$
352.45 g/mol

222 mg (0.63 mmol, 63 %) of **5ff** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:10)).

Mp. 75 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 2.36 (t, ^3J = 7.8 Hz, 2 H), 2.78 (t, ^3J = 7.8 Hz, 2 H), 4.22 (d, ^3J = 5.7 Hz, 2 H), 5.88 (s, 2 H), 7.14-7.41 (m, 10 H), 7.22 (s, 1 H), 8.32 (br t, ^3J = 5.60 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 31.0 (CH_2), 34.0 (CH_2), 36.8 (CH_2), 51.4 (CH_2), 122.4 (CH), 125.9 (CH), 127.5 (CH), 128.2 (CH), 128.2 (CH), 129.2 (CH), 130.3 (CH), 132.6 (C_{quat}), 141.2 (C_{quat}), 145.5 (C_{quat}), 171.3 (C_{quat}). **EI-MS:** *m/z* (%) = 353 ([M + H]$^+$, 3.3), 352 (M^+, 12.6), 234 (13.1), 215 (5.8), 188 ($C_{12}H_{14}NO^+$, 12.2), 149 ($C_9H_{11}NO^+$, 9.3), 150 ($C_9H_{12}NO^+$, 4.3), 134 ($C_9H_{10}O^+$, 2.2), 133 ($C_9H_9O^+$, 2.9), 125 ($C_4H_5N_4O^+$, 100), 123 ($C_7H_7S^+$, 18.9), 105 ($C_8H_9^+$, 17.3), 91 ($C_7H_7^+$, 30.0), 83 ($C_4H_5NO^+$, 15.5), 77 ($C_6H_5^+$, 12.6), 68 ($C_2H_2N_3^+$, 11.1), 54 ($C_3H_4N^+$, 41.4). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3059 (w), 3003 (w), 2953 (w), 2926 (w), 2852 (w), 1635 (s), 1583 (w), 1543 (s), 1494 (w), 1483 (w), 1454 (w), 1438 (w), 1404 (w), 1386 (w), 1375 (w), 1334 (w), 1309 (w), 1278 (w), 1261 (w), 1226 (m), 1184 (w), 1161 (w), 1111 (w), 1085 (w), 1047 (m), 1026 (m), 993 (w), 968 (w), 827 (w), 777 (w), 740 (s), 721 (m), 692 (s), 659 (w), 613 (w). Anal. calcd. for $C_{19}H_{20}N_4OS$ (352.5): C 64.75, H 5.72, N 15.90, S 9.10. Found C 64.62, H 5.71, N 16.19, S 9.11.

7.6.18. 2-(Phenylamino)-*N*-((1-((phenylthio)methyl)-1*H*-1,2,3-triazol-4-yl)methyl) acetamide (5gg)

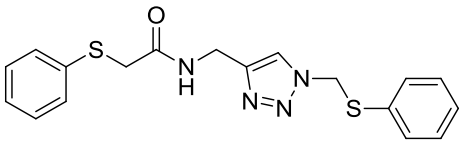


$C_{18}H_{19}N_5OS$
353.44 g/mol

276 mg (0.78 mmol, 78 %) of **5gg** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:20)).

Mp. 97 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 3.74 (d, ^3J = 4.9 Hz, 2 H), 4.43 (m, 2 H), 5.39 (br s, 1 H), 5.81 (s, 2 H), 6.56-6.67 (m, 3 H), 7.07-7.14 (m, 2 H), 7.27-7.43 (m, 5 H), 7.69 (s, 1 H), 7.78 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-acetone): δ = 35.3 (CH_2), 48.6 (CH_2), 53.5 (CH_2), 113.6 (CH), 118.2 (CH), 122.8 (CH), 128.9 (CH), 129.9 (CH), 130.2 (CH), 132.4 (CH), 133.7 (C_{quat}), 146.7 (C_{quat}), 149.2 (C_{quat}), 171.1 (C_{quat}). **EI-MS:** *m/z* (%) = 355 ($[M + 2H]^+$, 2.8), 354 ($[M + H]^+$, 8.9), 353 (M^+, 36.5), 230 (4.6), 228 (7.8), 221 ($C_{10}H_{13}N_4S^+$, 25.3), 205 ($C_{10}H_{11}N_3S^+$, 13.0), 167 (2.0), 151 (5.6), 149 (8.1), 133 (6.5), 123 ($C_7H_7S^+$, 38.2), 106 ($C_7H_8N^+$, 100), 83 (5.7), 77 ($C_6H_5^+$, 21.3), 51 (6.1), 43 ($C_2H_3O^+$, 9.0), 39 ($C_3H_3^+$, 3.4). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3383 (w), 3302 (w), 3126 (w), 2980 (w), 2970 (w), 2889 (w), 1637 (m), 1604 (m), 1552 (w), 1512 (m), 1485 (w), 1471 (w), 1436 (w), 1421 (w), 1396 (w), 1357 (w), 1338 (w), 1317 (m), 1288 (w), 1257 (w), 1232 (w), 1180 (w), 1155 (w), 1111 (w), 1074 (w), 1045 (m), 1020 (w), 877 (w), 854 (w), 788 (w), 746 (s), 723 (w), 686 (m), 651 (w). Anal. calcd. for $C_{18}H_{19}N_5OS$ (353.4): C 61.17, H 5.42, N 19.81, S 9.07. Found: C 61.06, H 5.41, N 19.51, S 9.17.

7.6.19. 2-(Phenylthio)-*N*-((1-((phenylthio)methyl)-1*H*-1,2,3-triazol-4-yl)methyl) acetamide (5hh)

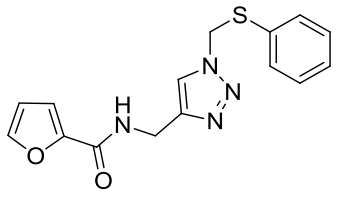


$C_{18}H_{18}N_4OS_2$
370.49 g/mol

218 mg (0.59 mmol, 59 %) of **5hh** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:10)).

Mp. 102 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 3.68 (s, 2 H), 4.40 (d, ^3J = 5.8 Hz, 2 H), 5.81 (s, 2 H), 7.17-7.42 (m, 10 H), 7.60 (s, 1 H), 7.85 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-acetone): δ = 35.8 (CH_2), 37.8 (CH_2), 53.5 (CH_2), 122.8 (CH), 127.1 (CH), 128.9 (CH), 129.4 (CH), 129.9 (CH), 130.2 (CH), 132.3 (CH), 133.7 (C_{quat}), 136.9 (C_{quat}), 146.4 (C_{quat}), 168.6 (C_{quat}). **EI-MS:** *m/z* (%) = 372 ($[M + 2H]^+$, 5.0), 371 ($[M + H]^+$, 10.8), 370 (M^+, 45.2), 261 (3.8), 247 (8.9), 221 ($C_{10}H_{13}N_4S^+$, 71.6), 205 (4.4), 176 (2.9), 151 (2.2), 147 (6.1), 135 (2.0), 125 ($C_7H_9S^+$, 14.6), 123 ($C_7H_7S^+$, 100), 111 ($C_6H_7S^+$, 13.2), 109 ($C_6H_5S^+$, 15.0), 96 (7.6), 83 ($C_4H_5NO^+$, 24.3), 84 ($C_4H_6NO^+$, 9.7), 65 ($C_5H_5^+$, 6.7), 54 ($C_3H_4N^+$, 10.0), 45 (26.2), 39 ($C_3H_3^+$, 6.7). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3008 (w), 2924 (w), 2902 (w), 2852 (w), 2681 (w), 1645 (s), 1517 (m), 1481 (w), 1471 (w), 1436 (w), 1408 (w), 1392 (w), 1332 (w), 1284 (w), 1257 (w), 1240 (w), 1230 (w), 1217 (w), 1049 (w), 1026 (w), 759 (m), 732 (s), 752 (w), 690 (s), 665 (w), 630 (w). Anal. calcd. for $C_{18}H_{18}N_4OS_2$ (370.5): C 58.35, H 4.90, N 15.12, S 17.31. Found: C 58.50, H 4.95, N 15.07, S 17.18.

7.6.20. *N*-((1-((Phenylthio)methyl)-1*H*-1,2,3-triazol-4-yl)methyl)furan-2-carboxamide (5ii)



$C_{15}H_{14}N_4O_2S$
314.36 g/mol

267 mg (0.85 mmol, 85 %) of **5ii** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (1:1), product obtainment with *n*-hexane:ethylacetate (1:20)).

