

Development of sustainable approaches for the synthesis of biologically relevant molecules

**Thesis submitted in fulfillment of the requirements for the award of the
degree of Doctor of Philosophy**

by

PARMJEET KAUR

(Reg. No. 902009010)



Under the Supervision of

Dr. Vikas Tyagi

Associate Professor

**Department of Chemistry and Biochemistry
Thapar Institute of Engineering and Technology
Patiala-147004, Punjab**

2026

Dedicated to my

mother

Mrs. Ravinder Kaur

Acknowledgements



First and foremost, I bow my head in deep gratitude to the Almighty for His infinite blessings and mercy, for guiding my footsteps and illuminating my path with strength, patience, and wisdom throughout the course of my research. This long-awaited day has finally arrived, and my heart overflows with thankfulness for all those whose contributions made this thesis a reality.

I extend my deepest gratitude to my research supervisor, **Dr. Vikas Tyagi**, for his unwavering support, patience, and guidance during my doctoral journey. His profound expertise, inspiring mentorship, and ever-encouraging words taught me not only how to conduct research but also how to present it with clarity and purpose. His understanding and receptive nature inspired me to push my boundaries and aim higher. Working under his supervision has been an honour and an invaluable learning experience.

My sincere thanks go to **Prof. Padmakumar Nair**, Director, **Prof. R. S. Kaler**, Dean of Research and Sponsored Projects, and **Dr. Bhupendrakumar Chudasama**, Associate Dean of Research and Sponsored Projects, Thapar Institute of Engineering and Technology, Patiala, for their constant support and encouragement. I am profoundly grateful to **Prof. Manmohan Chhibber**, Head of the Department of Chemistry and Biochemistry, for his ever-supportive attitude and good wishes.

I deeply appreciate the insightful discussions and valuable suggestions of my doctoral committee members - **Dr. Kamaldeep Paul**, **Dr. Manmohan Chhibber**, and **Dr. Siddharth Sharma**, which have greatly enriched my work. My heartfelt thanks also go to all the faculty members and staff of the Department of Chemistry and Biochemistry for their cooperation and kind assistance.

I extend my gratitude to **Mr. Chander Thakur**, **Mr. Hemant**, **Mr. Mayank Sharma**, and all the non-teaching staff for their support and help. My sincere thanks to the **Department of Science and Technology** (DST/INSPIRE Fellowship/2019/IF190908) and **Science and Engineering Research Board (SERB), India**, for their financial support throughout this journey. I am also grateful to **Mr. Mukesh Aggarwal**, Laboratory In-charge of SAI labs, for his kind cooperation during the operation of the NMR facility.

My special thanks to **Dr. Kuljit Singh**, Scientist, Infectious Diseases Division at CSIR-Indian Institute of Integrative Medicine (IIIM), Jammu, for performing the biological evaluations of my synthesized compounds.

I feel truly blessed to have shared this journey with my senior colleagues and friends, **Dr. Priya Kamboj** and **Dr. Pooja Soam**, whose guidance, constant support, and encouraging words uplifted me in moments of doubt. Their kindness and companionship will always remain close to my heart. I am equally grateful to **Ms. Kirti Singh** for her cheerful spirit and constant efforts to lift me up during tough times, and to **Mr. Abinash Mohapatra** for his selfless assistance, problem-solving skills, and significant contributions to the computational aspects of my research. I also wish to thank my labmates, **Mr. Ashok Jangra** and **Ms. Simran**, and my seniors, **Dr. Sunil Dutt** and **Dr. Meenakshi Budhiraja**, for their support and cooperation.

I acknowledge with gratitude the guidance and assistance of my seniors and friends: **Dr. Priyanka Gautam**, **Dr. Avneet Kaur**, **Dr. Abida**, **Dr. Aastha Palta**, **Dr. Priti Rohilla**, **Anshu Tyagi**, **Mallika Phull**, **Akanksha Ranade**, **Kamya Jasuja**, **Anuva Mondal**, **Simran Guleria**, **Vaishali** and other research fellows who enriched my journey in countless ways.

Words cannot fully express the depth of gratitude I hold for my beloved mother, **Mrs. Ravinder Kaur**, whose unconditional love, countless sacrifices, and unwavering support have been the cornerstone of my aspirations. Her wisdom and prayers have been a guiding light, carrying me through every challenge. I am deeply thankful to my younger brother and sister, **Satnam Singh** and **Gurpreet Kaur**, for their constant care and for ensuring our mother's well-being in my absence, which gave me the peace of mind to dedicate myself fully to this journey. Their love, encouragement, and silent sacrifices have been a source of strength and motivation throughout this period.

To my husband, **Mr. Gurinder Singh**, I owe a gratitude beyond words. Thank you for being my unwavering support, my strength, and my greatest encouragement - for believing in me when I doubted myself, for lifting my spirits during difficult times, and for inspiring me to pursue my goals with determination. Your love, patience, and quiet strength have been a guiding light, helping me navigate every challenge and making this journey truly possible.

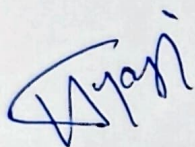
I am deeply thankful to my father-in-law and mother-in-law, **Mr. Chamkaur Singh** and **Mrs. Paramjit Kaur**, for embracing me as their own daughter and showering me with unconditional love, blessings, and encouragement. Their warmth, prayers, and support became a source of strength and peace of mind, helping me stay focused and motivated to complete this journey.

I express my gratitude to all individuals who, despite not being acknowledged here, have provided assistance, whether consciously or unconsciously, in the successful compilation of my thesis.

Paramjeet Kaur
Paramjeet Kaur

Certificate

This is to certify that the thesis entitled "**Development of sustainable approaches for the synthesis of biologically relevant molecules**" submitted by **Parmjeet Kaur**, Department of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala, Punjab for the award of the degree of Doctor of Philosophy, is a bonafide piece of the research work carried out by her. **Parmjeet Kaur** has worked under my guidance and has fulfilled the requirements for the submission of the thesis. The results embodied in the thesis have not been submitted in a part or full to any other University or Institute for the award of any other degree or diploma.



Dr. Vikas Tyagi

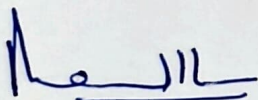
(Supervisor)

Associate Professor

Department of Chemistry and Biochemistry

Thapar Institute of Engineering and Technology, Patiala

Punjab (INDIA)



Dr. Manmohan Chhibber

Professor and Head

Department of Chemistry and Biochemistry

Thapar Institute of Engineering and Technology, Patiala

Punjab (INDIA)

Candidate's Declaration

I, hereby declare that the work presented in the thesis entitled "**Development of sustainable approaches for the synthesis of biologically relevant molecules**" in fulfilment of the requirement for the award of the degree of Philosophy, Department of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala, Punjab is an authentic record of my own work carried under the supervision of **Dr. Vikas Tyagi**, Associate Professor, Department of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala, India. The research work embodied in the thesis has not been submitted in a part or full to any other University or Institute for the award of any other degree or diploma in India or abroad.

Parmjeet Kaur
Parmjeet Kaur

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LIST OF ABBREVIATIONS

| | |
|--------------------------------|--|
| PPL | Lipase from <i>Porcine pancreas</i> |
| CALB | Lipase from <i>Candida antarctica</i> |
| CRL | Lipase from <i>Candida rugosa</i> |
| RML | Lipase from <i>Rhizomucor miehei</i> |
| PFL | Lipase from <i>Pseudomonas fluorescens</i> |
| PCL | Lipase from <i>Pseudomonas cepacia</i> |
| HNTs | Halloysite nanotubes |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| CLEA | Cross-linked enzyme aggregates |
| PdNPs | Pd-nanoparticles |
| DMSO | Dimethyl sulfoxide |
| EtOH | Ethanol |
| MeOH | Methanol |
| THF | Tetrahydrofuran |
| EtOAc | Ethyl acetate |
| K ₂ CO ₃ | Potassium carbonate |
| NaOH | Sodium hydroxide |
| KO ^t Bu | Potassium tert-butoxide |
| ¹ H NMR | Proton Nuclear Magnetic Resonance |
| ¹³ C NMR | Carbon Nuclear Magnetic Resonance |
| ¹⁹ F NMR | Fluorine Nuclear Magnetic Resonance |
| TMS | Trimethyl silane |
| D ₂ O | Deuterium oxide |
| CDCl ₃ | Deuterated chloroform |
| TLC | Thin Layer Chromatography |
| HPLC | High-Performance Liquid Chromatography |
| UV | Ultra-violet Spectroscopy |
| HRMS | High-resolution mass spectrometry |
| FT-IR | Fourier Transform Infra-Red Spectroscopy |

LIST OF ABBREVIATIONS

| | |
|--------------------------------|---|
| FT-NMR | Fourier Transform-Nuclear Magnetic Resonance Spectroscopy |
| XRD | X-Ray Diffraction |
| EDS | Energy Dispersive X-ray Spectroscopy |
| FE-SEM | Field Emission Scanning Electron Microscopy |
| HR-TEM | High-Resolution Transmission Electron Microscopy |
| KPi | Potassium phosphate |
| MOFs | Metal Organic Frameworks |
| KMnO ₄ | Potassium Permanganate |
| ppm | Parts per million |
| rpm | Revolutions per minute |
| mmol/g | Millimole per gram |
| kcal mol ⁻¹ | Kilocalories per mole |
| ⁿ Bu ₄ N | Tetrabutyl ammonium |
| TEMPO | 2,2,6,6-tetramethylpiperidin-1-yl)oxyl |
| BHT | Butylated hydroxytoluene |
| CV | Cyclic voltammetry |
| r.t. | Room temperature |
| n.r. | No reaction |

LIST OF SYMBOLS

| Symbols | Description |
|----------------|--------------------|
| Å | Angstrom |
| C | Celsius |
| T | Temperature |
| g | Gram |
| mg | Milligram |
| nm | Nanometre |
| ° | Degree |
| J | Coupling constant |
| Min | Minute |
| h | Hour |
| s | Second |
| % | Percentage |
| Y | Yield |
| C | Conversion |
| α | Alpha |
| β | Beta |
| μ | Micro |
| γ | Gamma |
| ν | Frequency |
| λ | Wavelength |
| o | Ortho |
| m | Meta |
| p | Para |
| L | Litre |
| δ | Chemical shift |
| V | Volts |
| F | Faraday |
| Pt | Platinum |
| MHz | Megahertz |
| eV | Electron volt |
| v/v | Volume by volume |

LIST OF SYMBOLS

| | |
|--------|---------------------|
| Equiv. | Equivalent |
| M | Molar |
| MM | Molar Mass |
| mA | Milliampere |
| e.r. | Enantiomeric ratio |
| e.e. | Enantiomeric excess |
| E | Enantioselectivity |

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A major goal in modern synthetic chemistry is to develop sustainable and cost-efficient methodologies for the synthesis of complex, chiral molecules-particularly those with pharmaceutical importance. There are number of approaches being used for the sustainable synthesis of organic molecules, including biocatalysis, electrosynthesis, and photocatalysis. Enzymes, or biocatalysts, are integral to synthetic chemistry, offering exceptional chemo-, regio-, and enantioselectivity while operating under mild and environmentally benign conditions. Despite these advantages, their application is often limited by narrow substrate specificity and restricted reactivity. To overcome these limitations, one promising approach is to integrate biocatalysis with other catalytic techniques. These integrated strategies offer new opportunities for selective and green synthesis. However, a significant challenge lies in the potential deactivation of enzymes when used alongside other catalysts, which can compromise the overall catalytic efficiency. To address this challenge, it is essential to identify reaction conditions that preserve the stability and activity of each catalytic component. Achieving such compatibility is key to enabling efficient multi-catalytic cascade processes for the synthesis of drug-like compounds. The first chapter of thesis emphasizes recent progress particularly from the last three years in the field of hydrolase catalyzed reactions integrated with chemical, electro-, and photocatalysis, highlighting their emerging role in advanced synthetic methodologies.

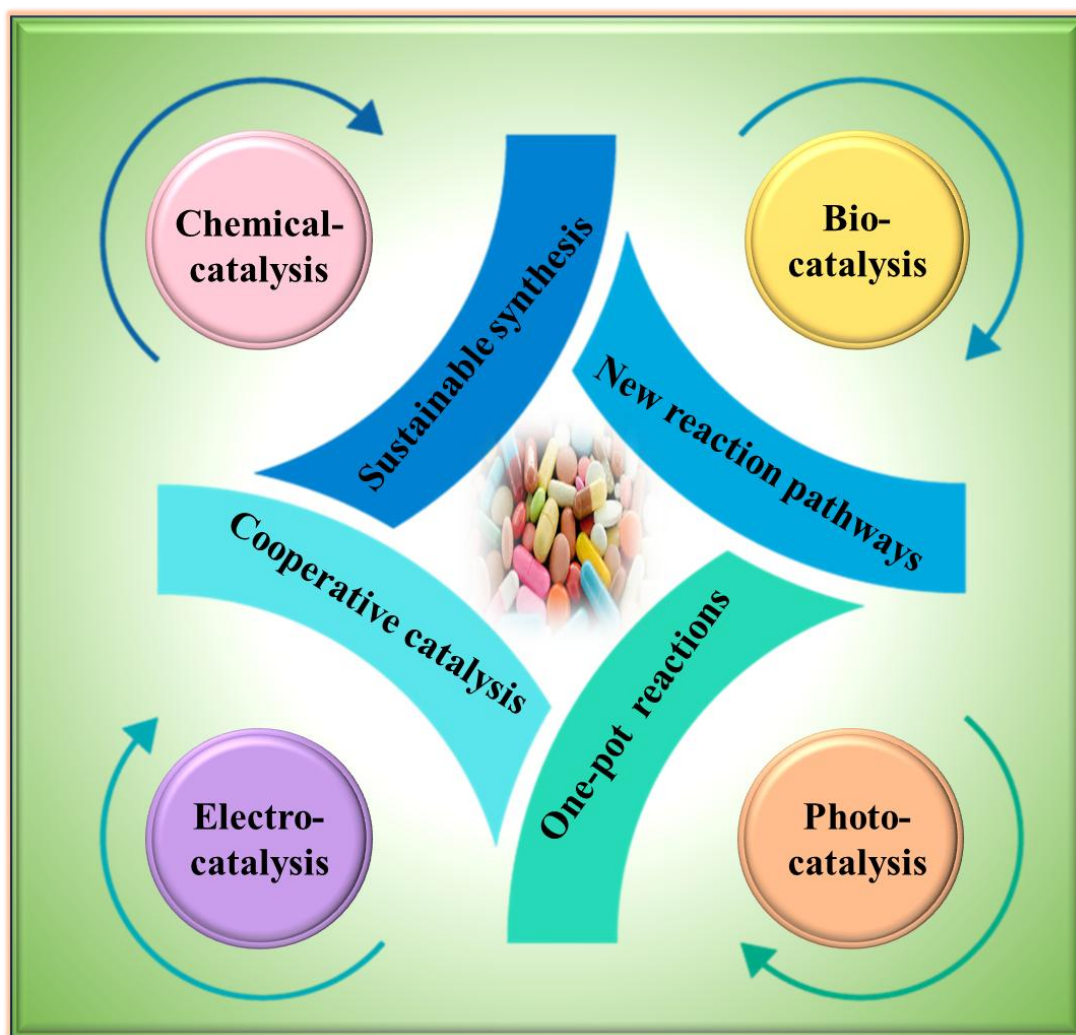
In the second chapter, we have developed a chemoenzymatic approach that combines chemical and bio-catalyst and has proven very useful in synthetic chemistry. However, mutual deactivation of chemical and bio-catalyst when employed in the same pot is still a challenge. In this context, the development of nanobiohybrid catalysts has played an important role and overcoming the issue of mutual deactivation between catalysts to a certain extent. Herein, we design and synthesize a novel heterogeneous recyclable nanobiohybrid catalyst comprising palladium nanoparticles and α -amylase from *Aspergillus oryzae* immobilized onto halloysite nanotubes as a solid heterogeneous support. Further, the wider applicability of the developed nanobiohybrid catalyst is revealed in the one-pot chemoenzymatic synthesis of functionalized biphenyls and bis(indolyl)methanes which consists of Pd-catalyzed Suzuki-Miyaura coupling and α -amylase mediated aza-Michael addition or electrophilic substitution reactions respectively. Further, the robustness and generality of the developed one-pot chemoenzymatic synthesis are demonstrated by incorporating different substitutions at the starting materials and obtaining the corresponding products in moderate to good yields.

In the third chapter of this thesis, we have developed a sustainable process to synthesize chiral sulfur-based organofluorine compounds by integrating electrosynthesis and biocatalysis within a single vessel while using water as a solvent. In this context, differently substituted 2-fluoro-3-mercaptopropionic acids have been synthesized in good isolated yields using thiophenols and fluorine-containing α,β -unsaturated alkenes. In addition, molecular docking and control experiments were carried out that suggest the formation of radical species during the electrolysis and participation of the lipase active site during the biocatalysis. The scalability and applicability of the developed protocol have been illustrated through the synthesis of a key intermediate of the MMP-3 inhibitor and by performing a gram-scale reaction. Further, the compatibility of the lipase enzyme with electricity highlights the promising potential of enzymatic electrosynthesis in advancing environmentally friendly organic transformations.

In the fourth chapter, we reported a novel metal-free electrochemical strategy for the regioselective *N*-sulfonylation of *in-situ* generated indole-based hydrazones using readily available sodium sulfinates. The feasibility of the protocol was demonstrated by employing differently substituted aldehydes, hydrazines, and sodium sulfinates to access *N*-sulfonylated products up to 81% isolated yield. Further, various control experiments and cyclic voltammetry studies have been performed to get valuable mechanistic insights. These studies suggested the formation of indole-phenyldiazonium as an intermediate while ruling out the possibility of any radical formation during this transformation. In addition, the synthesized compounds were tested for antibacterial activity against Gram-positive and Gram-negative pathogens. Among them, compounds **5d**, **5e**, **5l**, and **5q** were found to have strong and selective antibacterial activity against *Staphylococcus aureus*, with **5d** being the most potent (MIC = 6.87 μ M), while showing only moderate activity against Gram-negative pathogens. Furthermore, scanning electron microscopy (SEM) analysis revealed that the most promising hit (**5d**) causes significant morphological alternations and exerts its effects by causing considerable cellular damage.

CHAPTER 1

Introduction and Literature Review



1.1: Introduction

In recent years, the concept of sustainability has gained significant attention across diverse industries, including pharmaceuticals, agrochemicals, polymers, energy, and fuels.¹ In chemistry, sustainability refers to the design and development of chemical methodologies that reduce environmental effects, conserve resources, and ensure long-term ecological stability.² It primarily relies on the twelve principles of green chemistry to develop safe, efficient, and environmentally friendly methods for the safer production of pharmaceuticals, chemicals, materials, and beyond.³ Moreover, sustainability offers several advantages in organic synthesis, such as enhanced selectivity and reactivity, reduced environmental impact, access to novel chemical space, utilization of renewable resources, waste minimization, etc.⁴

In the past decades, synthetic methodologies have focused primarily on achieving high yields and product purity, often overlooking energy consumption, toxicity, and waste generation.⁵ Additionally, the traditional synthetic approaches, particularly at industrial scales, rely heavily on harmful chemicals and solvents, leading to massive waste production and a negative impact on both the environment and human health.⁶ Therefore, rising environmental and economic concerns have prompted the scientific community to concentrate on inventing new and replacing old synthetic methods that are not only efficient and scalable, but also cost-effective and environmentally beneficial. Furthermore, sustainable synthesis in organic chemistry can be achieved in different ways, such as by using green solvents and catalysts, biomass-derived chemicals, and generating no toxic waste during reactions.⁷

In the last decade, biocatalysis has gained recognition as an environmentally benign and sustainable catalysis in organic synthesis.⁸ Moreover, biocatalysis refers to the use of whole cells, isolated enzymes, or other biomolecules to accelerate the rate of chemical reactions.⁹ It offers several notable advantages. First, enzymes are exceptionally efficient catalysts, capable of increasing reaction rates by factors of 10^8 to 10^{10} compared to uncatalyzed processes.¹⁰ Second, unlike metal catalysts, enzymes are biodegradable and environmentally safe. Third, they function under mild conditions, typically at temperatures between 20-50 °C and often in aqueous media, without the need to protect functional groups, thereby minimizing undesired side reactions such as isomerization, racemization, decomposition, and rearrangement, which are common in traditional chemical methods. Fourth, enzymes demonstrate high levels of chemo-, regio-, and stereoselectivity when used as a catalyst in the reactions (**Figure 1.1**).¹¹ As a result, biocatalysis has increasingly become the preferred method for synthesizing chiral molecules and key precursors in the chemical and pharmaceutical industries in recent years.¹²

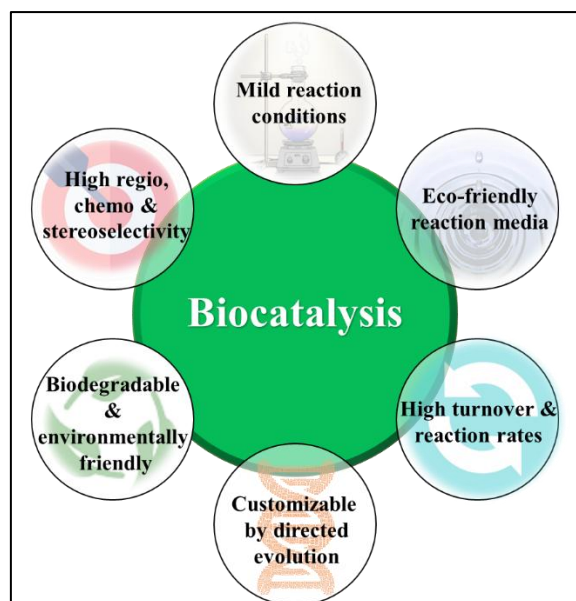


Figure 1.1: Advantages of biocatalysis in sustainable organic synthesis

Further, one of the key breakthroughs in the area of biocatalysis, which has attracted a lot of attention in recent years, has been directed evolution.¹³ This technology enables the efficient engineering and customization of enzymes to adapt the diverse reaction conditions. Through this technique, enzymes can be tailored to accept non-natural substrates and operate under a broad range of pH values and extreme temperatures.¹⁴ This has significantly expanded their applicability, allowing for stereoselective, specific, and complex chemical transformations.¹⁵ Enzymes being used in biocatalysis are broadly classified into seven categories based on their catalytic activity, which have been presented in **Table 1.1**.¹⁶

Table 1.1: Classification of enzymes based on their catalysis

| EC class | Enzyme | General catalytic action | Examples |
|----------|-----------------|--|--|
| EC1 | Oxidoreductases | Redox reactions (electron transfer) | Cytochrome oxidase, NADH dehydrogenase, etc. |
| EC2 | Transferases | Functional group transfer | Kinase, aminotransferase, etc. |
| EC3 | Hydrolases | Water mediated hydrolytic bond cleavage | Lipase, amylase, etc. |
| EC4 | Lyases | Addition/removal of groups to form double bonds without hydrolysis | Aldolase, fumarase, etc. |
| EC5 | Isomerases | Isomerization (rearrangement within the molecule) | Racemase, Phosphoglucose isomerase, etc. |
| EC6 | Ligases | Bond formation using ATP | DNA ligase, synthetase etc. |
| EC7 | Translocases | Movement of ions/molecules across membranes | ATP synthase, Sodium/Potassium ATPase, etc. |

Among different classes of enzymes, hydrolases are widely utilized biocatalysts in industrial organic synthesis, valued for their impressive features such as high stability in organic solvents,

high chemo-, regio-, and stereoselectivity, broad substrate scope, commercial availability, and the ability to operate without cofactors.¹⁷ Their functionality in both aqueous and non-aqueous environments, along with their operational simplicity, makes them highly appealing to chemists. In addition to their well-established roles in hydrolysis, esterification, acylation, and amidation reactions, hydrolases have also demonstrated the ability to catalyse non-natural transformations that extend beyond their classical hydrolytic functions.¹⁸ The discovery and exploration of such unusual reactivities present new opportunities to further expand the scope of hydrolase-mediated transformations in organic synthesis.¹⁹

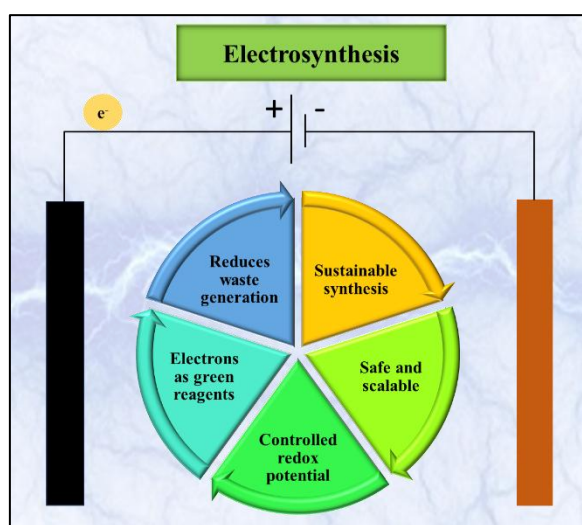


Figure 1.2: Key features of electrocatalysis

On the other hand, organic electrocatalysis is another novel, sustainable, and eco-friendly strategy for synthesizing organic compounds, utilizing inexpensive and potentially renewable electrical energy to drive redox reactions.²⁰ Since electrochemical processes generally require mild conditions, they eliminate the need for external oxidants. They often produce the desired products in shorter times while conserving energy, as hazardous oxidizing and reducing agents are replaced by electrons (**Figure 1.2**).²¹ Moreover, by simply adjusting the applied current, it is possible to precisely control the oxidation and reduction potentials of the system.²² This tunability offers a significant advantage over conventional methods. This flexibility helps minimize side reactions and byproduct formation, enabling cleaner reactions with high efficiency.²³ However, enantioselective organic synthesis remains a challenge in electrocatalysis due to the high reactivity of free radicals generated during the process.²⁴ It often requires chiral ligands, catalysts, or transition metals, but the limited compatibility of these chiral catalysts within electrochemical cells makes it difficult to obtain chiral molecules.²⁵ Therefore, developing new approaches for asymmetric synthesis is crucial to achieving more

sustainable catalytic systems in organic electrosynthesis. In addition, the development of multistep, one-pot cascade reactions has become a central focus in modern green chemistry and synthetic organic methodologies.⁵ By combining distinct catalytic modes within a single process, these cascades enhance substrate tolerance, reactivity, and stereoselectivity.²⁶ Importantly, they improve overall synthetic efficiency by increasing yields, reducing reaction times, and minimizing the need for intermediate purification.²⁷ In this context, to achieve multistep synthesis in a one-pot fashion, researchers in recent decades have explored the integration of biocatalysis with other catalytic disciplines such as chemocatalysis, electrocatalysis, and photocatalysis.²⁸ This interdisciplinary approach aims to expand the accessible chemical space and enable the synthesis of a broader range of valuable chiral compounds (**Figure 1.3**).²⁹ However, merging biocatalysis with other catalytic strategies in a one-pot setup presents significant challenges, primarily due to incompatibilities in optimal reaction conditions.³⁰ Enzymes typically require mild, aqueous, and neutral pH environments, while chemical catalysts often operate under a broader range of conditions such as elevated temperatures, extreme pH values, or organic solvents, or electricity generally necessitate redox mediators and uncontrollable free radical species.³¹ As a result, simultaneous operation of both catalytic systems within a single pot is very limited, as most successful examples rely on sequential strategies that allow each catalyst to operate under its preferred conditions.³²

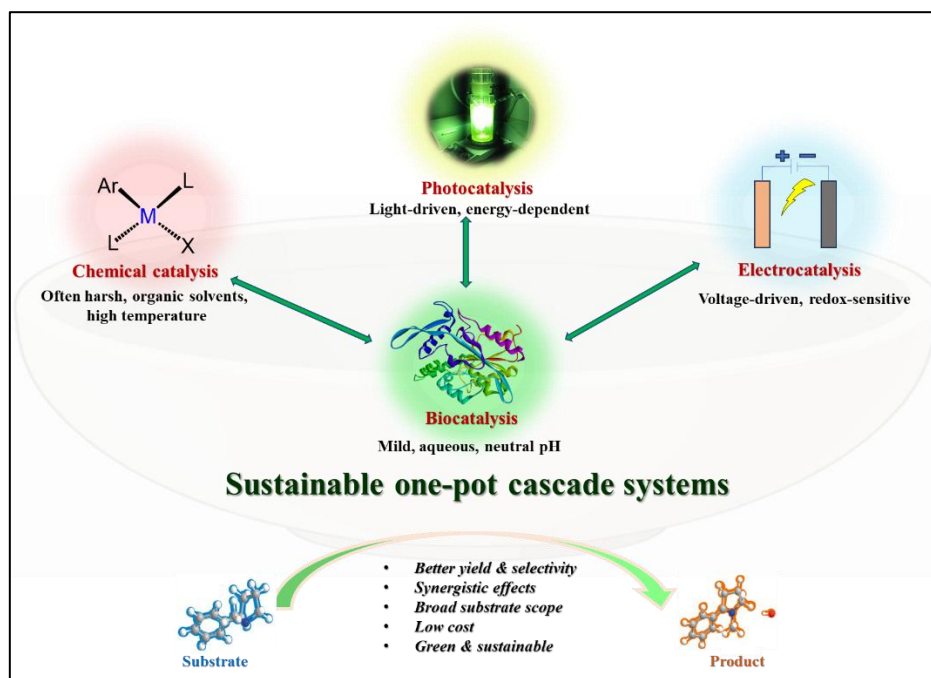


Figure 1.3: A representation of the integration of biocatalysis with chemocatalysis, photocatalysis, or electrocatalysis in a one-pot reaction system and its advantages

This chapter highlights recent advancements mainly reported in the last three years in the designing and execution of hydrolase-based cascade systems in synergy with other catalytic approaches, emphasizing their potential for efficient, sustainable, and environmentally friendly synthesis of complex organic compounds.

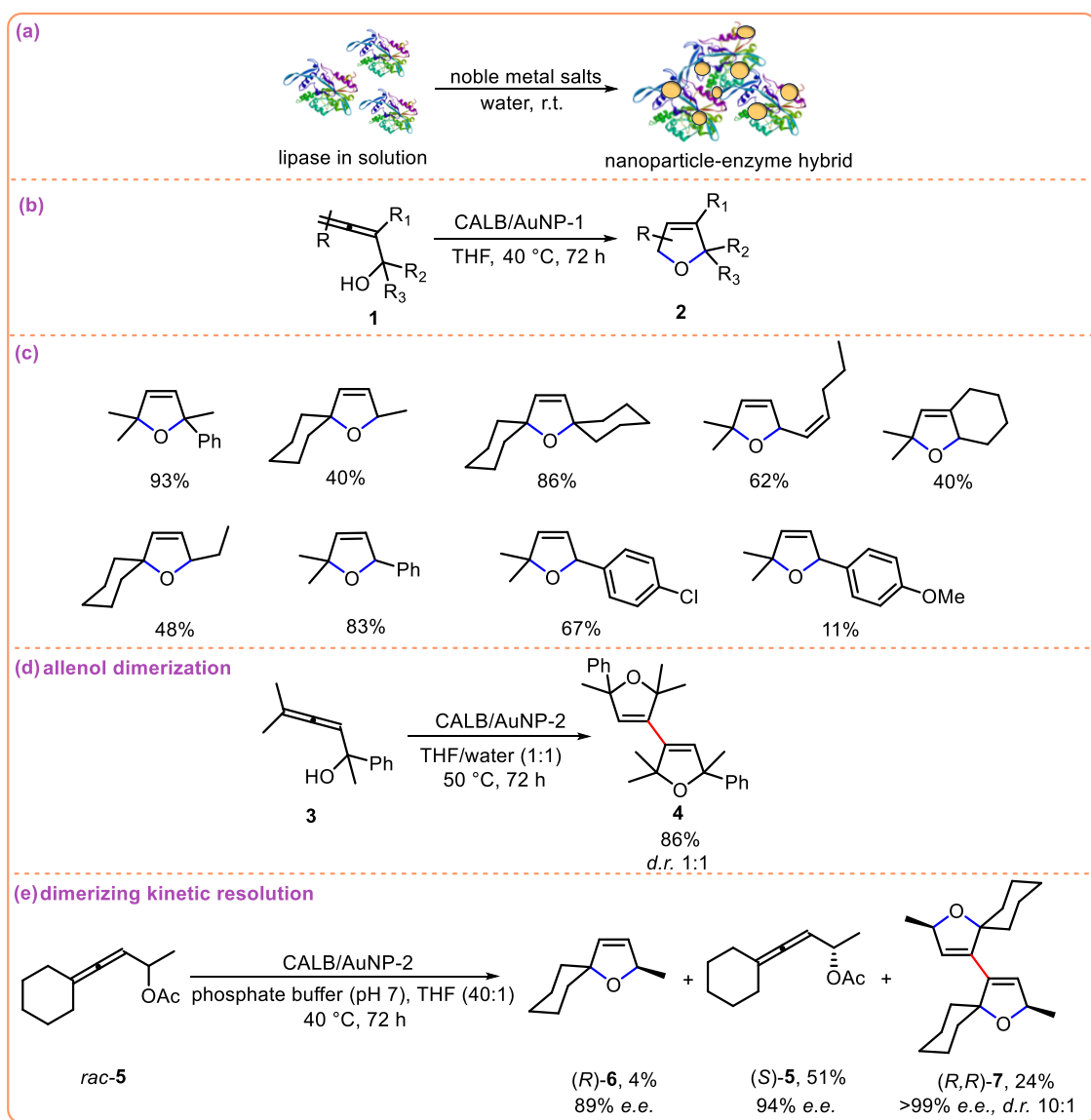
1.2: Literature Review

1.2.1: Integration of bio- and chemical-catalysts

The integration of biocatalysis and chemical catalysis offers notable advantages, as it eliminates the need for additional efforts in setting up multicatalytic reactions. In this approach, the product of one step seamlessly serves as the reactant for the next, ultimately saving time, solvents, and costs.³³ However, certain limitations persist, such as low final product conversions and mutual deactivation of enzymes and chemical catalysts in one-pot systems. To address these challenges, chemists have increasingly adopted the metal-enzyme nanohybrid strategy in recent years.³⁴ In this approach, the hybrid catalysts are designed to spatially separate the chemical catalyst and the enzyme, minimizing direct contact and thereby reducing the risk of deactivating their individual catalytic activities.³⁵

In this context, Deska and co-workers reported the design and synthesis of nanobiohybrids comprising gold or silver nanoparticles embedded within a protein matrix (**Scheme 1.1a**).³⁶ This heterogeneous catalyst, having dual activity, was prepared by incubating the Lipase from *Candida antarctica* (CALB) enzyme with the desired metal salts in aqueous solution at room temperature, followed by centrifugation and lyophilization. The dual functionality of the resulting hybrid catalysts was evaluated via a one-pot cascade synthesis of chiral 2,5-dihydrofurans from allenic acetates (**Scheme 1.1b**). In this reaction, the lipase catalyzed the hydrolytic kinetic resolution, while the metal component facilitated allene cycloisomerization. To demonstrate the applicability of the hybrid catalysts, CALB/AuNP-1 was employed for the synthesis of 2,5-dihydrofurans using tetrahydrofuran (THF) as the solvent at 40 °C. Interestingly, more α -substituted allenes underwent cyclization more efficiently than α -monosubstituted allenic alcohols. The CALB/gold nanomaterial provided moderate yields of the desired products i.e. 40-48%, when using allenes containing saturated hydrocarbon chains (**Scheme 1.1c**). In contrast, allenes bearing methyl or aromatic groups in the allylic olefin moiety produced dihydrofurans more efficiently. Additionally, the formation of bisdihydrofurans through dimerization was observed with all hybrid catalysts, but it occurred more prominently with dextran-based polymer-modified CALB/AuNP-2 (**Scheme 1.1d**). The

utility and versatility of this catalytic system were further demonstrated through the synthesis of stereoselective multicyclic structures with excellent enantiopurity (**Scheme 1.1e**).

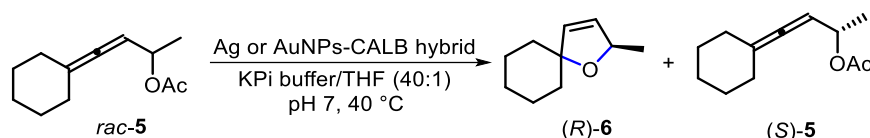


Scheme 1.1: CALB/gold nanobiohybrid catalyzed synthesis of various 2,5-dihydrofurans

Similarly, Palomo and co-workers devised the synthesis of nanohybrids composed of gold (Au) or silver (Ag) nanoparticles integrated with the lipase enzyme (**Table 1.2**).³⁷ The formation of these metallic nanohybrids is highly influenced by the choice of protein, bioconjugate, and pH. When lipase was modified with a bioconjugate-specifically dextran-aspartic acid polymer (Dext6kDa) and used in the synthesis of Au or Ag nanohybrids, smaller metal nanoparticles were observed. These nanobiohybrids exhibited dual catalytic activity, enabling the synthesis of enantiopure oxygen containing-heterocycles through a cascade reaction involving lipase-catalyzed and metal-catalyzed cycloisomerization of allenic acetates. Among the catalysts, Ag-

based nanobiohybrids generated the (*R*)-2,5-dihydrofuran product with good enantioselectivity, though only moderate conversion. In contrast, the AuNPs-CALB-pH4.5 catalyst produced the desired cyclized product with excellent enantioselectivity in nearly half the time compared to the AgNPs-CALB catalyst, while maintaining a similar conversion rate. The lower conversion observed with AgNPs-CALB-Dext6kDa was attributed to a side reaction, as a significant amount of unreacted allenic acetate was recovered with 99% enantiomeric excess (*e.e.*) (Table 1.2).

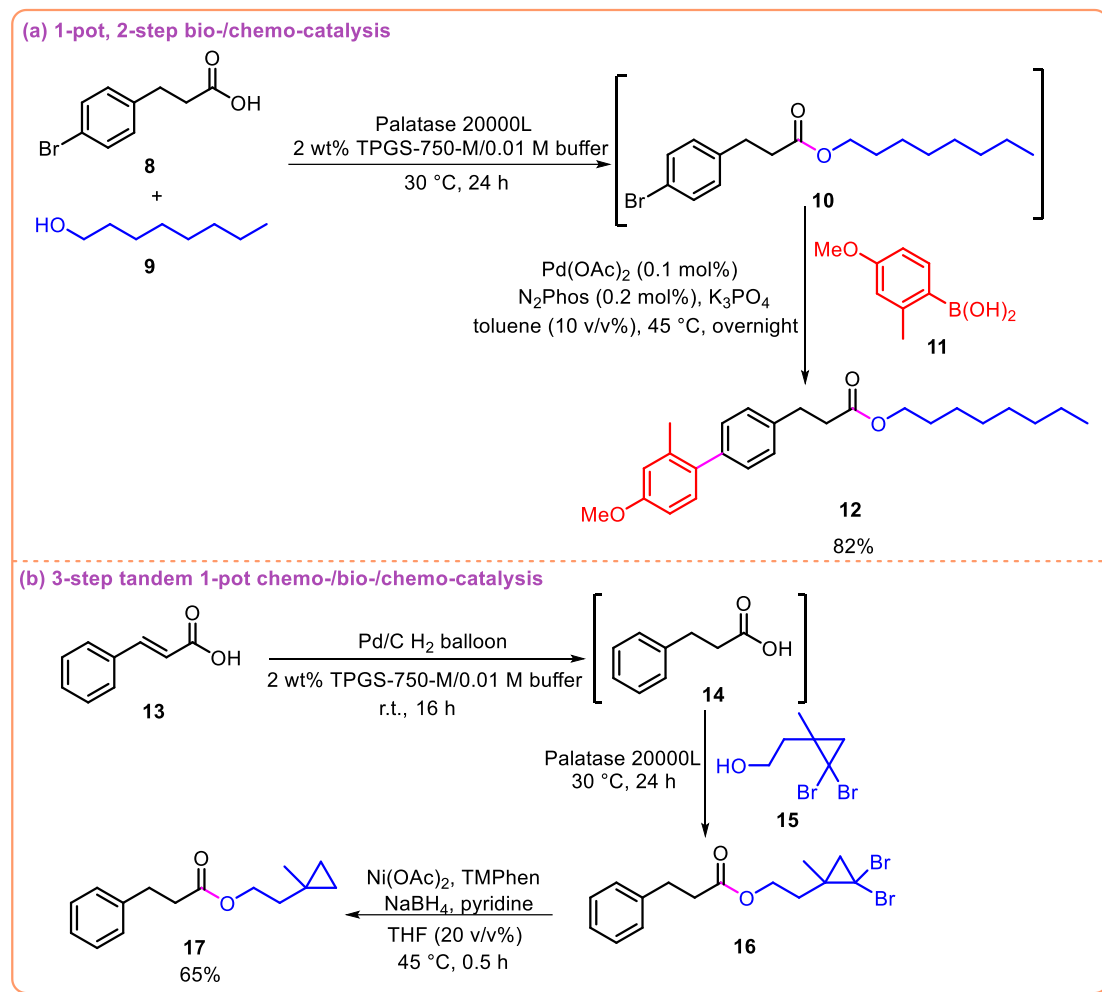
Table 1.2: Screening of metallic nanohybrids towards cycloisomerization of allenic acetates



| Entry | Catalyst | Time (d) | Conversion (%) | (<i>R</i>) <i>e.e.</i> (%) | (<i>S</i>) <i>e.e.</i> (%) |
|-------|------------------------|----------|----------------|------------------------------|------------------------------|
| 1 | AgNPs-CALB | 4 | 26 | 88 | 88 |
| 2 | AgNPs-CALB-Dext6kDa | 3 | 43 | 94 | 94 |
| 3 | AgNPs-CALB-Dext2000kDa | 3 | 42 | 95 | 84 |
| 4 | AuNPs-CALB-pH4.5 | 2 | 21 | 93 | 64 |
| 5 | AuNPs-CALB-Dext6kDa | 3 | 2 | 93 | 99 |

Next, Singhania et al. reported a novel enzymatic protocol employing a commercially available lipase, Palatase 20000L, in combination with the surfactant TPGS-750-M (DL- α -tocopherol methoxypolyethylene glycol succinate) to catalyze esterification reactions in water without the need for any cofactors.³⁸ This biocatalytic process was integrated into a one-pot which consist a lipase-catalyzed esterification to produce an intermediate (**10**), followed by subsequent palladium-catalyzed cross-coupling reaction to afford the final product (**12**) in 82% yield (Scheme 1.2a). Notably, the produced ester (**12**) was proposed to localize within the lipophilic core of micelles rather than the aqueous phase. This hypothesis was supported by observations made when the pH was increased during the coupling step through the addition of a base. The protocol was further extended to mixed-catalysis sequences. In one example, cinnamic acid (**13**) was first catalytically hydrogenated to produce an intermediate acid (**14**), which then underwent esterification with an alcohol (**15**) to generate a gem-dibromocyclopropane (**16**). This intermediate was subsequently reduced using nickel to yield the final product (**17**) in 65%

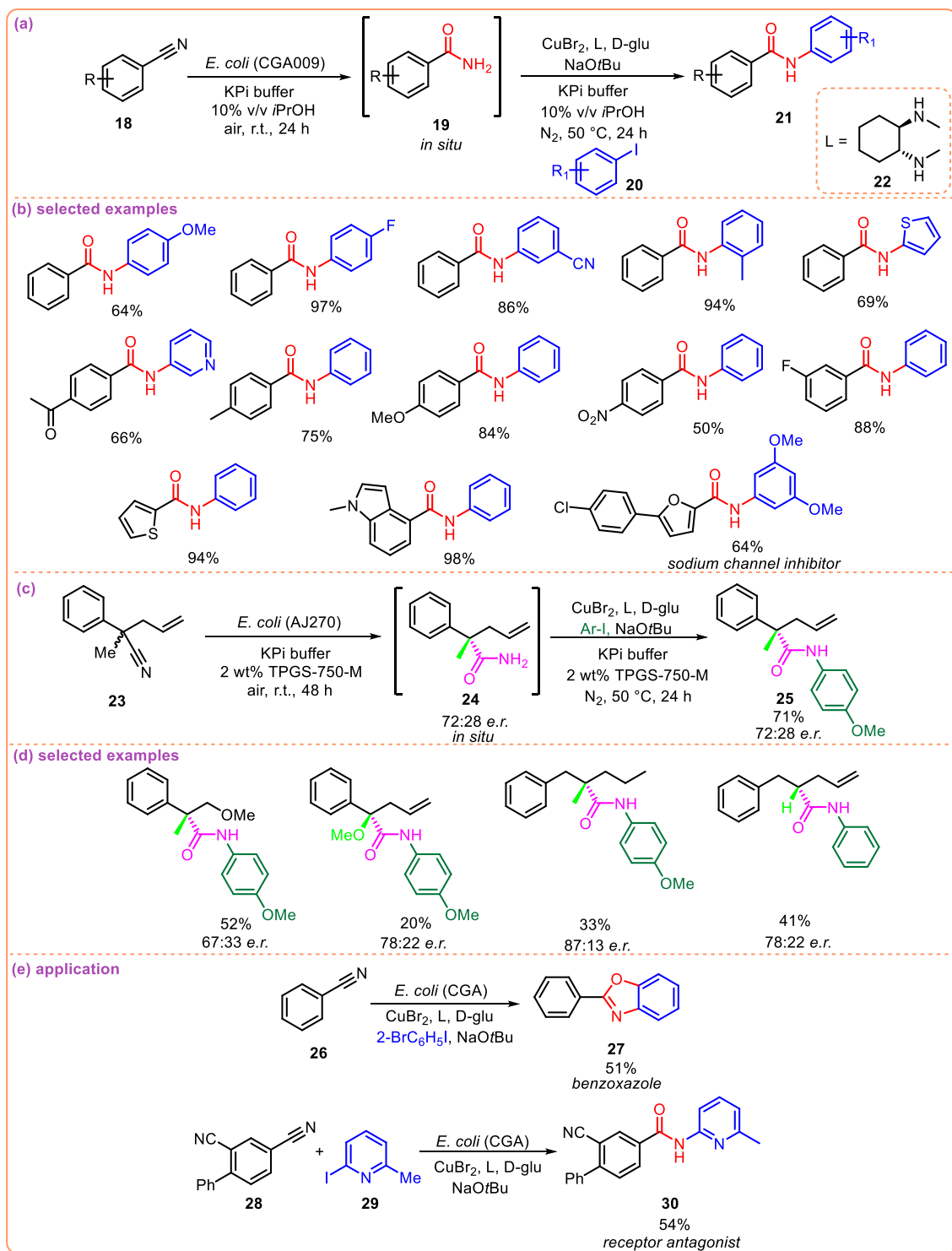
isolated yield. Overall, this one-pot lipase-catalyzed protocol demonstrates significant potential as an environmentally friendly approach for tandem catalysis in aqueous media (**Scheme 1.2b**).



Scheme 1.2: Lipase-catalyzed esterification combined with metal-catalyzed reactions in a one-pot

A one-pot amide bond synthesis involving nitrile hydratase from *E. coli* CGA009, which catalyzed the hydration of nitriles, followed by a copper-catalyzed N-arylation reaction was reported by Micklefield and co-workers (**Scheme 1.3a**).³⁹ This integrated protocol demonstrated broad substrate scope and excellent functional group tolerance. Various ortho-, meta-, and para-substituted iodoarenes were well tolerated, yielding the desired amides in good yields (**Scheme 1.3b**). Additionally, iodinated heterocycles were smoothly converted to the target products with high efficiency. The versatility of this method was further illustrated using nitriles bearing diverse substituents at the ortho, meta, and para positions, and various heterocyclic nitriles were effectively transformed using the *E. coli* CGA009 enzyme, giving excellent yields of the corresponding amides (**Scheme 1.3b**). The practical utility of this chemoenzymatic strategy was highlighted by the successful synthesis of a sodium channel

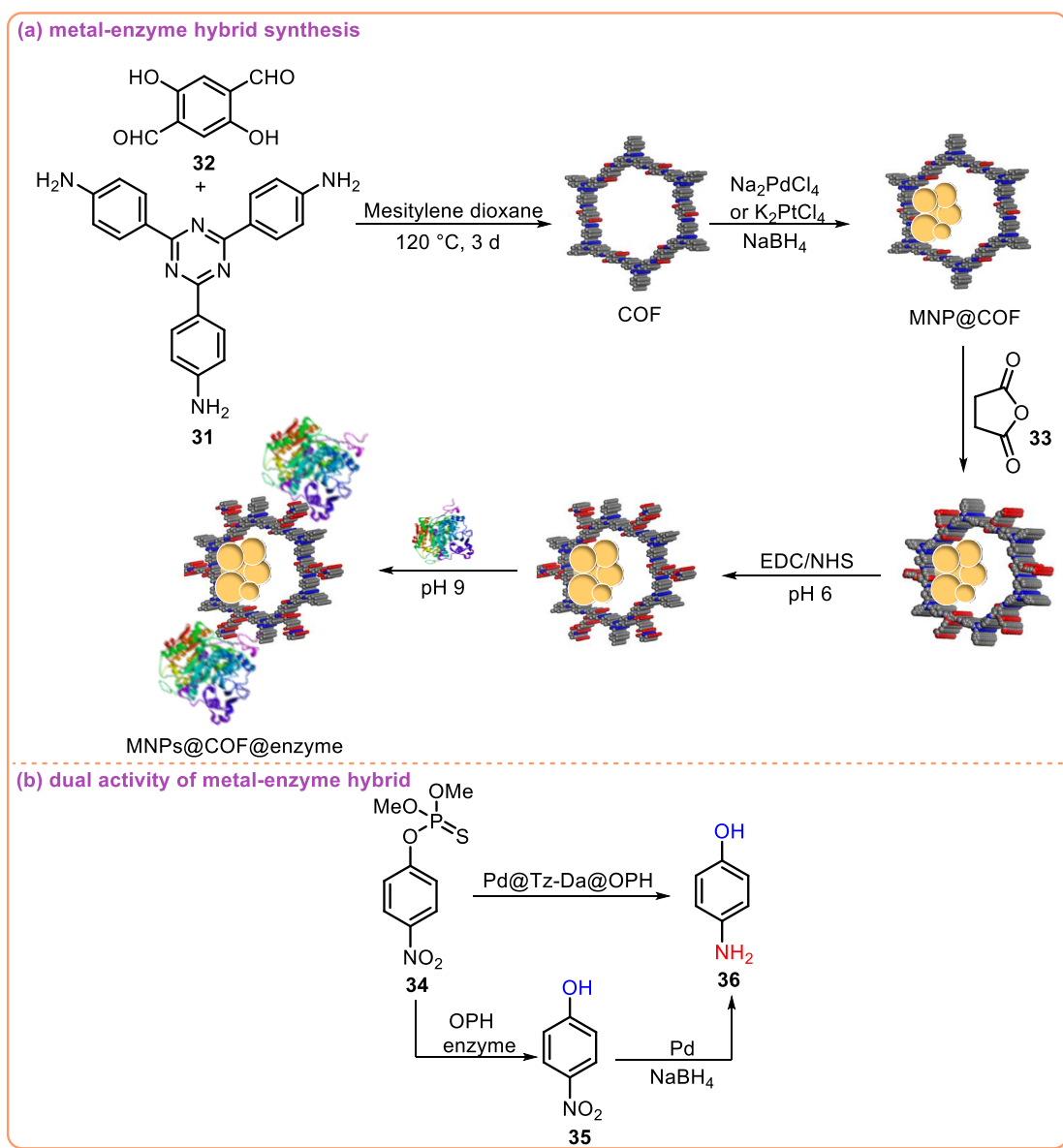
inhibitor, suggesting its potential for the preparation of other pharmaceutical intermediates (Scheme 1.3b). Notably, nitrile hydratase (AJ270) also enabled asymmetric synthesis of amides within this integrated approach, affording ether-functionalized chiral amides in good



Scheme 1.3: One-pot amide bond synthesis via *E. coli* CGA009-derived nitrile hydratase-catalyzed hydration followed by copper-catalyzed N-arylation.

yields and with favorable diastereomeric ratios (**Scheme 1.3c**). Furthermore, allyl- and propyl-substituted amides were synthesized with good yields and selectivity (**Scheme 1.3d**). The synthetic utility of the method was also demonstrated by the preparation of heterocyclic scaffolds and extended to the synthesis of a drug target (**30**) in 54% overall yield, significantly improving upon a previously reported six-step linear route that gave much lower yield (**Scheme 1.3e**).

In 2022, Jiang and co-workers disclosed the synthesis of a multifunctional nanocatalyst through the co-immobilization of metal nanoparticles and enzymes onto the pores and surface of a stable covalent organic framework (COF) (**Scheme 1.4a**).⁴⁰ The COF was prepared via a solvothermal method using triazinyl (Tz) and 2,5-dihydroxyterephthalaldehyde (Da) as building blocks. The nitrogen-rich structure of Tz enabled the in-situ reduction and uniform dispersion



Scheme 1.4: Synthesis of multifunctional Pd@Tz-Da@OPH nanocatalyst and its application

of palladium (Pd) nanoparticles within the COF through the double solvent method, facilitated by strong electronic interactions between nitrogen atoms and Pd. The hydroxyl groups on Da were further functionalized to carboxyl groups using succinic anhydride (SA), allowing for the covalent immobilization of organophosphorus hydrolase (OPH) via EDC/NHS coupling chemistry. The resulting integrated nanocatalyst, Pd@Tz-Da@OPH, exhibited excellent catalytic performance in the degradation of the toxic organophosphate nerve agent methyl parathion. In this cascade reaction, the OPH enzyme first hydrolyzed methyl parathion (**34**) to 4-nitrophenol (**35**), a still-toxic intermediate that is challenging to degrade. Subsequently, the Pd nanoparticles further reduced 4-nitrophenol to the less harmful compound 4-aminophenol (**36**) in the presence of NaBH₄. The overall reaction rate was primarily governed by the enzymatic hydrolysis step (**Scheme 1.4b**). This recyclable and stable nanocatalyst demonstrates high versatility and holds significant promise for future applications in environmental remediation.

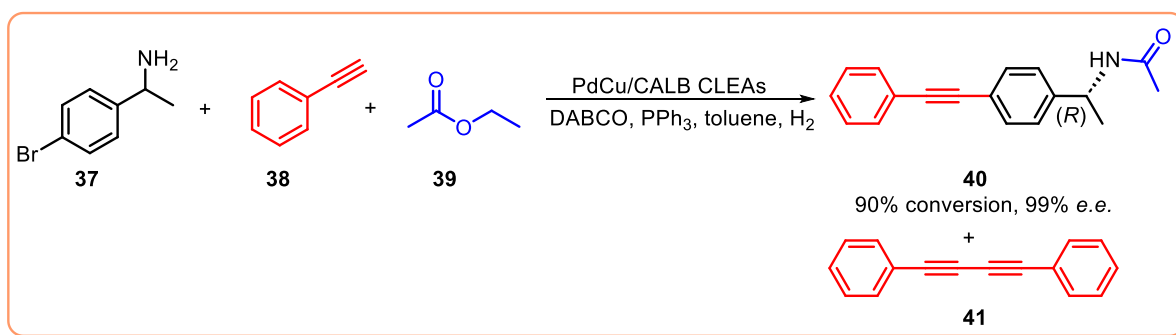
Further, the synthesis of a nanobiohybrid catalyst composed of glucose oxidase (GOx) and glucoamylase (GA) immobilized onto mesoporous silica nanoparticles (SiO₂) was demonstrated by Sun and colleagues.⁴¹ The presence of both enzymes on the catalyst surface was essential for the efficient production of gluconic acid from starch, as they enable the timely removal of hydrogen peroxide (H₂O₂) and glucose. This synergy was further confirmed through experiments using various catalyst combinations, as detailed in the accompanying **Table 1.3**. In the first three systems, where enzymes were absent on the surface of Au@SiO₂ particles, efficient starch consumption was observed, but gluconic acid formation remained limited. However, when both GA and GOx were co-immobilized onto Au@SiO₂ particles (GA&GOx@Au-SiO₂), there was a significant increase in gluconic acid yield along with improved starch degradation. This system outperformed previously reported enzyme immobilization methods, such as those using graphene as a support. Additionally, varying the GA/GOx ratio influenced the overall reaction yield. Increasing the amount of glucoamylase enhanced starch degradation and glucose generation, thereby improving the efficiency of the entire chemoenzymatic process. Notably, the catalyst exhibited excellent recyclability, maintaining its catalytic activity for up to 10 cycles, making it a promising candidate for the industrial-scale production of gluconic acid.

In 2023, Ge and colleagues reported the synthesis of a hybrid catalyst by immobilizing a Pd-Cu nanoalloy onto cross-linked enzyme aggregates (CLEAs) of *Candida antarctica* lipase B

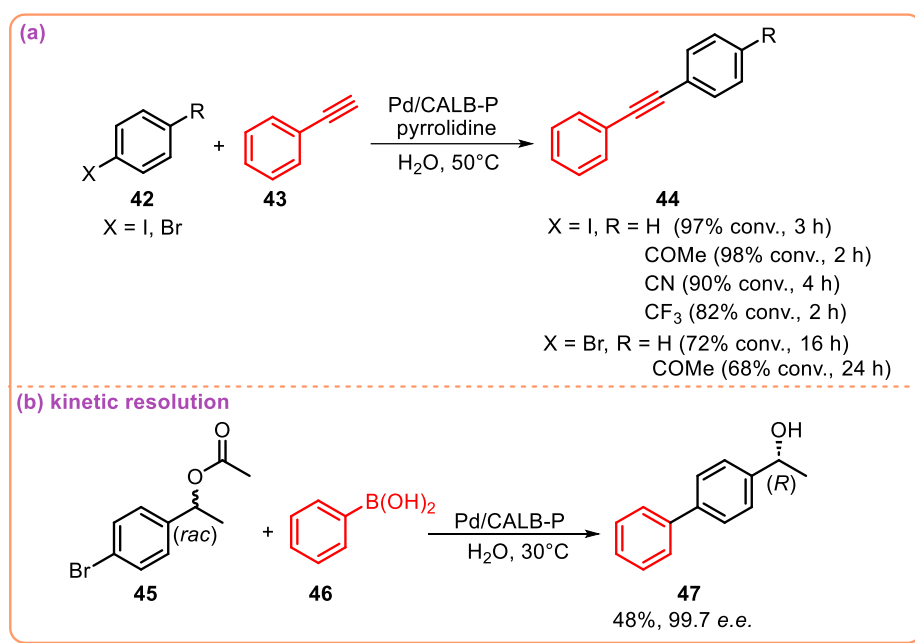
Table 1.3: Examination of different nanobiohybrids for the production of gluconic acid from starch

| Catalytic systems | GA ($\mu\text{g mL}^{-1}$) | GOx ($\mu\text{g mL}^{-1}$) | Au-SiO ₂ ; (mg mL^{-1}) | Starch consumption efficiency (%) | Yield of gluconic acid (%) |
|---|---------------------------------|----------------------------------|--|---|----------------------------------|
| GA+ GOx | 31.1 | 21.6 | 0 | 99.7 | 60.8 |
| GA+ GOx + Au-SiO ₂ | 31.1 | 21.6 | 1.8 | 99.3 | 74.9 |
| GA&GOx@SiO ₂ + Au-SiO ₂ | 26.6 | 18.4 | 1.8 | 73.6 | 63.6 |
| GA&GOx@Au-SiO ₂ (GA/GOx = 1/1.48) | 22.5 | 33.3 | 1.8 | 93.6 | 93.0 |
| GA&GOx@Au-SiO ₂ (GA/GOx = 1.06/1) | 29.1 | 27.5 | 1.8 | 96.4 | 93.8 |
| GA&GOx@Au-SiO ₂ (GA/GOx = 1.44/1) | 31.1 | 21.6 | 1.8 | 98.7 | 97.8 |

(CALB).⁴² The incorporation of copper alongside palladium in the nanoalloy was intended to enhance the hybrid catalyst's performance in chemoenzymatic reactions (**Scheme 1.5**). This improvement was attributed to a synergistic effect between the two metals, which significantly boosted the catalytic activity. The resulting hybrid catalyst demonstrated excellent efficiency in Sonogashira coupling reactions, yielding the desired products in high amounts. In this cascade process, the CALB enzyme catalyzed the selective acylation of the (*R*)-enantiomer of an amine, while the Pd-Cu nanoclusters facilitated both the Sonogashira coupling and the racemization of the (*S*)-enantiomer. Overall, the Pd-Cu/CALB CLEAs exhibited remarkable dual functionality, showing high enzymatic as well as metallic catalytic activity in the cascade reaction.

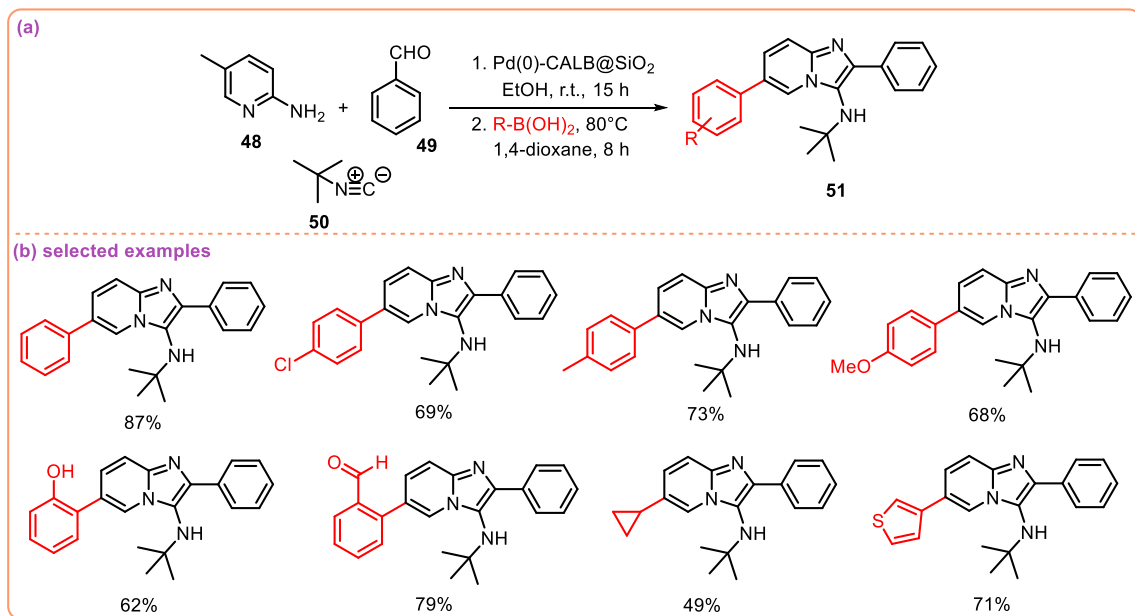
**Scheme 1.5:** Pd-Cu/CALB CLEAs-catalyzed one-pot selective acylation and Sonogashira coupling reaction

Next, the same group designed a hybrid catalyst by anchoring palladium (Pd) single atoms onto the lipase enzyme (CALB), resulting in the formation of Pd/CALB-P (**Scheme 1.6a**).⁴³ The catalytic activity of this system was first assessed through the Sonogashira coupling of aryl halides with alkynes, where it demonstrated superior efficiency compared to traditional homogeneous Pd-catalysts. Further investigation of its substrate scope revealed broad compatibility, accommodating both electron-donating and electron-withdrawing groups. The hybrid catalyst was employed in a one-pot chemoenzymatic reaction integrating lipase-catalyzed hydrolysis and Pd-catalyzed C–C coupling, efficiently producing a chiral biphenyl alcohol at 30 °C with moderate yield and excellent enantioselectivity (**Scheme 1.6b**).



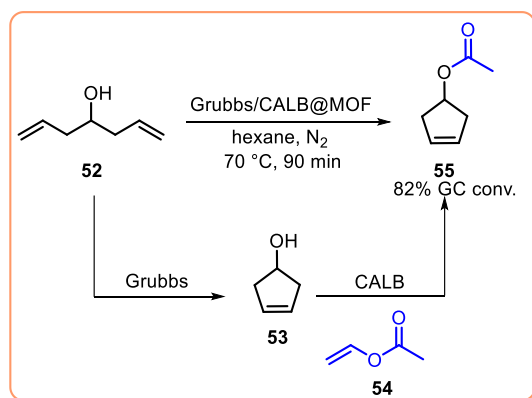
Scheme 1.6: Application of Pd/CALB-P integrated catalyst towards lipase-catalyzed hydrolysis and Pd-catalyzed C–C coupling reaction

Our group has recently developed a nanobiohybrid catalyst by integrating palladium (Pd) metal and the CALB enzyme within a silica matrix, resulting in the formation of Pd(0)-CALB@SiO₂ (**Scheme 1.7a**).⁴⁴ This catalyst was employed in a two-step, one-pot chemoenzymatic reaction sequence involving a CALB-catalyzed Groebke-Blackburn-Bienayme (GBB) multicomponent reaction, followed by a Pd-catalyzed Suzuki-Miyaura coupling. The substrate scope was explored by varying substituents on the phenylboronic acids, successfully yielding a series of imidazo[1,2-a]pyridine derivatives in good to excellent yields (**Scheme 1.7b**). The practical applicability of the catalyst was demonstrated through a gram-scale reaction, and the hybrid system proved reusable for up to five cycles. Although a slight decrease in isolated yield was observed after the second cycle, the catalyst retained considerable activity across multiple runs.



Scheme 1.7: Construction of Pd(0)-CALB@SiO₂ nanocatalyst and its application in the synthesis of imidazo[1,2-a]pyridine derivatives

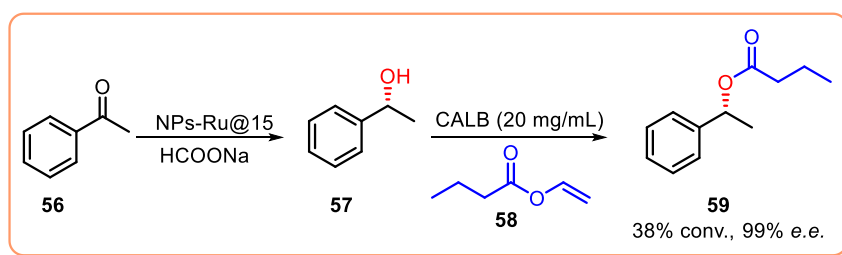
In 2023, Yang et al. constructed a multicompartmental metal-organic framework (MOF) microreactor using the Pickering double emulsion methodology.⁴⁵ This approach leverages multiple liquid-liquid interfaces to guide the controlled growth of dense MOF layers, resulting in microreactors with tailored internal architectures and selective permeability (**Scheme 1.8**). Two distinct catalytic entities—hydrophilic CALB enzyme and hydrophobic Grubb's catalyst—were successfully co-encapsulated within a single microreactor. The catalytic performance was evaluated through a cascade reaction involving Grubb's catalyst-mediated ring-closing metathesis of 1,6-heptadien-4-ol (**52**), followed by CALB-catalyzed transesterification to produce 3-cyclopentenyl acetate (**55**). The resulting MOF microreactor demonstrated a 2-5 fold



Scheme 1.8: Synthesis of 3-cyclopentenyl acetate from constructed microreactor containing Grubbs catalyst and CALB enzyme

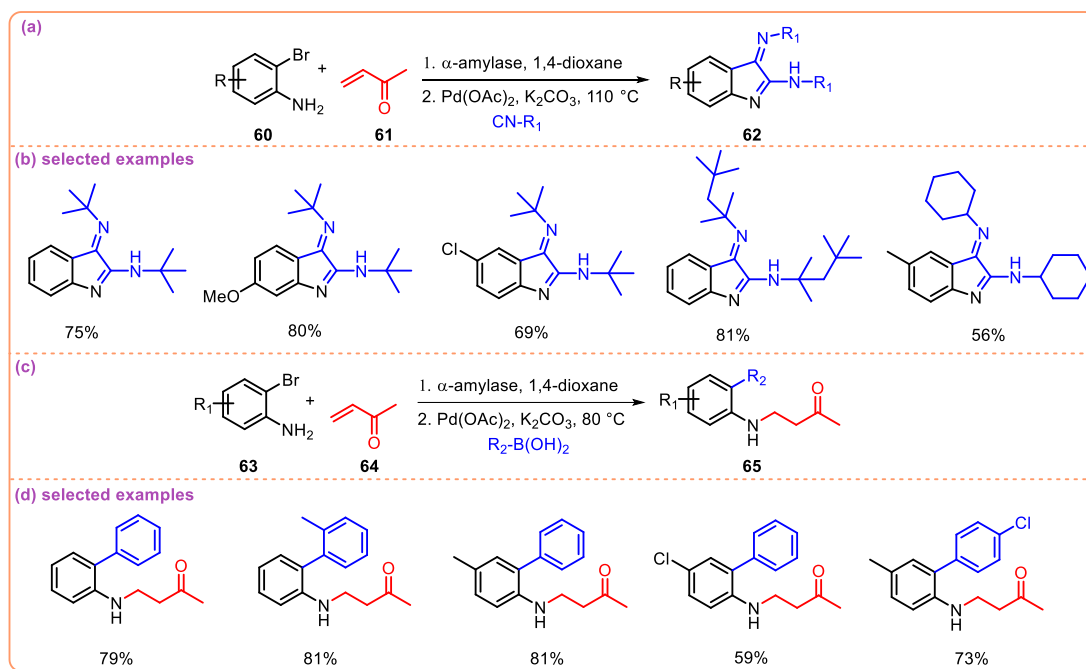
enhancement in catalytic activity compared to individual catalysts, attributed to controlled mutual deactivation and improved substrate channelling.

A simple multistep one-pot platform that integrates chemo- and biocatalysis using nanoparticle-based catalysts was developed by Wu and group members.⁴⁶ These catalysts were synthesized in two steps: first, chiral ligand monomers were polymerized onto silica nanoparticles via atom transfer radical polymerization (ATRP); second, the resulting particles were coordinated with $[\text{RuCl}_2(\text{p-cymene})]_2$ to form the final catalysts. They efficiently catalyzed the asymmetric transfer hydrogenation of acetophenone (**56**) to 1-phenylethanol (**57**) in water, achieving up to 99% conversion and 93% enantiomeric excess (**Scheme 1.9**). Additionally, the nanoparticles served as emulsifiers to form stable Pickering emulsions. Incorporation of CALB enabled a second-step enantioselective acylation with vinyl butyrate (**58**), reaching up to 38% conversion and 99% *e.e.* This strategy demonstrates a versatile and effective approach for sequential chemoenzymatic cascades in aqueous and emulsion systems.



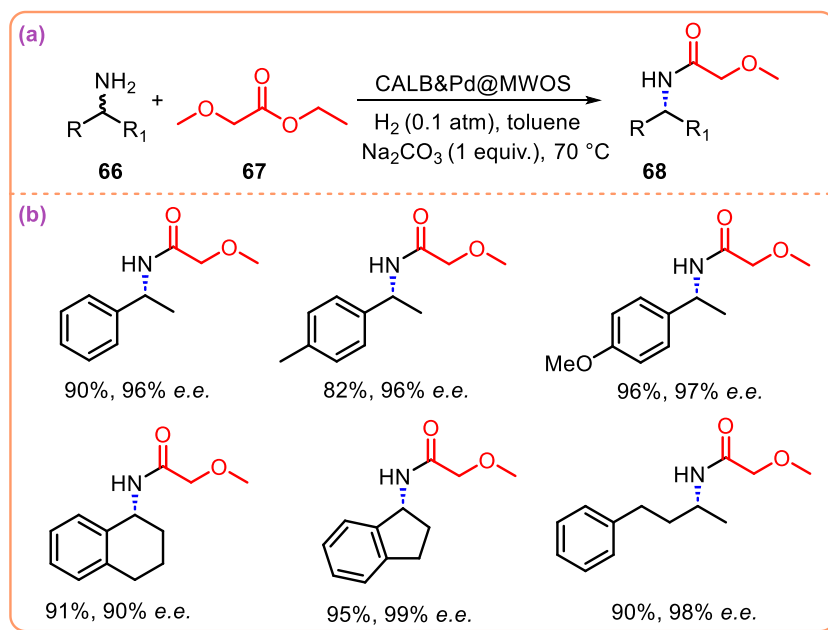
Scheme 1.9: Sequential chemoenzymatic cascade asymmetric synthesis of (*R*)-1-phenylethyl butyrate in water

Also, we have developed a one-pot synthesis for substituted indole derivatives and amino-substituted biaryls in good yields using α -amylase and a Pd-catalyst (**Scheme 1.10a**).⁴⁷ Initially, we synthesized indole derivatives via an α -amylase-mediated aza-Michael addition, followed by a transition-metal-catalyzed isocyanide insertion reaction. The substrate scope was then expanded by employing various bromoanilines and isocyanides (**Scheme 1.10b**). It was observed that electronic effects of the substituents had minimal impact on the yields of the desired products. However, when cyclohexyl isocyanide was used, a slight decrease in yield was noted. Encouraged by these results, we extended our methodology to a one-pot sequence combining the enzyme-mediated aza-Michael addition and a metal-catalyzed Suzuki–Miyaura coupling reaction (**Scheme 1.10c**). Under the same optimized conditions, the substrate scope was evaluated using different substituted boronic acids and bromoanilines. In all cases, good yields of the desired amino-substituted biaryl products were obtained (**Scheme 1.10d**).



Scheme 1.10: One-pot synthesis and substrate-controlled diversification of β -aminocarbonyl compounds

Recently, Jiang and co-workers reported an innovative strategy for constructing a magnetic wrinkled organosilica (MWOS) core-shell catalyst by co-immobilizing palladium (Pd) nanoparticles and lipase (CALB) within the porous structure of MWOS.⁴⁸ This metal-enzyme hybrid catalyst was evaluated for its performance in the dynamic kinetic resolution (DKR) of

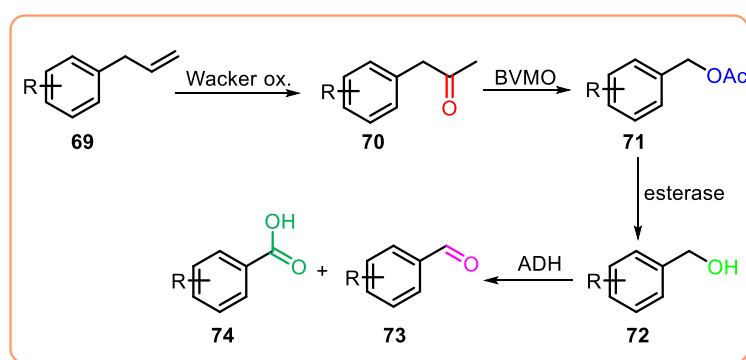


Scheme 1.11: Synthesis of chiral amides from core-shell nanocatalyst CALB&Pd@MWOS

chiral primary amines (**Scheme 1.11a**). Owing to the close proximity of the two catalytic components within the MWOS framework, the integrated catalyst exhibited significantly enhanced reactivity compared to the individual Pd@MWOS and CALB@MWOS systems. The hybrid catalyst demonstrated excellent recyclability, retaining high activity and selectivity over five consecutive cycles. Furthermore, it exhibited a broad substrate scope, producing chiral amides from various primary amines with yields of up to 96% along with enantiomeric excess (*e.e.*) of up to 99% (**Scheme 1.11b**). Notably, the overall catalytic performance, particularly the selectivity and activity, was strongly influenced by the efficiency of the racemization step.

Last year, Rudroff and his team reported a multistep, one-pot cascade synthesis of aldehydes containing flavor and fragrance properties.⁴⁹ The process begins with the conversion of phenylpropenes to phenylacetones via Wacker oxidation. This step employs a catalytic system comprising palladium (II) trifluoroacetate [Pd(TFA)₂] and sodium trifluoroacetate (NaTFA), with iron(III) chloride (FeCl₃) serving as the terminal oxidant. Subsequently, the resulting phenylacetones undergo Baeyer–Villiger oxidation catalyzed by phenylacetone monooxygenase from *Thermobifida fusca*, yielding the corresponding acetate esters. These esters are then hydrolyzed by esterase from *Pseudomonas fluorescens* to form aromatic alcohols. In the final step, enzymatic oxidation using alcohol dehydrogenase converts the alcohols into aldehydes. This four-step cascade reaction successfully produced eight different aldehydes in moderate to good yields (**Table 1.4**). The authors highlighted the green chemistry aspect of their approach, emphasizing the use of Fe(III) as a benign terminal oxidant for the Pd-catalyzed Wacker oxidation. This is in contrast to conventional methods that rely on copper salts, which are considered hazardous.