Mp. 138 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 4.55-4.58 (m, 2 H), 5.84 (s, 2 H), 6.58 (dd, ^3J = 1.8 Hz, 1 H), 7.06 (dd, ^3J = 2.7 Hz, ^4J = 0.8 Hz, 1 H), 7.25-7.34 (m, 3 H), 7.35-7.42 (m, 2 H), 7.66 (dd, ^3J = 1.8 Hz, ^4J = 0.8 Hz, 1 H), 7.80 (s, 1 H), 8.01 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-acetone): δ = 35.2 (CH_2), 53.5 (CH_2), 112.6 (CH), 114.3 (CH), 123.2 (CH), 128.9 (CH), 130.1 (CH), 132.5 (CH) 133.7 (C_{quat}), 145.4 (CH), 146.6 (C_{quat}), 149.4 (C_{quat}), 158.7 (C_{quat}). **EI-MS:** *m/z* (%) = 315 ($[M + H]^+$, 1.8), 314 (M^+, 7.3), 268 (2.8), 204 (4.7), 205 ($C_{10}H_{11}N_3S^+$, 23.6), 206 (2.7), 177 ($[M - N_2 - C_6H_5S]^+$, 22.3), 191 (1.3), 148 ($[M - C_7H_8N_3S]^+$, 11.4), 136 (1.8), 123 ($C_6H_5NO_2^+$, 10.7), 124 ($C_6H_6NO_2^+$, 7.8), 109 (6.1), 95 ($C_5H_3O_2^+$, 100), 84 (5.6), 67 (3.1), 55 (3.6), 45 (5.2), 39 ($C_3H_3^+$, 9.4). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3051 (w), 3010 (w), 2954 (w), 2918 (w), 2837 (w), 2779 (w), 1643 (s), 1595 (m), 1571 (w), 1539 (m), 1469 (w), 1427 (w), 1402 (w), 1355 (w), 1303 (m), 1282 (m), 1236 (m), 1226 (w), 1188 (m), 1122 (m), 1053 (s), 1006 (m), 981 (w), 910 (w), 885 (w), 837 (w), 794 (w), 769 (w), 759 (s), 744 (s), 713 (m), 690 (s). Anal. calcd. for $C_{15}H_{14}N_4O_2S$ (314.4): C 57.31, H 4.49, N 17.82, S 10.20. Found: C 57.43, H 4.56, N 17.60, S 10.23.

7.7. General Procedure for Chemoenzymatic One-pot Amidation-Coupling Sequence

To a solution of 55 mg (1.00 mmol) of propargylamine (**2**), in 2.0 mL dry MTBE in a screw-cap Schlenk vessel, were added 1.20 mmol of ester **1** and Novozyme® 435 (50 % w/w of respective ester substrate **1**) and reaction was allowed to shake for 4-24 h (depending upon the nature of ester **1** used) in an incubating shaker at 45 °C. After the completion of aminolysis, 2.0 mL of DMF was added in the same reaction vessel and reaction mixture was flushed with argon for 15 min. Then 1.00 mmol of respective iodoaryl **4h-m**, 115 mg (1.00 mmol) of TMG, 23 mg (2 mol%) of $Pd(PPh_3)_4$ and 8 mg (4 mol%) of CuI were added to the reaction mixture under argon and the reaction was allowed to shake for 1 h at 45 °C. After 1 h of reaction time, the reaction mixture was filtered to remove the enzyme beads. Then, to the filtered reaction mixture, was added 5.0 mL of brine followed by extraction with ethylacetate (3 x 10.0 mL). The combined organic layers were dried with anhydrous Na_2SO_4 and pure product was obtained after column chromatography on silica gel using *n*-hexane:ethylacetate as eluent to get analytically pure product **6**.

Table 7.4. Experimental details of chemoenzymatic one-pot synthesis of coupled product **6**.

Entry	Ester Substrate 1	Iodoaryl (**4h-m**)	Coupled Product 6
1	233 mg (1.20 mmol) of **1a**	204 mg (1.00 mmol) of **4h**	170 mg (58 %) of **6c**[a]
2	233 mg (1.20 mmol) of **1a**	234 mg (1.00 mmol) of **4i**	200 mg (62 %) of **6d**[a]
3	197 mg (1.20 mmol) of **1b**	204 mg (1.00 mmol) of **4h**	142 mg (54 %) of **6e**[a]
4	180 mg (1.20 mmol) of **1c**	204 mg (1.00 mmol) of **4h**	132 mg (53 %) of **6f**[a]
5	180 mg (1.20 mmol) of **1c**	262 mg (1.00 mmol) of **4j**	227 mg (74 %) of **6g**[a]
6	151 mg (1.20 mmol) of **1s**	210 mg (1.00 mmol) of **4k**	164 mg (71 %) of **6h**[a]
7	151 mg (1.20 mmol) of **1s**	222 mg (1.00 mmol) of **4l**	151 mg (62 %) of **6i**[a]
8	171 mg (1.20 mmol) of **1t**	210 mg (1.00 mmol) of **4k**	109 mg (44 %) of **6j**[a]

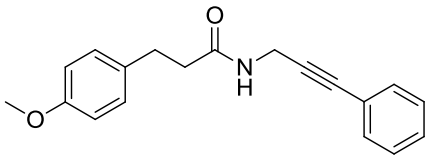
[a]Reaction time of 24 h for Novozyme® 435 catalyzed aminolysis. [b]Reaction time of 4 h for Novozyme® 435 catalyzed aminolysis.

Entry	Ester Substrate 1	Iodoaryl 4g-4n	Coupled Product 6
9	205 mg (1.20 mmol) of **1u**	246 mg (1.00 mmol) of **4m**	191 mg (64 %) of **6k**[b]
10	192 mg (1.20 mmol) of **1aa**	204 mg (1.00 mmol) of **4h**	132 mg (51 %) of **6l**[a]
11	222 mg (1.20 mmol) of **1dd**	204 mg (1.00 mmol) of **4h**	202 mg (71 %) of **6m**[a]

[a]Reaction time of 24 h for Novozyme® 435 catalyzed aminolysis. [b]Reaction time of 4 h for Novozyme® 435 catalyzed aminolysis.

7.8. Analytical Data of Arylated Propargylamides 6

7.8.1. 3-(4-Methoxyphenyl)-*N*-(3-phenylprop-2-yn-1-yl)propanamide (6c)



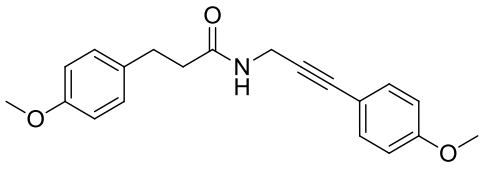
$C_{19}H_{19}NO_2$
293.36 g/mol

170 mg (0.58 mmol, 58 %) of **6c** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (5:1)).

Mp. 101 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 2.48 (t, ^3J = 7.6 Hz, 2 H), 2.93 (t, ^3J= 7.6 Hz, 2 H), 3.75 (s, 3 H), 4.24 (d, ^3J = 5.2 Hz, 2 H), 5.63 (br s, 1 H), 6.81 (d, ^3J = 8.7 Hz, 2 H), 7.12 (d, ^3J = 8.7 Hz, 2 H), 7.28-7.43 (m, 5H). **^{13}C-NMR** (75 MHz , $CDCl_3$): δ = 30.1 (CH_2), 30.9 (CH_2), 38.7 (CH_2), 55.3 (CH_3), 83.5 (C_{quat}), 84.9 (C_{quat}), 114.1 (CH), 122.7 (C_{quat}), 128.5 (CH), 128.6 (CH), 129.5 (CH), 131.8 (CH), 132.8 (C_{quat}), 158.2 (C_{quat}), 171.9 (C_{quat}). **EI-MS:** *m/z* (%) = 294 ($[M+H]^+$, 2.7), 293 (M^+, 13.7), 292 ($[M - H]^+$, 12.4), 216 ($[M - C_6H_5]^+$, 1.7), 172 ($[M - C_8H_9O]^+$, 100), 135 ($C_9H_{11}O^+$, 20.8), 121 ($C_8H_9O^+$, 61.4), 115 ($C_9H_7^+$, 18.2), 105 ($C_7H_5O^+$, 12.9), 77 ($C_6H_5^+$, 11.7), 43 ($C_3H_7^+$, 4.9). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3059 (w), 2995 (w), 2954 (w), 2937 (w), 2922 (w), 2906 (w), 2866 (w), 2835 (w), 1635 (s), 1610 (w), 1535 (m), 1508 (w), 1489 (w), 1446 (w), 1421 (w), 1377 (w), 1340 (w), 1319 (w), 1300 (w), 1251 (m), 1240 (s), 1211 (w), 1180 (w), 1157 (w), 1111 (w), 1078 (w), 1035 (m), 1020 (w), 941 (w), 914 (w), 883

(w), 831 (m), 813 (w), 759 (s), 713 (w), 690 (s), 661 (m). Anal. calcd. for $C_{19}H_{19}NO_2$ (293.4): C 77.79, H 6.53, N 4.77. Found C 77.56, H 6.38, N 4.68.

7.8.2. 3-(4-Methoxyphenyl)-*N*-(3-(4-methoxyphenyl)prop-2-yn-1-yl)propanamide (6d)

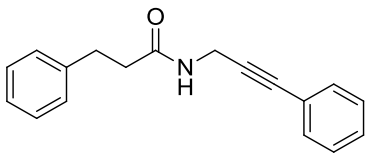


$C_{20}H_{21}NO_3$
323.39 g/mol

200 mg (0.62 mmol, 62 %) of **6d** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (5:1)).

Mp. 114 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 2.37 (t, ^3J = 7.7 Hz, 2 H), 2.76 (t, ^3J = 7.5 Hz, 2 H), 3.68 (s, 3 H), 3.76 (s, 3 H), 4.06 (d, ^3J = 5.4, 2 H), 6.80 (d, ^3J = 8.7 Hz, 2 H), 6.92 (d, ^3J = 8.8 Hz, 2 H), 7.10 (d, ^3J = 8.7 Hz, 2 H), 7.34 (d, ^3J = 8.8 Hz, 2 H), 8.32 (t, ^3J = 5.3 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): 28.5 (CH_2), 30.1 (CH_2), 37.1 (CH_2), 54.9 (CH_3), 55.2 (CH_3), 81.5 (C_{quat}), 85.5 (C_{quat}), 113.7 (CH), 114.3 (CH, C_{quat}), 129.2 (CH), 132.9 (CH), 133.1 (C_{quat}), 157.5 (C_{quat}), 159.3 (C_{quat}), 171.2 (C_{quat}). **EI-MS:** *m/z* (%) = 324 ($[M + H]^+$, 3.1), 323 (M^+, 15.2), 281 (1.3), 221 (1.6), 202 ($[M - C_8H_9O]^+$, 100), 188 ($C_{11}H_{10}NO_2^+$, 4.2), 163 ($C_{10}H_{11}O_2^+$, 2.5), 162 ($C_{10}H_{12}NO^+$, 10.1), 161 ($C_{10}H_9O_2^+$, 20.2), 145 ($C_{10}H_9O^+$, 15.6), 135 ($C_9H_{11}O^+$, 23.6), 121 ($C_8H_9O^+$, 36.4), 102 (4.8), 91 ($C_7H_7^+$, 6.4), 89 (2.3), 77 ($C_6H_5^+$, 5.9), 73 (2.0), 43 ($C_3H_7^+$, 5.0), 40 (3.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3032 (w), 2962 (w), 2920 (w), 2843 (w), 1633 (m), 1604 (w), 1529 (w), 1510 (m), 1481 (w), 1462 (w), 1440 (w), 1417 (w), 1373 (w), 1344 (w), 1294 (w), 1244 (s), 1220 (w), 1176 (w), 1149 (w), 1101 (m), 1076 (m), 1029 (s), 1016 (s), 1006 (m), 939 (w), 923 (w), 860 (w), 846 (w), 802 (s), 790 (s), 767 (m), 759 (m), 729 (w), 692 (m), 651 (w), 640 (w), 623 (w). Anal. calcd. for $C_{20}H_{21}NO_3$ (323.4): C 74.28, H 6.55, N 4.33. Found C 74.41, H 6.47, N 4.36.

7.8.3. 3-Phenyl-*N*-(3-phenylprop-2-yn-1-yl)propanamide (6e)

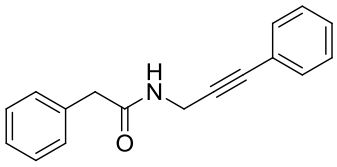


$C_{18}H_{17}NO$
263.33 g/mol

142 mg (0.54 mmol, 54 %) of **6e** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (5:1)).

Mp. 102 °C. **^{1}H-NMR** (300 MHz, d_6-acetone): δ = 2.51 (t, ^3J = 8.1 Hz, 2 H), 2.93 (t, ^3J = 8.2 Hz, 2 H), 4.21 (d, ^3J = 5.4 Hz, 2 H), 7.13-7.29 (m, 5 H), 7.34-7.43 (br m, 5 H), 7.50 (br s, 1 H). **^{13}C-NMR** (75 MHz, d_6-acetone): δ = 29.8 (CH_2), 32.3 (CH_2), 38.3 (CH_2), 82.7 (C_{quat}), 87.3 (C_{quat}), 124.0 (C_{quat}), 126.9 (CH), 129.3 (CH), 129.3 (CH), 129.4 (CH), 132.4 (CH), 142.5 (C_{quat}), 172.0 (C_{quat}). **EI-MS:** *m/z* (%) = 264 ($[M + H]^+$, 3.5), 263 (M^+, 17.2), 262 ($[M - H]^+$, 3.6), 203 (2.5), 172 ($[M - C_7H_7]^+$, 100), 158 ($[M - C_8H_9]^+$, 5.3), 130 ($C_9H_8N^+$, 30.2), 132 ($C_9H_{10}N^+$, 5.5), 115 ($C_9H_7^+$, 17.1), 105 ($C_8H_9^+$, 18.2), 91 ($C_7H_7^+$, 19.4), 77 ($C_6H_5^+$, 7.6), 65 ($C_5H_5^+$, 3.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3028 (w), 2927 (w), 2862 (w), 1633 (s), 1533 (s), 1489 (w), 1429 (w), 1379 (w), 1346 (w), 1303 (w), 1294 (w), 1263 (w), 1226 (m), 1072 (w), 1028 (w), 1012 (w), 754 (s), 688 (s), 609 (s). Anal. calcd. for $C_{18}H_{17}NO$ (263.3): C 82.10, H 6.51, N 5.32. Found C 81.99, H 6.55, N 5.31.

7.8.4. 2-Phenyl-*N*-(3-phenylprop-2-yn-1-yl)acetamide (6f)

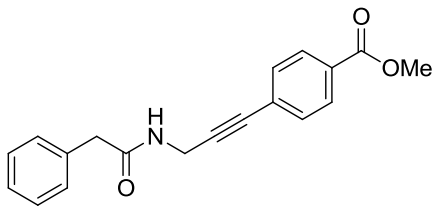


$C_{17}H_{15}NO$
249.31 g/mol

132 mg (0.53 mmol, 53 %) of **6f** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (5:1)).

Mp. 96 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 3.62 (s, 2 H), 4.24 (d, ^3J = 5.3 Hz, 2 H), 5.70 (br s, 1 H), 7.27-7.41 (br m, 10 H). **^{13}C-NMR** (75 MHz , $CDCl_3$): δ = 30.3 (CH_2), 43.7 (CH_2), 83.5 (C_{quat}), 84.7 (C_{quat}), 122.6 (C_{quat}), 127.6 (CH), 128.4 (CH), 128.6 (CH), 129.2 (CH), 129.6 (CH), 131.8 (CH), 134.6 (C_{quat}), 170.7 (C_{quat}). **EI-MS:** *m/z* (%) = 250 ($[M + H]^+$, 7.1), 249 (M^+, 36.6), 248 ($[M - H]^+$, 2.0), 174 (11.9), 158 ($[M - C_7H_7]^+$, 100), 130 ($C_9H_8N^+$, 49.4), 119 ($C_8H_7O^+$, 28.7), 115 ($C_9H_7^+$, 53.2), 91 ($C_7H_7^+$, 98.3), 77 ($C_6H_5^+$, 14.0), 65 ($C_5H_5^+$, 16.9), 43 ($C_3H_7^+$, 8.9). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3026 (w), 2939 (w), 2914 (w), 2856 (w), 2773 (w), 2735 (w), 2601 (w), 1946 (w), 1874 (w), 1805 (w), 1637 (s), 1598 (w), 1537 (s), 1490 (w), 1452 (w), 1442 (w), 1413 (w), 1386 (w), 1365 (w), 1330 (w), 1292 (w), 1278 (w), 1242 (w), 1232 (w), 1199 (w), 1182 (w), 1153 (w), 1120 (w), 1097 (w), 1070 (w), 1049 (w), 1014 (w), 999 (w), 968 (w), 939 (w), 904 (w), 871 (w), 829 (w), 802 (w), 771 (w), 754 (s), 721 (m), 686 (s), 623 (w). Anal. calcd. for $C_{17}H_{15}NO$ (249.3): C 81.90, H 6.06, N 5.62. Found C 82.08, H 6.11, N 5.58.