Table 1.4: Multistep, one-pot cascade synthesis of flavor and fragrance containing aldehydes

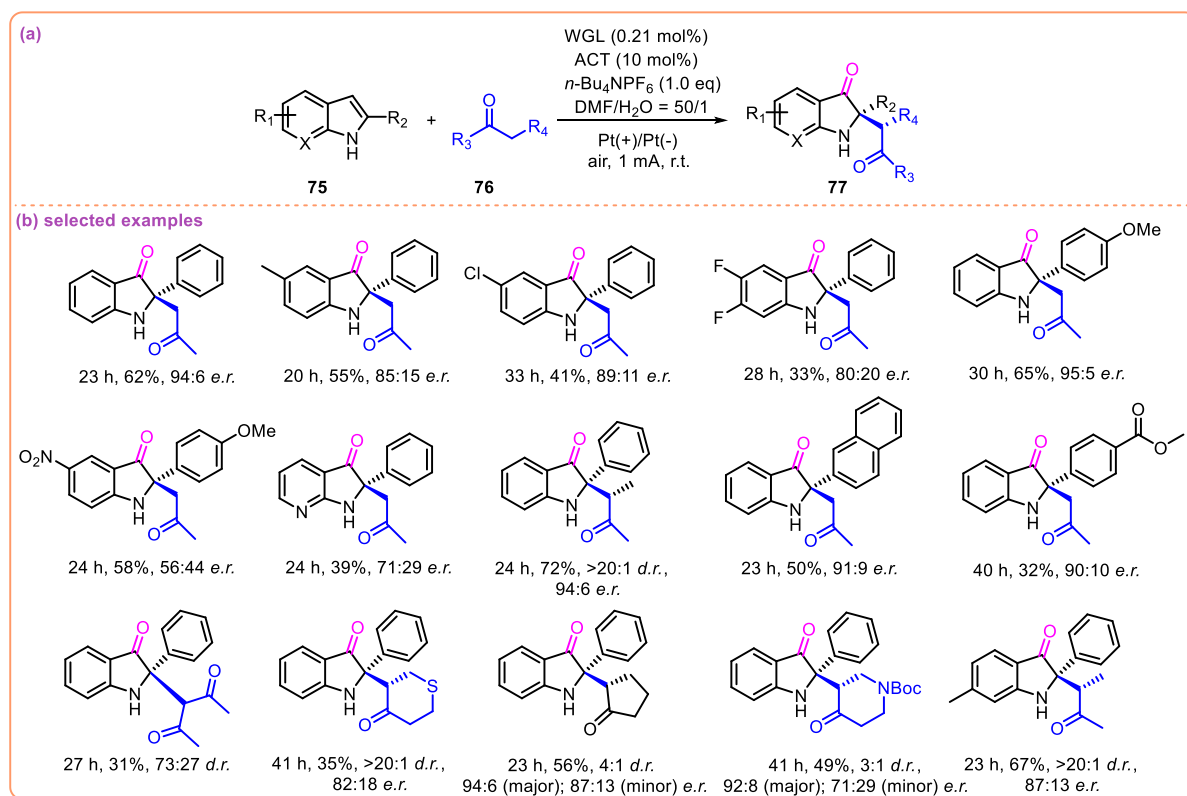


| Entry | R | Yield of enzymatic cascade, t = 24 h | Ratio of aldehyde/acid |
|-------|---------------------------------------|--------------------------------------|------------------------|
| 1 | H | 53 ± 11% | 2.7/1 |
| 2 | 3-CH ₃ | 61 ± 22% | 2.9/1 |
| 3 | 4-CH ₃ | 86 ± 11% | 6.2/1 |
| 4 | 4-OCH ₃ | 62 ± 19% | 11.1/1 |
| 5 | 1,2-OCH ₃ | 58 ± 7% | 16.6/1 |
| 6 | 1,2-CH ₂ -OCH ₂ | 89 ± 7% | 14.2/1 |
| 7 | 1-OH, 2-OCH ₃ | 20 ± 10% | 500/1 |
| 8 | 1-OAc, 2-OCH ₃ | 16 ± 4% | - |

1.2.2: A combined electro-biocatalysis approaches

The integration of electrocatalysis with biocatalysis opens new avenues in organic synthesis.⁵⁰ However, enzymes often exhibit a limited substrate scope, and achieving stereoselective synthesis under electrochemical conditions can be challenging due to the high reactivity of free radicals.^{51,12a} By carefully optimizing the reaction parameters, the synergy between both catalytic systems can be harnessed to produce the desired compounds efficiently.⁵²

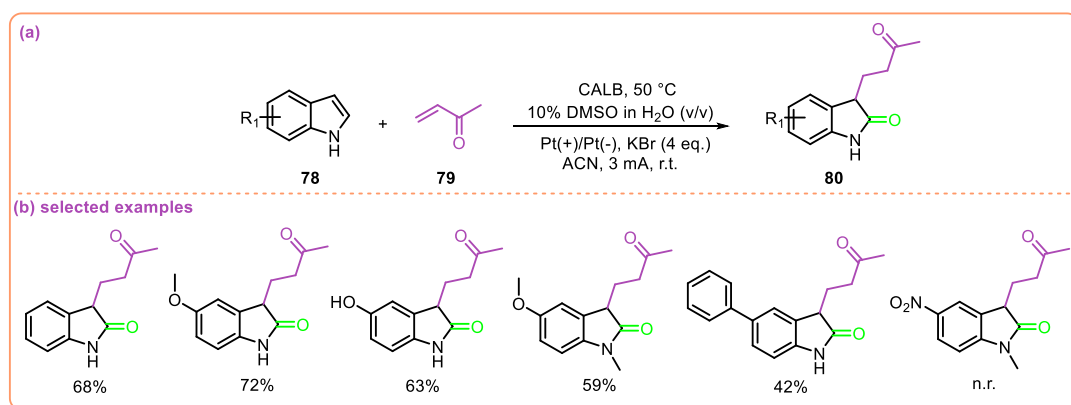
Guan and colleagues introduced an innovative strategy that integrates organic electrocatalysis with the non-natural catalytic function of wheat germ lipase (WGL) (Scheme 1.12a).⁵³ They demonstrated the effectiveness of this method by successfully synthesizing a range of



Scheme 1.12: Bioelectrosynthesis of various asymmetric 2,2-disubstituted 3-carbonyl indoles

asymmetric 2,2-disubstituted 3-carbonyl indoles from 2-substituted indoles and various ketones. The introduction of a redox mediator, 4-acetamido-2,2,6,6-tetramethyl-1-piperidinyloxy (ACT), significantly enhanced the oxidation process by operating at lower potentials, thereby minimizing the formation of undesired by-products. This combined catalytic approach proceeds efficiently under mild conditions and delivers products in notable yields. Indole derivatives bearing both electron-donating and electron-withdrawing substituents on the indole core showed good reactivity with acetone (**Scheme 1.12b**). Among the 2-phenylindoles, those with electron-donating groups at the phenyl position produced higher yields compared to their electron-withdrawing counterparts. Furthermore, the reaction was shown to be compatible with a variety of ketones, including cyclic, acyclic, and heterocyclic types. Importantly, this groundbreaking work offers the first demonstration of the compatibility between hydrolase catalysis and organic electrochemical synthesis.

Building on this strategy, our group has recently reported a novel one-pot protocol that merges electrosynthesis with the non-natural catalytic activity of lipase.⁵⁴ In this system, CALB facilitates the regioselective C-3 alkylation of indoles, followed by electrochemical C-2 oxidation within the same reaction vessel (**Scheme 1.13a**). This bio-electro-synthetic approach demonstrated its versatility with indole derivatives bearing electron-donating groups, yielding the desired products in moderate to good isolated yields (**Scheme 1.13b**). However, no product formation was observed when indoles containing electron-withdrawing substituents were used. To validate the essential roles of both the enzyme and the electric current, a series of control experiments were performed, confirming that both components are critical for the successful formation of 3-alkylated oxindoles in this integrated one-pot system.

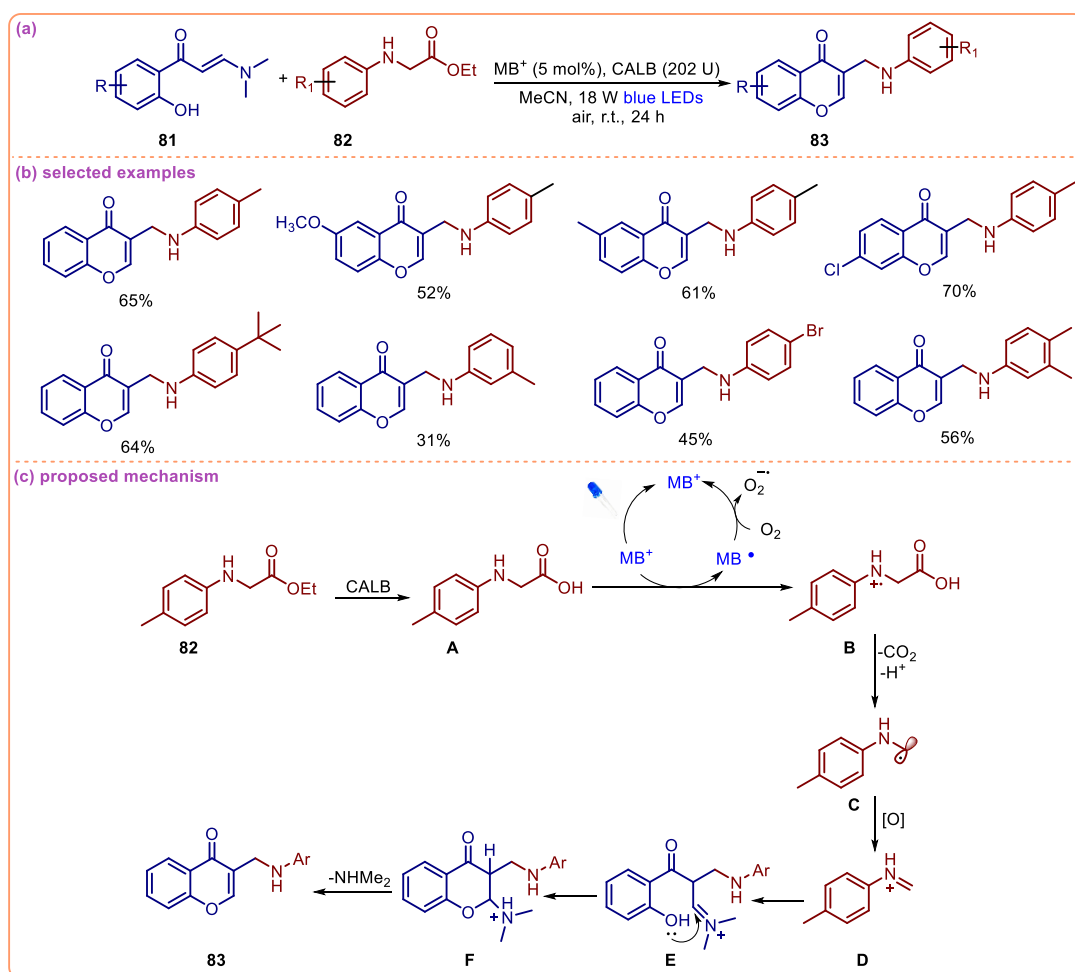


Scheme 1.13: Synthesis of various 3-alkylated oxindoles via an integrated biocatalysis and electrochemical approach

1.2.3: One-pot integration of Bio- and Photo-catalysis

Photocatalysis and biocatalysis have both attracted significant attention in organic synthesis due to their remarkable features, including flexibility, selectivity, atom economy, and strong potential for reaction optimization.⁵⁵ Biocatalysis enables the production of highly selective products under mild reaction conditions, while photocatalysis utilizes visible light as an energy source to access a broad range of reactivities.⁵⁶ Combining biocatalysis with photocatalysis expands the scope of biocatalytic transformations, offering a sustainable approach for the synthesis of high-value compounds.⁵⁷

In 2022, Zhu and colleagues reported an integrated catalytic strategy combining photocatalysis and biocatalysis for the efficient one-pot synthesis of 3-aminoalkyl chromones (**Scheme 1.14a**).⁵⁸ This method employed photoredox catalysis in tandem with enzymatic catalysis, using *o*-hydroxyaryl enaminones and *N*-aryl glycine esters as starting materials. Methylene blue (MB⁺) was identified as the optimal photocatalyst, while CALB was chosen as the biocatalyst. The reaction was carried out in acetonitrile under 18 W blue LED irradiation at room temperature.

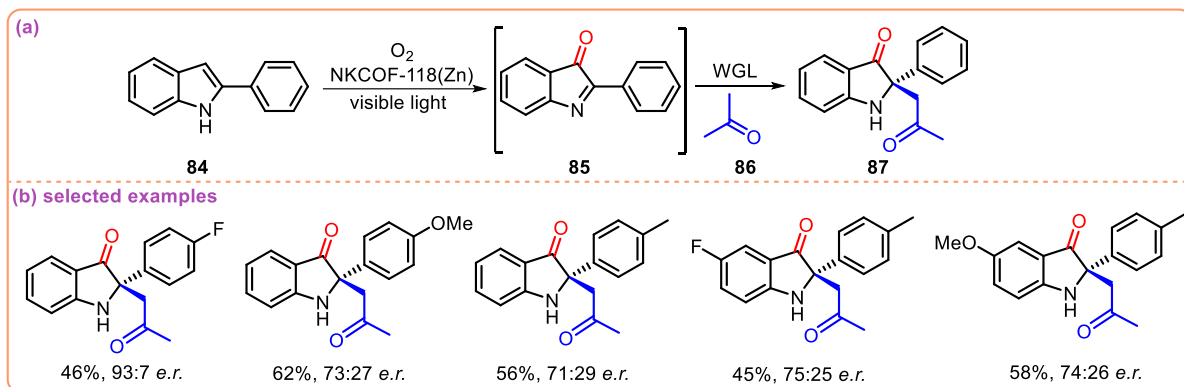


Scheme 1.14: Photobiocatalytic one-pot synthesis of various 3-aminoalkyl chromones

Under these mild conditions, various substituted chromones were synthesized with good yields (**Scheme 1.14b**). Good product yields were obtained when *N*-aryl glycine ethyl ester was reacted with enaminones bearing electron-donating groups or halo substituents. Additionally, the substrate scope was further explored by varying the *N*-aryl glycine esters. It was observed that esters bearing electron-donating groups performed well, while the presence of a methyl group at the *meta*-position led to reduced yields. Based on previous literature and control experiments, the authors proposed a plausible mechanism for this photobiocatalytic transformation. Initially, enzymatic hydrolysis of the *N*-aryl glycine ester by CALB generates the corresponding *N*-aryl glycine (**A**) (**Scheme 1.14c**). Under blue light irradiation, MB⁺ mediates a single-electron transfer (SET) process, leading to the formation of a radical cation intermediate (**B**), which undergoes deprotonation and decarboxylation to yield an aminoalkyl radical (**C**). Oxidation of the resulting radical intermediate (**C**) produces an imine intermediate (**D**), which reacts with the enaminone to form an iminium species (**E**). Intramolecular nucleophilic cyclization followed by the elimination of dimethylamine affords the desired 3-aminoalkyl chromone product (**85**).

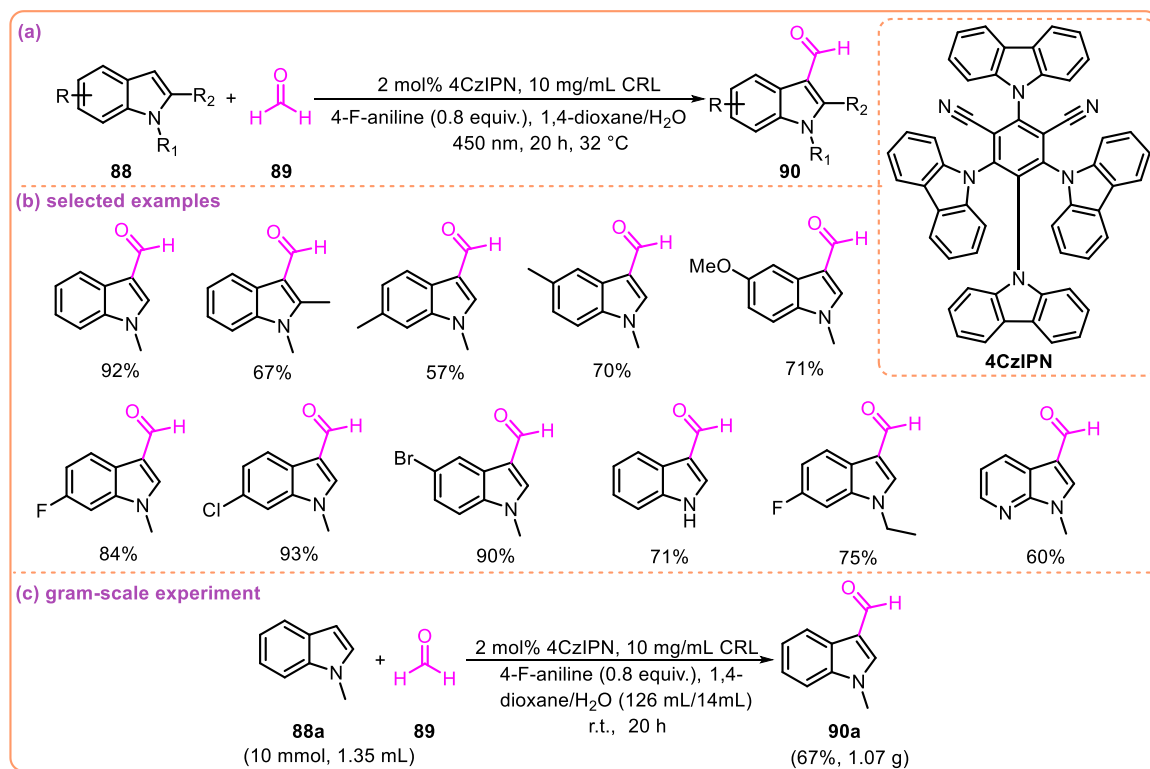
Jin et al. established a robust photoenzymatic platform by immobilizing wheat germ lipase (WGL) onto mesoporous porphyrin-based covalent organic frameworks (COFs) for asymmetric catalysis (**Scheme 1.15a**).⁵⁹ The resulting hybrid photobiocatalysts, referred to as WGL@NKCOF-118(M) (where M = H, Ni, Cu, or Zn), were thoroughly characterized, confirming the successful integration of the enzyme into the photocatalytic COF matrix. These hybrid catalysts were evaluated using asymmetric Mannich reactions as a model to assess their catalytic performance. A notable feature of this system is the close spatial proximity between the photocatalyst and the enzyme's active site, which significantly enhances the selectivity and efficiency of radical-mediated transformations. This proximity effect facilitates precise substrate binding and localized radical generation within the same catalytic microenvironment. Remarkably, this synergistic system enabled the enantioselective formation of C(sp³)-C(sp³) bonds-reactions that cannot be accomplished by WGL or COFs alone. Mechanistic studies, supported by a range of analytical techniques, revealed that singlet oxygen plays a key role in mediating the observed asymmetric catalysis. Furthermore, the substrate scope was explored using various 2-phenylindole derivatives (**Scheme 1.15b**). It was observed that substrates bearing electron-donating groups on the indole core afforded higher yields, likely due to their ability to better stabilize cationic intermediates. Overall, the WGL@COF composites

demonstrated excellent enzymatic activity, operational stability, and high reusability under visible light irradiation.



Scheme 1.15: One-pot photobiocatalytic C–C bond formation using wheat germ lipase and a COF matrix

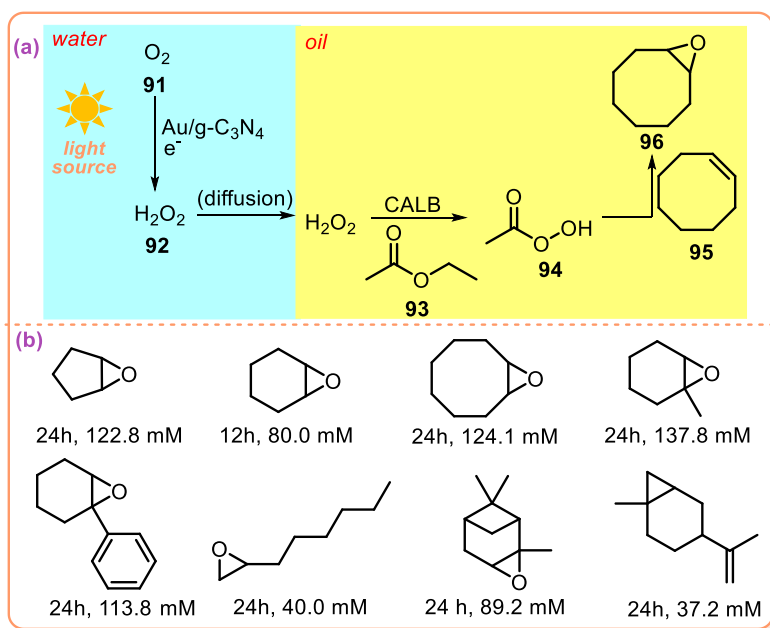
Yu and co-workers devised a hybrid photocatalytic–biocatalytic strategy for the efficient synthesis of diverse C-3 acylated indole derivatives, delivering products in good to excellent yields (**Scheme 1.16a**).⁶⁰ The synthetic sequence begins with the introduction of an aminomethyl group onto the indole ring via a multicomponent Mannich reaction involving amines, aldehydes, and indoles. This Mannich product then undergoes photocatalysis



Scheme 1.16: Synthesis of various C-3 acylated indoles via combinative approach using photocatalyst 4CzIPN and CRL enzyme

to afford the desired C-3 acylated indole product. In addition, the substrate scope was thoroughly explored using *N*-methyl indoles bearing both electron-donating and electron-withdrawing substituents, all of which reacted smoothly under the mild photobiocatalytic conditions (**Scheme 1.16b**). Control experiments confirmed that the cascade reaction proceeds via a radical pathway, with the lipase enzyme playing a dual role in both the formation and hydrolysis of key intermediates. To demonstrate the practicality of the method, the reaction was successfully scaled up to gram-scale under optimized conditions, affording the desired cascade acylated product in a 67% yield (**Scheme 1.16c**).

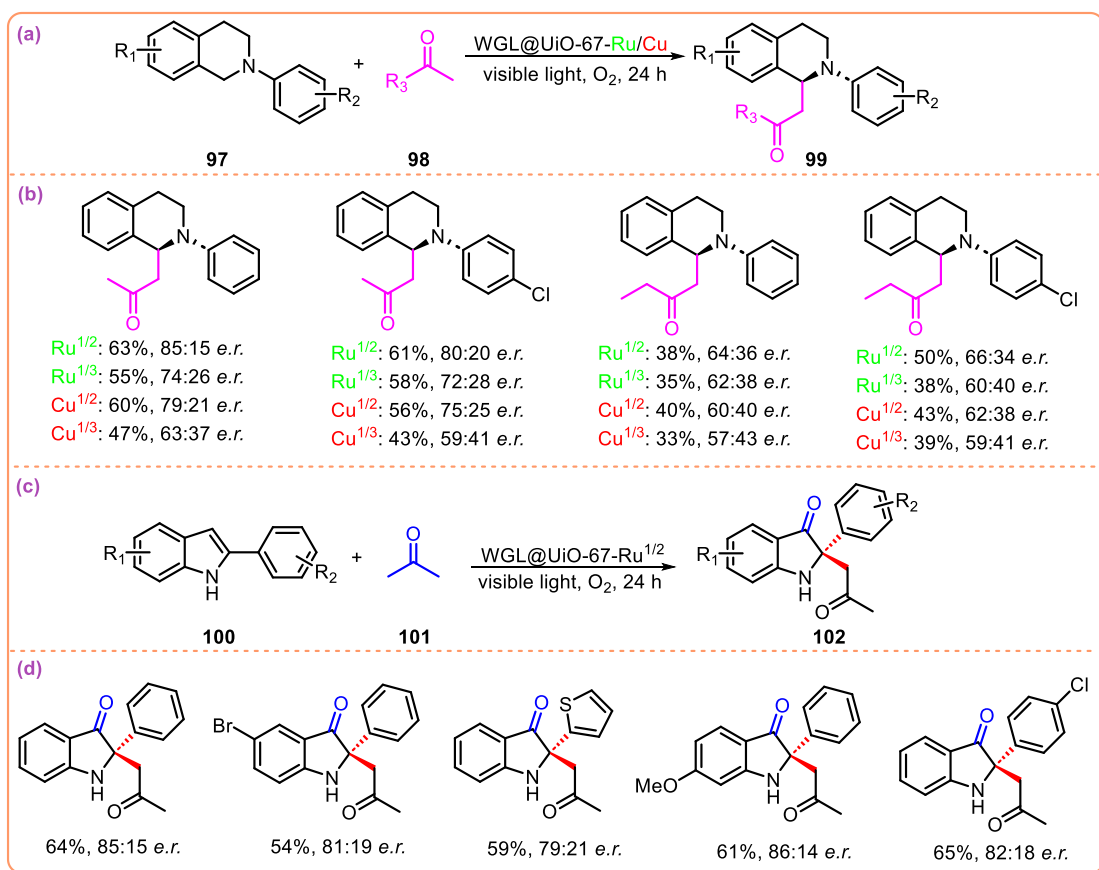
Last year, Li et al. developed a novel strategy to facilitate photobiocatalytic cascade reactions, effectively overcoming the differing behaviours of enzymes and photocatalysts by spatially regulating their distances on Pickering droplet interfaces (**Scheme 1.17a**).⁶¹ Gold nanoparticles immobilized on ultrathin graphitic carbon nitride nanosheets were positioned at the outer layer of oil droplets, where they generated hydrogen peroxide under light irradiation (xenon lamp). Concurrently, CALB was precisely localized at the inner interfacial layer to catalyze enzyme-mediated biooxidations, utilizing the in situ generated H_2O_2 as the oxidant. This methodology was successfully applied to the epoxidation of various alkenes, including aliphatic olefins, challenging α -olefins, and biomass-derived olefins such as α -pinene and limonene (**Scheme 1.17b**). The engineered interfacial system achieved 2-5 times higher reactivity compared to reactions conducted in the bulk aqueous phase. Moreover, this approach not only enhanced the efficiency of the cascade reaction and the transport of H_2O_2 across interfaces but also provided



Scheme 1.17: Photobiocatalytic cascade epoxidation of various alkenes at Pickering droplet interfaces

protection to enzymes against oxidative inactivation due to photogenerated reactive species.

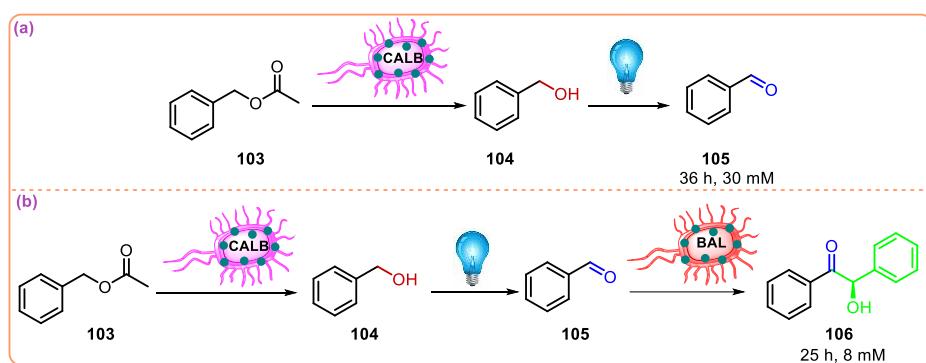
Chen and co-workers designed and constructed a heterogeneous Zr-based metal–organic framework (MOF) catalyst, within which both a photocatalyst and wheat germ lipase (WGL) enzyme were simultaneously encapsulated (**Scheme 1.18a**).⁶² The rapid synthesis of this photobiocatalyst, achieved without the use of strong acids or organic solvents, demonstrated excellent performance in the asymmetric Mannich reaction, along with good reusability and operational stability. The zirconia-based MOF, UiO-67, was employed to co-immobilize two photosensitizers (Ru- and Cu-based) alongside the WGL enzyme (**Scheme 1.18b**). Depending on the ratio of the two photosensitizers (either 1/2 or 1/3), four different photobiocatalysts were designed. Next, the activity of these catalysts was evaluated for the asymmetric synthesis of various *N*-aryl-substituted tetrahydroisoquinoline (THIQ) derivatives under visible light irradiation (**Scheme 1.18c**). Among them, WGL@UiO-67-Ru1/2 exhibited the best catalytic performance and was subsequently used for the transformation of different 2-phenylindole



Scheme 1.18: Synthesis of *N*-aryl-substituted tetrahydroisoquinoline derivatives by newly constructed WGL@UiO-67-Cu/Ru photobiocatalyst

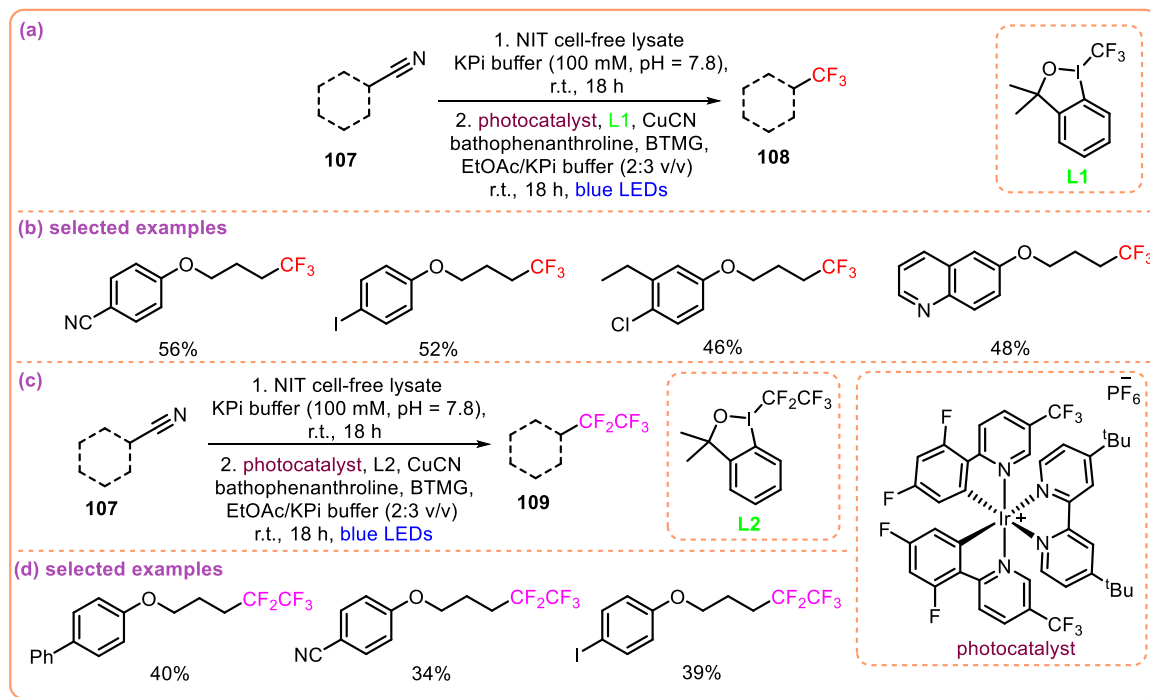
derivatives with acetone. Impressively, the photobiocatalyst maintained high activity regardless of the electronic properties of the substrates (**Scheme 1.18d**). Overall, the UiO-67 framework provided a protective environment for the enzyme, shielding it from the detrimental effects of organic solvents while preserving its catalytic activity.

Very recently, Wu and co-workers developed a photobiocatalyst by encapsulating anthraquinone-2-sulfonate (AQS) as the photocatalyst within the core of cationic liposomes comprised of dioleoyl-3-trimethylammonium propane (DOTAP) and helper cholesterol lipids (**Scheme 1.19a**).⁶³ Interestingly, electrostatic stabilization was achieved through interactions between the positively charged liposome surface and the negatively charged membrane of *E. coli* cell. Liposome-coated bacterial cells overexpressing enzymes such as CALB and benzaldehyde lyase (BAL) enabled the efficient synthesis of value-added compounds through multistep cascade reactions. These cascades included two step or three step CALB-catalyzed hydrolysis, BAL-catalyzed condensation, and AQS-mediated photo-oxidation processes (**Scheme 1.19a,b**).



Scheme 1.19: Combining liposomal-coated enzymatic activity with a photocatalyst for multistep cascade reactions

Next, Micklefield and co-workers developed a new strategy combining nitrilase enzyme catalysis with photoredox catalysis to convert inexpensive organonitrile compounds into multifluorinated products (**Scheme 1.20a**).⁶⁴ Their approach involved the enzymatic hydrolysis of nitriles using nitrilase in potassium phosphate (KPi) buffer at room temperature for 18 hours, followed by light irradiation for an additional 18 hours after the addition of solvents and chemical reagents. Using this method, they successfully transformed various nitriles into trifluoromethylated compounds with moderate to good isolated yields (**Scheme 1.20b**). Notably, the enzyme exhibited high regioselectivity by selectively hydrolyzing aliphatic nitriles while leaving aromatic nitriles intact. Halogen-substituted substrates further demonstrated the broad applicability of this methodology. Moreover, by employing a hypervalent iodine



Scheme 1.20: Nitrilase enzyme and photoredox catalysis combined for the efficient synthesis of multifluorinated products

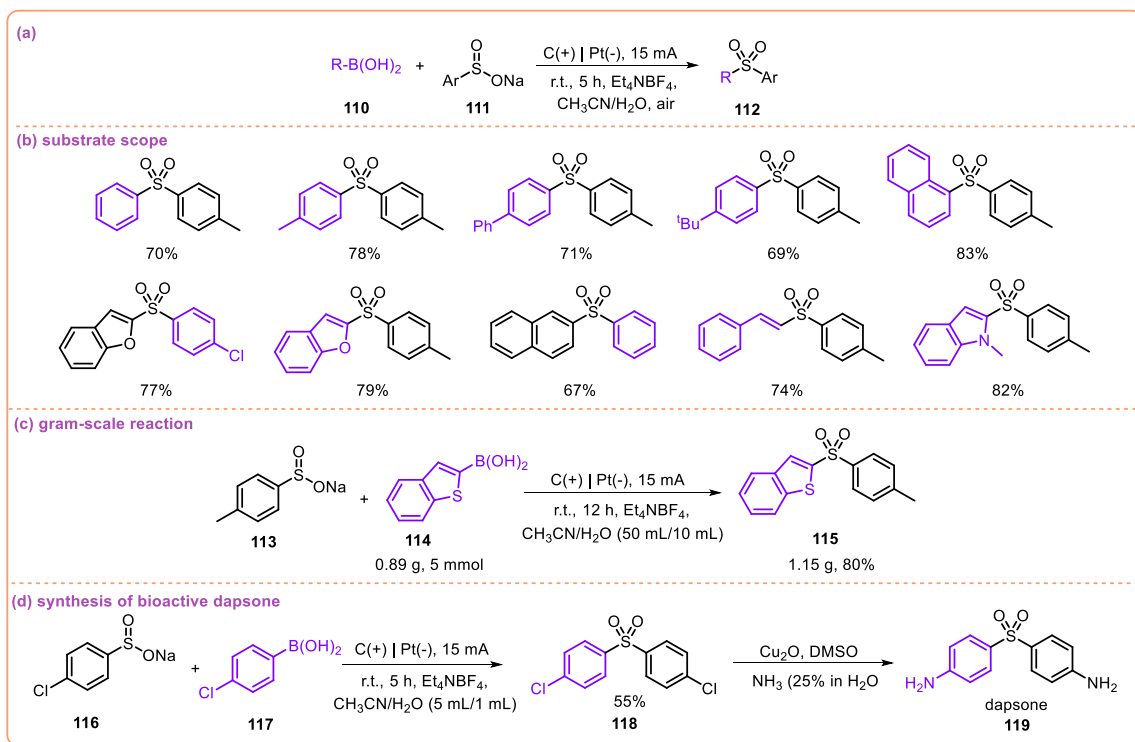
compound (L2) instead of the traditional Togni's reagent (L1), they achieved the synthesis of pentafluoroethyl phenol derivatives with yields ranging from 34 to 40% (Scheme 1.20c,d).

1.2.4: Electrochemical strategies for the synthesis of bioactive molecule

Electrochemistry has emerged as a key tool in modern synthetic strategies, aligning with green chemistry principles and offering an alternative to traditional methodologies.²¹ It enables the direct synthesis of both simple and complex molecules within a highly functionalized chemical space.²² Thus, electrochemistry effectively bridges the gap between sustainable practices and the synthesis of bioactive molecules.⁶⁵

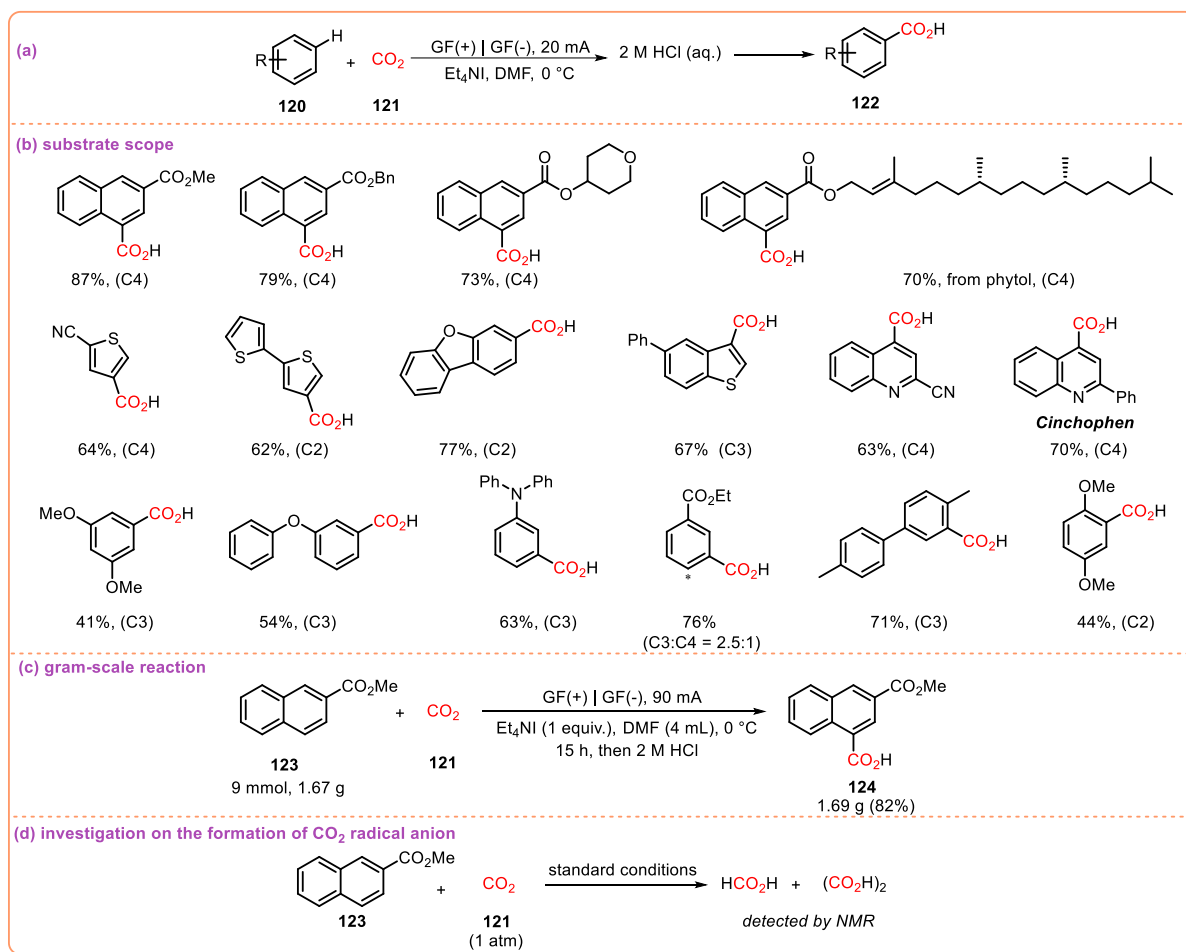
Yao et al. reported a straightforward electrochemical methodology for the sulfonylation of organoboronic acids using sodium sulfonates (Scheme 1.21a).⁶⁶ This green approach enables the synthesis of a broad range of organosulfones from aryl, heteroaryl, and alkenyl boronic acids in good to excellent yields at room temperature within 5 hours (Scheme 1.21b). The reaction was performed in an undivided electrochemical cell using carbon and platinum as the anode and cathode, respectively. Notably, this sustainable protocol operates without the need for additives or catalysts. Mechanistic studies, including radical trapping and cyclic voltammetry experiments, revealed that the reaction proceeds via the formation of sulfonyl

radicals. Furthermore, the utility of the method was demonstrated through scale-up reactions and the synthesis of the antibacterial drug dapsone (**Scheme 1.21c,d**).