7.8.5. Methyl 4-(3-(2-phenylacetamido)prop-1-yn-1-yl)benzoate (6g)

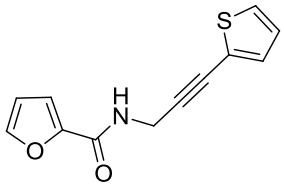


$C_{19}H_{17}NO_3$
307.34 g/mol

227 mg (0.74 mmol, 74 %) of **6g** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (3:1)).

Mp. 147 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 3.47 (s, 2 H), 3.85 (s, 3 H), 4.16 (d, ^3J = 5.3 Hz, 2 H), 7.22-7.33 (m, 5 H), 7.54 (d, ^3J = 8.3 Hz, 2 H), 7.94 Hz (d, ^3J = 8.3 Hz, 2 H), 8.64 (t, ^3J = 5.0 Hz, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 28.8 (CH_2), 42.0 (CH_2), 52.3 (CH_3), 80.9 (C_{quat}), 90.4 (C_{quat}), 126.4 (CH_2), 127.0 (C_{quat}), 128.2 (CH), 129.0 (CH), 129.2 (C_{quat}), 129.4 (CH), 131.7 (CH), 136.0 (C_{quat}), 165.6 (C_{quat}), 170.0 (C_{quat}). **EI-MS:** *m/z* (%) = 307 (M^+, 3.4), 293 ([M - CH_3 + 1H]$^+$, 13.9), 275 ([M - MeOH]$^+$, 1.6), 263 ([M - CO_2]$^+$, 7.7), 216 ([M - C_7H_7]$^+$, 1.7), 199 (2.3), 184 (2.7), 183 (8.7), 174 (3.6), 167 (17.3), 150 ($C_9H_{12}NO^+$, 10.3), 149 ($C_9H_{11}NO^{\bullet+}$, 100), 127 (13.9), 111 (3.3), 97 (7.5), 91 ($C_7H_7^+$, 7.6), 85 ($C_4H_5O_2^+$, 16.9), 83 ($C_4H_3O_2^+$, 7.5), 77 ($C_6H_5^+$, 2.5), 71 ($C_3H_3O_2^+$, 28.3), 69 ($C_3HO_2^+$, 11.3), 65 ($C_5H_5^+$, 2.3), 43 ($C_3H_7^+$, 19.1), 39 ($C_3H_3^+$, 1.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3068 (w), 2954 (w), 2924 (w), 2850 (w), 2821 (w), 1716 (m), 1639 (m), 1598 (w), 1543 (w), 1490 (w), 1454 (w), 1436 (w), 1408 (w), 1328 (w), 1303 (w), 1273 (m), 1257 (m), 1228 (w), 1192 (w), 1174 (w), 1147 (w), 1109 (w), 1099 (w), 1080 (w), 1024 (w), 1014 (w), 980 (w), 916 (w), 908 (w), 860 (w), 848 (w), 829 (w), 765 (w), 746 (w), 732 (w), 694 (s), 642 (w). Anal. calcd. for $C_{19}H_{17}NO_3$ (307.3): C 74.25, H 5.58, N 4.56. Found C 74.03, H 5.39, N 4.48.

7.8.6. *N*-(3-(Thiophen-2-yl)prop-2-yn-1-yl)furan-2-carboxamide (6h)

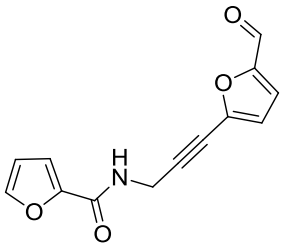


$C_{12}H_9NO_2S$
231.27 g/mol

164 mg (0.71 mmol, 71 %) of **6h** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (6:1)).

Mp. 67 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.47 (d, ^3J = 5.4 Hz, 2 H), 6.50 (dd, ^3J = 3.5 Hz, ^4J = 1.8 Hz, 1 H), 6.62 (br s, 1 H), 6.95 (dd, ^3J = 5.2 Hz, ^3J = 3.7 Hz, 1 H), 7.15 (dd, ^3J = 3.5 Hz, ^4J = 0.8 Hz, 1 H), 7.20 (m, 1 H), 7.24 (m, 1 H), 7.45 (m, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 29.9 (CH_2), 77.1 (C_{quat}), 88.6 (C_{quat}), 112.3 (CH), 114.9 (CH), 122.5 (C_{quat}), 127.1 (CH), 127.4 (CH), 132.5 (CH), 144.3 (CH), 147.6 (C_{quat}), 158.0 (C_{quat}). **EI-MS:** *m/z* (%) = 232 ([M + H]$^+$, 2.8), 231 (M$^+$, 17.7), 230 ([M - H]$^+$, 3.9), 202 ([M - CHO]$^+$, 100), 186 ([M - CH_2S]$^+$, 2.7), 184 (10.4), 174 ($C_{10}H_8NO_2^+$, 8.2), 170 (1.2), 159 (3.3), 148 ([M - C_4H_3S]$^+$, 2.1), 136 ($C_7H_6NS^+$, 8.6), 134 ($C_7H_4NS^+$, 2.9), 121 ($C_7H_5S^+$, 8.9), 109 (19.0), 95 ($C_5H_3O_2^+$, 55.6), 77 (7.9), 69 (5.1), 45 (3.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3076 (w), 3041 (w), 2968 (w), 2935 (w), 2779 (w), 1672 (w), 1651 (m), 1591 (m), 1523 (m), 1477 (w), 1381 (w), 1357 (w), 1348 (w), 1294 (w), 1257 (w), 1226 (w), 1186 (s), 1143 (w), 1082 (w), 1307 (w), 1008 (m), 987 (w), 935 (w), 906 (w), 885 (w), 848 (w), 831 (w), 825 (w), 781 (w), 750 (m), 692 (s), 607 (m). Anal. calcd. for $C_{12}H_9NO_2S$ (231.3): C 62.32, H 3.92, N 6.06, S 13.86. Found C 62.40, H 3.95, N 6.05, S 14.11.

7.8.7. *N*-(3-(5-Formylfuran-2-yl)prop-2-yn-1-yl)furan-2-carboxamide (6i)

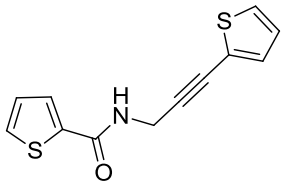


$C_{13}H_9NO_4$
243.21 g/mol

151 mg (0.62 mmol, 62 %) of **6i** was obtained as yellow solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (3:1)).

Mp. 118 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 4.34 (d, ^3J = 5.7, 2 H), 6.64 (dd, ^3J = 3.5 Hz, ^4J = 1.7 Hz, 1 H), 7.03 (d, ^3J = 3.7 Hz, 1 H), 7.16 (dd, ^3J = 3.5 Hz, ^4J = 0.7 Hz, 1 H), 7.56 (d, ^3J = 3.7 Hz, 1 H), 7.87 (m, 1 H), 8.99 (t, ^3J = 5.6 Hz, 1 H), 9.56 (s, 1 H). **^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 28.6 (CH_2), 71.0 (C_{quat}), 95.0 (C_{quat}), 112.0 (CH), 114.1 (CH), 117.7 (CH), 123.4 (CH), 140.1 (C_{quat}), 145.5 (CH), 147.3 (C_{quat}), 152.1 (C_{quat}), 157.6 (C_{quat}), 178.1 (C_{quat}). **EI-MS:** *m/z* (%) = 244 ([M + H]$^+$, 5.1) , 243 (M$^+$, 37.3), 214 ([M - CHO]$^+$, 64.0), 215 ([M - CO]$^+$, 10.0), 186 ([M - C_2HO_2]$^+$, 39.0), 168 (11.9), 148 ($C_8H_6NO_2^+$, 12.9), 140 (8.3), 131 (5.6), 130 (11.5), 121 ($C_7H_5O_2^+$, 5.3), 115 (10.1), 103 (11.9), 95 ($C_5H_3O_2^+$, 100), 77 (5.6), 75 (7.0), 67 ($C_4H_3O^+$, 6.1), 63 (5.1), 43 ($C_3H_7^+$, 5.4), 39 ($C_3H_3^+$, 13.1). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3103 (w), 2985 (w), 2825 (w), 1668 (m), 1647 (m), 1597 (w), 1521 (w), 1506 (m), 1471 (m), 1408 (w), 1390 (m), 1348 (w), 1309 (w), 1284 (w), 1267 (w), 1230 (w), 1188 (w), 1147 (w), 1103 (w), 1080 (w), 1053 (w), 1033 (m), 1020 (m), 1006 (w), 985 (w), 966 (m), 916 (w), 896 (w), 812 (s), 798 (w), 775 (w), 756 (s), 659 (w), 626 (w), 601 (w). Anal. calcd. for $C_{13}H_9NO_4$ (243.2): C 64.20, H 3.73, N 5.76. Found C 64.12, H 4.02, N 5.82.

7.8.8. *N*-(3-(Thiophen-2-yl)prop-2-yn-1-yl)thiophene-2-carboxamide (6j)

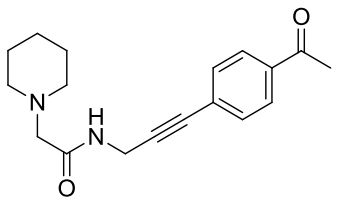


$C_{12}H_9NOS_2$
247.34 g/mol

109 mg (0.44 mmol, 44 %) of **6j** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (6:1)).

Mp. 120 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.48 (d, ^3J = 5.3 Hz, 2 H), 6.36 (br, 1 H), 6.96 (dd, ^3J = 5.2 Hz, 3.7 Hz, 1 H), 7.08 (dd, ^3J = 5.0 Hz, 3.8 Hz, 1 H), 7.20 (dd, ^3J = 3.6 Hz, ^4J = 1.9 Hz, 1 H), 7.24 (dd, ^3J = 5.2 Hz, ^4J = 1.1 Hz, 1 H), 7.49 (dd, ^3J = 5.0 Hz, ^4J = 1.1 Hz, 1 H), 7.56 (dd, ^3J = 3.7 Hz, ^4J = 1.1 Hz, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 30.8 (CH_2), 77.4 (C_{quat}), 88.7 (C_{quat}), 122.5 (C_{quat}), 127.1 (CH), 127.5 (CH), 127.8 (CH), 128.7 (CH), 130.5 (CH), 132.6 (CH), 138.3 (C_{quat}), 161.6 (C_{quat}). **EI-MS:** *m*/*z* (%) = 247 (M^+, 8.5), 246 ($[M - H]^+$, 4.6), 202 ($[M - CH_2S + H]^+$, 45.7), 186 (6.0), 183 (4.9), 136 ($C_7H_6NS^+$, 9.8), 121 ($C_7H_5S^+$, 10.6), 111 ($C_5H_3OS^+$, 100), 109 (19.8), 108 (8.9), 83 ($C_4H_3S^+$, 17.8), 69 (11.0), 45 (11.6). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3097 (w), 3010 (w), 2981 (w), 2924 (w), 2872 (w), 2848 (w), 2358 (w), 2127 (w), 1797 (w), 1708 (w), 1624 (m), 1595 (w), 1541 (m), 1512 (w), 1479 (w), 1458 (w), 1417 (w), 1381 (w), 1359 (w), 1344 (w), 1300 (m), 1284 (m), 1253 (m), 1247 (m), 1188 (w), 1143 (m), 1095 (m), 1080 (w), 1060 (w), 1047 (w), 1031 (w), 1020 (w), 968 (w), 954 (w), 906 (w), 885 (w), 854 (m), 842 (m), 827 (w), 785 (w), 771 (w), 752 (w), 719 (m), 700 (s), 657 (w), 634 (m). Anal. calcd. for $C_{12}H_9NOS_2$ (247.3). C 58.27, H 3.67, N 5.66, S 25.93. Found C 58.52, H 3.94, N 5.70, S 26.07.

7.8.9. *N*-(3-(4-Acetylphenyl)prop-2-yn-1-yl)-2-(piperidin-1-yl)acetamide (6k)

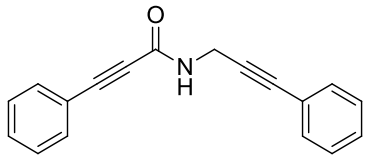


$C_{18}H_{22}N_2O_2$
298.38 g/mol

191 mg (0.64 mmol, 64 %) of **6k** was obtained as viscous yellow liquid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (2:1)).

Viscous yellow liquid. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 1.38 (m, 2 H), 1.53 (m, 4 H), 2.37 (t, ^3J = 5.0 Hz, 4 H), 2.57 (s, 3 H), 2.91 (s, 2 H), 4.17 (d, ^3J = 5.9 Hz, 2 H), 7.53 (d, ^3J = 8.5 Hz, 2 H), 7.94 (d, ^3J = 8.6 Hz, 2 H), 8.19 (t, ^3J = 5.7 Hz, 1 H). **^{13}C-NMR** (75 MHz , d_6-DMSO): δ = 23.5 (CH_2), 25.4 (CH_2), 26.7 (CH_3), 28.5 (CH_2), 54.1 (CH_2), 61.9 (CH_2), 80.5 (C_{quat}), 90.8 (C_{quat}), 126.9 (C_{quat}), 128.4 (CH_2), 131.5 (CH_2), 136.1 (C_{quat}), 169.6 (C_{quat}), 197.2 (C_{quat}). **EI-MS:** *m*/*z* (%) = 277 (2.9), 155 ([M - $C_{10}H_7O$]$^+$, 2.5), 155 (2.5), 149 (3.4), 113 (2.6), 112 (5.0), 98 ($C_6H_{12}N^+$, 100), 84 ($C_5H_{10}N^+$, 8.5), 73 ($C_3H_7NO^{\bullet+}$, 20.1), 70 (6.4), 67 (2.6), 43 ($C_2H_3O^+$, 6.9). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 2933 (w), 2854 (w), 2810 (w), 2794 (w), 2756 (w), 1678 (s), 1600 (m), 1552 (w), 1504 (m), 1467 (w), 1452 (w), 1402 (w), 1386 (w), 1355 (w), 1334 (w), 1301 (w), 1259 (s), 1178 (w), 1161 (w), 1126 (w), 1111 (w), 1087 (w), 1074 (w), 1039 (w), 1014 (w), 997 (w), 983 (w) 958 (w), 902 (w), 864 (w), 839 (m), 810 (w), 794 (w), 765 (w), 746 (w), 723 (w), 696 (w), 634 (w). Anal. calcd. for $C_{18}H_{22}N_2O_2$ (298.4): C 72.46, H 7.43, N 9.39. Found C 72.15, H 7.31, N 9.02.

7.8.10. 3-Phenyl-*N*-(3-phenylprop-2-yn-1-yl)propiolamide (6l)

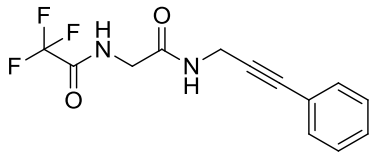


$C_{18}H_{13}NO$
259.30 g/mol

132 mg (0.51 mmol, 51 %) of **6l** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (6:1)).

Mp. 119 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): δ = 4.38 (d, ^3J = 5.3 Hz, 2 H), 6.23 (br, 1H), 7.27-7.62 (br m, 10 H).**^{13}C-NMR** (75 MHz , $CDCl_3$): δ = 30.6 (CH_2), 82.6 (C_{quat}), 83.8 (C_{quat}), 84.1 (C_{quat}), 85.7 (C_{quat}), 120.1 (C_{quat}), 122.4 (C_{quat}), 128.5 (CH), 128.7 (CH), 128.8 (CH), 130.4 (CH), 131.9 (CH), 132.7 (CH), 153.1 (C_{quat}). **EI-MS:** *m/z* (%) = 260 ($[M + H]^+$, 5.2), 259 (M^+, 29.4), 258 ($[M - H]^+$, 30.5), 241 (25.7), 230 (74.2), 215 (54.8), 182 ($[M - C_6H_5]^+$, 15.6), 154 (11.0), 129 ($C_9H_5O^+$, 100), 130 ($C_9H_8N^+$, 11.9), 115 ($C_9H_7^+$, 12.8), 101 ($C_8H_5^+$, 16.1), 89 (6.1), 77 ($C_6H_5^+$, 11.7), 63 (4.5). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3012 (w), 2989 (w), 2929 (w), 2900 (w), 2615 (w), 2594 (w), 2517 (w), 2436 (w), 2222 (w), 2185 (w), 2146 (w), 2100 (w), 2042 (w), 2015 (w), 1984 (w), 1969 (w), 1950 (w), 1897 (w), 1818 (w), 1737 (w), 1718 (w), 1647 (w), 1618 (m), 1597 (w), 1533 (m), 1487 (m), 1442 (w), 1417 (w), 1394 (w), 1384 (w), 1355 (w), 1303 (m), 1273 (w), 1257 (w), 1219 (m), 1178 (w), 1157 (w), 1097 (w), 1070 (w), 1058 (w), 1016 (w), 997 (w), 987 (w), 960 (w), 916 (w), 902 (w), 877 (w), 842 (w), 788 (w), 754 (s), 731 (w), 686 (w), 623 (w), 605 (w). Anal. calcd. for $C_{18}H_{13}NO$ (259.3): C 83.37, H 5.05, N 5.40, Found C 83.60, H 5.11, N 5.44.