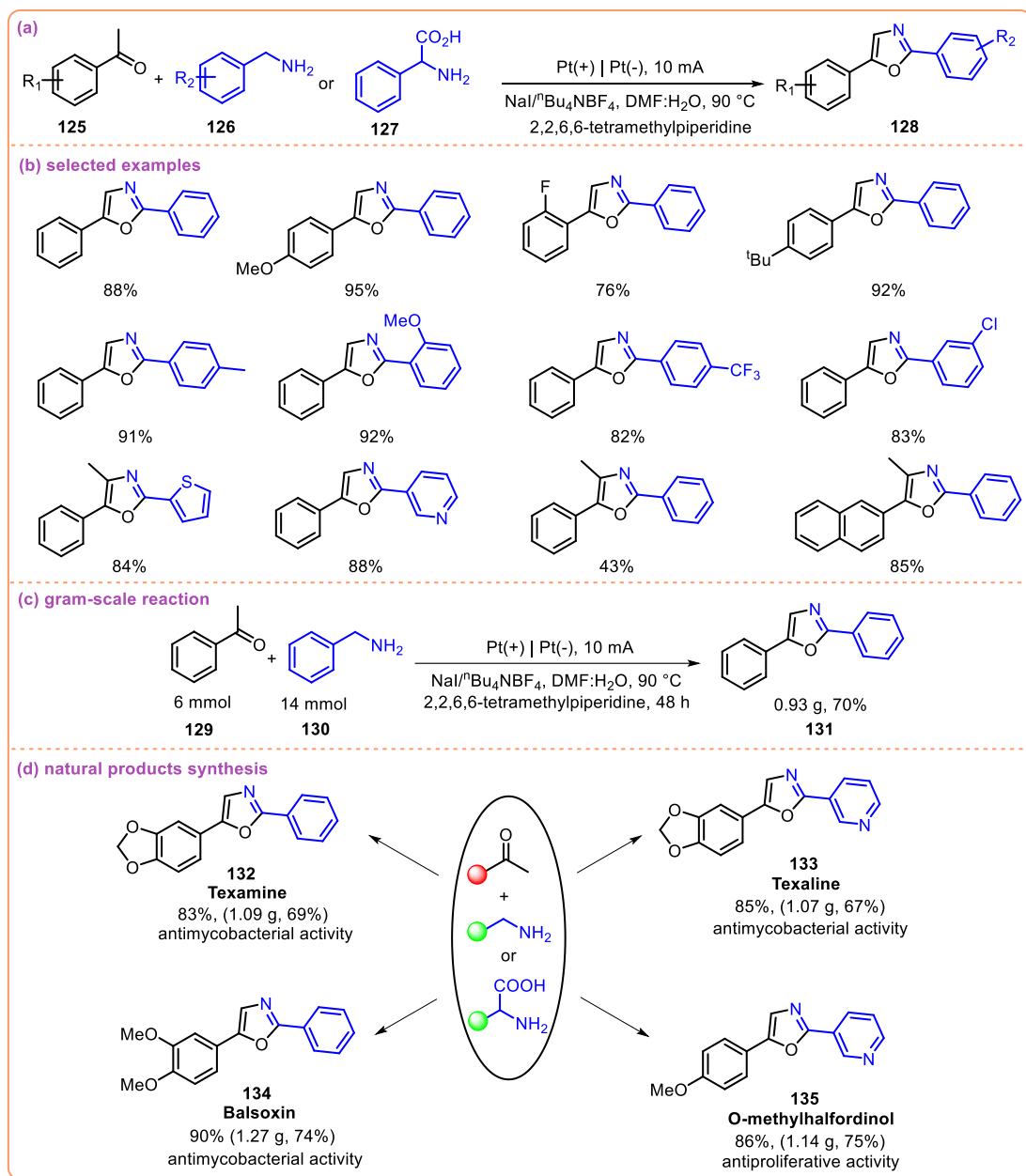
Scheme: 1.21: Electrochemical synthesis of organosulfones

In 2023, Qiu and co-workers developed a metal-free electrocarboxylation strategy that employs electricity and CO_2 as the energy source and carboxylic acid precursor, respectively, for the site-selective C–H carboxylation of unactivated arenes (**Scheme 1.22a**).⁶⁷ This versatile approach demonstrated broad substrate scope, efficiently transforming polycyclic aromatic hydrocarbons, challenging heteroarenes, simple phenyl derivatives, and substituted arenes with excellent chemo- and regioselectivity as well as high atom economy. Notably, the method enabled the reductive activation of challenging substrates such as naphthalenes, pyridines, pyrimidines, and substituted quinolines (**Scheme 1.22b**). The scalability of the protocol was illustrated through the gram-scale carboxylation of methyl-2-naphthoate (9 mmol) with CO_2 , affording the desired product in 82% yield (**Scheme 1.22c**). Mechanistic investigations, including cyclic voltammetry and control experiments, suggested the formation of CO_2 radical anions at the cathode (**Scheme 1.22d**). Further DFT studies revealed that these radical anions play a key role in dictating the regioselectivity of the reaction.



Scheme 1.22: Electrochemical site-selective C-H carboxylation of arenes

Wang and co-workers reported an electrochemical strategy for the synthesis of 2,5-diaryloxazoles from aryl ketones and benzyl amines or amino acids (**Scheme 1.23a**).⁶⁸ This metal-, base-, and chemical oxidant-free protocol enables N–H and C(sp³)–H functionalization to afford a wide range of functionalized oxazoles using various benzyl amines, α -amino acids, and phenyl glycines in moderate to excellent yields (**Scheme 1.23b**). The versatility of the method was further demonstrated through gram-scale reactions and the synthesis of several bioactive natural products (**Scheme 1.23c,d**). Mechanistic studies, including control experiments, revealed that the transformation proceeds via a radical pathway.



Scheme 1.23: Synthesis of different 2,5-diaryloxazoles using electricity

1.3 Conclusion

In summary, to achieve sustainability in organic synthesis has become a key focus for chemists in recent years. The primary aim is to design organic reactions that minimize waste, employ green solvents, and utilize catalytic strategies aligned with the principles of green chemistry. Major green catalytic approaches include biocatalysis, electrocatalysis, photocatalysis, and organocatalysis, each offering distinct advantages and limitations. Integrating these diverse catalytic methods expands the accessible chemical space and enables the synthesis of complex molecules that are often unattainable through individual catalytic strategies. Such integrated systems operate in sequential or concurrent multicatalytic cascades, facilitating the efficient

production of pharmaceuticals and their precursors. This review focuses on hydrolase-catalyzed integration strategies that combine biocatalysis with other catalytic disciplines. It also highlights how interdisciplinary, integrative cascade approaches enable the synthesis of clinically important molecules by addressing the challenges inherent in designing multicatalytic systems.

1.4: References

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LITERATURE GAPS AND RESEARCH OBJECTIVES

Literature gaps

By going through the literature, the following gaps have been observed:

1. The area of integration of enzyme catalysis with other catalytic systems, like photo, electro, or chemical catalysts, to synthesize biologically relevant molecules is still underexplored.
2. Metal-based catalysts and enzymes have distinguishable natures; the former often operate under comparatively more forcing reaction parameters, while the latter typically function under mild reaction conditions. So, the use of both of the catalysts in a one-pot reaction is a big challenge to synthetic chemists.
3. There is no integrated electro-bio-catalytic approach documented previously to catalyze the combination of Michael addition reaction with other synthetic transformations in one-pot fashion.

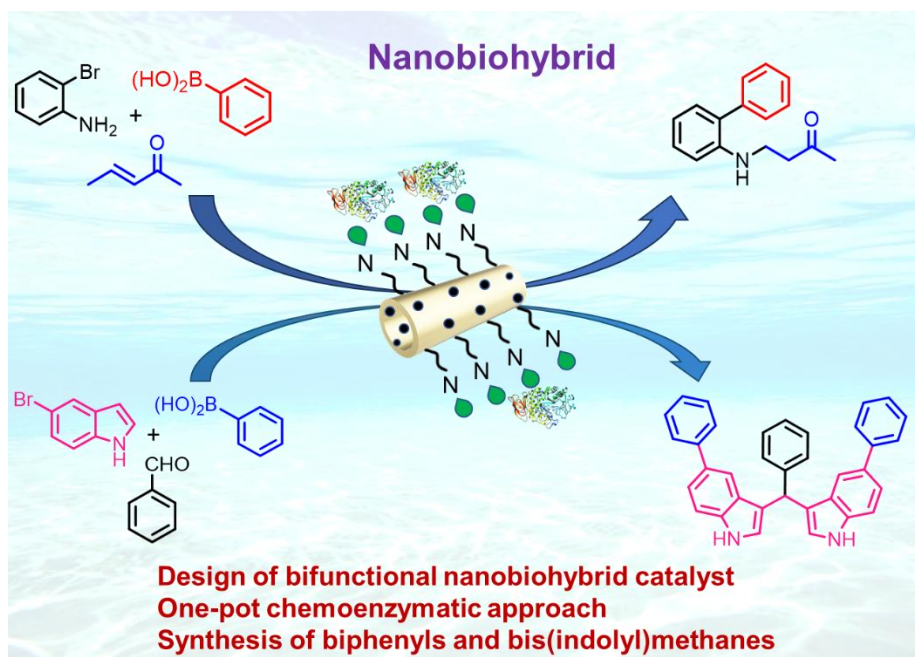
Objectives

To overcome the aforementioned gaps, we are proposing the following objectives:

1. Development of one-pot chemo-enzymatic approach for catalyzing the combination of Suzuki-Miyaura and Michael-addition reactions.
2. Exploration of thia-Michael addition reaction using enzyme-based catalyst and further application in the synthesis of pharmaceutical precursors.
3. Investigation of an electrochemical strategy for regioselective *N*-sulfonylation of heterocycles.

CHAPTER 2

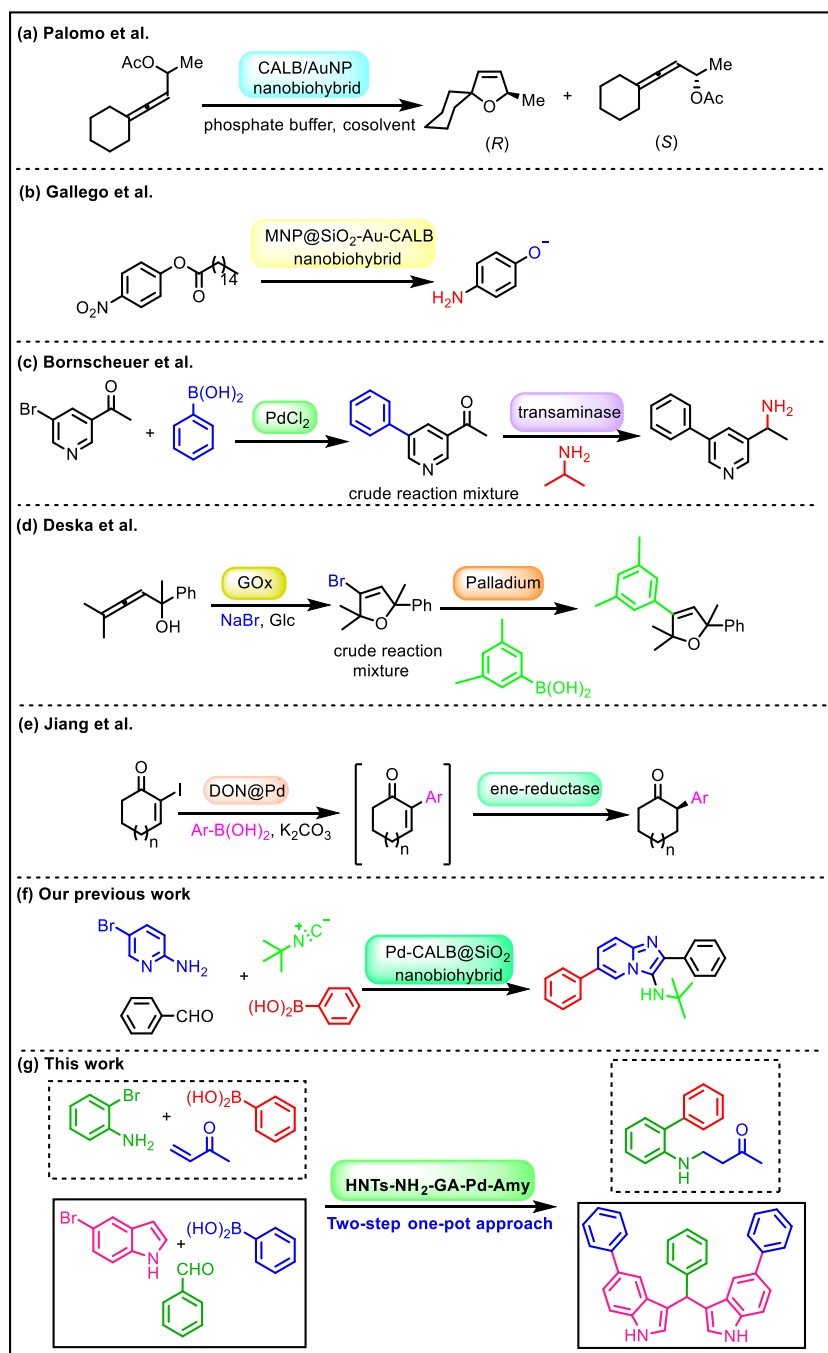
Design and Preparation of Novel Bifunctional Nanobiohybrid Catalyst by Combining Palladium and α -Amylase Enzyme: Application in the One-pot Chemoenzymatic Catalysis



2.1: Introduction

The combining of chemical and bio-catalysis to catalyze dual chemical reactions known as a chemoenzymatic procedure, provides phenomenal opportunities in organic synthesis.¹ Moreover, chemoenzymatic catalysis has the potential to boost overall synthetic productivity by reducing waste production, solvent consumption as well as purification steps.² Besides, chemoenzymatic reactions are often classified in two ways either sequential or concurrent. In sequential reactions, usually, a second catalyst and subsequent reagents are added after the completion of the first catalytic transformation. Sometimes, protein removal, dilution/concentration, degassing, and alteration of solvents are also required in this approach. In contrast, in concurrent chemoenzymatic reactions, all reactants and catalysts are added simultaneously in a single pot at the beginning of the reaction.³ Despite that, it is very challenging to conduct a successful concurrent chemoenzymatic reaction in a single-pot due to the differences in chemical and enzymatic reaction conditions followed by mutual deactivation of both catalysts.^{3a, 4} Alternatively, the immobilization of both catalysts on solid surfaces avoids incompatibility between both catalysts and allows their recyclability, physical segregation, and separation.⁵ Thus, the construction of heterogeneous nanobiohybrid catalyst fills the gap between chemical and enzymatic catalysis, allowing the chemoenzymatic process to perform in a concurrent manner within a single vessel.⁶ The hybrid catalyst enables efficient, green chemoenzymatic reactions by bringing the metal and enzyme active sites into close proximity, which is difficult to achieve with separately immobilized catalysts.⁷ For instance, the interfacial interactions can impact the catalytic activities of both catalysts which might influence the diffusion of reactants and reaction rates of chemoenzymatic processes. Therefore, while designing the heterogeneous nanobiohybrid catalyst it becomes important to understand the interfacial interactions between both of the catalysts to control the challenges of inherent complexity of chemoenzymatic processes.⁸ Recently, certain developments have been made in the field of nanotechnology in the designing of nano-biohybrids.⁹ However, among the various approaches used to generate nano-biohybrid catalysts, the immobilization of both entities on the same solid supports has been proven more successful since it offers a strong covalent interaction between the support and enzyme, reducing the likelihood of enzyme leakage.^{9h} Additionally, after immobilization, there would be a lower chance of enzyme agglomeration on the support.⁹ⁱ On the other hand, in the CLEA (cross-linked enzyme aggregates) methodology, the problem of diffusion might arise due to the uncontrollable immobilization of metal nanoparticles.^{6d} In addition, immobilizing the enzyme onto solid support enhances the catalyst's storage stability, and reusability, along with

operational and thermal stability.^{9j} One such example of CLEA methodology illustrates the formation of enzyme polymer conjugates in which protein acts as a matrix for the generation of silver or gold nanoparticles retaining the catalytic activities of both entities towards the utilization in the chemoenzymatic process (**Scheme 2.1a**).¹⁰ Likewise, Gallego and group members gave an idea to co-immobilize the gold nanoparticles and CALB onto a magnetic silica shell (**Scheme 2.1b**). They tried to investigate the synergistic kinetic effects of both constituents towards the cascade chemoenzymatic hydrolysis and reduction of para-nitrophenyl esters.^{3a}



Scheme 2.1: Recently reported chemoenzymatic approaches

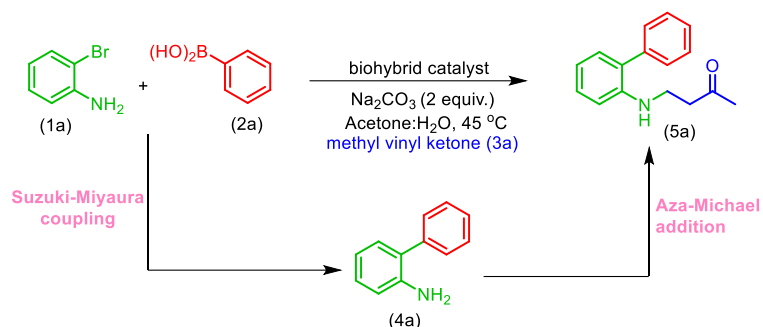
On the other hand, certain efforts were made to combine Suzuki-Miyaura coupling with other biocatalytic transformations to meet the chemoenzymatic approach. In this context, Bornscheuer et al. introduced chemoenzymatic two-step one-pot enantioselective synthesis of chiral amines via the Pd-catalyzed Suzuki cross-coupling followed by transaminase catalyzed bioreduction (**Scheme 2.1c**).¹¹ Furthermore, a combination of glucose oxidase matrix with palladium heterogeneous biohybrid has been disclosed by Deska and coworkers to integrally catalyze the enzyme-mediated biocyclization and metallic Suzuki cross-coupling (**Scheme 2.1d**).¹² In addition, Jiang and group members developed a one-pot synthetic route for the enantioselective synthesis of tertiary α -aryl cyclo ketones by merging the Pd-catalyzed Suzuki-Miyaura coupling and enzymatic stereoselective C-C bond reduction (**Scheme 2.1e**).¹³ Moreover, we recently developed a bifunctional Pd-CALB@SiO₂ nanohybrid catalyst by encapsulation of both catalysts within silica (**Scheme 2.1f**). This biohybrid catalyst was utilized for the one-pot synthesis of imidazo[1,2]pyridine derivatives via Groebke-Blackburn-Bienayme (GBB) multicomponent reaction and Suzuki coupling.¹⁴ Besides, HNTs are natural aluminosilicate clay material having a versatile solid surface. They are highly biocompatible, inert to organic solvents & reagents, easier to functionalize towards the immobilization of metal NPs and enzymes.¹⁵ Drawing encouragement from the advantages of biohybrid catalysts and our previous work in this area, herein, we scrutinize the rational design of heterogeneous bifunctional catalyst by immobilization of palladium nanoparticles (PdNPs) and α -amylase from *Aspergillus oryzae* onto halloysite nanotubes (HNTs) in order to maximize the efficiency of chemoenzymatic synthesis of functionalized biphenyls and bisindoles (**Scheme 2.1g**).

2.2: Results and Discussion

2.2.1: Designing and Preparation of Hybrid Catalyst

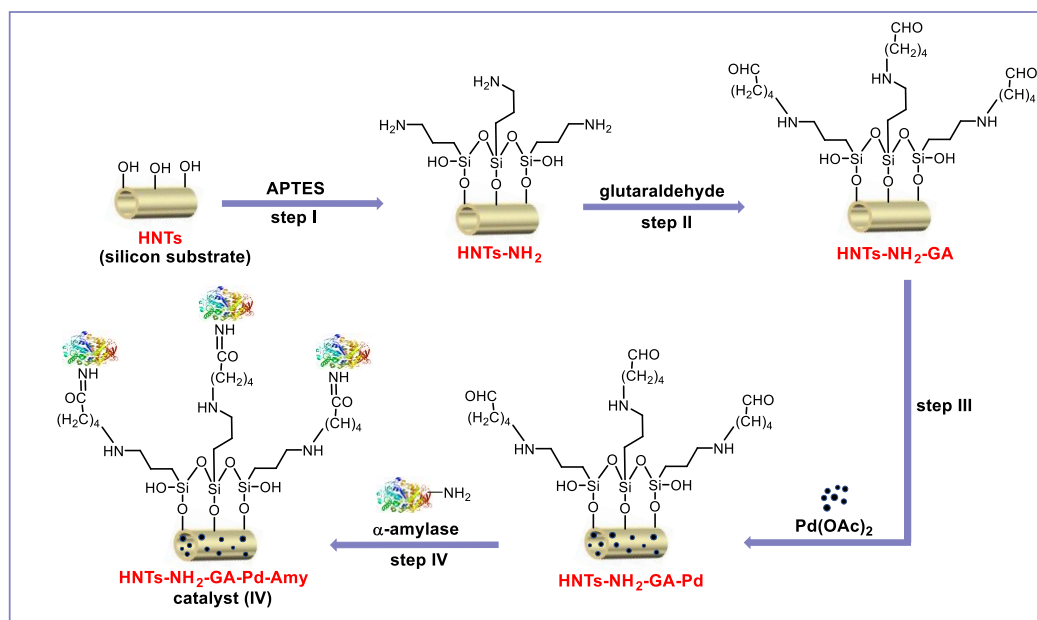
We started this study by designing and preparing of heterogeneous hybrid catalyst by immobilizing the Pd-nanoparticles (PdNPs) and α -amylase enzyme (Amy) on the surface of halloysite nanotubes (HNTs). First, PdNPs were immobilized at HNTs, followed by the immobilization of α -amylase to deliver the hybrid catalyst i.e., HNTs-Pd-Amy (I) (**Scheme 2.5 & Figure 2.5**). Next, the reactivity of the developed hybrid catalyst (I) was tested to catalyze the one-pot chemoenzymatic reaction consisting of Suzuki-Miyaura and aza-Michael addition reactions respectively. Unfortunately, the prepared hybrid catalyst (I) was not able to perform the model chemoenzymatic reaction (**entry 1, Table 2.1**). Subsequently, we altered the immobilization order, first, α -amylase was immobilized at HNTs followed by the immobilization of PdNPs and as a result, obtained the hybrid catalyst HNTs-Amy-Pd (II) (**Scheme 2.6 & Figure 2.6**). The catalytic activity of the developed hybrid catalyst (II) was

tested, but still, there was no reaction (**entry 2, Table 2.1**). As both of the catalysts were unable to produce the desired product (**5a**), it indicated that both the constituents i.e., enzyme and metal of the hybrid catalyst were in direct contact with each other which might be leading to their mutual deactivation. To actualize our hypothesis, we functionalize the HNTs using OH-bonds situated at the surface. In this context, after the immobilization of PdNPs, the HNTs-Pd were treated with 3-aminopropyl triethoxy silane (APTES) which makes strong Si-O-Si bonds and provides a free NH₂ group away from the surface of HNTs.¹⁶ Further, the amine-functionalized HNTs-Pd-NH₂ were treated with glutaraldehyde (GA) which provided a covalent binder to immobilize the α -amylase enzyme and afforded the hybrid catalyst HNTs-Pd-NH₂-GA-Amy (III) (**Scheme 2.7 & Figure 2.7**). Further, the hybrid catalyst (III) was employed in the chemoenzymatic reaction i.e., Suzuki-Miyaura coupling followed by the aza-Michael addition reaction and no reaction occurred (**entry 3, Table 2.1**). However, when we employed the hybrid catalyst (III) to catalyze the chemoenzymatic reaction in reverse order i.e., aza-Michael addition reaction first followed by Suzuki-Miyaura coupling, we obtained only Michael addition product in 58 % yield (**entry 3, Table 2.8**). This indicated that the immobilized enzyme was working well but the catalytic activity of PdNPs was completely lost. Then, we made changes in the immobilization order, first HNTs were functionalized using APTES and GA followed by the immobilization of PdNPs and enzyme respectively which afforded the hybrid catalyst HNTs-NH₂-GA-Pd-Amy (IV) as depicted in **Scheme 2.2**. Fortunately, the developed catalyst (IV) successfully catalyzed the one-pot chemoenzymatic reaction consisting of Suzuki-Miyaura coupling followed by an aza-Michael addition reaction and furnished the product (**5a**) in 47 % yield (**entry 4, Table 2.1**). This indicated that both catalysts were physically segregated over the HNTs, forcing the intermediate to diffuse from one catalyst to another to perform a successful one-pot chemoenzymatic reaction. Further, the hybrid catalyst HNTs-NH₂-GA-Pd-Amy (IV) was characterized by using several techniques like FTIR, XRD, SEM, EDS, HR-TEM, and elemental mapping analysis. When HNTs, HNTs-Pd, and pure α -amylase along with Pd(OAc)₂ were employed as a catalyst in the one-pot reaction, product **5(a)** was not observed (**entries 5-7, Table 2.1**). However, the Suzuki-Miyaura product was obtained while using HNTs-Pd as a catalyst (**entry 6, Table 2.8**). Furthermore, we also examined the relative activity of the biohybrid catalyst (IV) for individual reactions i.e. aza-Michael addition and Suzuki-Miyaura coupling reaction (**Table 2.7**). Likewise, the results demonstrated that the biohybrid catalyst (IV) was more effective than the catalysts in their native forms (**entries 1-3, Table 2.7**).

Table 2.1. One-pot chemoenzymatic approach^a

| Entry | Catalyst | Product (5a) ^c |
|-------|--|---------------------------|
| 1 | HNTs-Pd-Amy (I) | - |
| 2 | HNTs-Amy-Pd (II) | - |
| 3 | HNTs-Pd-NH ₂ -GA-Amy (III) | - ^b |
| 4 | HNTs-NH ₂ -GA-Pd-Amy (IV) | 47% ^c |
| 5 | HNTs | - |
| 6 | HNTs-Pd | - ^d |
| 7 | α -amylase+Pd(OAc) ₂ | - ^e |

^a**Reaction conditions:** 2-Bromoaniline (**1a**) (0.06 mmol, 1.0 equiv.), phenylboronic acid (**2a**) (0.06 mmol, 1.0 equiv.), methyl vinyl ketone (**3a**) (0.06 mmol, 1.0 equiv.), catalyst 5 mg, Na₂CO₃ (0.12 mmol, 2 equiv.), acetone: H₂O (1:1) as a solvent, 45 °C, overnight. ^bOnly aza-Michael product was obtained in 58% yield, ^c%conversion using HPLC, ^dOnly Suzuki-Miyaura product was obtained in 89% yield, ^epure enzyme and Pd-catalyst were added in the same pot.

Scheme 2.2: Schematic representation of HNT-NH₂-GA-Pd-Amy (IV) synthesis

2.2.2: Characterization of Catalyst HNTs-NH₂-GA-Pd-Amy (IV)

2.2.2a: FTIR analysis

The immobilization of α -amylase on functionalized HNTs was initially analyzed by FTIR spectroscopy over the range of 4000-400 cm^{-1} . The absorption bands at 3630 and 903 cm^{-1} are attributed to inner Al-OH groups of HNTs.¹⁷ In addition, the stretching bands between 1000-1200 cm^{-1} are due to Si-O-Si bonds of HNTs.¹⁸ The broad peak at 3000 cm^{-1} is assigned to the stretching vibrations of the O-H bond. Further, the absorption peaks at 1580 and 1491 cm^{-1} in catalyst (IV) spectra were allocated to the amide II band as shown in **Figure 2.1**. Additionally, the absorption band positioned at 1286 cm^{-1} is assigned to the amide III bond which is due to the C-O-C stretching vibration. In the literature, similar findings were observed, and the appearance of bands 1400-1600 cm^{-1} and 1100-1300 cm^{-1} were attributed to the amide II and amide III bands respectively.¹⁹ The detected spectral vibrations of catalyst (IV) validate the stable binding of α -amylase over functionalized HNTs. Along with this, it verifies the actual structural integrity of HNTs after profitable anchoring of α -amylase over functionalized HNTs.

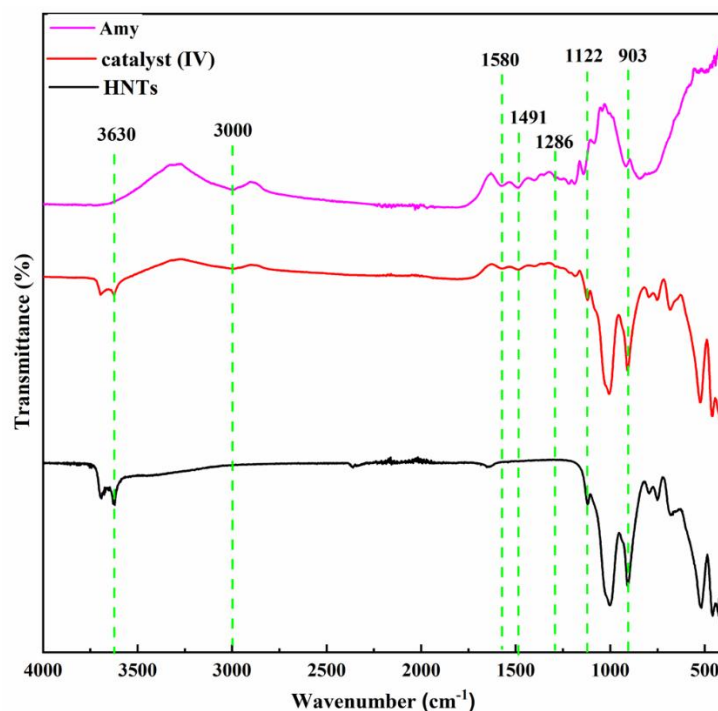


Figure 2.1: FTIR spectra of catalyst (IV)

2.2.2b: XRD analysis

To investigate the core structure or crystalline nature of the prepared nano biohybrid catalyst (IV), XRD analyses were carried out. The incorporation of successful PdNPs on HNTs in catalyst (IV) was confirmed by the appearance of diffraction peaks at $2\theta = 40.04^\circ$, 45.83° , 68.23° , 82.32° , and 86.64° , corresponding to (111), (200), (220), (311), and (222) planes of PdNPs (JCPDS card no. 05-0681), as is documented in the literature.²⁰ Additionally, the above characteristic peaks of XRD pattern may be assigned to the face-centered cubic phase of Pd.^{20a}

Moreover, proof of the HNTs matrix's sustained crystalline structure in the hybrid catalyst (IV) even after α -amylase immobilization can be seen in **Figure 2.2**, where 2θ diffraction peaks at 11.89° , 19.98° and 24.50° were indexed to the basal planes (001), (020), and (002) of HNTs respectively. However, the immobilization of the enzyme may have caused a slight decrease in peak intensity in the case of hybrid catalyst (IV) compared to pure HNTs powder. A similar modest decrease in peak intensity of the catalyst after enzyme immobilization was also noticed by Ali et al.²¹ Overall, the absence of any additional peak indicated that fabricated PdNPs did not alter the structural characteristics of HNTs.

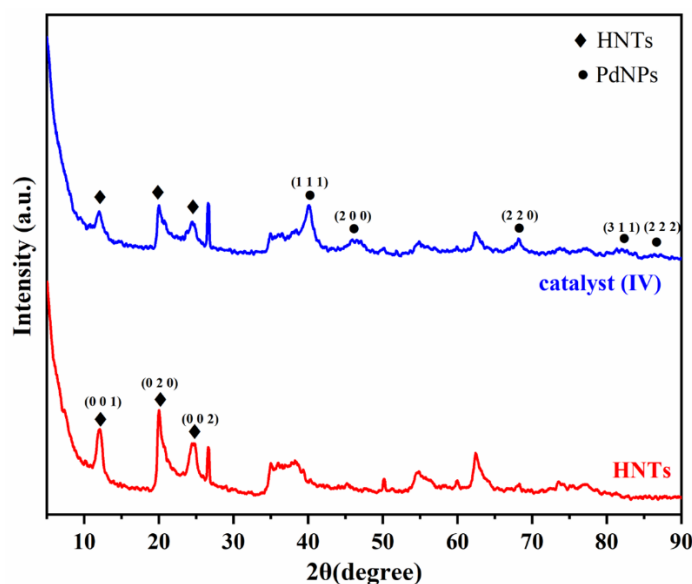


Figure 2.2: XRD analysis of catalyst (IV)

2.2.2c: FE-SEM-EDS, HR-TEM, elemental mapping analysis

The energy-dispersive spectroscopy (EDS) data of hybrid catalyst (IV) are presented in **Figure 2.3f**, which confirmed the successful fabrication of palladium nanoparticles on HNTs. Furthermore, the presence of element nitrogen depicted the loading of α -amylase over HNTs. The appearance of elements carbon, aluminum, silica, and oxygen corroborated towards aluminosilicates of HNTs. The catalyst (IV) was further investigated towards its outer structural morphology through FE-SEM where the uniform existence of PdNPs was found on HNTs (**Figure 2.3a**). Adding to this, no cluster formation of metal PdNPs was observed in the nanobiohybrid catalyst.

Moreover, the elemental mapping shown in **Figure 2.3b** depicted the even distribution of PdNPs and nitrogen on the prepared binary catalyst. Next, to know the inner morphology of the catalyst, HRTEM studies were conducted, which illustrated the presence of cylindrical tubes of HNTs in the range of 400-500 nm. This technique exemplified the existence of spherical nanoparticles of palladium that range in size from \sim 4-5 nm on the HNTs. The darker

region present over the HNTs might be due to the attachment of the enzyme (**Figure 2.3c-e**). Similar kinds of observations were also reported in the literature.¹⁷ Thus, all these above characterization techniques validate the immobilization of enzyme and metal NPs on HNTs. Furthermore, in order to increase the loading of Pd onto the HNTs, we also tuned the amount of Pd(OAc)₂, and discovered that 10 mg of salt provided the highest Pd mass percentage, as shown in **Table 2.6**. Next, we altered the amount of α -amylase to achieve maximum loading onto the HNTs. We discovered that the best immobilization efficiency was 72.11% when enzyme concentration was kept at 3 mg/ml in the reaction (more details are given in section **2.2.8b**, **Figure 2.4**).

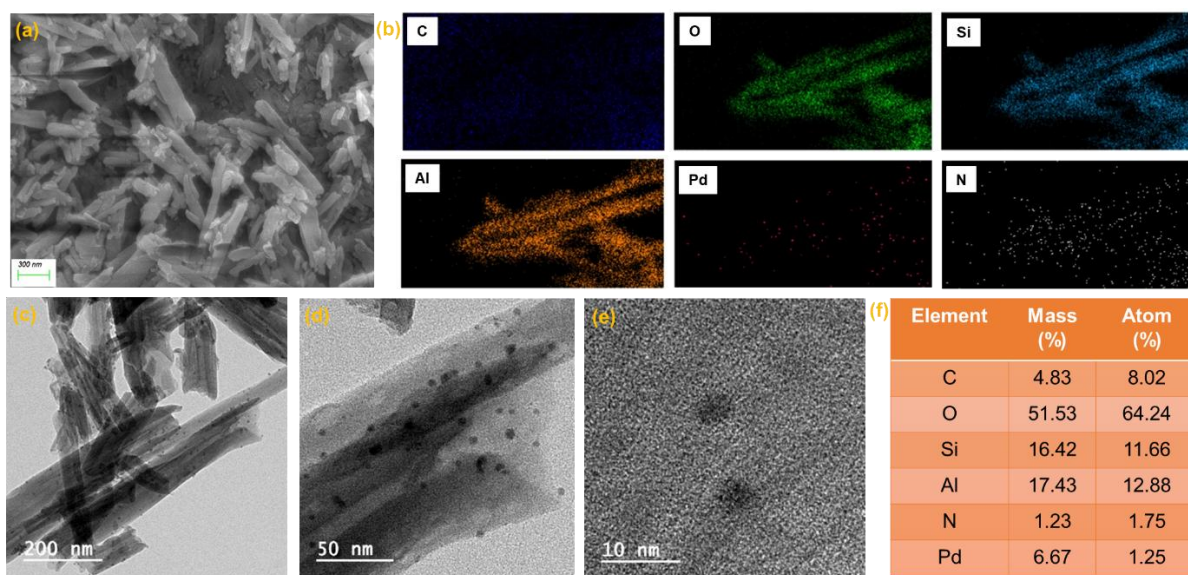


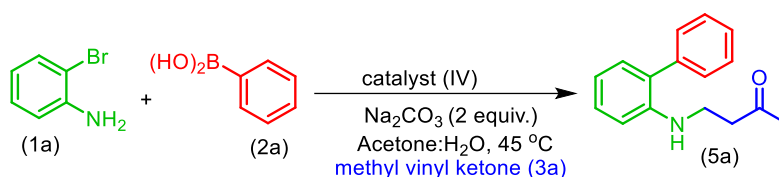
Figure 2.3: (a) FE-SEM image of catalyst (IV) predicting the HNTs matrix remain intact even after immobilization of both metal and enzyme. (b) All elemental mapping images of the corresponding catalyst demonstrate the uniform fabrication of PdNPs on HNTs. (c-e) High-resolution TEM images of the hybrid catalyst (IV) Showing the presence of palladium & enzyme onto the halloysite nanotubes. (f) EDS data of the prepared catalyst HNTs-NH₂-GA-Pd-Amy (IV).

2.2.3: Optimization of the reaction conditions

After successfully developing a nano-biohybrid catalyst (IV), we explored its applications toward the synthesis of functionalized biphenyls using a one-pot chemoenzymatic approach consisting Suzuki-Miyaura and aza-Michael addition reactions. As we have obtained 47% conversion for the one-pot chemoenzymatic reaction (**entry 1, Table 2.2**), next, we decided to optimize the reaction conditions to improve the yield of this transformation. In this context, first, we changed the amount of catalyst (IV) from 5 mg to 2.5 mg, 10 mg, and 15 mg and found that 5 mg was a sufficient amount to obtain the maximum conversion in the model reaction (**entries 2-4, Table 2.2**). Then, we screened the ratio of substrates and found that 1:1.5:1.5

equiv. of 1a:2a:3a respectively was the best combination to achieve the best conversion (**entries 5-10, Table 2.2**). Further, we replaced Na₂CO₃ with Cs₂CO₃ and K₂CO₃, however, Na₂CO₃ remained the best base for this transformation (**entries 11-12, Table 2.2**). In the last, we screened different solvents and their combinations, however, we observed that the reaction gave maximum conversion when 50% acetone in H₂O (v/v) was used as a solvent (**entries 13-16, Table 2.2**). Also, there was no improvement when the temperature was increased, however, the yield dropped if the temperature goes below than 45°C.

Table 2.2: Optimization of reaction conditions^a



| Entry | Deviation from standard reaction conditions | Conversion (5a) ^b |
|-------|--|------------------------------|
| 1 | No deviation | 47% |
| 2 | 2.5 mg catalyst used | 33% |
| 3 | 10 mg catalyst used | 48% |
| 4 | 15 mg catalyst used | 43% |
| 5 | 1.5 equiv. of 2a used | 62% |
| 6 | 2.0 equiv. of 2a used | 63% |
| 7 | 3.0 equiv. of 2a used | 62% |
| 8 | 1.0 equiv. of 3a used | 65% |
| 9 | 1.2 equiv. of 3a used | 69% |
| 10 | 1.5 equiv. of 3a used | 71% |
| 11 | Cs ₂ CO ₃ instead of Na ₂ CO ₃ | 37% |
| 12 | K ₂ CO ₃ instead of Na ₂ CO ₃ | 43% |
| 13 | H ₂ O only as a solvent | trace |
| 14 | Acetone only as a solvent | trace |
| 15 | EtOH in water (1:1, v/v) | 19% |
| 16 | DMSO in water (1:1, v/v) | 27% |

^aStandard Reaction conditions: 2-Bromoaniline (**1a**) (0.06 mmol, 1 equiv.), phenylboronic acid (**2a**) (0.06 mmol, 1.0 equiv.), methyl vinyl ketone (**3a**) (0.06 mmol, 1.0 equiv.), catalyst (IV)=5 mg, Na₂CO₃ (0.12 mmol, 2 equiv.), acetone: H₂O (1:1) as a solvent, 45°C, overnight. ^b%conversion using HPLC.