7.8.11. 2,2,2-Trifluoro-*N*-(2-oxo-2-((3-phenylprop-2-yn-1-yl)amino)ethyl) acetamide (6m)



$C_{13}H_{11}F_3N_2O_2$
284.23 g/mol

202 mg (0.71 mmol, 71 %) of **6m** was obtained as colorless solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (10:1), product obtainment with *n*-hexane:ethylacetate (4:1)).

Mp. 137 °C. **^{1}H-NMR** (300 MHz , $CDCl_3$): δ = 4.07 (d, ^3J = 4.9 Hz, 2 H), 4.31 (d, ^3J = 5.2 Hz, 2 H), 6.45 (br, 1 H), 7.28-7.42 (br m, 5 H), 7.53 (br, 1 H). **^{13}C-NMR** (75 MHz , $CDCl_3$): δ = 30.5 (CH_2), 42.8 (CH_2), 83.7 (C_{quat}), 84.1 (C_{quat}), 115.8 (d, ^1J = 287.2 Hz, C_{quat}), 122.3 (C_{quat}), 128.5 (CH), 128.9 (CH), 131.9 (CH), 157.7 (d, ^2J = 37.9 Hz, C_{quat}), 166.5 (C_{quat}). **EI-MS:** m/z (%) = 209 (3.0), 171 ($C_4H_6F_3N_2O_2^+$, 100), 172 ([M - C_2HF_3NO]$^+$, 14.2), 158 ($C_{10}H_8NO^+$, 9.1), 143 (15.1), 130 ($C_9H_8N^+$, 25.1), 115 ($C_9H_7^+$, 48.9), 103 ($C_8H_7^+$, 12.4), 97 ($C_2F_3O^+$, 3.1), 89 (6.8), 77 ($C_6H_5^+$, 7.8), 69 (CF_3^+, 7.4), 43 ($C_3H_7^+$, 1.9). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3099 (w), 2916 (w), 2854 (w), 1701 (s), 1654 (s), 1556 (m), 1490 (w), 1442 (w), 1423 (w), 1386 (w), 1355 (w), 1344 (w), 1284 (w), 1213 (m), 1188 (s), 1157 (s), 1103 (w), 1091 (w), 1070 (w), 1041 (w), 1006 (w), 964 (w), 916 (w), 885 (w), 846 (w), 754 (s), 731 (w), 688 (s), 621 (w), 601 (w). Anal. calcd. for $C_{13}H_{11}F_3N_2O_2$ (284.2): C 54.93, H 3.90, N 9.86. Found C 54.68, N 3.93, H 9.79.

7.9. General Procedure for Chemoenzymatic One-pot Triazole ligation to Arylated Propargylamides (Synthesis of 8a and 8b)

To a solution of 55 mg (1.00 mmol) of propargylamine (**2**), in 2.0 mL dry MTBE in a screw-cap Schlenk vessel, were added 1.20 mmol of ester **1** and Novozyme® 435 (50 % w/w of respective ester substrate **1**) and the reaction was allowed to shake for 4-24 h (depending upon the nature of ester **1** used) in an incubating shaker at 45 °C. After the aminolysis, 2.0 mL of DMF was added in the same reaction vessel and reaction mixture was flushed with argon for 15 minutes followed by the addition of 300 mg (1.00 mmol) of (4-iodophenyl)-ethynyl) trimethylsilane (**4p**), 115 mg (1.00 mmol) of TMG, 23 mg (2 mol %) of $Pd(PPh_3)_4$ and 8 mg (4 mol %) of CuI under argon and the reaction was allowed to shake for 1 h at 45 °C. After 1 h of reaction time, 58 mg (1.00 mmol) of potassium fluoride (KF) was added into the reaction vial and was allowed to run for 10 minutes after which 133 mg (1.00 mmol) of benzyl azide (**4a**) was added. After 1 h of shaking, the reaction mixture was filtered to remove the enzyme beads. Then, to the filtered reaction mixture, was added 5.0 mL of brine followed by extraction with ethylacetate (3 x 10.0 mL). The combined organic layers were dried with anhydrous Na_2SO_4 followed by column chromatography (*n*-hexane:ethylacetate) to get analytically pure product **8**.

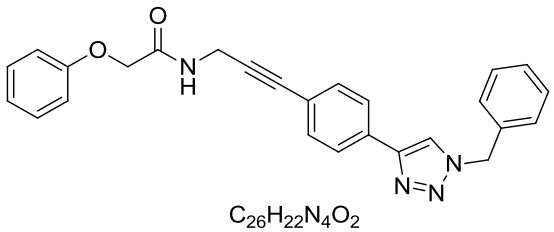
Table 7.5. Experimental details of chemoenzymatic one-pot synthesis of triazole ligated product **8**.

Entry	Ester Substrate 1	Aryl Iodide (**4p**)	Benzyl azide (**4a**)	Product 8
1	166 mg (1.20 mmol) of **1e**	300 mg (1.00 mmol)	133 mg (1.00 mmol)	245 mg (58 %) of **8a**[a]
2	192 mg (1.20 mmol) of **1aa**	300 mg (1.00 mmol)	133 mg (1.00 mmol)	259 mg (62 %) of **8b**[b]

[a]Reaction time of 24 h for Novozyme® 435 catalyzed aminolysis. [b]Reaction time of 4 h for Novozyme® 435 catalyzed aminolysis.

7.10. Analytical Data of Triazole ligated Systems 8

7.10.1. *N*-(3-(4-(1-Benzyl-1H-1,2,3-triazol-4-yl)phenyl)prop-2-yn-1-yl)-2-phenoxy acetamide (8a)

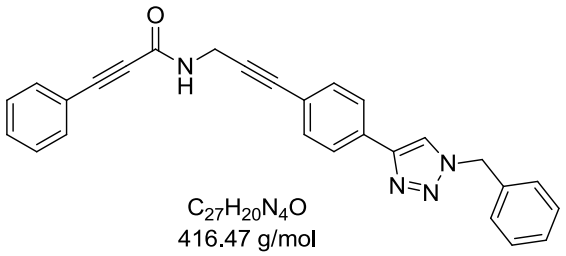


$C_{26}H_{22}N_4O_2$
422.48 g/mol

245 mg (0.58 mmol, 58 %) of **8a** was obtained as yellow solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (4:1), product obtainment with *n*-hexane:ethylacetate (1:1)).

Mp. 147 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 4.21 (d, ^3J = 5.6 Hz, 2 H), 4.54 (s, 2 H), 5.65 (s, 2 H), 6.97-7.00 (m, 3 H), 7.28-7.39 (br m, 7 H), 7.45 (d, ^3J = 8.4 Hz, 2 H), 7.59-7.62 (m, 1 H), 7.86 (d, ^3J = 8.4 Hz, 2 H), 8.69 (s, 1 H).**^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 28.5 (CH_2), 53.1 (CH_2), 66.8 (CH_2), 81.3 (C_{quat}), 87.7 (C_{quat}), 114.7 (CH), 121.2 (CH), 121.5 (C_{quat}), 122.1 (CH), 125.3 (CH), 127.9 (CH), 128.2 (CH), 129.5 (CH), 130.7 (C_{quat}), 132.0 (CH), 135.9 (C_{quat}), 145.9 (C_{quat}), 157.6 (C_{quat}), 167.7 (C_{quat}). **MALDI-MS:** *m/z* = 423 ($[M + H]^+$). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3034 (w), 2922 (w), 2910 (w), 2856 (w), 1662 (s), 1598 (w), 1587 (w), 1519 (m), 1489 (s), 1456 (w), 1435 (w), 1409 (w), 1350 (w), 1286 (w), 1242 (s), 1226 (s), 1170 (w), 1080 (w), 1060 (w), 1047 (w), 1028 (w), 1018 (w), 1001 (w), 835 (m), 798 (m), 756 (s), 721 (s), 657 (w), 603 (w). Anal. calcd. for $C_{26}H_{22}N_4O_2$ (422.5): C 73.92, H 5.25, N 13.26. Found C 74.06, N 5.32, H 13.47.

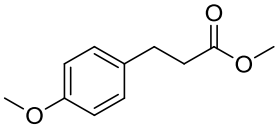
7.10.2. *N*-(3-(4-(1-Benzyl-1H-1,2,3-triazol-4-yl)phenyl)prop-2-yn-1-yl)-3-phenyl propiolamide (8b)



258 mg (0.62 mmol, 62 %) of **8b** was obtained as yellow solid after purification by silica gel column chromatography (elution started with *n*-hexane:ethylacetate (4:1), product obtainment with *n*-hexane:ethylacetate (1:1)).