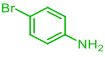
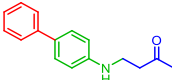
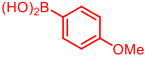
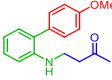
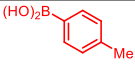
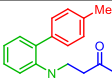
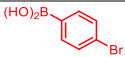
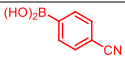
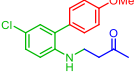
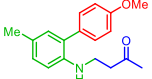
2.2.4: Substrate scope for Suzuki-Miyaura coupling/aza-Michael addition reaction

Having the best reaction conditions, we explored the substrate scope for the one-pot chemoenzymatic reaction using a catalyst (IV) as shown in **Table 2.3**. In this regard, we checked the effect of substitutions at bromoaniline (**entries 1-6, Table 2.3**). The reaction gave good conversion when electron-donating groups like -CH₃ and halides were installed at the para position of 2-bromoaniline (**entries 2-4, Table 2.3**). However, there was no reaction took place when 4-NO₂ containing 2-bromoaniline was employed (**entry 5, Table 2.3**). Also, the reaction worked well in the case of 4-bromoaniline instead of 2-bromoaniline (**entry 6, Table 2.3**). Next, we employed differently substituted phenylboronic acid along with 2-bromoaniline and methyl vinyl ketone. The electron-donating group such as -CH₃ and -OMe were tolerated well but surprisingly, there was no reaction in the case of 4-CN and 4-Br substitutions (**entries 7-10, Table 2.3**). Next, we evaluated the compatibility of substitutions at both 2-bromoaniline and phenylboronic acid and obtained the corresponding products in 55% and 58% isolated yield (**entries 11-12, Table 2.3**). In the last, we replaced methyl vinyl ketone with cyclohexenone and cyclopentenone respectively, however, there was no reaction observed and it might be due increment in the steric hindrance during the aza-Michael addition reaction.

Table 2.3: Substrate scope of one-pot chemoenzymatic reaction^a

Reaction scheme: Bromoaniline (1) + Phenylboronic acid (2) + Methyl vinyl ketone (3a) $\xrightarrow[\text{Acetone:H}_2\text{O (1:1)}]{\text{Catalyst (IV), Na}_2\text{CO}_3 \text{ (2 equiv.)}}$ Product (5)

| Entry | Bromoaniline (1) | Phenylboronic acid (2) | Product (5), yield ^b |
|-------|------------------|------------------------|---------------------------------|
| 1 | | | 5a, 63% |
| 2 | | | 5b, 68% |
| 3 | | | 5c, 59% |
| 4 | | | 5d, 53% |
| 5 | | | |

| | 1e | 2a | nr ^c |
|----|---|---|--|
| 6 |  1f | 2a |  5e, 71% |
| 7 | 1a |  2b |  5f, 64% |
| 8 | 1a |  2c |  5g, 61% |
| 9 | 1b |  2d | nr ^c |
| 10 | 1a |  2e | nr ^c |
| 11 | 1c | 2b |  5h, 55% |
| 12 | 1b | 2b |  5i, 58% |

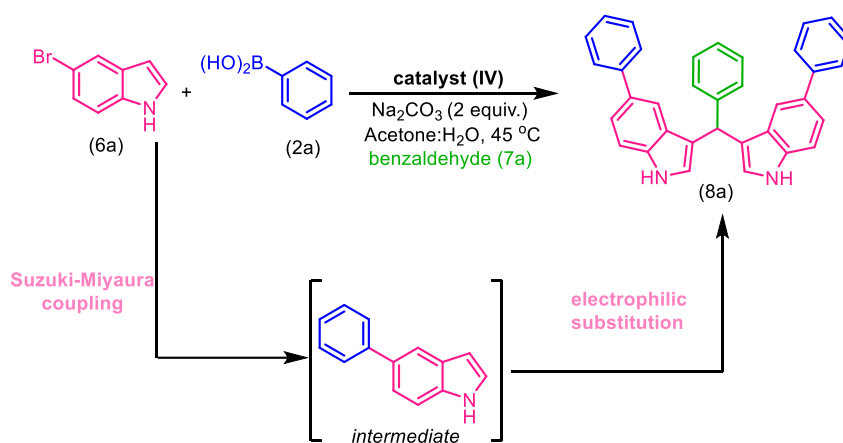
^aReaction conditions: Bromoaniline (**1**) (1 equiv.), phenylboronic acid (**2**) (1.5 equiv.), methyl vinyl ketone (**3a**) (1.5 equiv.), catalyst (IV)=50 mg, Na₂CO₃ (2 equiv.), acetone: H₂O (1:1) as a solvent, 45 °C, overnight. ^bisolated yield, ^cnr=no reaction.

2.2.5: Reaction condition optimization for bis(indolyl)methanes synthesis

In the second phase of our endeavors, we planned to extend the application of constructed nano-biohybrid catalyst in the one-pot synthesis and functionalization of pharmacologically important bis(indolyl)methane derivatives. Initially, one-pot synthesis consisting Pd-catalyzed functionalization of 5-bromoindole using Suzuki-Miyaura coupling reaction followed by enzyme-catalyzed electrophilic synthesis of bis(indolyl)methane was set up using previously optimized reaction conditions (**Table 2.2**). Although, no bis(indolyl)methane product was observed in the one-pot chemoenzymatic reaction (**entry 1, Table 2.4**). Afterward, we changed the solvent system from 50% acetone in water (v/v) to 20% DMSO in H₂O which again did not lead to desired product (**entry 2, Table 2.4**). Subsequently, we put efforts towards the exchange

of solvent from 50% acetone in water (v/v) to 20% DMSO in H₂O after the completion of the first step i.e., Suzuki-Miyaura coupling. As it has been reported previously that 20% DMSO in H₂O is the better solvent system for enzyme-catalyzed synthesis of bis(indolyl)methane.²² To our delight, we were successful this time to synthesize bis(indolyl)methane derivatives in one pot using chemoenzymatic approach (**entry 3, Table 2.4**). Also, we tried to change the loading of catalyst (IV) and molar ratio of substrates along with the reaction temperature but there was no improvement in the outcome of the reaction (**entries 4-7, Table 2.4**).

Table 2.4: Reaction conditions optimization^a

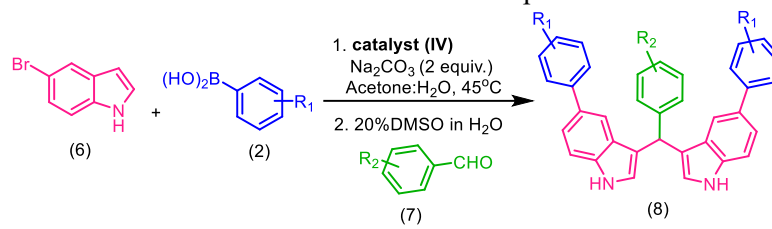


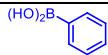

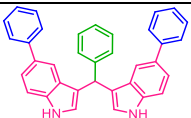
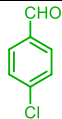
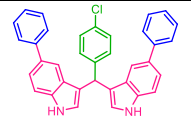
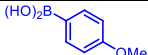

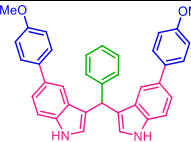
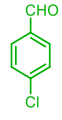
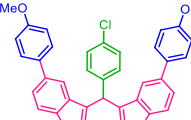
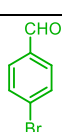
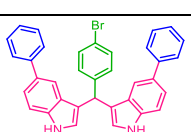
| Entry | Deviation from standard reaction conditions | Yield (8a) ^b |
|-------|---|-------------------------|
| 1 | No deviation | 0% |
| 2 | 20% DMSO in H ₂ O (v/v) | 0% |
| 3 | First 50% acetone in H ₂ O (v/v) then 20%DMSO in H ₂ O(v/v) | 57% |
| 4 | Catalyst (IV) 10 mg | 53% |
| 5 | Molar ratio of 6a:2a:7a =1:2:2 equiv. | 56% |
| 6 | Temperature 60°C | 53% |
| 7 | Temperature 25°C | - |

^a**Standard Reaction conditions:** 5-bromoindole (**1a**) (0.05 mmol, 1 equiv.), phenylboronic acid (**2a**) (0.08 mmol, 1.5 equiv.), benzaldehyde (**7a**) (0.03 mmol, 1.5 equiv.), catalyst (IV)=5 mg, Na₂CO₃ (0.09 mmol, 2 equiv.), acetone: H₂O (1:1) as a solvent, 45 °C, overnight. ^b%conversion using HPLC.

2.2.6: Substrate scope

Next, to extend the substrate scope of one-pot synthesis of functionalized bis(indolyl)methane, we examined differently substituted boronic acids and benzaldehydes as depicted in **Table 2.5**. Gratifyingly, there was no effect of different substitutions on the outcome of the reaction and we obtained the desired products in good yields (**entries 1-5, Table 2.5**).

Table 2.5: Substrate scope^a

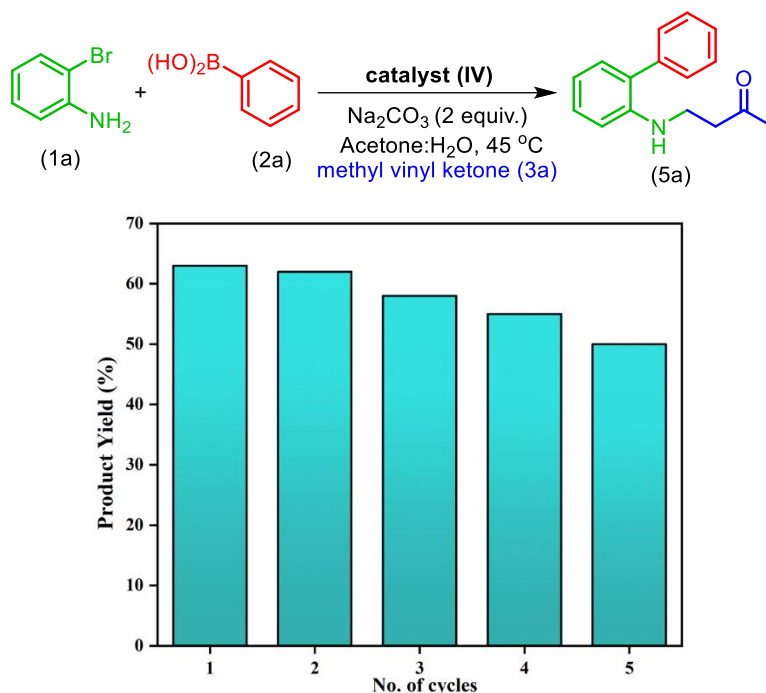
| Entry | Phenyl boronic acid | Benzaldehyde | Product |
|-------|---|--|--|
| 1 |  2a |  7a |  8a, 53% |
| 2 | 2a |  7b |  8b, 55% |
| 3 |  2b |  7a |  8c, 62% |
| 4 | 2b |  7b |  8d, 59% |
| 5 | 2a |  7c |  8e, 53% |

^aReaction conditions: 5-bromoindole (6) (100 mg, 0.5 mmol, 1 equiv.), phenyl boronic acid (2) (98 mg, 0.8 mmol, 1.5 equiv.), benzaldehyde (7) (30 μ L, 0.3 mmol, 1.5 equiv.), catalyst (IV) =50 mg, Na₂CO₃ (105 mg, 1.0 mmol, 2 equiv.), acetone: H₂O (1:1) as a solvent which altered to DMSO:H₂O (20%) after completion of Suzuki reaction, 45 °C, overnight. ^bisolated yield.

2.2.7: Catalyst (IV)'s reusability, thermostability and shelf life

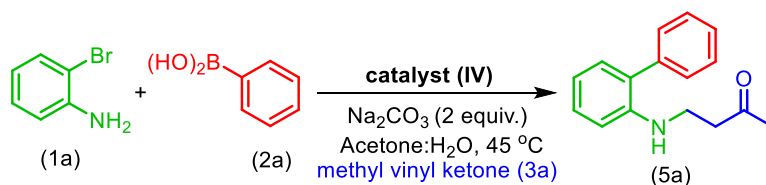
Next, we tested the catalyst (IV)'s ability to be reused in the one-pot cascade reaction. In this context, after the completion of the first cycle, the catalyst was separated by centrifugation and the resultant mixture was decanted. The above-separated catalyst was washed with isopropyl alcohol dried in a desiccator and used for a further catalytic cycle to produce the desired

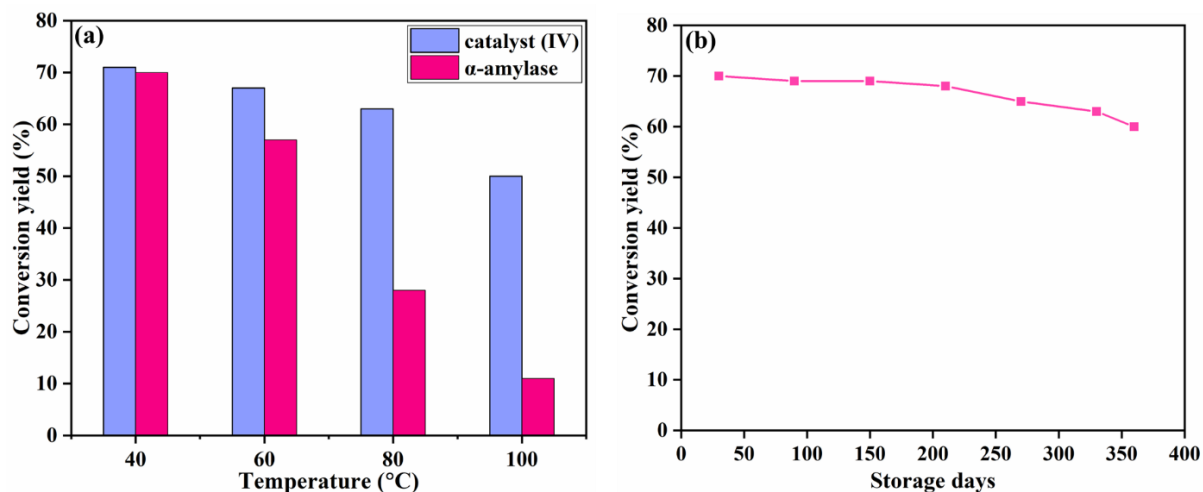
functionalized biphenyls (**5a**). Gratifyingly, there was only a slight decrease in the yield of the desired product in consecutive cycles and catalyst was reusable up to five catalytic cycles (**Scheme 2.3**).



Scheme 2.3: Assessment of catalyst (IV)'s recyclability for the gram scale model reaction. Reaction conditions: 2-Bromoaniline (**1**) (1.00 g, 1 equiv.), phenylboronic acid (**2**) (1.06 g, 1.5 equiv.), methyl vinyl ketone (**3a**) (0.76 mL, 1.5 equiv.), catalyst (IV)=500 mg, Na₂CO₃ (1.23 g, 2 equiv.), acetone: H₂O (1:1) as a solvent, 45 °C, overnight. %conversion yield using HPLC.

Moreover, we have tested the thermostability of hybrid catalyst (IV) and pure α -amylase enzyme for the model reaction at different reaction temperatures ranging from 40 to 100 °C (**Scheme 2.4a**). Gratifyingly, there was a slight decrement in the yield in the case of catalyst (IV) whereas the reaction almost diminished in the case of pure enzyme when temperature increased up to 100 °C. These experiments suggested the increment in the thermostability of α -





Scheme 2.4. Thermostability and shelf life of hybrid catalyst (IV) (a) Comparison of thermostability of catalyst (IV) with pure α -amylase enzyme. (b) Shelf life of hybrid catalyst (IV).

amylase enzyme after immobilization. Further, after 12 months of storage at room temperature in an airtight container, we examined the catalyst's effectiveness for the model reaction (**Scheme 2.4b**). Gratifyingly, the activity of the biohybrid catalyst (IV) did not much decline, and we successfully produced the cascade product (**5a**) with a 62% yield.

2.2.8: Optimization of Pd and α -amylase loading over HNTs

The immobilization efficiency of both Pd-nanoparticles and α -amylase over HNTs was optimized as given below.

2.2.8a: Effect of palladium concentration on immobilization: The PdNPs immobilization efficiency was checked by adjusting the amount of palladium salt i.e. Pd(OAc)₂ from 5 mg to 20 mg. At the very first, in a 50 mL beaker, a solution of Pd(OAc)₂ (5 mg to 20 mg, 0.07 equiv. to 0.27 equiv.) in deionized water (D.I.) was made. This solution was then treated with a solution of L-sodium ascorbate (235 mg, 3.5 equiv.) in D.I. water (5 mL), followed by the addition of trisodium citrate.2H₂O (135 mg, 1.35 equiv.) in D.I. water (3.3 mL). The resulting mixture was stirred at room temperature for 15 min which induced black color solution due to the formation of PdNPs. Afterward, the solution was allowed to rest at rt for ~80 min. Then, the PdNPs solution was mixed with HNTs (100 mg, 1 equiv.), and the colloid combination was agitated for 15 min. After the rest of 10 min, the reaction mixture was subjected to centrifugation and the residue was washed with D.I water and isopropyl alcohol.²³ The resultant catalysts were characterized by EDS after drying at room temperature for 24 h in a desiccator. Thus, it was depicted by EDS studies (**Table 2.6**) that whenever we increase the amount of Pd-salt, the immobilization efficiency increases. Also, we tested Pd-HNTs with different loading

of Pd for catalyzing the Suzuki-Miyaura coupling reaction, however, we observed that Pd-HNTs prepared using 10 mg of Pd(OAc)₂ per 100 mg of HNTs was sufficient to complete the Suzuki-Miyaura coupling reaction, so we opted 10 mg of Pd(OAc)₂ per 100 mg of HNTs for the preparation of catalysts (1-IV)

Table 2.6: mass (%) ratio of Pd detected by EDS

| Sr. No. | Pd salt (mg) | Mass (%) |
|---------|--------------|----------|
| 1 | 5 | 1.27 |
| 2 | 10 | 2.51 |
| 3 | 15 | 3.78 |
| 4 | 20 | 6.64 |

2.2.8b: Effect of enzyme concentration: Different enzyme dosages, ranging from 1 mg/mL to 9 mg/mL, have been immobilized with the goal to calculate the amount of α -amylase immobilized onto HNTs. Initially, varied enzyme concentrations were made in a potassium phosphate buffer, and measured the absorbance at 289 nm. Further, 30 mg of imine functionalized HNTs-NH₂-GA support was added to the reaction mixture in order to get enzyme immobilization. Afterward, the solid support was separated by centrifugation and washed with phosphate buffer. Then, used the following formula (equation 1) to determine the enzyme immobilization efficiency of the support, taking into account the absorbance of residual and washings conducted after immobilization.

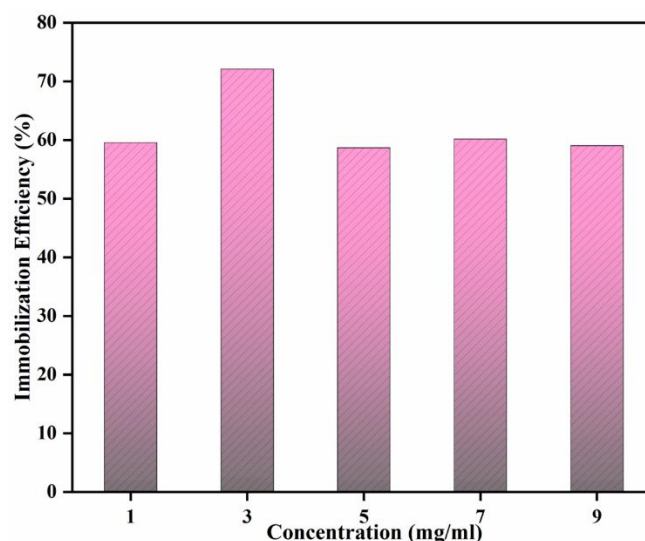


Figure 2.4: impact of α -amylase loading on immobilization efficiency

$$\text{Immobilization Efficiency (\%)} = \frac{A_{(pre)} - A_{(post)}}{A_{(pre)}} \times 100 \quad (1)$$

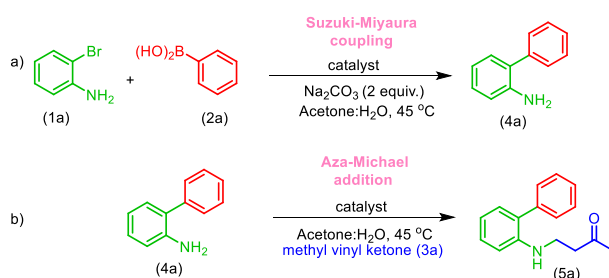
Where A is α -amylase's 289 nm absorbance. The absorbance of the solution prior to enzyme immobilization is designated as $A_{(pre)}$, and the absorbance of the solution following enzyme immobilization is designated as $A_{(post)}$.²⁴

As shown in the **figure 2.4** higher loading of α -amylase was noted at 3 mg/mL, afterward immobilization saturation occurred.

2.2.9: Comparison of relative activity of the biohybrid catalyst and native catalysts

To compare the catalytic effectiveness of a hybrid catalyst with an individual catalyst, we set up different experiments as displayed in **Table 2.7**. First, the Suzuki-Miyaura coupling reaction was set up using biohybrid catalyst (IV) under the optimized conditions and got the respective product (**4a**) with a conversion of 90% (**entry 1, Table 2.7**). Then, we set up the Michael addition reaction of (**4a**) with methyl vinyl ketone in the presence of biohybrid catalyst (IV) and obtained the resulting product (**5a**) in 71% yield (**entry 1, Table 2.7**). However, when we tested the catalytic effectiveness of the individual catalysts for the corresponding reactions, Pd(OAc)₂ yielded the Suzuki-Miyaura coupling product (**4a**) in 92% yield (**entry 2, Table 2.7**) and α -amylase as a biocatalyst for the aza-Michael addition reaction afforded the product (**5a**) in 63% yield (**entry 3, Table 2.7**). These experiments show the effectiveness of biohybrid catalyst (IV) in a sequential manner as compared to the individual catalysts.

Table 2.7: Comparative studies on the relative activity of the biohybrid catalyst (IV) and native catalysts^a



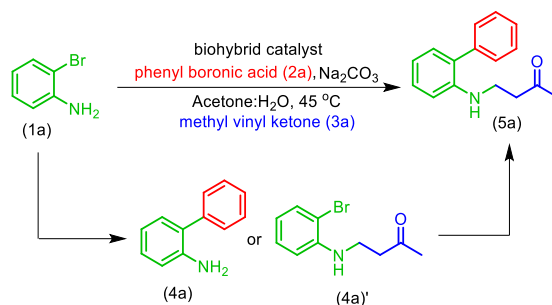
| Entry | Catalyst | Conversion (4a) ^b | Conversion (5a) ^b |
|-------|----------------------|------------------------------|------------------------------|
| 1 | Biohybrid (IV) | 90% | 71% |
| 2 | Pd(OAc) ₂ | 92% | - |
| 3 | α -amylase | - | 63% |

^a**Reaction conditions:** (i) Suzuki-Miyaura coupling reaction: catalyst 5 mg, 2-bromoaniline (**1a**) (1 equiv.), phenylboronic acid (**2a**) (1.5 equiv.), Na₂CO₃ (2 equiv.), 2 ml of acetone:H₂O (1:1) as a solvent, 45 °C, overnight; (ii) aza-Michael addition reaction: **4a** (1 equiv.), methyl vinyl ketone (**3a**) (1.5 equiv.), catalyst 5 mg, 2 ml of acetone:H₂O (1:1) as a solvent, 45 °C. [b]%conversion using HPLC.

2.2.10: Quantification of the chemoenzymatic cascade's intermediates

We have quantified the by-product formed in the one-pot chemoenzymatic approach as shown in **Table 2.8**. We observed only the formation of Suzuki product (**4a**) in 89% when employing HNTs-Pd as a catalyst (**entry 6, Table 2.8**). Also, we observed only the aza-Michael addition product (**4a'**) in 58% yield when using catalyst HNTs-Pd-NH₂-GA-Amy (III) (**entry 3, Table 2.8**).

Table 2.8: Quantification of intermediates for chemoenzymatic cascade synthesis^a



| Entry | Catalyst | Product (4a) ^b | Product (4a') ^b | Product (5a) ^b |
|-------|--|------------------------------|-------------------------------|------------------------------|
| 1 | HNTs-Pd-Amy (I) | - | - | - |
| 2 | HNTs-Amy-Pd (II) | - | - | - |
| 3 | HNTs-Pd-NH ₂ -GA-Amy (III) | - | 58% | - |
| 4 | HNTs-NH ₂ -GA-Pd-Amy (IV) | - | - | 47% ^b |
| 5 | HNTs | - | - | - |
| 6 | HNTs-Pd | 89% | - | - |
| 7 | α -amylase+Pd(OAc) ₂ | - | - | - ^c |

^a**Reaction conditions:** 2-Bromoaniline (**1a**) (0.06 mmol, 1.0 equiv.), phenylboronic acid (**2a**) (0.06 mmol, 1.0 equiv.), methyl vinyl ketone (**3a**) (0.06 mmol, 1.0 equiv.), catalyst 5 mg, Na₂CO₃ (0.12 mmol, 2 equiv.), acetone: H₂O (1:1) as a solvent, 45 °C, overnight. ^b%conversion using HPLC, ^cpure enzyme and Pd-catalyst were added in the same pot.

2.3: Experimental Section

2.3.1: General information

All solvents and chemicals were purchased commercially and used as such without further purification. HNTs and α -amylase were purchased from Sigma aldrich. The thin layer chromatography technique was used to monitor the progress of the cascade reaction. The desired products were purified by column chromatography using particular ratios of ethyl acetate and hexane as mobile phase over stationary phase silica (60-120 mesh size). The product conversion (%) was analyzed from Thermo scientific Dionex, Ultimate 3000 high

performance liquid chromatography (HPLC). JEOL NMR 400 MHz spectrometer was used to collect NMR spectra of synthesized compounds using CDCl_3 having TMS as an internal standard as a deuterated solvent. The coupling constant (J) and chemical shift (δ) is expressed in hertz (Hz) and parts per million (ppm) subsequently. The splitting of signals is abbreviated as; singlet (s), doublet (d), triplet (t), doublet of doublet (dd), triplet of doublet (td), doublet of triplet (dt), broad singlet (bs) and multiplet (m). The Fourier transform infrared FTIR spectrum was collected on IRT racer-100 spectrophotometer. The X-ray diffraction (XRD) analysis was performed on Rigaku SmartLab SE instrument with angle range 5 to 90 degree. Field emission scanning electron microscopy (Fe-SEM) and energy dispersive X-ray spectroscopy (EDS) studies were done on Zeiss device. The high-resolution images of the catalyst were captured by JEOL JEM 2100 PLUS instrument.

2.3.2: Procedure for the preparation of catalyst (IV)

2.3.2a: Surface modification of halloysite nanotubes: For the functionalization of HNTs, 200 mg HNTs was combined with 1 mL of 3-aminopropyltriethoxysilane. Then 20 mL of ethanol/acetic acid (3/5; v/v) was added as a co-solvent in a nitrogen environment. The resulting mixture was subjected to a 30 min sonication followed by heating for one hour at 110 °C. After the allotted time, the white-colored amine-modified HNTs were cooled and centrifuged at 7000 rpm, washed with ethanol (3 x 10 mL), and dried for an overnight period at 60 °C. The following stage involved adding amine functionalized HNTs to a 2.5% (v/v) glutaraldehyde solution and shaking it vigorously for 24 h at room temperature. The resulting mixture was centrifuged (7000 rpm) and the supernatant was separated from the white solid residue. The residue was rinsed multiple times with ethanol to get rid of any remaining glutaraldehyde molecules, then dried at 60 °C.

2.3.2b: Procedure for immobilization of palladium onto functionalized HNTs

In a beaker, $\text{Pd}(\text{OAc})_2$ salt (15 mg, 0.13 equiv.) was dissolved in deionized water (2.5 mL). The resulted colloidal solution was subsequently treated with a solution of L-sodium ascorbate (350 mg, 3.5 equiv.) in deionized water followed by the addition of trisodium citrate.2H₂O (200 mg, 1.35 equiv.) in deionized water. At room temperature, the mixed solution was allowed to stir for 15 min. The palladium acetate solution mixture's initial orange color changed to black during the first 10 min, signifying the emergence of Pd nanoparticles. The solution was allowed to rest at room temperature and exposed to air for 80 min.²³ Afterward, the resulting solution was mixed with surface functionalized HNTs (150 mg, 1 equiv.), and the colloid combination was stirred for 15 min. After stirring, it was left undisturbed for 10 min. The

reaction mixture was subjected to centrifugation and the residue was washed with D.I water (3 x 10 mL) and isopropyl alcohol (3 x 10 mL). The resultant grey powder was dried at 25 °C in the desiccator for 24 h.

2.3.2c: Procedure for immobilization of α -amylase onto functionalized HNTs

The α -amylase was immobilized by combining 150 mg of HNT-NH₂-GA-Pd support with an optimized enzyme concentration of 3 mg/mL in 25 mL of potassium phosphate (KPi) buffer (100mM, pH 7.4) at 25 °C with gentle stirring for overnight. After the mentioned time, it was transferred in a falcon tube and centrifuged at 8000 rpm. The obtained residue was washed with KPi buffer (3 x 10 mL) and then with distilled water (3 x 10 mL). The resultant grey coloured catalyst (IV) was dried at 40 °C and kept in an air-tight container for further use.

2.3.3: General procedure for the one-pot chemoenzymatic synthesis of functionalized biphenyls

A mixture of 2-bromoaniline (**1a**) (100 mg, 1 equiv.), phenyl boronic acid (**2a**) (106 mg, 1.5 equiv.), catalyst (IV) (50 mg), and sodium carbonate (123 mg, 2 equiv.) in acetone:water (1:1) (10 mL) as a solvent was stirred for 5 hours in a round bottom flask at 45 °C. The formation of Suzuki-Miyaura product was monitored by TLC, afterwards methyl vinyl ketone (**3a**) (70 μ L, 1.5 equiv.) was added to the same reaction vessel and stirred the reaction mixture at 45 °C for overnight. After completion of aza-Michael addition reaction, as indicated by TLC, the catalyst was separated by centrifugation and the reaction mixture was subjected to work-up using ethyl acetate and water. The final product was purified by column chromatography using silica as a stationary phase and ethyl acetate in hexane as a mobile phase. The resultant yellowish-brown viscous product (**5a**) was obtained in 63% isolated yield.

2.3.4: General procedure for the one-pot chemoenzymatic synthesis of bis(indolyl)methane derivatives

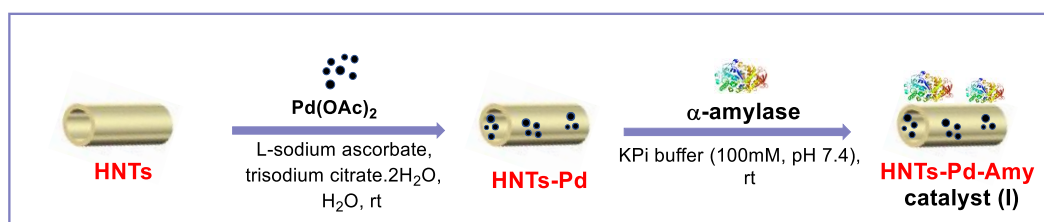
The 50 mg of biohybrid catalyst (IV) in 10 mL of acetone:H₂O (1:1) was added into a round bottom flask equipped with a stirrer bar. Then, 5-bromo indole (100 mg, 0.5 mmol, 1 equiv.), phenylboronic acid (98 mg, 0.8 mmol, 1.5 equiv.), and sodium carbonate (105 mg, 1.0 mmol, 2.0 equiv.) were added and agitated the reaction mixture at 45°C. The formation of the Suzuki-Miyaura product was checked by TLC. Thereafter, the catalyst was recovered by centrifugation and the supernatant (reaction mixture) was poured into a separatory funnel for work-up. The crude combination was then re-added to the round bottom flask containing the recovered catalyst along with 20% DMSO in water as a solvent and benzaldehyde (30 μ L, 0.3 mmol, 1.5

equiv.). After the overnight stirring at 45 °C, the reaction mixture was subjected to work-up followed by column chromatography using ethyl acetate and hexane as an eluting solvent (20:80) to get the pure product.

2.3.5: Synthesis and characterization of nano-biohybrid catalysts

2.3.5a: HNTs-Pd-Amy (Catalyst I)

HNTs were used as solid support for the preparation of catalyst I and they were treated with freshly made PdNPs according to the procedure followed for the preparation of catalyst (IV). After that, α -amylase was reacted with HNTs-Pd support at room temperature in phosphate buffer to get it immobilize (**Scheme 2.5**).



Scheme 2.5: Schematic representation of catalyst (I) preparation

Further, catalyst (I) was characterized by Fe-SEM and EDS to know the outer morphology and elemental composition of the catalyst respectively. The Fe-SEM image as shown in **Figure 2.5(a)** depicted the presence of Pd-nanoparticles in cluster form. Further EDS data provided insight into the presence of Pd and enzyme over HNTs by knowing the weight percentage (**Figure 2.5(b,c)**).

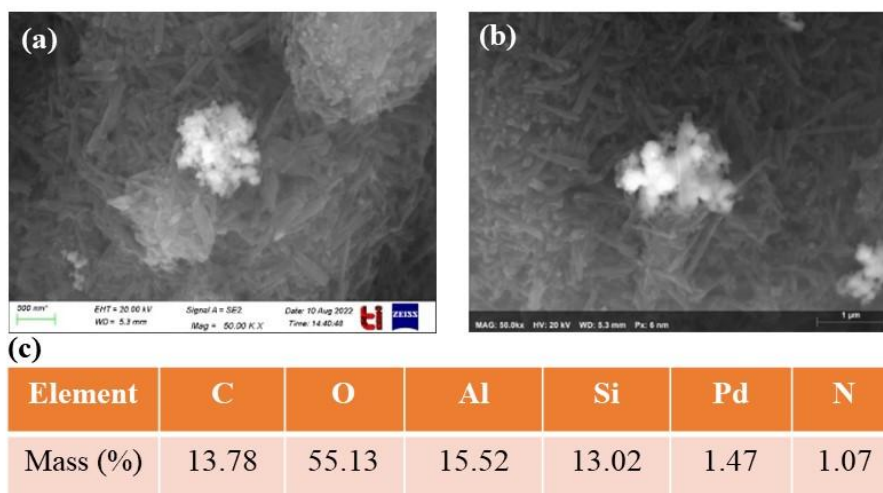
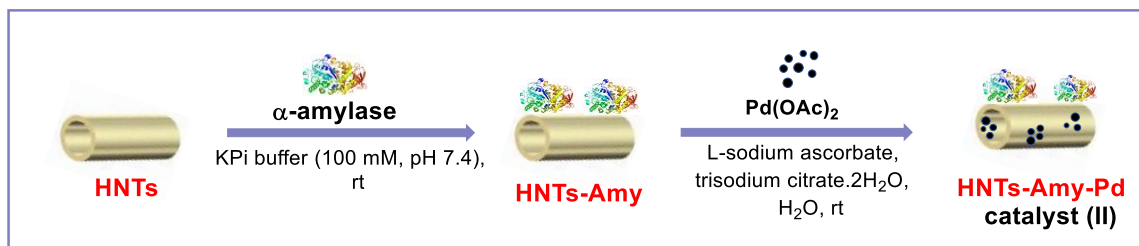


Figure 2.5: (a) Fe-SEM image and (b, c) EDS data of catalyst (I)

2.3.5b: HNTs-Amy-Pd (Catalyst II)

For the preparation of catalyst II, HNTs were treated with a freshly prepared α -amylase solution in phosphate buffer at room temperature. Next, enzyme-supported HNTs were treated with PdNPs in water for Pd-immobilization (**Scheme 2.6**).



Scheme 2.6: Schematic representation of catalyst (II) preparation

The biohybrid catalyst was characterized by Fe-SEM and EDS for the confirmation of Pd and α -amylase enzyme onto HNTs. Again, PdNPs were in the form of clusters over the HNTs as shown in **Figure 2.6(a)**. Further, the EDS data depicted the presence of Pd (8.06%) and N (1.41%) which gave a clear indication of the successful immobilization of Pd and α -amylase enzyme onto HNTs (**Figure 2.6(b,c)**).

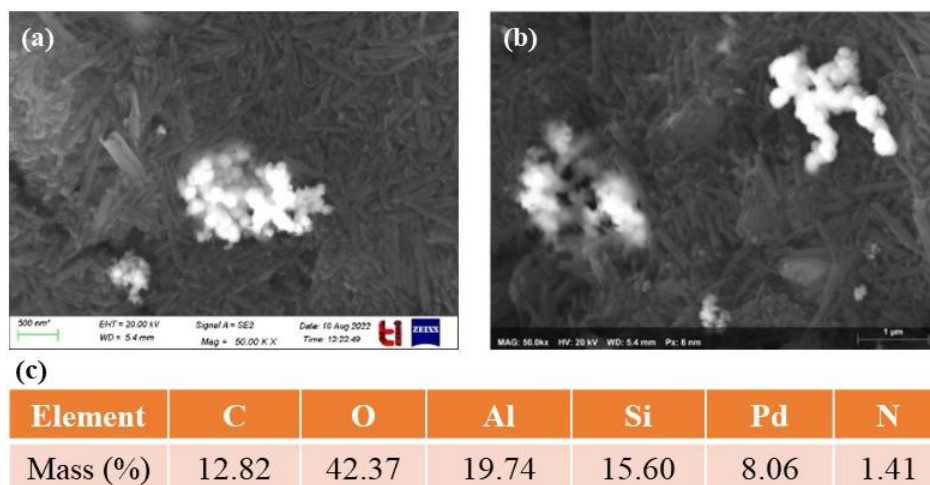
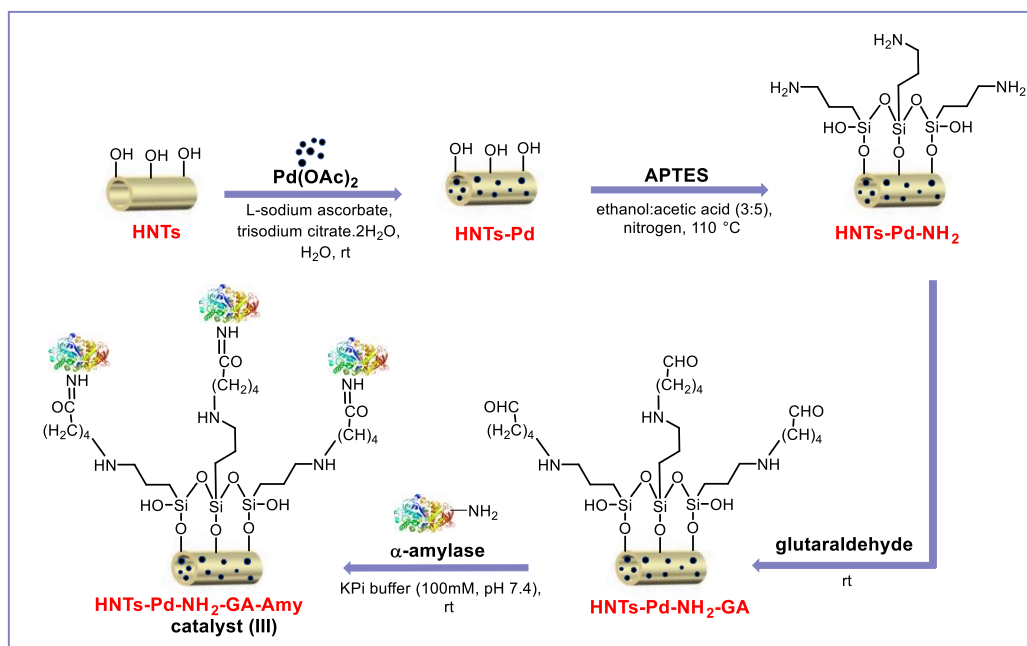


Figure 2.6: (a) FeSEM image and (b, c) EDS data of catalyst (II)

2.3.5c: HNTs-Pd-NH₂-GA-Amy (Catalyst III)

For the construction of catalyst (III), initially, HNTs were mixed with freshly produced PdNPs (according to the procedure used for catalyst (IV)) and then HNTs-Pd were dispersed with APTES solution under a nitrogen atmosphere for the amino-functionalization of HNTs. Afterward, the resulting amino-functionalized HNTs were subjected to glutaraldehyde solution at room temperature to generate HNTs-Pd-NH₂-GA which was further treated with α -amylase for immobilization of enzyme (**Scheme 2.7**).

Next, Fe-SEM and EDS techniques were utilized to know the successful loading of Pd and α -amylase (**Figure 2.7**). In this catalyst (III), uniform distribution of palladium was noticed under the scanning electron microscope. Moreover, EDS data gave an estimation of the presence of Pd and nitrogen in the catalyst.



Scheme 2.7: Schematic representation of catalyst (III) preparation

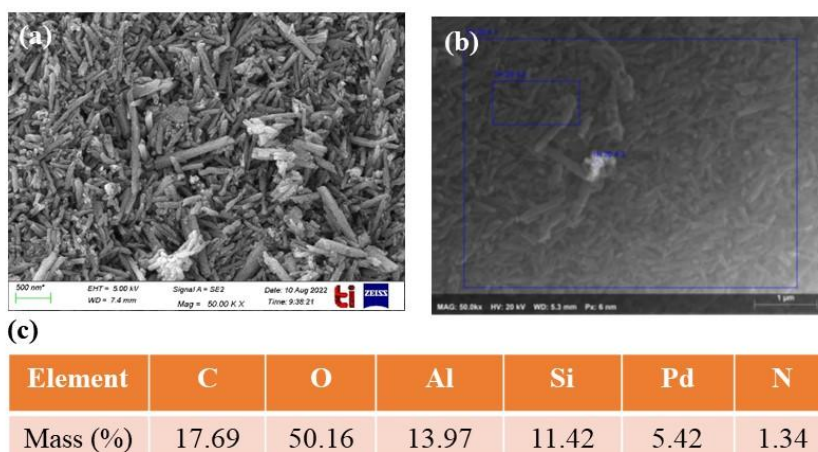


Figure 2.7: (a) FeSEM image and (b, c) EDS data of catalyst (III)

2.3.6: Characterization data of synthesized compounds

4-([1,1'-biphenyl]-2-ylamino)butan-2-one (5a): ^1H NMR (400 MHz, CDCl_3): δ 7.452-7.414 (m, 2H), 7.372-7.314 (m, 3H), 7.256-7.212 (m, 1H), 7.085 (dd, $J = 7.2$ Hz, 1.6 Hz, 1H), 6.770 (t, $J = 7.6$ Hz, 1H), 6.705 (d, $J = 8.0$ Hz, 1H), 4.167 (bs, 1H), 3.392 (t, $J = 7.2$ Hz, 2H), 2.689 (t, $J = 6.4$ Hz, 2H), 2.114 (s, 3H) ppm. ^{13}C NMR: δ 207.767, 144.577, 139.343, 130.532, 129.378, 129.006, 128.787, 128.234, 127.376, 117.288, 110.413, 42.732, 38.527, 30.422 ppm.

4-((5-methyl-[1,1'-biphenyl]-2-yl)amino)butan-2-one (5b): ^1H NMR (400 MHz, CDCl_3): δ 7.440-7.403 (m, 2H), 7.364-7.304 (m, 3H), 7.049 (dd, $J = 8.4$ Hz, 2.4 Hz, 1H), 6.917 (d, $J = 2$ Hz, 1H), 6.634 (d, $J = 8$ Hz, 1H), 4.014 (bs, 1H), 3.368 (t, $J = 6.4$ Hz, 2H), 2.674 (t, $J = 6.4$ Hz, 2H), 2.265 (s, 3H), 2.105 (s, 3H) ppm. ^{13}C NMR: δ 207.920, 142.298, 139.457, 131.276,

129.369, 129.130, 128.940, 128.415, 127.300, 126.527, 110.842, 42.799, 38.909, 30.413, 20.420 ppm.

4-((5-chloro-[1,1'-biphenyl]-2-yl)amino)butan-2-one (5c): ^1H NMR (400 MHz, CDCl_3): δ 7.453-7.415 (m, 2H), 7.376-7.309 (m, 3H), 7.169 (dd, $J = 8.4$ Hz, 2.8 Hz, 1H), 7.048 (d, $J = 2.4$ Hz, 1H), 6.603 (d, $J = 8.4$ Hz, 1H), 4.156 (bs, 1H), 3.351 (t, $J = 6.4$ Hz, 2H), 2.671 (t, $J = 6.4$ Hz, 2H), 2.112 (s, 3H) ppm. ^{13}C NMR: δ 207.614, 143.271, 138.027, 130.132, 129.569, 129.188, 129.159, 128.329, 127.872, 121.903, 111.500, 42.465, 38.584, 30.441 ppm.

4-((5-fluoro-[1,1'-biphenyl]-2-yl)amino)butan-2-one (5d): ^1H NMR (400 MHz, CDCl_3): δ 7.460-7.420 (m, 2H), 7.377-7.322 (m, 3H), 6.932 (td, $J = 8.8$ Hz, 3.2 Hz, 1H), 6.837 (dd, $J = 9.2$ Hz, 2.8 Hz, 1H), 6.622 (dd, $J = 9.2$ Hz, 5.2 Hz, 1H), 3.981 (bs, 1H), 3.339 (t, $J = 6.4$ Hz, 2H), 2.663 (t, $J = 6.4$ Hz, 2H), 2.110 (s, 3H) ppm. ^{13}C NMR: δ 207.700, 156.830, 154.484, 141.059, 138.322, 129.188, 129.111, 127.815, 117.288, 117.059, 114.828, 114.618, 111.557, 111.481, 42.637, 39.271, 30.394 ppm.

4-([1,1'-biphenyl]-4-ylamino)butan-2-one (5e): ^1H NMR (400 MHz, CDCl_3): δ 7.525 (d, $J = 6.8$ Hz, 2H), 7.433 (dt, $J = 8.8$ Hz, 2.8 Hz, 2H), 7.382 (t, $J = 7.6$ Hz, 2H), 7.248 (t, $J = 8.4$ Hz, 1H), 6.668 (dt, $J = 8.8$ Hz, 3.2 Hz, 2H), 4.087 (bs, 1H), 3.452 (t, $J = 6.0$ Hz, 2H), 2.769 (t, 6.0 Hz, 2H), 2.170 (s, 3H) ppm. ^{13}C NMR: δ 208.253, 147.171, 141.250, 130.627, 128.759, 128.110, 126.394, 126.222, 113.331, 42.656, 38.413, 30.460 ppm.

4-((4'-methoxy-[1,1'-biphenyl]-2-yl)amino)butan-2-one (5f): ^1H NMR (400 MHz, CDCl_3): δ 7.284 (dt, $J = 8.8$ Hz, 2.8 Hz, 2H), 7.214 (td, $J = 8.0$ Hz, 1.6 Hz, 1H), 7.065 (dd, $J = 7.2$ Hz, 1.2 Hz, 1H), 6.968 (dt, $J = 8.4$ Hz, 2.8 Hz, 2H), 6.755 (td, $J = 7.2$ Hz, 0.8 Hz, 1H), 6.687 (d, $J = 8.0$ Hz, 1H), 4.265 (bs, 1H), 3.842 (s, 3H), 3.390 (t, $J = 6.4$ Hz, 2H), 2.693 (t, $J = 6.4$ Hz, 2H), 2.119 (s, 3H) ppm. ^{13}C NMR: δ 207.881, 158.890, 144.778, 131.514, 130.599, 130.484, 128.520, 127.910, 117.250, 114.408, 110.298, 55.395, 42.751, 38.518, 30.432 ppm.

4-((4'-methyl-[1,1'-biphenyl]-2-yl)amino)butan-2-one (5g): ^1H NMR (400 MHz, CDCl_3): δ 7.256-7.207 (m, 5H), 7.083 (dd, $J = 7.6$ Hz, 2.0 Hz, 1H), 6.766 (td, $J = 7.6$ Hz, 1.2 Hz, 1H), 6.7 (d, $J = 8.0$ Hz, 1H), 4.184 (bs, 1H), 3.398 (t, $J = 6.4$ Hz, 2H), 2.696 (t, $J = 6.4$ Hz, 2H), 2.399 (s, 3H), 2.125 (s, 3H) ppm. ^{13}C NMR: δ 207.834, 144.654, 137.054, 136.320, 130.561, 129.731, 129.226, 128.616, 128.205, 117.269, 110.327, 42.761, 38.537, 30.422, 21.326 ppm.

4-((5-chloro-4'-methoxy-[1,1'-biphenyl]-2-yl)amino)butan-2-one (5h): ^1H NMR (400 MHz, CDCl_3): δ 7.242 (dt, $J = 8.8$ Hz, 2.8 Hz, 2H), 7.145 (dd, $J = 8.8$ Hz, 2.8 Hz, 1H), 7.023 (d, $J = 2.8$ Hz, 1H), 6.959 (dt, $J = 8.4$ Hz, 3.2 Hz, 2H), 6.580 (d, $J = 8.4$ Hz, 1H), 4.137 (bs, 1H), 3.836 (s, 3H), 3.345 (t, $J = 6.4$ Hz, 2H), 2.672 (t, $J = 6.4$ Hz, 2H), 2.115 (s, 3H) ppm. ^{13}C

NMR: δ 207.589, 159.206, 143.448, 130.317, 130.170, 129.314, 128.008, 121.846, 114.526, 111.359, 55.400, 42.483, 38.606, 30.405 ppm.

4-((4'-methoxy-5-methyl-[1,1'-biphenyl]-2-yl)amino)butan-2-one (5i): ^1H NMR (400 MHz, CDCl_3): δ 7.276 (dt, $J = 8.8$ Hz, 2.8 Hz, 2H), 7.029 (dd, $J = 8.0$ Hz, 2.4 Hz, 1H), 6.957 (dt, $J = 8.4$ Hz, 2.8 Hz, 2H), 6.897 (d, $J = 2.4$ Hz, 1H), 6.615 (d, $J = 8.4$ Hz, 1H), 3.990 (bs, 1H), 3.839 (s, 3H), 3.367 (t, $J = 6.4$ Hz, 2H), 2.675, (t, $J = 6.4$ Hz, 2H), 2.258 (s, 3H), 2.110 (s, 3H) ppm. ^{13}C NMR: δ 207.920, 158.852, 142.489, 131.657, 131.362, 130.456, 128.835, 128.101, 126.480, 114.351, 110.728, 55.395, 42.837, 38.937, 30.394, 20.429 ppm.

3,3'-(phenylmethylene)bis(5-phenyl-1H-indole) (8a): ^1H NMR (400 MHz, CDCl_3): δ 7.951 (bs, 2H), 7.593 (s, 2H), 7.519 (d, $J = 2.4$ Hz, 2H), 7.498 (s, 2H), 7.413 (t, $J = 1.6$ Hz, 4H), 7.381 (d, $J = 2.8$ Hz, 2H), 7.366 (t, $J = 2.8$ Hz, 3H), 7.340 (s, 1H), 7.294 (d, $J = 6.8$ Hz, 2H), 7.265 (s, 1H), 7.228-7.194 (m, 2H), 6.707 (d, $J = 2.8$ Hz, 2H), 5.959 (s, 1H) ppm. ^{13}C NMR: δ 143.977, 142.594, 136.358, 132.935, 128.835, 128.663, 128.406, 127.652, 127.500, 126.318, 124.506, 121.931, 120.110, 118.518, 111.405, 40.320 ppm.

3,3'-((4-chlorophenyl)methylene)bis(5-phenyl-1H-indole) (8b): ^1H NMR (400 MHz, CDCl_3): δ 7.943 (bs, 2H), 7.578 (s, 2H), 7.528 (d, $J = 8.0$ Hz, 4H), 7.444 (d, $J = 8.8$ Hz, 3H), 7.405 (s, 2H), 7.375 (d, $J = 7.2$ Hz, 4H), 7.309 (s, 1H), 7.272-7.239 (m, 4H), 6.656 (s, 2H), 5.940 (s, 1H) ppm. ^{13}C NMR: δ 142.575, 142.499, 136.358, 133.097, 132.000, 130.198, 128.749, 128.568, 127.509, 127.462, 126.441, 124.544, 122.103, 119.567, 118.356, 111.548, 39.719 ppm.

3,3'-(phenylmethylene)bis(5-(4-methoxyphenyl)-1H-indole)(8c): ^1H NMR (400 MHz, CDCl_3): δ 7.940 (bs, 2H), 7.527 (s, 2H), 7.435 (t, $J = 3.2$ Hz, 2H), 7.413 (t, $J = 2.4$ Hz, 2H), 7.373 (s, 4H), 7.358 (s, 1H), 7.275 (t, $J = 7.2$ Hz, 3H), 7.223-7.179 (m, 1H), 6.912 (t, $J = 3.2$ Hz, 2H), 6.891 (t, $J = 2.0$ Hz, 2H), 6.694 (d, $J = 2.8$ Hz, 2H), 5.936 (s, 1H), 3.805 (s, 6H) ppm. ^{13}C NMR: δ 158.461, 144.015, 136.053, 135.252, 132.573, 128.835, 128.415, 128.368, 127.767, 126.298, 124.439, 121.693, 120.034, 118.003, 114.103, 111.328, 55.424 ppm.

3,3'-((4-chlorophenyl)methylene)bis(5-(4-methoxyphenyl)-1H-indole) (8d): ^1H NMR (400 MHz, CDCl_3): δ 7.944 (bs, 2H), 7.500 (s, 2H), 7.433 (d, $J = 8.8$ Hz, 4H), 7.384 (s, 4H), 7.290 (d, $J = 8.8$ Hz, 2H), 7.237 (d, $J = 8.4$ Hz, 2H), 6.916 (d, $J = 8.8$ Hz, 4H), 6.664 (d, $J = 2.8$ Hz, 2H), 5.912 (s, 1H), 3.812 (s, 6H) ppm. ^{13}C NMR: δ 158.527, 142.594, 136.034, 135.138, 132.754, 131.943, 130.198, 128.530, 128.444, 127.452, 124.468, 121.865, 119.490, 117.850, 114.151, 111.433, 55.443 ppm.

3,3'-((4-bromophenyl)methylene)bis(5-phenyl-1H-indole) (8e): ^1H NMR (400 MHz, CDCl_3): δ 7.983 (bs, 2H), 7.553 (s, 2H), 7.517 (d, $J = 1.6$ Hz, 2H), 7.495 (s, 2H), 7.427-7.403

(m, 5H), 7.386 (s, 2H), 7.367 (s, 2H), 7.348 (s, 1H), 7.270-7.248 (m, 2H), 7.231 (s, 2H), 6.700 (d, $J = 2.8$ Hz, 2H), 5.918 (s, 1H) ppm. ^{13}C NMR: δ 158.527, 142.594, 136.034, 135.138, 132.754, 131.943, 130.198, 128.530, 128.444, 127.452, 124.468, 121.865, 119.490, 117.850, 114.151, 111.433, 55.443 ppm.

2.4: Conclusion

In summary, we have designed and prepared a bifunctional nano-biohybrid catalyst by immobilization of PdNPs and α -amylase on halloysite nanotubes. Further, the successful incorporation of both the catalysts i.e. PdNPs and α -amylase on halloysite nanotubes was confirmed by using FTIR, XRD, FE-SEM, EDS, HR-TEM techniques. Besides, the synthetic utility of developed nanobiohybrid catalyst was demonstrated by catalyzing one-pot chemoenzymatic reaction consisting Pd-catalyzed Suzuki-Miyaura coupling and α -amylase mediated aza-Michael addition reaction to synthesized functionalized biphenyls. Next, the wider applicability of the constructed nanobiohybrid catalyst was examined on the Pd-catalyzed Suzuki-Miyaura coupling of 5-bromo indoles with aryl boronic acids and subsequent α -amylase catalyzed electrophilic substitution of different benzaldehydes to furnish biologically active substituted bis(indolyl)methanes in good yields. These results indicates that the nanobiohybrid approach has an ample potential to conduct multi-catalytic cycles in a single pot in an efficient manner. Furthermore, the prepared nanobiohybrid catalyst (IV) was found to be recyclable up to five consecutive cycles and highly stable when stored at room temperature for the period of 12 months.

2.5: References

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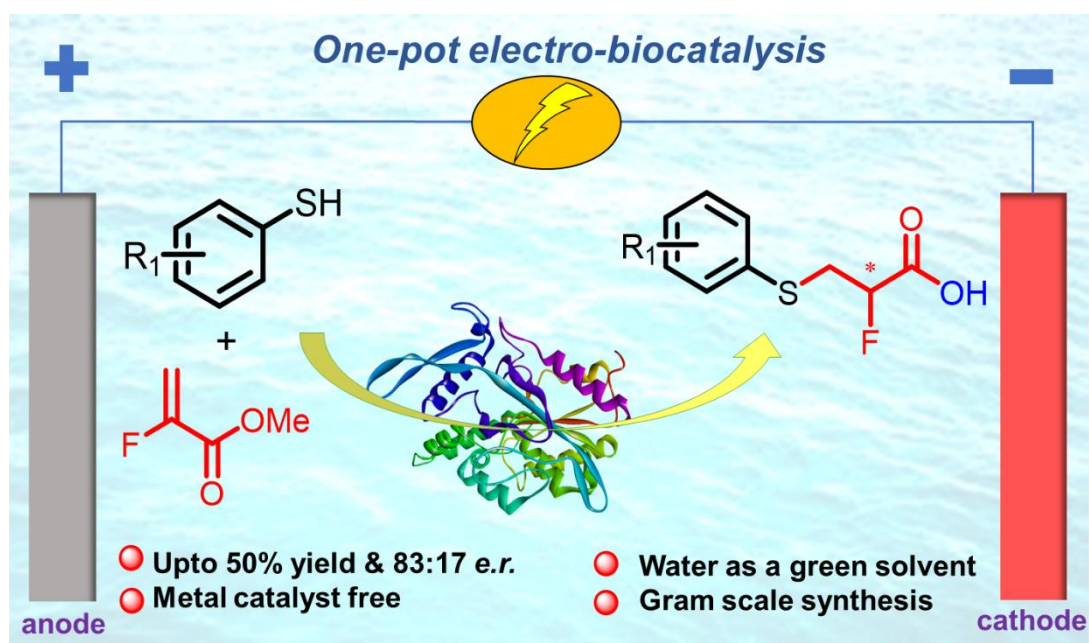
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CHAPTER 3

Merging Electrosynthesis and Biocatalysis to Access Sulfur-Based Chiral α -Fluorinated Carboxylic Acids



3.1: Introduction

In recent years, the importance of organofluorine compounds has expanded significantly due to their wide range of applications in different areas such as materials science, drug discovery, agrochemicals, and the polymer industry.¹ The incorporation of one or more fluorine atoms into organic compounds often leads to unique physical, chemical, and biological properties, ascribed to the small size, high electronegativity, and low polarizability of the fluorine atom. It is noteworthy that approximately 20 per cent of marketed drugs contain at least one fluorine atom.² In particular, chiral sulfur-based organofluorine compounds have gained significant attention as they are used as precursors of numerous drugs or clinically important molecules (**Figure 3.1**).³ Previously, these compounds have been predominantly synthesized through sulfa-Michael addition reactions of substituted thiols and fluorine-containing electron-deficient alkenes as starting materials. In this context, Wang and colleagues documented the asymmetric sulfa-Michael addition of thiols and 4,4,4-trifluorocrotonates to obtain organofluorine compounds in high yields and enantioselectivities (**Scheme 3.1a**).⁴ Subsequently, Couve-Bonnaire and group members unveiled the use of the cinchona derivative (DHQ)₂PYR as a catalyst for the sulfa-Michael addition of thiophenols with trisubstituted α -fluoroacrylates to synthesize chiral sulfur-based organofluorinated compounds (**Scheme 3.1b**).⁵ Interestingly, Haufe *et al.* observed the removal of HF as a side product when a base-catalyzed sulfa-Michael addition reaction of 4-methyl thiophenol and methyl-2-fluoro acrylate was performed, as a result, they were unable to synthesize sulfur-based organofluorine compounds.⁶ Notwithstanding, most of the methodologies present certain limitations such as the use of organic solvents, complex work-up procedures often required, and the challenges of preserving the fluorine atom in the final product under the established reaction conditions. As a consequence, there is a significant need for the development of an environmentally friendly and efficient methodology to streamline the one-pot synthesis of chiral sulfur-based organofluorine compounds.

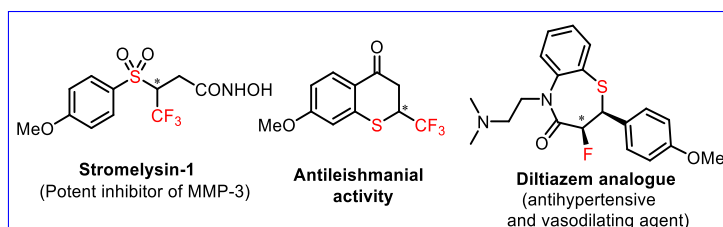
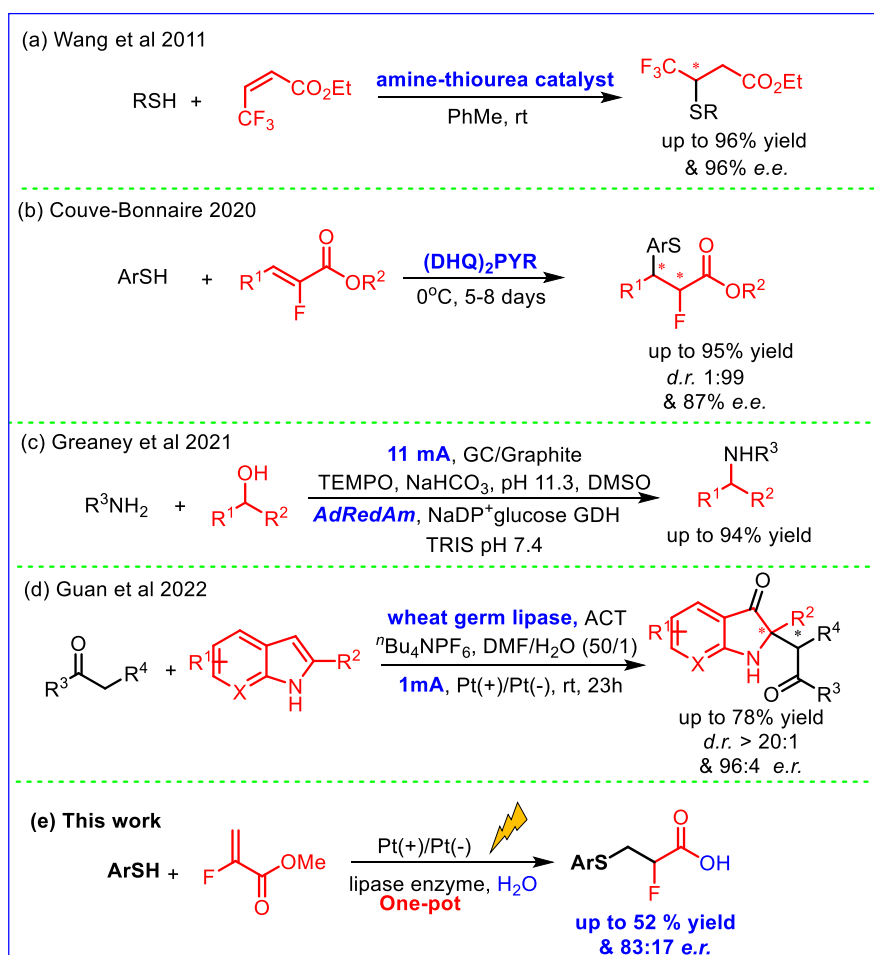


Figure 3.1: Clinically important sulfur-based organofluorine compounds

In recent years, substantial advancements have been made in synthesizing clinically significant molecules using greener methodologies by replacing traditional approaches.⁷ Moreover, the

development of greener methodologies involves careful planning of synthetic routes, aiming to avoid the use of hazardous and toxic substances, as well as minimizing the generation of byproducts and chemical waste.⁸ In this context, the use of enzymes as catalysts in water has proven highly advantageous in realizing the objectives of green chemistry.⁹ Additionally, employing enzymes in organic synthesis has proven highly effective in generating molecules with exceptional chemo-, regio-, and stereoselectivities.¹⁰ On the other hand, organic electrosynthesis is an alternative strategy for replacing the traditional synthetic approaches to achieve green chemistry objectives.¹¹ Particularly, utilizing electricity in organic synthesis provides numerous benefits such as the use of electron(s) as a reagent by avoiding exogenous oxidants or reductants, fine-tuning of reactions by adjusting the electrode potential, easy scale-up of the reactions without substantial modifications in the reaction conditions, etc.¹² Despite these advantages, achieving enantioselective organic electrosynthesis remains highly challenging due to factors such as the high reactivity of free radicals or intermediates and the



Scheme 3.1: Recent approaches to produce sulfur-based organofluorine compounds (a-b); One-pot integration of electro and biocatalytic approaches (c-e).

limited compatibility of chiral catalysts within an electrochemical cell.¹³ To address these challenges, the integration of electrosynthesis and biocatalysis in the same pot offers a promising solution to obtain the compounds in a stereoselective manner.

Previously, the integration of electrosynthesis and biocatalysis was mainly focused either on the production of fuels or chemicals from carbon dioxide or to facilitate electron transfer through electrocatalysis to regenerate the cofactors necessary for enzymatic transformations.¹⁴ However, the integration of electrosynthetic and biocatalytic approaches, which aims to catalyze dual chemical reactions in the same vessel where the product of one reaction serves as the starting material for the next reaction to synthesize biologically important molecules, requires more attention. In this context, Greaney and colleagues reported a one-pot integration of an organic electrosynthesis and biocatalytic approach for amine alkylation with alcohols, comprising TEMPO-mediated electro-catalytic oxidation followed by biocatalytic reductive amination using the *AdRedAm* enzyme (**Scheme 3.1c**).¹⁵ Very recently, Guan and co-workers discovered enantioselective oxidative cross-coupling of secondary amines with ketones by merging the non-natural catalytic activity of lipase and electrosynthesis (**Scheme 3.1d**).¹⁶

Drawing encouragement from the importance of chiral sulfur-based organofluorine compounds, herein, we hypothesized to combine electrochemical sulfa-Michael addition of thiophenols and 2-fluoroacrylate followed by lipase-catalyzed ester hydrolysis reaction in the same pot to produce chiral sulfur-based organofluorine compounds (**Scheme 3.1e**). While the sulfa-Michael addition of thiyl radicals to alkenes, alkynes and α , β -unsaturated acid has been previously demonstrated through flash photolysis or using iodine as a chemical oxidant.^{3f-g} The electrochemical sulfa-Michael addition involving thiophenol and an α , β -unsaturated ester has not yet been explored. Also, the lipase-catalyzed hydrolysis of 2-fluoro-3-thiopropionate is still undocumented. Taking this into account, initially, we plan to explore both the electrochemical sulfa-Michael addition and the lipase-catalyzed hydrolysis reactions separately and subsequently proceed toward the integration of both approaches in the same vessel.

3.2: Results and Discussion

3.2.1: Optimization of reaction conditions

We started this study by setting up a reaction of thiophenol (**1a**) and methyl 2-fluoro acrylate (**2a**) as model substrates by using tetrabutylammonium iodide ($n\text{Bu}_4\text{NI}$) as a supporting electrolyte in ethanol under a constant current of 10 mA in an undivided cell with two platinum electrodes at 50 °C and gratifyingly obtain the corresponding product (**3a**) with conversion of 65% from this reaction. To enhance the conversion of the model reaction, we screened

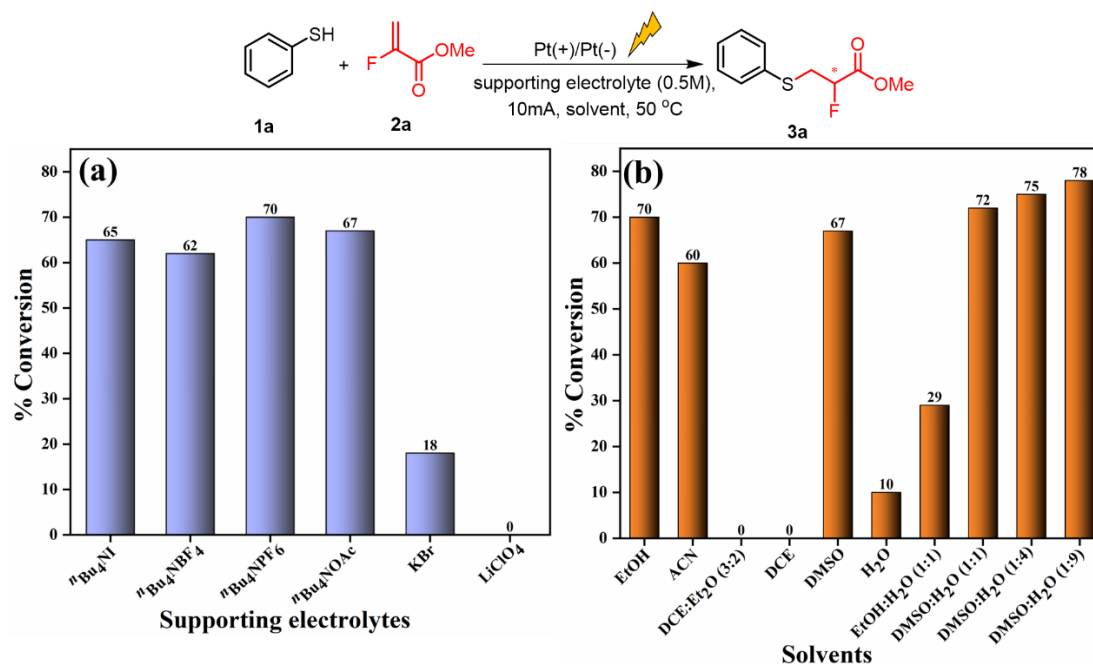


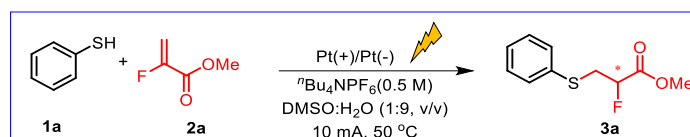
Figure 3.2 (a) Screening of supporting electrolytes for the model reaction (b) Solvent screening for model reaction; **Reaction Conditions:** (a) **1a** (38 μ L, 0.4 mmol, 2 equiv.), **2a** (18 μ L, 0.2 mmol, 1 equiv.), supporting electrolyte (0.5 M), solvent (2 mL), under a constant current of 10 mA in an undivided cell with two platinum electrodes at 50 °C for 1 hour; charge passed with respect to starting material (**2a**) is 2F, % conversion determined by HPLC as given in supporting information, the product **3a** was obtained as a racemic mixture.

tetrafluoroborate (ⁿBu₄NBF₄), tetrabutylammonium hexafluorophosphate (ⁿBu₄NPF₆), and tetrabutylammonium acetate (ⁿBu₄NOAc), however, no notable improvement in the conversion was observed (**Figure 3.2a**). Moreover, when KBr was employed as a supporting electrolyte, the product was obtained with only 18% conversion. Additionally, no reaction was observed when lithium perchlorate (LiClO₄) was utilized. Consequently, ⁿBu₄NPF₆ was found as the best supporting electrolyte for the model reaction. Next, we checked the effect of the solvents to improve the efficiency of the model reaction and first switched the solvent from ethanol to acetonitrile, however, there was a slight decrease in the % conversion (**Figure 3.2b**). Afterward, the product was obtained only in trace amounts when DCE (dichloroethane) or the mixture of DCE with diethyl ether (3:2, v/v) was used as a solvent. It is noteworthy that there was an increment in the % conversion when the reaction was conducted in DMSO (**Figure 3.2b**). Subsequently, in an attempt to make the electrochemical sulfa-Michael addition process greener, the model reaction was set up using water as a solvent; however, the formation of the product (**3a**) was only observed in trace amounts, possibly due to the very low solubility of the starting materials in water. Then, we explored the mixture of DMSO or EtOH with water (1:1,

v/v), but the % conversion of the model reaction remained low. Fortunately, an improved % conversion was attained when utilizing a mixture of DMSO in water (1:9 v/v) as the solvent. Consequently, this combination was selected as the best solvent for the electrochemical sulfa-Michael addition reaction. (**Figure 3.2b**).

Afterward, we reduced the supporting electrolyte concentration from 0.5 M to 0.05 M (**entries 1-4, Table 3.1**), as a result, the maximum conversion was obtained when 0.1 M ⁿBu₄NPF₆ was used (**entry 3, Table 3.1**). Further, we varied the current and found that the reaction provides maximum conversion in the case of 5 mA current (**entries 5-6, Table 3.1**). Subsequently, a decrease in the % conversion was noted when the reaction was conducted with an equal amount of both substrates (**entry 7, Table 3.1**). Moreover, there was no enhancement in the % conversion observed when (**1a**) and (**3a**) were employed in a 3:1 molar ratio (**entry 8, Table 3.1**). Next, no improvement in % conversion was observed when the temperature was either decreased or increased from 50 °C (**entries 9-10, Table 3.1**). A control reaction was set up to check the role of electricity, and interestingly there was no reaction in the absence of current (**entry 11, Table 3.1**).

Table 3.1: Optimization of reaction conditions for sulfa-Michael addition reaction^a



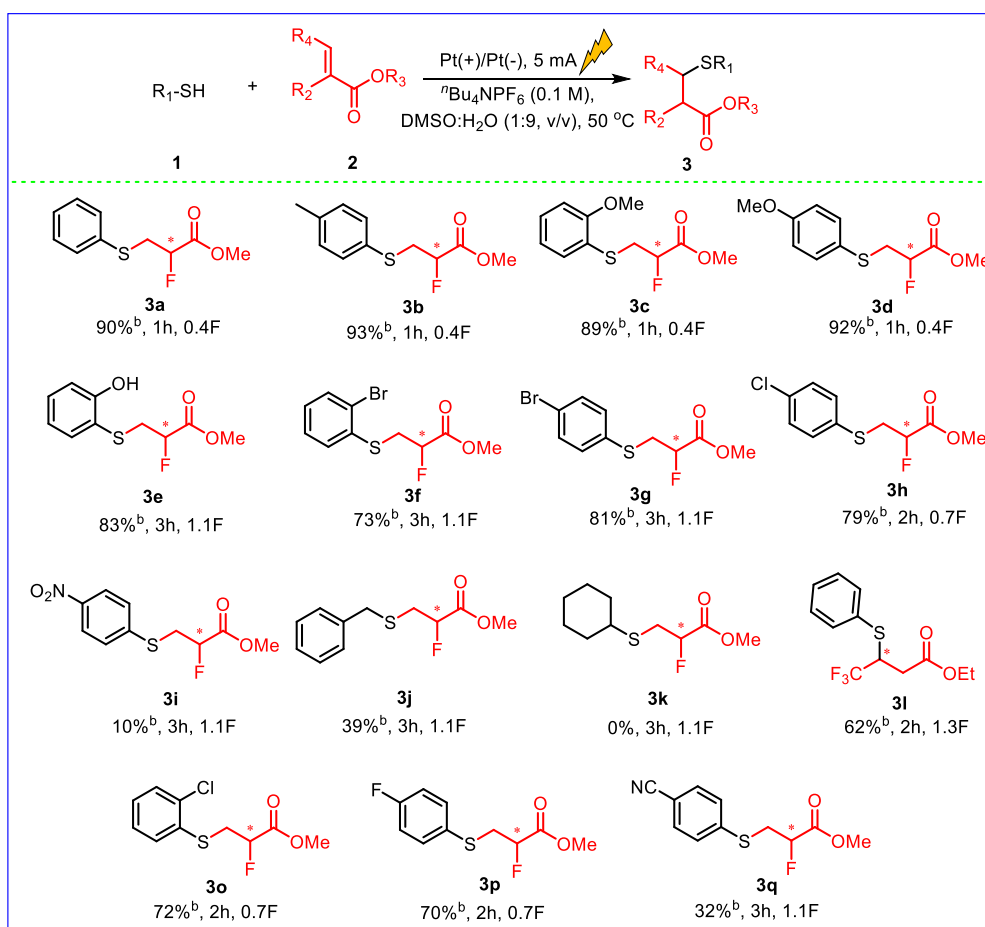
| entry | deviation from standard reaction conditions | % conversion (3a) ^b | Charge passed (F) |
|-------|---|--------------------------------|-------------------|
| 1 | none | 78 | 2 |
| 2 | 0.3 M supporting electrolyte used | 82 | 2 |
| 3 | 0.1 M supporting electrolyte used | 88 | 2 |
| 4 | 0.05 M supporting electrolyte used | 57 | 2 |
| 5 | 15 mA current used | 61 | 3 |
| 6 | 5 mA current used | 98 | 1 |
| 7 | 1 equiv. of 1a used | 46 | 2 |
| 8 | 3 equiv. of 1a used | 96 | 2 |
| 9 | Temperature 25 °C | 41 | 2 |
| 10 | Temperature 60 °C | 89 | 2 |
| 11 | no electricity | 0 | 0 |

^aStandard reaction conditions: **1a** (38 μL, 0.4 mmol, 2 equiv.), **2a** (18 μL, 0.2 mmol, 1 equiv.),

ⁿBu₄NPF₆ (0.5 M), DMSO:H₂O (2 mL), under a constant current of 10 mA flowed in an undivided cell with two platinum electrodes at 50 °C for 1 hour. ^b% conversion determined by HPLC.

3.2.2: Substrate scope for electrochemical sulfa-Michael addition reaction

Once the optimized reaction conditions were obtained, the substrate scope for the electrochemical sulfa-Michael addition reaction was investigated to demonstrate the generality of this transformation (**Scheme 3.2**). The reaction provided a very good isolated yield when unsubstituted thiophenol was employed in the reaction (**3a**, **Scheme 3.2**). Then, we tested the scope of the electron-donating group such as methyl-, methoxy-, and hydroxy-containing thiophenols, and obtained the corresponding products in excellent yield (**3b-e**, **Scheme 3.2**). Next, the reaction provided a slightly lower yield when halide-substituted thiophenols were



Scheme 3.2: Substrate scope of electrochemical sulfa-Michael addition reaction^a

^a**Reaction Conditions:** **1** (1 mmol, 2 equiv.), **2** (0.5 mmol, 1 equiv.), $t\text{Bu}_4\text{NPF}_6$ (0.1 M), DMSO: H₂O (5 mL), ^bisolated yield.

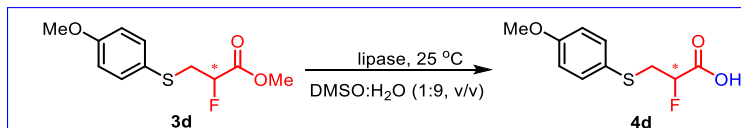
employed in the reaction (**3f-h**, **3o-p**, **Scheme 3.2**), also reaction took little longer time to get completed. Surprisingly, a very low yield was obtained in the case of 4-NO₂ thiophenol, which might be due to the weakened stabilization of the thiyl radical caused by the strong electron-withdrawing nature of the nitro group, which ultimately impaired the radical's reactivity in the reaction. (**3i**, **Scheme 3.2**).^{13c} Also, the reaction yielded the product (**3q**) with an isolated yield

of 32% in the case of 4-CN substituted thiophenol. The reaction provided sulfa-Michael addition product (**3j**) only in 39% isolated yield when thiophenol was replaced with benzyl thiol. Additionally, the current proved insufficient to drive the reaction of cyclohexyl thiol with 2-fluoroacrylate, resulting in no product formation (**3k**, **Scheme 3.2**). The low yield or no reaction observed in the case of (**3j**) and (**3k**) might be due to the low stability of the aliphatic thiyl radical as compared to the aromatic radical.^{13c} Gratifyingly, the reaction afforded the product (**3l**) in a good yield when we replaced methyl-2-fluoro acrylate (**2a**) with a CF₃-containing Michael acceptor i.e. methyl-4,4,4-trifluorobut-2-enoate (**3l**, **Scheme 3.2**).