Mp. 150 °C. **^{1}H-NMR** (300 MHz, d_6-DMSO): δ = 4.23 (d, ^3J = 5.5 Hz, 2 H), 5.65 (s, 2 H), 7.33-7.61 (br m, 12 H), 7.86 (d, ^3J = 8.3 Hz, 2 H), 8.70 (s, 1 H), 9.36 (t, ^3J = 5.3 Hz, 1 H).**^{13}C-NMR** (75 MHz, d_6-DMSO): δ = 30.7 (CH_2), 53.1 (CH_2), 81.8 (C_{quat}), 83.4 (C_{quat}), 83.9 (C_{quat}), 86.8 (C_{quat}), 119.6 (C_{quat}), 122.1 (CH), 123.3 (C_{quat}), 125.3 (CH), 127.9 (CH), 128.2 (CH), 128.8 (CH), 129.0 (CH), 130.4 (CH), 130.8 (C_{quat}), 132.0 (CH), 132.2 (CH), 135.9 (C_{quat}), 145.9 (C_{quat}), 152.1(C_{quat}). **MALDI-MS:** *m/z* = 417 ($[M + H]^+$). **IR (ATR)** $\tilde{\nu}$ [cm^{-1}] = 3037 (w), 2993 (w), 2954 (w), 2922 (w), 2360 (w), 2343 (w), 2331 (w), 2225 (w), 1637 (s), 1535 (m), 1419 (w), 1296 (w), 1217 (w), 1190 (w), 1074 (w), 1049 (w), 1020 (w), 977 (w), 844 (w), 800 (m), 754 (s), 713 (m), 686 (m), 617 (w). Anal. calcd. for $C_{27}H_{20}N_4O$ (422.5): C 77.87, H 4.84 N 13.45. Found C 77.59 H 4.66 N 13.35.

Molecule Content



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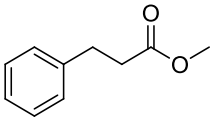

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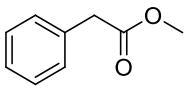

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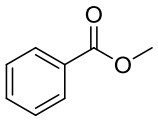

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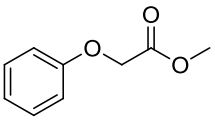

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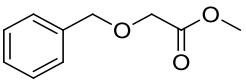

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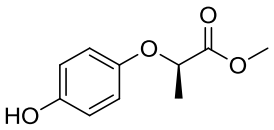

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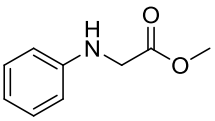

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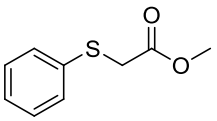

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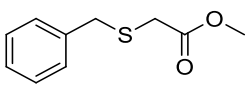

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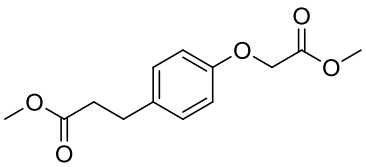

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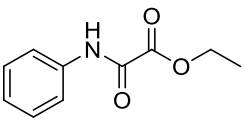

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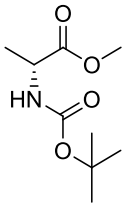

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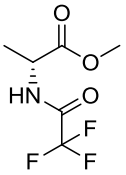

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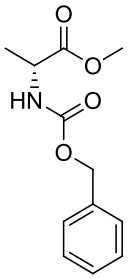

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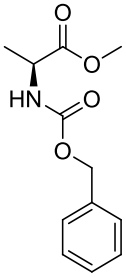

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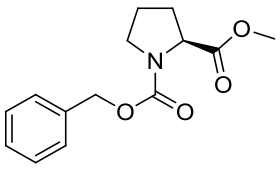

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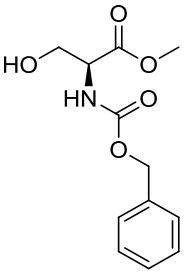

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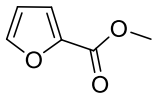
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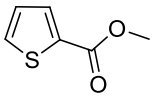
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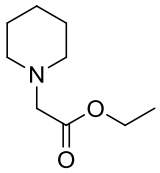
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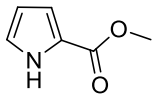
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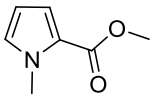
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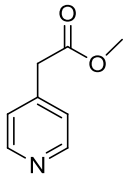
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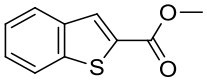
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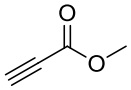
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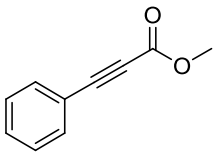
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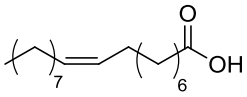
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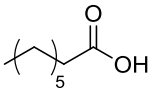
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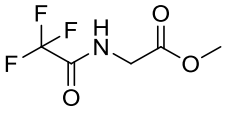
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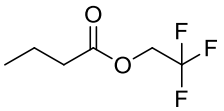
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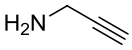
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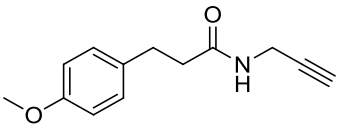
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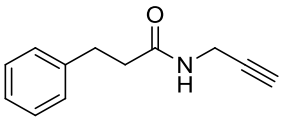
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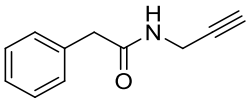
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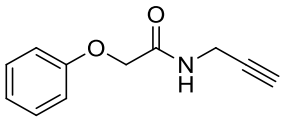
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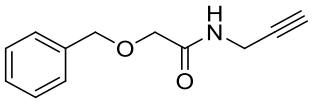
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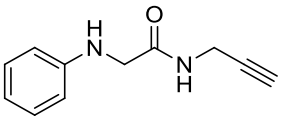
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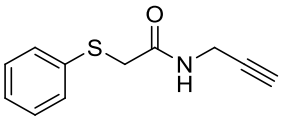
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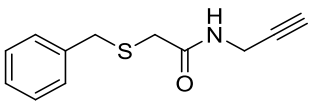
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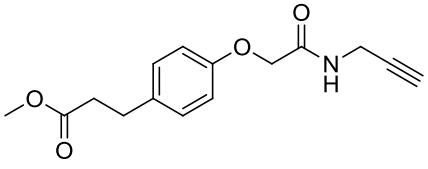
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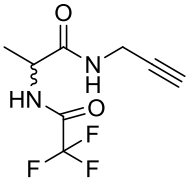
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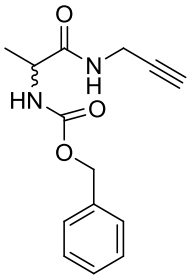

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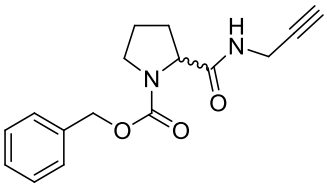

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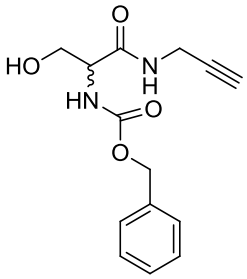

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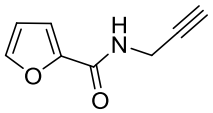

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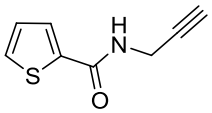

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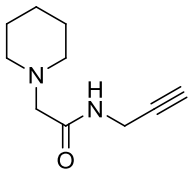

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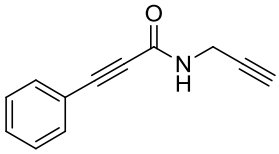

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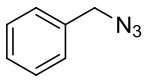

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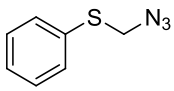

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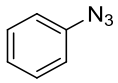

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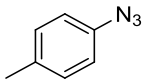

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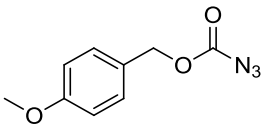

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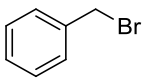

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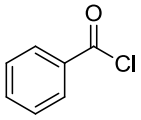

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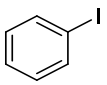

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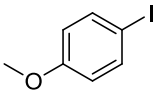

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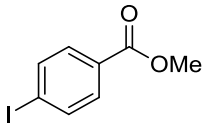

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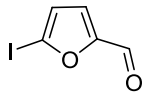

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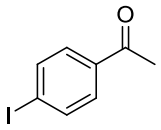

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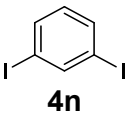

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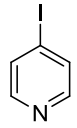

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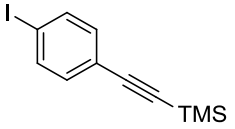

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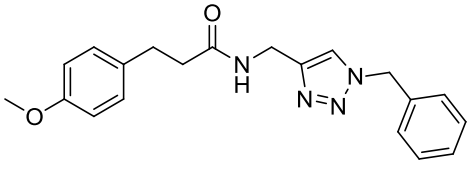

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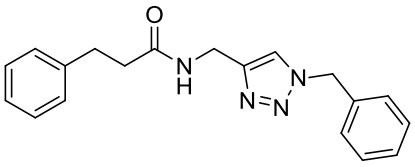

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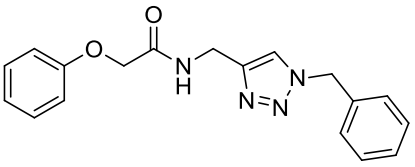

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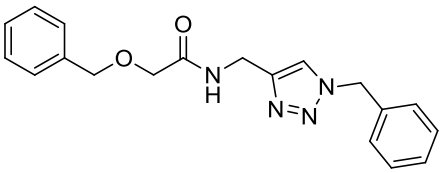

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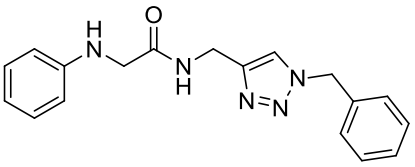

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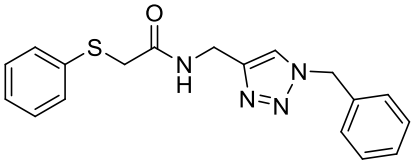

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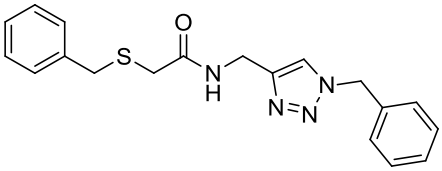

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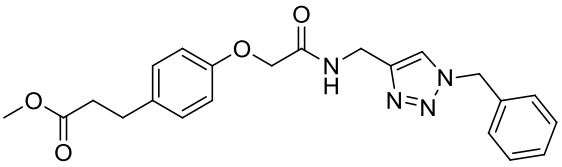

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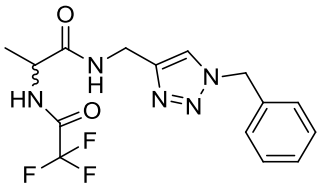

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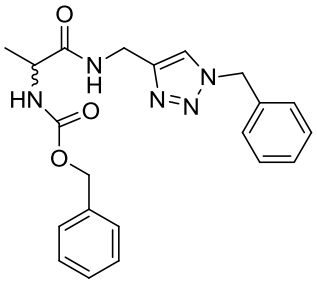

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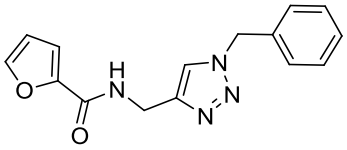

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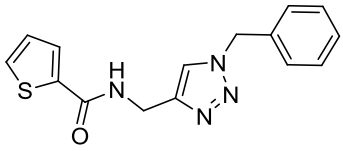

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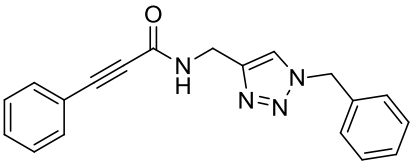

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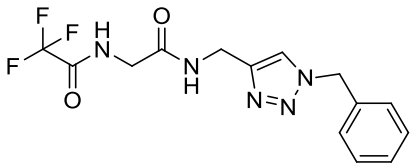

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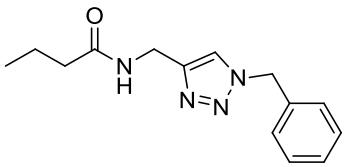

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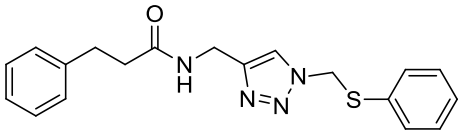

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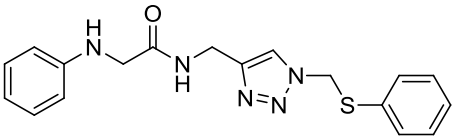

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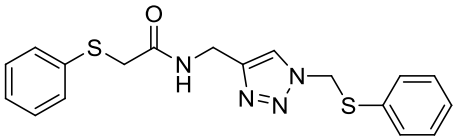

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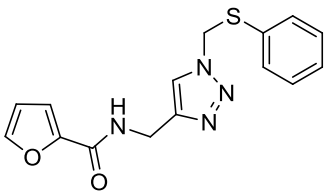

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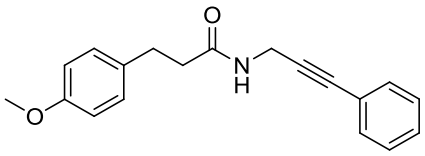

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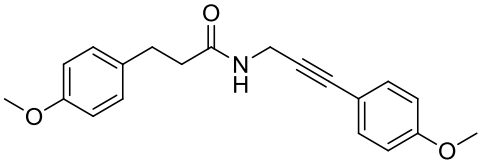

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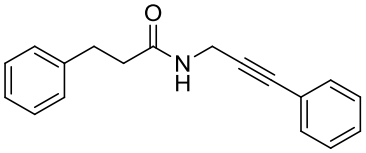

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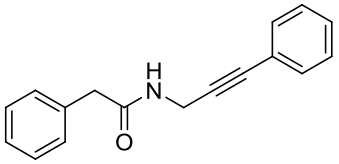

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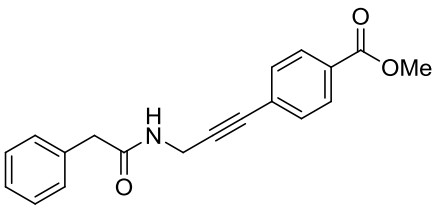

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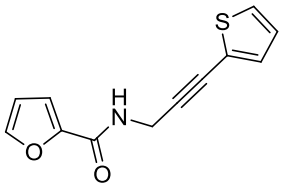

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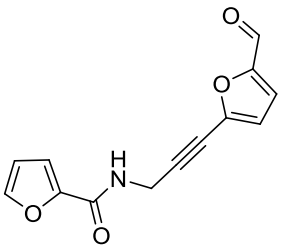

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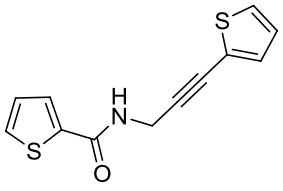

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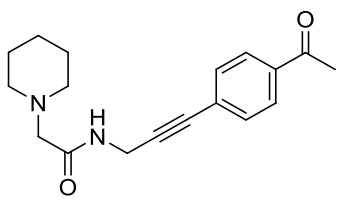

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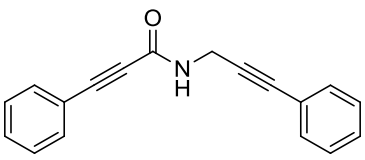

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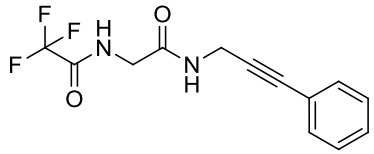

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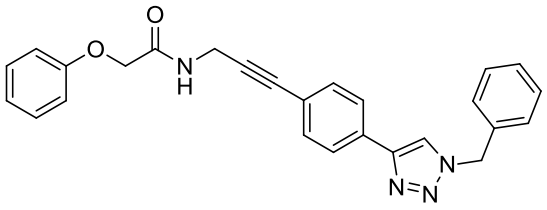

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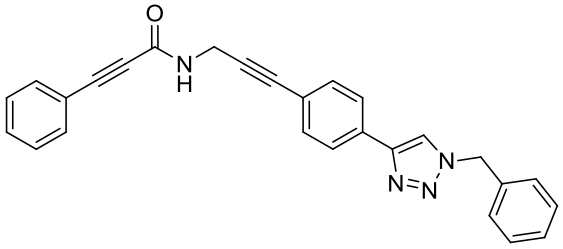

8b

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