3.2.3: Reaction condition optimization for enzymatic hydrolysis reaction

In the next phase of our endeavors, we focused on optimizing the reaction conditions for the enzymatic hydrolysis of 2-fluoro-3-mercaptopropanoate derivative (**3d**) to produce chiral 2-fluoro-3-mercaptopropionic acid (**4d**). First, various lipases from different sources were screened for the asymmetric hydrolysis of (**3d**), and the results were enclosed in **Table 3.2**. In this context, when lipases from *C. rugosa* and *A. niger* were employed as the catalyst, the desired product (**4d**) was obtained with low enantiomeric ratio and moderate % conversion (**entries 1-2**, **Table 3.2**). Then, other lipases such as lipase from porcine pancreas and *Candida*

Table 3.2: Screening of lipases for the hydrolysis reaction^a



| entry | lipase | % conversion ^b | <i>e.r.</i> ^c | % <i>ee</i> | <i>s</i> ^d |
|-------|--|---------------------------|--------------------------|-------------|-----------------------|
| 1 | lipase from <i>C. rugosa</i> | 39 | 62:38 | 24 | 2 |
| 2 | lipase from <i>A. niger</i> | 46 | 57:43 | 14 | 1 |
| 3 | lipase from Porcine pancreas | 62 | 60:40 | 20 | 2 |
| 4 | <i>Candida antarctica</i> lipase B | 59 | 70:30 | 40 | 4 |
| 5 | lipase from <i>Rhizomucor miehei</i> | 41 | 64:36 | 28 | 2 |
| 6 | lipase from <i>Burkholderia cepacia</i> | 37 | 72:28 | 44 | 3 |
| 7 | lipase from <i>P. Fluorescens</i> | 52 | 83:17 | 66 | 10 |

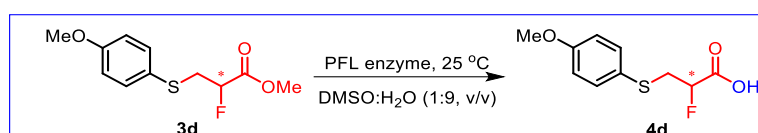
^aReaction conditions: **3d** (10 mg, 0.04 mmol, 1 equiv.), lipase (10 mg/mL), DMSO: H₂O (1 mL), incubated at 25°C on 200 rpm for three days; ^bdetermined by HPLC, ^cdetermined by chiral HPLC, ^dselectivity factor = $k_{\text{fast}}/k_{\text{slow}}$.

antarctica B were used and got product (**4d**) in improved % conversion however the enantiomeric ratios were moderate (**entries 3-4**, **Table 3.2**). Subsequently, lipase derived from *Rhizomucor miehei* and *Burkholderia cepacia* produced product (**4d**) with a reduced conversion

and modest enhancement in the enantiomeric ratios (**entries 5-6, Table 3.2**). Gratifyingly, when lipase from *P. fluorescens* (PFL) was used as the catalyst, the reaction provided the desired product (**4d**) in 52% conversion and improved enantiomeric ratio i.e. *e.r.* = 83:17 (**entry 7, Table 3.2**).

Consequently, PFL was selected as the optimal biocatalyst for further optimization of the reaction conditions to improve the enantioselectivity and conversion (**entry 7, Table 3.2**). In this context, a decrement in the % conversion was noticed along with a very slight change in the enantiomeric ratios as enzyme loading was decreased from 10 mg to 5, 3, and 1 mg respectively (**entries 1-4, Table 3.3**). Thus, 10 mg loading of the enzyme was opted as the best for achieving the maximum conversion. Additionally, to further improve the selectivity, water as a solvent was altered by THF, toluene, ethanol, and phosphate buffer (**entries 5-8, Table 3.3**). However, no noticeable rise in the selectivity was observed. Unfortunately, we did not

Table 3.3: Optimization of reaction conditions for enzymatic hydrolysis^a



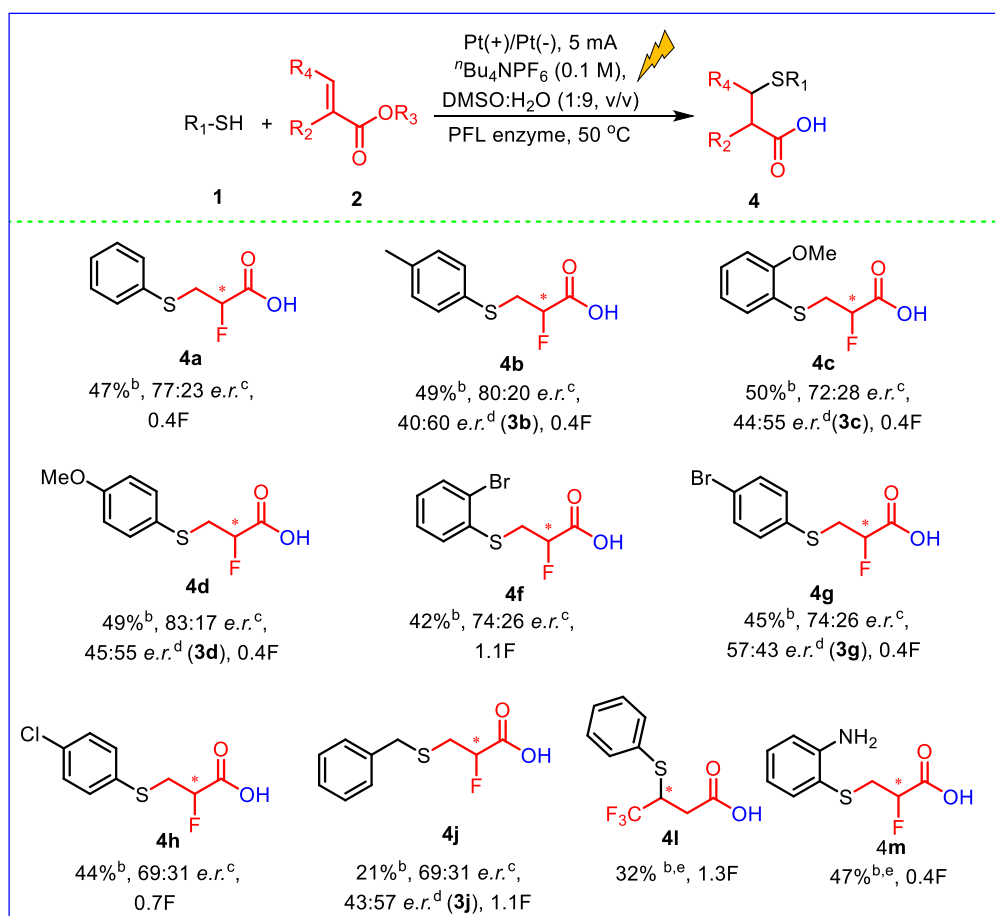
| entry | deviation from standard reaction conditions | % conversion ^b | <i>e.r.</i> ^c | % <i>e.e.</i> | <i>s</i> ^d |
|-------|--|---------------------------|--------------------------|---------------|-----------------------|
| 1 | no deviation | 52 | 83:17 | 66 | 10 |
| 2 | 5 mg lipase instead 10 mg | 31 | 81:19 | 62 | 6 |
| 3 | 3 mg lipase instead 10 mg | 23 | 80:20 | 60 | 5 |
| 4 | 1 mg lipase instead 10 mg | 19 | 77:23 | 54 | 4 |
| 5 | THF instead of H ₂ O | 39 | 75:25 | 50 | 4 |
| 6 | Toluene instead of H ₂ O | 31 | 69:31 | 38 | 3 |
| 7 | EtOH instead of H ₂ O | 50 | 67:33 | 34 | 3 |
| 8 | KPi buffer (pH 7, 100 mM), instead of H ₂ O | 32 | 55:45 | 10 | 1 |
| 9 | Temperature=10 °C instead of 25°C | 46 | 78:22 | 56 | 6 |
| 10 | Temperature=50 °C instead of 25°C | 45 | 77:23 | 54 | 5 |

^aReaction conditions: **3d** (10 mg, 0.04 mmol, 1 equiv.), enzyme (10 mg/mL), DMSO: H₂O (1 mL), incubated at 200 rpm for three days, ^bdetermined by HPLC, ^cdetermined by chiral HPLC ^dselectivity factor = k_{fast}/k_{slow} .

observe any positive change in enantioselectivity when we decreased or increased the temperature from room temperature to 10 °C or 50 °C, respectively (**entries 9-10, Table 3.3**).

3.2.4: Substrate scope for integrated electrochemical and hydrolysis reaction

In the final phase of our study, we integrate electrochemical sulfa-Michael addition and PFL-catalysed hydrolysis reaction in the same vessel using the optimized reaction conditions. In this context, we performed a one-pot reaction of thiophenol (**1a**) and methyl-2-fluoro acrylate (**2a**) and delightfully obtained the desired product (**4a**) in 47% isolated yield along with the non-hydrolyzed compound (**3a**). Also, we set up sequential electrosynthesis and biocatalysis reactions using the optimized reaction conditions to compare the outcome of a one-pot reaction, however, there was no significant change in the isolated yield of the reaction (**Scheme 3.10**).



Scheme 3.3: Substrate scope of integrated electrochemical and biocatalytic reaction^a

^a**Reaction conditions:** **1** (1 mmol, 2 equiv.), **2** (0.5 mmol, 1 equiv.), $n\text{Bu}_4\text{NPF}_6$ (0.1 M), DMSO: H₂O (5 mL), PFL enzyme (10 mg/mL), incubated at 200 rpm for three days after the completion of electrochemical reaction, ^bisolated yield, ^ce.r. of product (**4**) as determined by chiral HPLC, ^de.r. of non-hydrolyzed compound (**3**) as determined by chiral HPLC, ^eno enantiomer resolution.

Then, we tested the scope of substituents to show the generality and feasibility of the one-pot integrated electrochemical and biocatalytic transformation as depicted in **Scheme 3.3**. There

was no significant change observed in the yield and enantiomeric ratio of the product (**4b**) in comparison to product (**4a**) when thiophenol (**1a**) was replaced by 4-methyl thiophenol in the one-pot reaction (**Scheme 3.3**). Afterward, the effect of 2-methoxy and 4-methoxy substituent on the thiophenol was tested, and a slight increment in the yield, as well as the enantiomeric ratio, was observed (**4c-d**, **Scheme 3.3**). Next, halide-substituted thiophenols were employed in the one-pot reaction and obtained the corresponding products with good yield and enantiomeric ratios (**4f-h**, **Scheme 3.3**). Besides, the scope of benzyl thiol was tested, however, the desired product was obtained only in 21% isolate yield (**4j**, **Scheme 3.3**) accompanied by non-hydrolyzed compound (**3j**). Also, we replaced methyl 2-fluoro acrylate (**2a**) with ethyl 4,4,4-trifluorocrotonate and interestingly reaction provided the corresponding product (**4l**) in 32% isolated yield. The reaction provided the corresponding product (**4m**) in 47% isolated yield when 2-amino thiophenol was employed in the reaction. Noticeably, the lower yield of compounds (**4**) was attributed to the partial recovery of non-hydrolyzed esters intermediates (**3**). The enantiomeric ratios of these recovered esters are presented in **Scheme 3.3**.

3.2.5: Control experiments on electricity and enzyme roles

To understand the role of electricity and PFL enzyme in the one-pot procedure, several experiments were performed as depicted in **Figure 3.3**. First, we monitored the consumption of thiophenol (**1a**) under different reaction conditions and it was found that the concentration

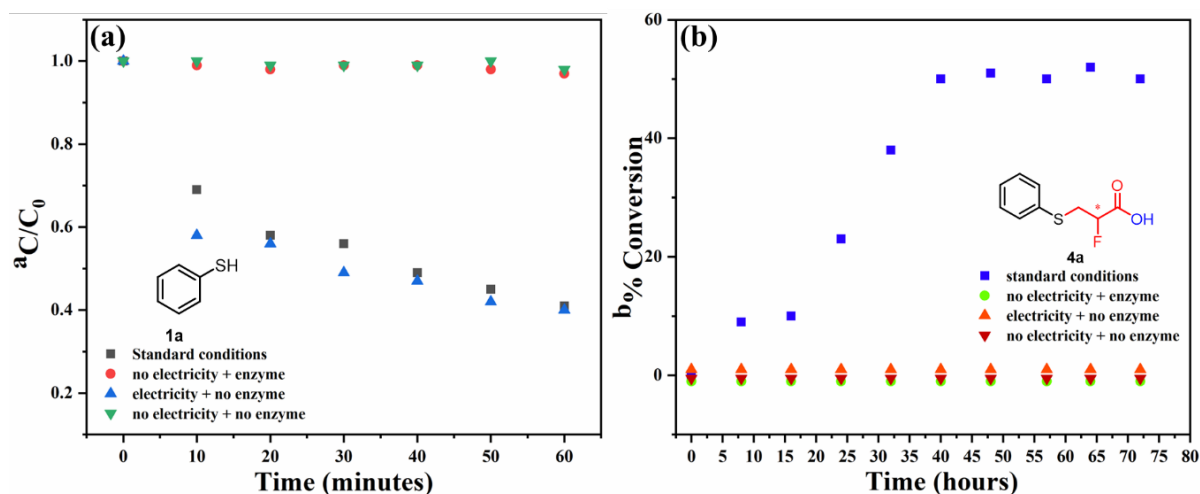
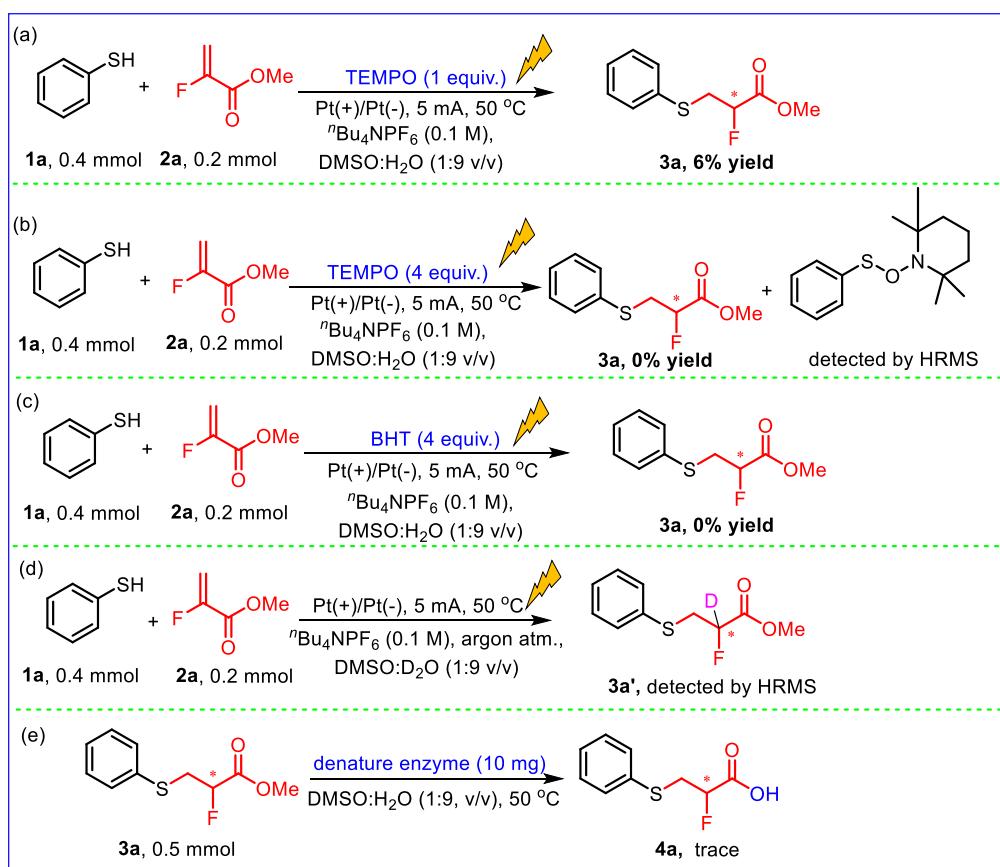


Figure 3.3: (a) Role of electricity and enzyme on the consumption of thiophenol (**1a**) with time (b) Role of electricity and enzyme on the formation of (**4a**) with time; **Standard conditions:** **1a** (38 μ L, 0.4 mmol, 2.0 equiv.), **2a** (18 μ L, 0.2 mmol, 1.0 equiv.), n Bu₄NPF₆ (0.1 M), DMSO: H₂O (1:9, v/v, 2 mL), PFL enzyme (10 mg/mL) under a constant current of 5 mA in an undivided cell with two platinum electrodes, maintaining the temperature at 50 °C using an oil bath, total reaction time=72 h, aC/C_0 = ratio of thiol concentration at different time intervals with respect to initial concentration (t=0); $b\%$ conversion determined by HPLC.

of thiophenol decreased gradually with time in the presence of electricity, and there was no change in the concentration of thiophenol in the presence of enzyme only (**Figure 3.3a**). Also, we monitored the formation of acid (**4a**) in different reaction conditions and found that acid was formed only in the presence of the PFL enzyme (**Figure 3.3b**). These experiments suggested that electricity is required for the sulfa-Michael addition reaction while the PFL enzyme plays a role only in the hydrolysis of ester formed *in-situ* through electrosynthesis.

3.2.6: Mechanistic insights via control experiments

Further, to get insight into the mechanism of electrochemical sulfa-Michael addition, a reaction of (**1a**) and (**2a**) was performed with commonly used radical trapping agent (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) (1 equiv.) and as a result, product (**3a**) was obtained only in 6% yield (**Scheme 3.4a**). By increasing the amount of TEMPO (4 equiv.), product formation was completely inhibited and we were able to detect the adducts formed by the reaction of thia radical (**II**) with TEMPO using HRMS techniques (**Scheme 3.4b**). Besides, we tried another radical scavenger butylated hydroxytoluene (BHT) under the electrochemical conditions, which again terminated the formation of the desired product (**3a**) (**Scheme 3.4c**). Also, we have detected the formation of diaryldisulfide as a side reaction when the reaction



Scheme 3.4: Control experiments

was conducted in the absence of fluoro-acrylate.^{18,21} These observations indicated that the reaction may follow a radical process, and thia radical (**II**) may be a possible intermediate in the electrolysis.

3.2.7: Cyclic voltammetry studies

Next, a cyclic voltammetry (CV) study was carried out to examine the redox potential of substrates (**1a**), (**2a**) and the mixture of substrates and electrolyte (**Figure 3.4I**). There was no oxidation peak in the CV of the blank DMSO:H₂O solution, ⁿBu₄NPF₆ and mixture of ⁿBu₄NPF₆ and (**2a**) (curves a, b & c, **Figure 3.4I**). The oxidation peak for (**1a**) and ⁿBu₄NPF₆ was observed at 0.41 V vs Ag/AgCl (curves c & d, **Figure 3.4I**). Whereas the CV of mixture of (**1a**), (**2a**) and ⁿBu₄NPF₆ exhibited an oxidation peak at 0.48 V. A possible explanation for the modest shift in oxidation potentials observed in the CV of mixtures is due to chemical interaction between (**1a**) and (**2a**) (curve e, **Figure 3.4I**). Xie et al. and Badsara et al. reported similar sorts of observations.¹⁷⁻¹⁸ Next, we conducted experiments using the radical scavenger TEMPO in a DMSO:H₂O (1:9) mixture (curve a **Figure 3.4II**). No appreciable oxidation peak was seen upon the addition of TEMPO to substrate (**2a**), demonstrating a lack of interaction between TEMPO and (**2a**) (curve b **Figure 3.4II**). However, when TEMPO was introduced with thiophenol (**1a**), an oxidation peak was observed at 1.20 V (curve c **Figure 3.4II**). Additionally, when TEMPO was present with both substrates, a new sharp peak appeared at 1.12 V (curve d **Figure 3.4II**), which likely indicates that TEMPO trapped the thiyl radical in the reaction mixture, providing further evidence for the proposed mechanism.

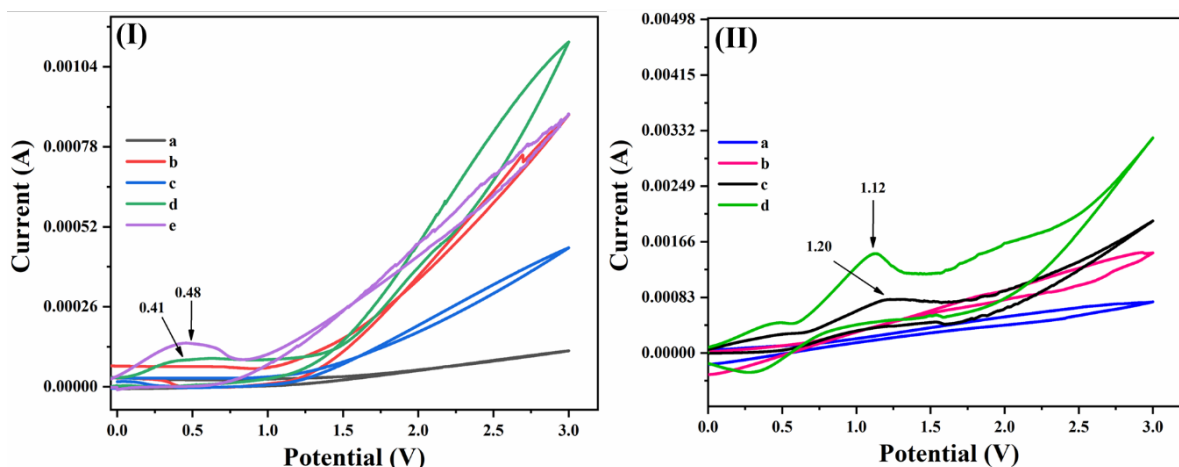


Figure 3.4: Cyclic voltammograms (IUPAC convention) of 0.1 M ⁿBu₄NPF₆ solution in DMSO:H₂O (1:9) at room temperature, using Pt wire as a counter electrode, Pt as working electrode, and Ag/AgCl as a reference electrode, at a scan rate of 0.05 V/s with initial potential = 0 V, the direction of scan = 0 to +3 (oxidative). (I) (a) DMSO:H₂O (1:9) (b) ⁿBu₄NPF₆ (0.1 M), (c) ⁿBu₄NPF₆ (0.1 M) and **2a** (0.001 M) (d) ⁿBu₄NPF₆ (0.1 M) and **1a** (0.002 M) (e) ⁿBu₄NPF₆ (0.1 M), **2a** (0.001 M) and **1a** (0.002 M). (II)

(a) TEMPO (0.004 M) and ${}^n\text{Bu}_4\text{NPF}_6$ (0.1 M) (b) TEMPO (0.004 M), ${}^n\text{Bu}_4\text{NPF}_6$ (0.1 M) and **2a** (0.001 M) (c) TEMPO (0.004 M), ${}^n\text{Bu}_4\text{NPF}_6$ (0.1 M) and **1a** (0.002 M) (d) TEMPO (0.004 M), ${}^n\text{Bu}_4\text{NPF}_6$ (0.1 M), **2a** (0.001 M) and **1a** (0.002 M).

3.2.8: Molecular docking studies

In addition, the molecular docking study was performed to get insights into the role of the active site of the PFL enzyme during the hydrolysis reaction (**Figure 3.5**). However, due to the non-availability of the crystal structure of the PFL enzyme, we have conducted the docking investigation using *P. cepacia* lipase (PCL) (PDB 3LIP), which is homologous and has sequence similarity with PFL.¹⁹ Previously, it has been reported that the catalytic triad comprising Ser87, Asp264, and His286 amino acids plays an important role in the ester hydrolysis, whereas Leu17 and Tyr29 residues are important for providing selectivity.^{19a,20} After performing the docking studies with (**3a**) as a ligand, we have obtained the best-docked pose with the lowest binding energy (-5.7 kcal/mol, **Figure 3.9**) which suggests that Ser87 along with His286 present in the active site may play an important role during the hydrolysis of substrate (**3a**) (**Figure 3.5**). Additionally, Tyr29 and Leu17 residues showed interaction with substrates (**3a**), which may facilitate selectivity during the hydrolysis.^{19a,b} To get more insight into the role of the active site of the PFL enzyme in the hydrolysis reaction, we set up the hydrolysis of (**3a**) with denatured PFL (*process for denaturing the enzyme is given in section 3.3.8*) and the desired acid (**4a**) was formed only in trace amounts (**Scheme 3.4**) which suggests that the natural active site (triad of Ser87, Asp264, and His286 residues) is playing a role in the selective hydrolysis reaction.

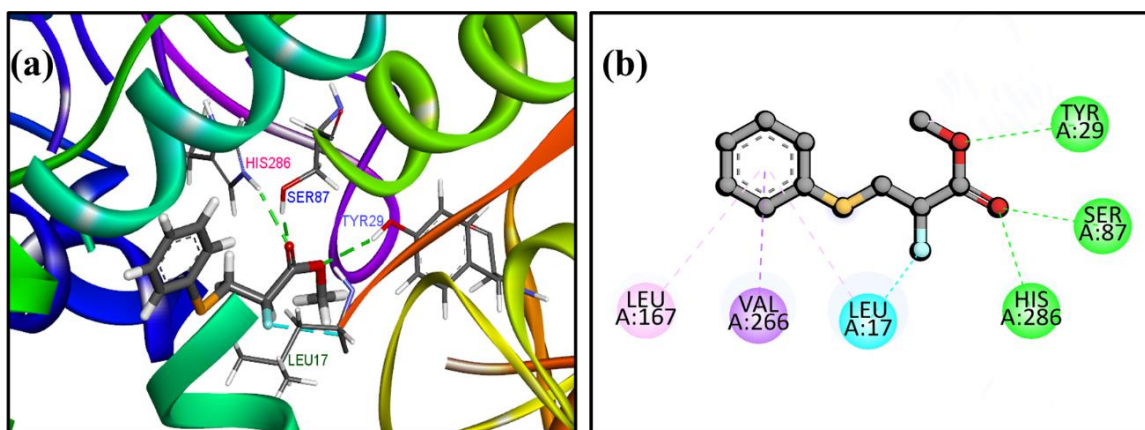
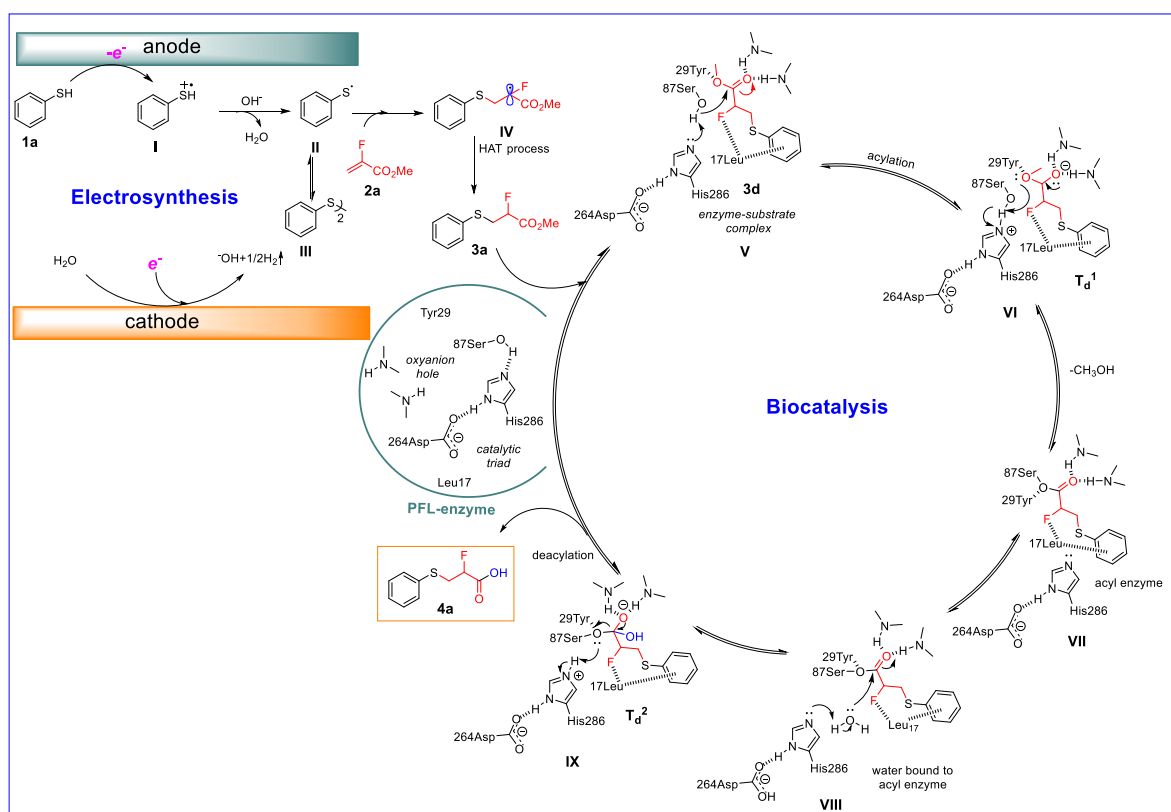


Figure 3.5: Predicted binding interactions of substrate (**3a**) with lipase, dotted lines represent the bonding interactions (a) represents the 3D interaction of active site amino acids Ser87 and His286 with the substrate (**3a**), whereas Tyr29 along with Leu17 helps in cooperative binding of substrate to the enzyme active site (b) 2D interaction of PCL binding to the substrate (**3a**) along with π -alkyl interactions.

3.2.9: Proposed Mechanism

Based on the above experimental results and literature findings,²¹ a plausible mechanism for the formation of (**4a**) was proposed as presented in the **scheme 3.5**. First, thiophenol (**1a**) is anodically oxidized to generate the radical cation species (**I**) which consequently undergo deprotonation to form thia radical (**II**).^{21,22} Subsequently, thia radical (**II**) combines with the methyl-2-fluoro acrylate (**2a**) to generate the carbon-centered radical intermediate (**IV**) which undergoes hydrogen abstraction to yield the electrochemical product (**3a**).^{21,23} Furthermore, when the reaction was conducted in the presence of deuterated water, the deuterated product (**3a'**) was detected using HRMS, with an $[M + OH]^+$ ion at 232.0563. This result confirmed the formation of (**3a**) from radical intermediate (**IV**) (**Scheme 3.4d**). The stability of the radical intermediate (**IV**) has also been reported by Ito and his group, attributing it to the interaction of the unpaired electron with the ester group.^{3f} Then, **3a** enters the active site of the enzyme and binds with the catalytic triad to generate the enzyme-substrate complex (**V**). Afterward, Ser87 attacks at the ester carbonyl atom followed by proton abstraction via His286 to furnish the first tetrahedral intermediate T_{d1} (**VI**).²⁴ After the restoration of the carbon-oxygen double bond, an acyl-enzyme intermediate (**VII**) is formed where His286 allows the release of methanol. Subsequently, water forms a bond with the intermediate acyl-enzyme (**VII**) to deliver (**VIII**). Next, His286 targets the carbonyl carbon of the acyl-enzyme and once again

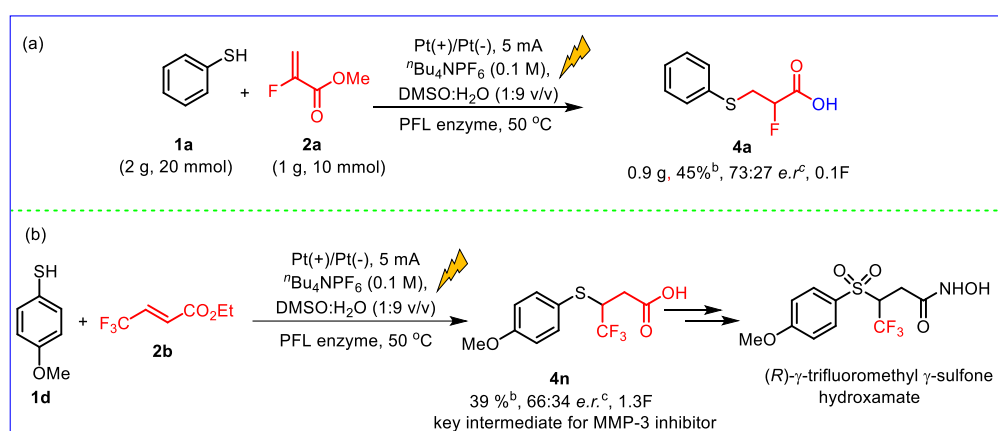


Scheme 3.5: Plausible mechanism for one-pot integrated electrosynthesis and biocatalysis

functions as a base to deprotonate the water as a result the second tetrahedral intermediate T_{d2} (IX) forms. In the end, 2-fluoro-3-(phenylthio)propanoic acid (**4a**) is released via deacylation, and the free enzyme state is restored after the reformation of the carbon-oxygen double bond.²⁵

3.2.10: Gram-scale experiment and application of integrated protocol

To demonstrate the utility of a one-pot integrated electrosynthesis and biocatalysis protocol, a gram-scale reaction involving methyl-2-fluoroacrylate (**2a**) and thiophenol (**1a**) was performed. This resulted in the production of (**4a**) with an isolated yield of 45% (Scheme 3.6a). Besides, we showed the application of an integrated electrosynthetic and biocatalytic approach by synthesizing the key intermediate of (R)- γ -trifluoromethyl γ -sulfone hydroxamate which is a potent MMP-3 inhibitor (stromelysin-1) in 39% isolated yield (Scheme 3.6b).



Scheme 3.6: Scale up and application of electrosynthetic and biocatalytic approach

Reaction conditions: (a) **1a** (1900 μ L, 20 mmol, 2 equiv.), **2a** (900 μ L, 10 mmol, 1 equiv.), ⁿBu₄NPF₆ (0.1 M), DMSO:H₂O (100 mL), PFL enzyme (10 mg/mL) under a constant current of 5 mA in an undivided cell with two platinum electrodes, maintaining the temperature at 50 °C using an oil bath; (b) **1d** (73 μ L, 0.6 mmol, 2 equiv.), **2b** (45 μ L, 0.3 mmol, 1 equiv.), ⁿBu₄NPF₆ (0.1 M), DMSO: H₂O (5 mL), PFL enzyme (10 mg/mL) under a constant current of 5 mA in an undivided cell with two platinum electrodes, maintaining the temperature at 50 °C using an oil bath; ^bisolated yield, ^cdetermined by chiral HPLC.

3.3: Experimental section

3.3.1: General information

All chemicals, including starting compounds and solvents, were purchased from commercial suppliers and utilized without any further purification. Lipase from *Candida rugosa* (≥ 700 U/mg, CAS No. 9001-62-1, Product No. L1754), lipase from *Aspergillus niger* (~ 200 U/g, CAS No. 9001-62-1, Product No. 62301) lipase from porcine pancreas (≥ 125 U/mg, CAS No. 9001-62-1, Product No. L3126), lipase from *Candida* sp. (recombinant, expressed in *Aspergillus niger*, Lipozyme CALB L) (≥ 5000 U/g, Product No. L3170), lipase from

Rhizomucor miehei ($\geq 20,000$ U/g, CAS No. 9001-62-1, Product No. L4277), and Amano lipase from *Pseudomonas Fluorescens* ($\geq 20,000$ U/g, CAS No. 9001-62-1, Product No. 534730) were purchased from Sigma-Aldrich. Amano lipase PS from *Burkholderia cepacia* (CAS No. 9001-62-1, Product No. GLR19.020344) bought from GLR innovations. The electrochemical reactions were conducted on an OWON (P4305) instrument equipped with an undivided cell and magnetic stirrer. The progress of the reaction was monitored by employing thin layer chromatography (TLC, thin layer of silica coated on a glass plates). The ^1H NMR, ^{13}C NMR and ^{19}F NMR spectra of the synthesized compounds were recorded on JEOL or Bruker spectrometer on 400 MHz (600/700 MHz), 100 MHz, and 376 MHz respectively. Tetramethylsilane (TMS) was employed as an internal standard in CDCl_3 and $\text{DMSO-}d_6$. The chemical shift is expressed as δ (ppm), whereas the coupling constant is symbolized by J (Hz). Chemical shifts were referenced to residual solvent peaks (CDCl_3 : 7.26 ppm for ^1H NMR, 77.16 ppm for ^{13}C NMR; $\text{DMSO-}d_6$: 2.50 ppm for ^1H NMR, 39.51 ppm for ^{13}C NMR. Additional acronyms utilized in NMR follow-up studies include: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; td, triplet of doublet; dt, doublet of triplet m, multiplet and dd, double doublet. High resolution mass spectra (HRMS) were acquired using a Water QTOF mass spectrometer (XEVO G2 XS) in ESI (-ve) mode. The % conversion (using 1,3-benzodioxole as the internal standard) and enantiomeric ratios (*e.r.*) were determined using a Shimadzu HPLC equipped with an LC-20AD pump, CTO-10AS column oven, and PDA detector. Cyclic voltammograms were acquired using a DY2300 potentiostat instrument.

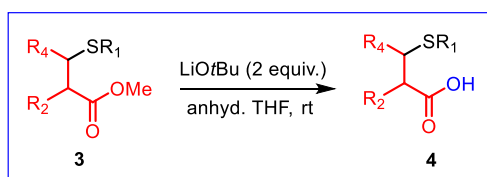
3.3.2: General procedure for the synthesis of 2-fluoro-3-mercaptopropanoate derivatives (3a-3l):

In a reaction vessel containing stirrer bar were added $^n\text{Bu}_4\text{NPF}_6$ (192 mg, 0.1 M), thiophenol (95 μL , 1.0 mmol, 2.0 equiv.), and methyl 2-fluoroacrylate (45 μL , 0.5 mmol, 1.0 equiv.) in a mixture of DMSO and deionized water (5 mL, 1:9, v/v) and placed on a preheated oil bath at 50 $^\circ\text{C}$. A constant current of 5 mA was applied to the reaction mixture in an undivided cell with two platinum sheet electrodes for certain hours as indicated for each substrate. The progress of the reaction was monitored by TLC. Afterward, the reaction mixture was poured into a separatory funnel and was extracted with an ethyl acetate. The resulting solution was then dried using anhydrous Na_2SO_4 . The filtrate was concentrated under vacuum and the residue was purified by column chromatography using silica gel with a mesh size of 60-120 as a stationary phase, while a mixture of ethyl acetate and hexane served as the mobile phase to afford the desired product.

3.3.3: General procedure for the one-pot synthesis of 2-fluoro-3-mercaptopropionic acids:

Thiol (2 equiv.), methyl-2-fluoroacrylate (1 equiv.) and $n\text{Bu}_4\text{NPF}_6$ (192 mg, 0.1 M) were dissolved in 0.5 mL of DMSO in a reaction tube. Subsequently, a suspension of PFL enzyme (10 mg/mL) in 4.5 mL of water was added to it. A steady current of 5 mA was supplied to the reaction mixture using two platinum electrodes while maintaining the temperature at 50 °C in an oil bath. Then, electricity was turned off after the completion of the sulfa-Michael addition reaction, as indicated by TLC. Afterward, the reaction mixture was stirred additionally at 50 °C for 72 h to facilitate the generation of 2-fluoro-3-mercaptopropionic acid derivatives. Next, the reaction mixture was transferred to a Buchner funnel with the sintered disc to remove the electrolyte. The crude residue in the funnel was then washed with ethyl acetate. Following that, the mixture underwent centrifugation at a speed of 9500 rpm to separate the enzyme from the reaction mixture. Next, a solution of saturated NaHCO_3 in water (3 x 20 mL) was employed to extract the residual acid present in the EtOAc layer. The pH of all the aqueous layers was adjusted to 2-3 by adding a 2M HCl solution. Following acidification, the desired compound (**4**) was extracted using EtOAc (3 x 20 mL), which was subsequently dried with Na_2SO_4 and evaporated using a rotary evaporator. The residue was once again rinsed with EtOAc into a separate round bottom flask. The pure solid product (**4**) was obtained by washing with a mixture of dichloromethane and hexane in a ratio of 1:9.

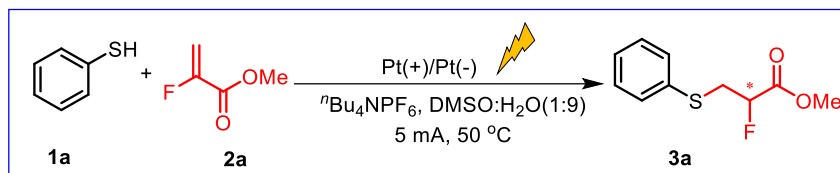
3.3.4: General procedure for preparing racemic 2-fluoro-3-mercaptopropionic acids (**4**) as HPLC standards²⁶



Scheme 3.7: Base-catalyzed racemic synthesis of (**4**)

A round bottom flask, fitted with a magnetic stirrer bar, was charged with lithium *tert*-butoxide (2 equiv.) and ester (**3**) (1 equiv.) dissolved in 2 mL of anhydrous tetrahydrofuran (THF) at room temperature. After the completion of the reaction, as confirmed by thin-layer chromatography (TLC), the tetrahydrofuran (THF) was removed using a rotary evaporator. The pure racemic product was obtained after washing the crude with DCM and hexane respectively.

3.3.5: Procedure to determine % conversion of (**3a**)



Scheme 3.8: Electrochemical synthesis of (3a)

To determine the % conversion, samples (100 μL) were collected after the completion of the reaction and extracted with 300 μL of ethyl acetate. Then, the extracted samples were dried and eluted with isopropyl alcohol (HPLC grade) for HPLC analysis (mobile phase: IPA and hexane) to determine percentage conversion. The following calibration curve was used to calculate the % conversion.

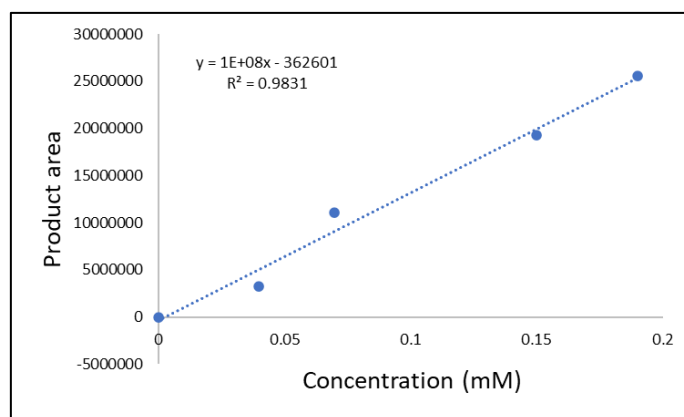
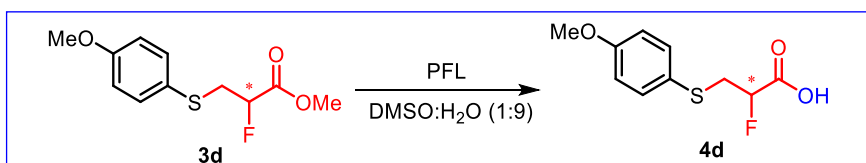


Figure 3.6: Calibration curve of (3a)

3.3.6: Procedure to determine the enantiomeric ratio and % conversion of (4d)



Scheme 3.9: Enzymatic synthesis of (4d)

After completion of the reaction, certain aliquots were collected and subjected to extraction using ethyl acetate. The extracted samples were then dried and diluted with isopropyl alcohol to obtain an enantiomeric ratio using Shimadzu HPLC equipped with a chiral column [Lux i-Cellulose-5].

To determine the % conversion, a 10 μL sample was taken and diluted using HPLC grade water followed by injection into Thermo Scientific Dionex Ultimate 3000 HPLC system. The mobile phase consisted of acetonitrile and water, while a C8 column (250 mm x 4.6 mm x 5 μm) served as the stationary phase. The analysis was conducted using the gradient mode. Following calibration curve was used to calculate % conversion.

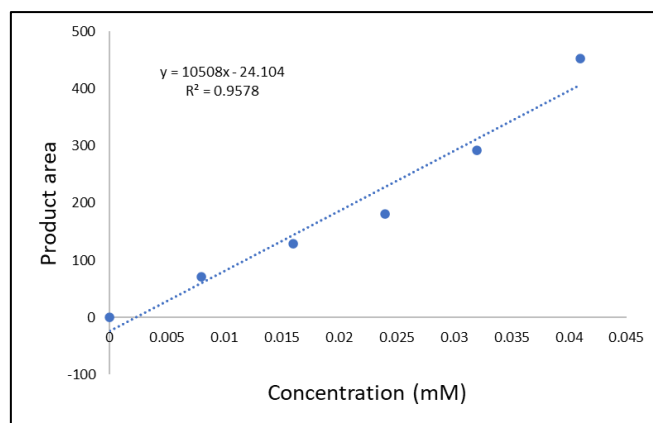


Figure 3.7: Calibration curve of (4d)

3.3.7: Representative HPLC chromatograms for one-pot electro-biocatalysis

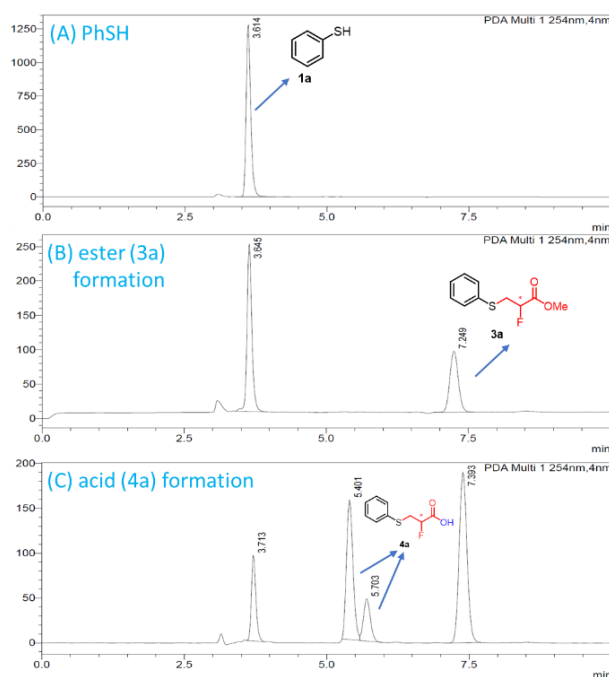


Figure 3.8: HPLC chromatogram for the product (4a) formation via electrobiocatalysis

3.3.8: Enzyme denature procedure

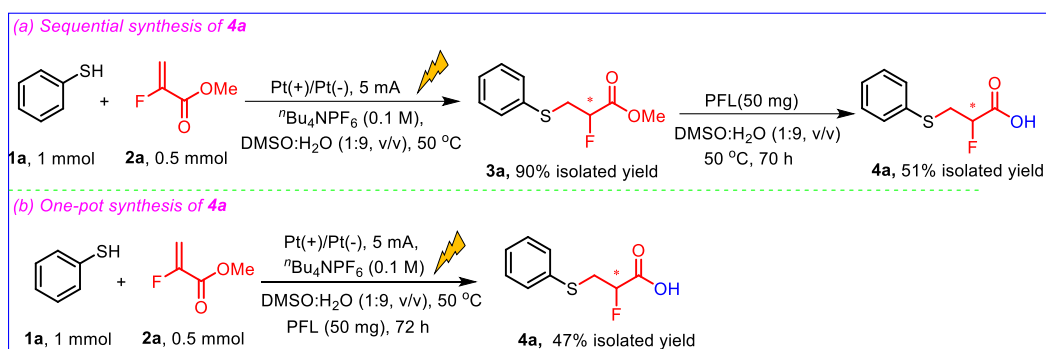
By heating: PFL was denatured by heating it to 100 °C for 1 h in *N,N*-dimethyl formamide.²⁷

By urea: PFL was denatured by pre-treatment of urea at 60 °C for 3 h.²⁸

3.3.9: Procedure for the set-up of sequential electro-biocatalysis

Thiophenol (**1a**) (95 μ L, 1.0 mmol, 2 equiv.), methyl-2-fluoroacrylate (**2a**) (45 μ L, 0.5 mmol, 1 equiv.) and n Bu₄NPF₆ (192 mg, 0.1 M) were dissolved in a mixture of DMSO:H₂O (1:9, v/v, 5 mL). The reaction was initiated by supplying a constant current of 5 mA in an undivided cell with two platinum electrodes at 50 °C. After the reaction completion as confirmed by TLC, the reaction mixture was poured into a separatory funnel, and extracted with ethyl acetate. The crude reaction mixture was dried with Na₂SO₄, concentrated under vacuum, and isolated (**3a**) by column chromatography with 90% yield. Afterward, (**3a**) was incubated with PFL enzyme

(10 mg/mL) at ~200 rpm in a DMSO: H₂O (1:9, v/v, 5 mL) for three days. Following that, the mixture underwent centrifugation at a speed of 9500 rpm to separate the enzyme from the reaction mixture. Next, a solution of saturated NaHCO₃ in water (3 x 20 mL) was employed to



Scheme 3.10: Comparison of sequential and one-pot synthesis of (**4a**) via electro- and biocatalysis

extract the residual acid present in the EtOAc layer. The pH of all the aqueous layers was adjusted to 2-3 by adding a 2M HCl solution. Following acidification, the desired compound (**4a**) was extracted using EtOAc (3 x 20 mL), which was subsequently dried with Na₂SO₄ and evaporated using a rotary evaporator. The residue was once again rinsed with EtOAc into a separate round bottom flask. The pure solid product (**4**) was obtained in 51% yield, after washing the crude with DCM and hexane respectively (**Scheme 3.10**).

3.3.10: Procedure for Gram scale one-pot synthesis of (**4a**)

Thiophenol (**1a**) (1900 μL , 20 mmol, 2 equiv.), methyl-2-fluoroacrylate (**2a**) (900 μL , 10 mmol, 1 equiv.) and ^tBu₄NPF₆ (0.1 M) were dissolved in 10 mL of DMSO in a round bottom flask. Subsequently, a suspension of PFL enzyme (10 mg/mL) in 90 mL of water was added to it. A steady current of 5 mA was supplied to the reaction mixture using two platinum electrodes at a temperature of 50 °C. Then, electricity was turned off after the completion of the sulfa-Michael addition reaction, as indicated by TLC. Afterward, the reaction mixture was stirred additionally at 50 °C for 72 h to facilitate the generation of (**4a**). Next, the reaction mixture was transferred to a Buchner funnel with the sintered disc to remove the electrolyte. The crude residue in the funnel was then washed with ethyl acetate. Following that, the mixture underwent centrifugation at a speed of 9500 rpm to separate the enzyme from the reaction mixture. Next, a solution of saturated NaHCO₃ in water (3 x 250 mL) was employed to extract the residual acid present in the EtOAc layer. The pH of all the aqueous layers was adjusted to 2-3 by adding a 2M HCl solution. Following acidification, the desired compound (**4**) was extracted using EtOAc (3 x 250 mL), which was subsequently dried with Na₂SO₄ and evaporated using a rotary

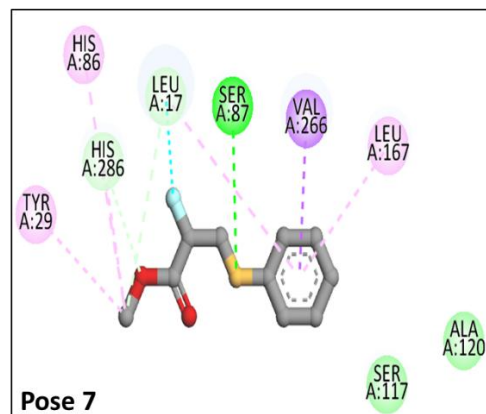
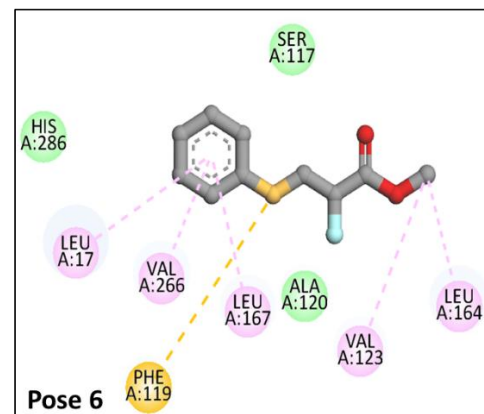
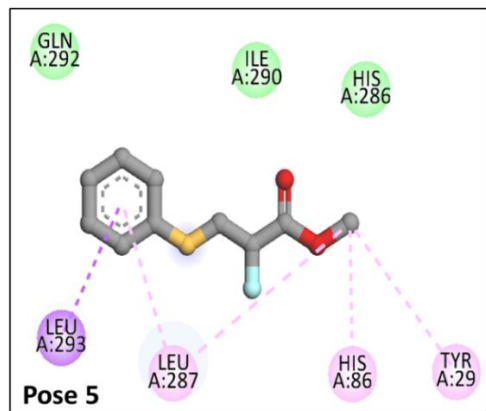
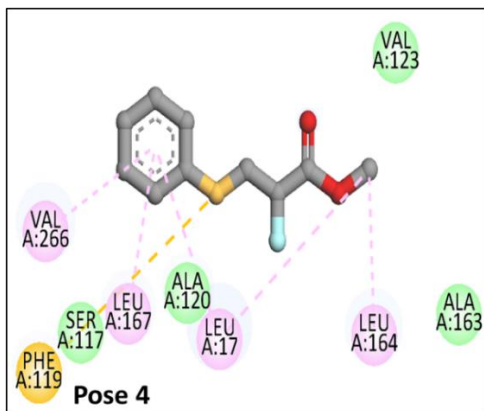
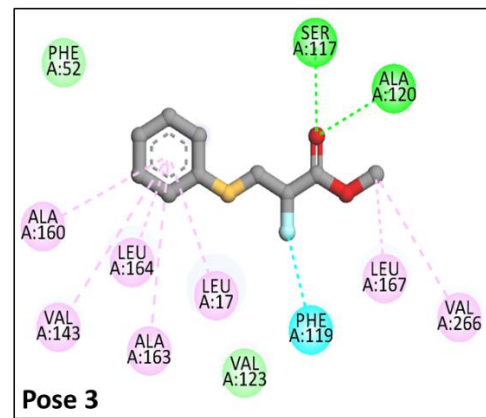
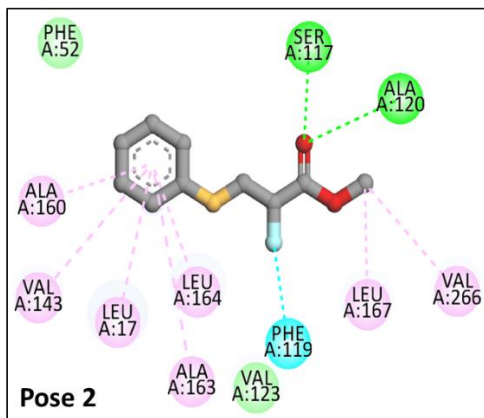
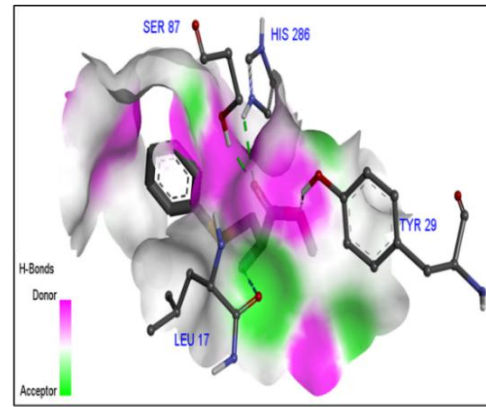
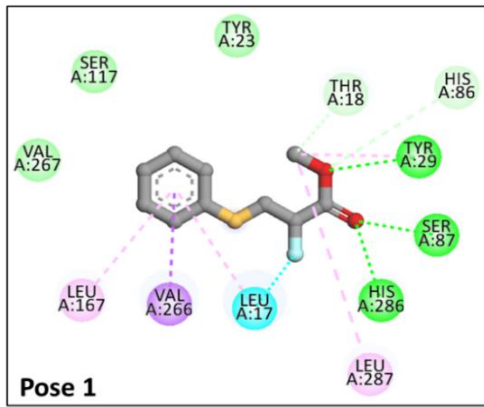
evaporator. The residue was once again rinsed with EtOAc into a separate round bottom flask. The pure solid product (**4**) was obtained in 45% yield by washing with a mixture of dichloromethane and hexane in a ratio of 1:9.

3.3.11: Docking studies

The binding interactions and approximate binding free energies were predicted using the advanced and extensively used molecular grid-based docking software Autodock (4.2.6).²⁹ PCL's X-ray crystal coordinates were acquired from the Protein Data Bank (<http://www.rcsb.org>) with PDB ID 3LIP.³⁰ Before docking, hydrogen atoms were added and the receptor's structure was changed. The receptor was given Gasteiger charges using AutoDock (MGL) Tools. Using the Discovery studio 2021 client, ligand structures were constructed.³¹ The substrate (**3a**) was docked at PCL's active site using an in-silico technique. The grid dimension with center_x,y,z are 10.966 ,0.552 ,17.911 respectively having each grid size spacing of 40 Å.

Table 3.4: Predicted binding affinity and root mean square deviations (R.S.M.D.) (both lower and upper binding) of substrate (**3a**) upto 9 best poses

| Mode | Binding Affinity (kcal/mol) | Distance from the best mode | |
|------|--------------------------------|-----------------------------|-------------|
| | | R.S.M.D L.B | R.S.M.D U.B |
| 1 | -5.7 | 0.000 | 0.000 |
| 2 | -5.7 | 6.287 | 8.071 |
| 3 | -5.6 | 0.401 | 1.323 |
| 4 | -5.4 | 2.803 | 6.462 |
| 5 | -5.2 | 6.381 | 7.752 |
| 6 | -5.0 | 2.690 | 6.260 |
| 7 | -4.9 | 10.783 | 11.500 |
| 8 | -4.9 | 3.051 | 6.485 |
| 9 | -4.9 | 6.345 | 7.755 |



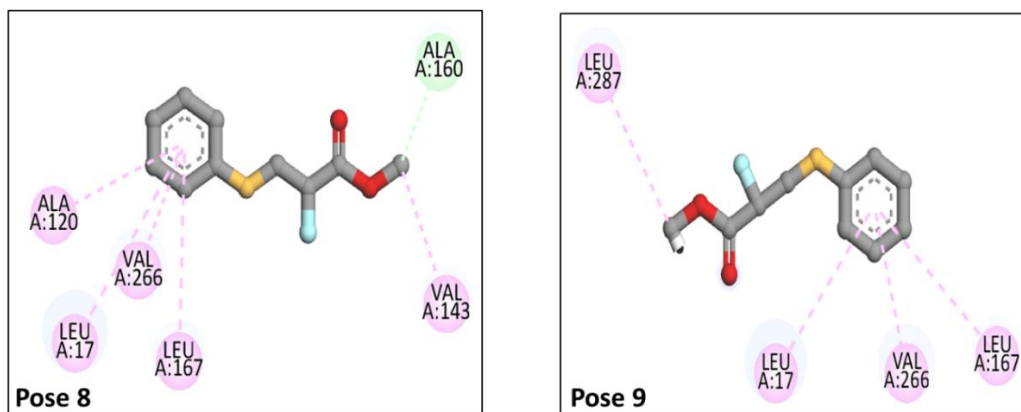


Figure 3.9: 2D interaction of substrate (3a) with various amino acids of PCL enzyme up to 9 poses

3.3.12: Procedure for electrolysis and cyclic voltammetry

The electrochemical reactions were carried out using an OWON (P4305) instrument equipped with an undivided cell and a magnetic stirrer. A platinum rectangular plate (25 mm × 6 mm × 1 mm) was used as both the counter and working electrodes, purchased from SmartChemSynths Machine PVT LTD, Hyderabad, India. The distance between the working and counter electrodes was maintained at 1 cm. The electrochemical sulfa-Michael addition reaction was not sensitive to stirring; however, during the hydrolysis reaction, the stirring speed (stir bar: 5 mm × 10 mm) was maintained between 200–300 rpm. Additionally, no homemade electrode was used.

The electrochemical workstation for cyclic voltammetry (CV) was manufactured by SmartChemSynths Machine PVT LTD, Hyderabad, India. A 30 mm cylindrical platinum wire with a 6 mm shaft, mounted on a Teflon rod and connected via a gold-coated copper rod (2 mm), was used as the working electrode. Before recording each CV, the electrodes were thoroughly cleaned and rinsed with acetone, and the solutions were purged with argon for at least 3 minutes. The initial potential was set to 0 V, and the scan direction ranged from 0 to +3 V (oxidative) at room temperature. The peak potential was measured against an Ag/AgCl reference electrode.

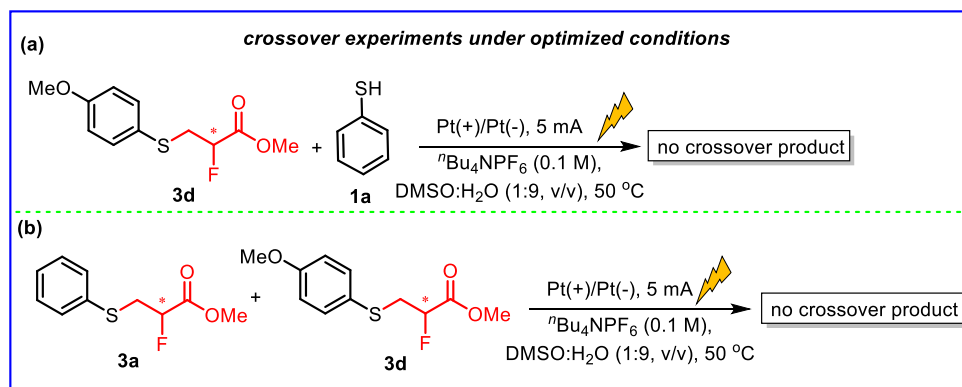
3.3.13: Experimental set-up



Figure 3.10: (A) Experimental-setup assembly (B) Electrochemical vial, magnetic stirrer bar, and platinum sheet electrodes (C) Gram-scale reaction set-up

3.3.14: Crossover experiments

To further investigate the origin of the observed low enantioselectivity, the possibility of background racemization via a retro-thia-Michael pathway³² was examined through crossover experiments³³ under the optimized electrochemical thia-Michael addition (TMA) conditions. In the first experiment, the isolated thio-Michael adduct (**3d**) derived from 4-methoxy thiophenol (**1d**) and methyl-2-fluoroacrylate (**2a**) was subjected to the reaction conditions with unsubstituted thiophenol (**1a**), and no evidence of reversibility was observed. In a second experiment, a mixture of two distinct thio-Michael adducts (**3a** and **3d**) generated from thiophenol and 4-methoxythiophenol was treated under identical conditions. In both cases, no crossover products were detected in either the starting materials or products, as confirmed by HRMS analysis. These results indicate that the electrochemical thia-Michael addition under the present conditions is not reversible. Based on these findings, the low enantioselectivity observed is therefore more likely attributable to limited enzymatic discrimination between the ester enantiomers rather than racemization arising from a reversible thia-Michael process.



Scheme 3.11: Crossover experiments to investigate retro-thia-Michael addition reaction

3.3.15: Characterization data of synthesized compounds

methyl 2-fluoro-3-(phenylthio)propanoate (3a). The product was prepared according to general procedure (3.3.2) from **1a** (95 μL , 1.0 mmol) and **2a** (45 μL , 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 1:99 v/v, stationary phase: silica 60-120 mesh), affording **3a** as a colorless liquid in 90% yield (96 mg, 0.45 mmol). ^1H NMR (400 MHz, CDCl_3): δ_{H} 7.42-7.39 (m, 2H), 7.28 (td, $J = 7.2$ Hz, 2.0 Hz, 2H), 7.23-7.19 (m, 1H), 5.06-4.92 (m, 1H), 3.69 (s, 3H), 3.46-3.24 (m, 2H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3): δ_{C} 168.6 (d, $^2J_{\text{C-F}} = 30.0$ Hz), 134.3, 131.0, 129.2, 127.3, 87.5 (d, $^1J_{\text{C-F}} = 190.0$ Hz),

52.6, 36.6 (d, $^2J_{C-F}$ = 20.0 Hz) ppm. HRMS (ESI-TOF) m/z: $[M + OH]^+$ Calcd for $C_{10}H_{12}FO_3S$ 231.0491; Found 231.0486.

methyl 2-fluoro-3-(*p*-tolylthio)propanoate (3b). The product was prepared according to general procedure (3.3.2) from **1b** (119 mg, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 1.5:98.5 v/v, stationary phase: silica 60-120 mesh), affording **3b** as a colorless liquid in 93% yield (106 mg, 0.46 mmol). 1H NMR (400 MHz, $CDCl_3$): δ_H 7.34 (dt, J = 8.4 Hz, 2.4 Hz, 2H), 7.11 (d, J = 8.4 Hz, 2H), 5.06-4.91 (m, 1H), 3.72 (s, 3H), 3.43-3.21 (m, 2H), 2.32 (s, 3H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ_C 168.7 (d, $^2J_{C-F}$ = 20.0 Hz), 137.7, 131.9, 130.5, 129.9, 87.5 (d, $^1J_{C-F}$ = 190.0 Hz), 52.5, 37.2 (d, $^2J_{C-F}$ = 30.0 Hz), 21.1 ppm. HRMS (ESI $^+$) m/z: $[M + OH]^+$ calcd for $C_{11}H_{14}FO_3S$: 245.0648, found: 245.0642.

methyl 2-fluoro-3-((2-methoxyphenyl)thio)-2 λ^3 -propanoate (3c). The product was prepared according to general procedure (3.3.2) from **1c** (116 μ L, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 3:97 v/v, stationary phase: silica 60-120 mesh), affording **3c** as a colorless liquid in 89% yield (108 mg, 0.44 mmol). 1H NMR (400 MHz, $CDCl_3$): δ_H 7.41 (dd, J = 7.6 Hz, 2.0 Hz, 1H), 7.26, (td, J = 7.6 Hz, 2.0 Hz, 1H), 6.92-6.87 (m, 2H), 5.06-4.91 (m, 1H), 3.89 (s, 3H), 3.69 (s, 3H), 3.45-3.24 (m, 2H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ_C 168.8 (d, $^2J_{C-F}$ = 20.0 Hz), 158.6, 133.1, 129.3, 121.5, 121.0, 111.0, 87.8 (d, $^1J_{C-F}$ = 180.0 Hz), 55.9, 52.6, 34.8 (d, $^2J_{C-F}$ = 20.0 Hz) ppm. HRMS (ESI-TOF) m/z: $[M + OH]^+$ Calcd for $C_{11}H_{14}FO_4S$ 261.0597; Found 261.0590.

methyl 2-fluoro-3-((4-methoxyphenyl)thio)-2 λ^3 -propanoate (3d). The product was prepared according to general procedure(3.3.2) from **1d** (117 μ L, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 4:96 v/v, stationary phase: silica 60-120 mesh), affording **3d** as a colorless liquid in 92% yield (112 mg, 0.46 mmol). 1H NMR (400 MHz, $CDCl_3$): δ_H 7.41 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 5.02-4.87 (m, 1H), 3.77 (s, 3H), 3.71 (s, 3H), 3.34-3.14 (m, 2H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ_C 168.6 (d, $^2J_{C-F}$ = 30.0 Hz), 159.7, 134.8, 124.3, 114.7, 87.5 (d, $^1J_{C-F}$ = 190.0 Hz), 55.3, 52.5, 38.3 (d, $^2J_{C-F}$ = 20.0 Hz) ppm. HRMS (ESI-TOF) m/z: $[M + OH]^+$ Calcd for $C_{11}H_{14}FO_4S$ 261.0597; Found 261.0596.

methyl 2-fluoro-3-((2-hydroxyphenyl)thio)-2 λ^3 -propanoate (3e). The product was prepared according to general procedure (3.3.2) from **1e** (97 μ L, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 5:95 v/v, stationary phase: silica 60-120 mesh), affording **3e** as a colorless liquid in 83% yield (95 mg,

0.41 mmol). ^1H NMR (400 MHz, CDCl_3): δ_{H} 7.46 (d, $J = 8.0$ Hz, 1H), 7.23 (t, $J = 8.4$ Hz, 1H), 6.95 (d, $J = 8.4$ Hz, 1H), 6.84 (t, $J = 6.0$ Hz, 1H), 6.69 (s, 1H), 4.97-4.82 (m, 1H), 3.70 (s, 3H), 3.22-3.05 (m, 2H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3): δ_{C} 168.5 (d, $^2J_{\text{C-F}} = 30.0$ Hz), 157.4, 136.5, 131.9, 121.2, 117.2, 115.5, 87.0 (d, $^1J_{\text{C-F}} = 180.0$ Hz), 52.8, 38.2 (d, $^2J_{\text{C-F}} = 20.0$ Hz) ppm. ^{19}F NMR (376 MHz, CDCl_3): δ_{F} -189.66 – -190.03 (m) ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{OH}]^+$ Calcd for $\text{C}_{10}\text{H}_{12}\text{FO}_4\text{S}$ 247.0440; Found 247.0453.

methyl 3-((2-bromophenyl)thio)-2-fluoro-2 λ^3 -propanoate (3f). The product was prepared according to general procedure (3.3.2) from **1f** (116 μL , 1 mmol) and **2a** (45 μL , 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 2.5:97.5 v/v, stationary phase: silica 60-120 mesh), affording **3f** as a colorless liquid in 73% yield (107 mg, 0.36 mmol). ^1H NMR (400 MHz, CDCl_3): δ_{H} 7.57 (dd, $J = 8.0$ Hz, 1.6 Hz, 1H), 7.43 (dd, $J = 7.6$ Hz, 1.2 Hz, 1H), 7.27 (td, 7.2 Hz, 1.2 Hz, 1H), 7.09 (td, $J = 7.2$ Hz, 1.2 Hz, 1H), 5.14-4.99 (m, 1H), 3.76 (s, 3H), 3.52-3.31 (m, 2H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3): δ_{C} 168.4 (d, $^2J_{\text{C-F}} = 30.0$ Hz), 135.5, 133.4, 131.1, 128.3, 128.0, 125.7, 87.5 (d, $^1J_{\text{C-F}} = 190.0$ Hz), 52.7, 35.5 (d, $^2J_{\text{C-F}} = 20.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[\text{M} - \text{HBr}]^+$ Calcd for $\text{C}_{10}\text{H}_{10}\text{FO}_2\text{S}$ 213.0386; Found 213.0377.

methyl 3-((4-bromophenyl)thio)-2-fluoro-2 λ^3 -propanoate (3g). The product was prepared according to general procedure (3.3.2) from **1g** (182 mg, 1 mmol) and **2a** (45 μL , 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 1.5:98.5 v/v, stationary phase: silica 60-120 mesh), affording **3g** as a colorless liquid in 81% yield (118 mg, 0.40 mmol). ^1H NMR (400 MHz, CDCl_3): δ_{H} 7.34 (dt, $J = 8.8$ Hz, 2.8 Hz, 2H), 7.24 (dt, $J = 8.8$ Hz, 2.4 Hz, 2H), 5.05-4.89 (m, 1H), 3.69 (s, 3H), 3.41-3.20 (m, 2H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3): δ_{C} 168.4 (d, $^2J_{\text{C-F}} = 20.0$ Hz), 133.6, 132.6, 132.2, 121.4, 87.6 (d, $^1J_{\text{C-F}} = 190.0$ Hz), 52.7, 36.6 (d, $^2J_{\text{C-F}} = 30.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[\text{M} - \text{H}]^+$ Calcd for $\text{C}_{10}\text{H}_9\text{BrFO}_2\text{S}$ 290.9491; Found 290.9505.

methyl 3-((4-chlorophenyl)thio)-2-fluoro-2 λ^3 -propanoate (3h). The product was prepared according to general procedure (3.3.2) from **1h** (138 mg, 1 mmol) and **2a** (45 μL , 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 2.5:97.5 v/v, stationary phase: silica 60-120 mesh), affording **3h** as a colorless liquid in 79% yield (98 mg, 0.39 mmol). ^1H NMR (700 MHz, CDCl_3): δ_{H} 7.35 (dt, $J = 9.1$ Hz, 2.8 Hz, 2H), 7.26 (dt, $J = 8.4$ Hz, 2.8 Hz, 2H), 5.05-4.96 (m, 1H), 3.73 (s, 3H), 3.42-3.36 (m, 1H), 3.32-3.26 (m, 1H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (175 MHz, CDCl_3): δ_{C} 168.4 (d, $^2J_{\text{C-F}} = 17.5$ Hz), 133.5, 132.9, 132.5, 129.3, 87.7 (d, $^1J_{\text{C-F}} = 192.5$ Hz), 52.7, 36.8 (d, $^2J_{\text{C-F}} = 17.5$ Hz) ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{OH}]^+$ Calcd for $\text{C}_{10}\text{H}_{11}\text{ClFO}_3\text{S}$ 265.0101; Found 265.0107.

methyl 2-fluoro-3-((4-nitrophenyl)thio)-2λ³-propanoate (3i). The product was prepared according to general procedure (3.3.2) from **1i** (149 mg, 1 mmol) and **2a** (45 μL, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 6:94 v/v, stationary phase: silica 60-120 mesh), affording **3i** as a yellow liquid in 10% yield (13 mg, 0.05 mmol). ¹H NMR (400 MHz, CDCl₃): δ_H 8.09 (dt, *J* = 9.2 Hz, 2.8 Hz, 2H), 7.42 (dt, *J* = 9.2 Hz, 2.8 Hz, 2H), 5.21-5.06 (m, 1H), 3.78 (s, 3H), 3.63-3.40 (m, 2H) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ_C 168.0 (d, ²*J*_{C-F} = 20.0 Hz), 145.8, 144.9, 127.7, 124.0, 87.5 (d, ¹*J*_{C-F} = 190.0 Hz), 52.9, 34.4 (d, ²*J*_{C-F} = 30.0 Hz) ppm. ¹⁹F NMR (376 MHz, CDCl₃): δ_F -188.84 (dt, ²*J*_{F-H} = 50.9 Hz, ³*J*_{F-H} = 25.4 Hz) ppm, HRMS (ESI-TOF) *m/z*: [M + OH]⁺ Calcd for C₁₀H₁₁FNO₅S 276.0342; Found 276.0346.

methyl 3-(benzylthio)-2-fluoro-2λ³-propanoate (3j). The product was prepared according to general procedure (3.3.2) from **1j** (119 μL, 1 mmol) and **2a** (45 μL, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 2:98 v/v, stationary phase: silica 60-120 mesh), affording **3j** as a colorless liquid in 44% yield (13 mg, 0.19 mmol). ¹H NMR (700 MHz, CDCl₃): δ_H 7.27-7.24 (m, 4H), 7.21-7.17 (m, 1H), 5.04-4.96 (m, 1H), 3.75 (d, *J* = 3.5 Hz, 2H), 3.74 (s, 3H), 2.87-2.74 (m, 2H) ppm. ¹³C{¹H} NMR (175 MHz, CDCl₃): δ_C 168.7 (d, ²*J*_{C-F} = 17.5 Hz), 137.6, 129.2, 128.7, 127.4, 89.7 (d, ¹*J*_{C-F} = 192.5 Hz), 52.6, 36.9, 32.4 (d, ²*J*_{C-F} = 17.5 Hz) ppm. HRMS (ESI-TOF) *m/z*: [M + OH]⁺ Calcd for C₁₁H₁₄FO₃S 245.0648; Found 245.0643.

ethyl 4,4,4-trifluoro-3-(phenylthio)-3λ³-butanoate (3l).⁴ The product was prepared according to general procedure (3.3.2) from **1a** (65 μL, 0.4 mmol) and **2b** (42 μL, 0.2 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 1:99 v/v, stationary phase: silica 60-120 mesh), affording **3l** as a colorless liquid in 62% yield (51 mg, 0.18 mmol). ¹H NMR (400 MHz, CDCl₃): δ_H 7.59-7.57 (m, 2H), 7.35-7.32 (m, 3H), 4.27-4.19 (m, 2H), 3.99-3.89 (m, 1H), 2.87 (dd, *J* = 16.8 Hz, 4.0 Hz, 1H), 2.65 (dd, *J* = 16.8 Hz, 10.8 Hz, 1H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ¹³C{¹H} NMR (100 MHz, CDCl₃): δ_C 169.5, 134.2, 132.3, 129.3, 129.0, 128.9, 127.7, 124.9, 61.6, 48.7 (q, ¹*J*_{C-F} = 30.0 Hz), 34.6, 14.2 ppm.

methyl 3-((2-chlorophenyl)thio)-2-fluoro-3λ³-propanoate (3o). The product was prepared according to general procedure (3.3.2) from **1o** (112 μL, 1 mmol) and **2a** (45 μL, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 2:98 v/v, stationary phase: silica 60-120 mesh), affording **3o** as a colorless liquid in 72% yield (89 mg, 0.36 mmol). ¹H NMR (300 MHz, CDCl₃): δ_H 7.49-7.41 (m, 2H), 7.28-7.18 (m, 2H), 5.17-4.99 (m, 1H), 3.77 (s, 3H), 3.56-3.32 (m, 2H) ppm. ¹³C{¹H} NMR (75 MHz, CDCl₃): δ_C 168.4 (d, ²*J*_{C-F} = 22.5 Hz), 135.5, 133.3, 131.6, 130.1, 128.4, 127.4, 87.6 (d, ¹*J*_{C-F} = 187.5 Hz), 52.7, 35.1

(d, $^2J_{C-F} = 22.5$ Hz) ppm. HRMS (ESI-TOF) m/z: $[M - HCl]^+$ Calcd for $C_{10}H_{10}FO_2S$ 213.0386; Found 213.0381.

methyl 2-fluoro-3-((4-fluorophenyl)thio)-2 λ^3 -propanoate (3p). The product was prepared according to general procedure (3.3.2) from **1p** (102 μ L, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 2:98 v/v, stationary phase: silica 60-120 mesh), affording **3p** as a colorless liquid in 70% yield (81 mg, 0.35 mmol). 1H NMR (300 MHz, $CDCl_3$): δ_H 7.44 (t, $J = 5.4$ Hz, 2H), 6.99 (t, $J = 8.7$ Hz, 2H), 5.08-4.89 (m, 1H), 3.72 (s, 3H), 3.41-3.18 (m, 2H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ_C 168.5 (d, $^2J_{C-F} = 22.5$ Hz), 162.5 (d, $^1J_{C-F} = 240.0$ Hz), 134.3 (d, $^4J_{C-F} = 7.5$ Hz), 129.2 (d, $^3J_{C-F} = 7.5$ Hz), 116.3 (d, $^2J_{C-F} = 15.0$ Hz), 87.6 (d, $^1J_{C-F} = 187.5$ Hz), 52.6, 37.7 (d, $^2J_{C-F} = 22.5$ Hz) ppm. HRMS (ESI-TOF) m/z: $[M + OH]^+$ Calcd for $C_{10}H_{11}F_2O_3S$ 249.0397; Found 249.0391.

methyl 3-((4-cyanophenyl)thio)-2-fluoropropanoate (3q). The product was prepared according to general procedure (3.3.2) from **1q** (130 mg, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude was purified by column chromatography (mobile phase: EtOAc/Hexane, 15:85 v/v, stationary phase: silica 60-120 mesh), affording **3q** as a yellow liquid in 32% yield (38 mg, 0.16 mmol). 1H NMR (300 MHz, $CDCl_3$): δ_H 7.55 (d, $J = 8.7$ Hz, 2H), 7.41 (d, $J = 8.1$ Hz, 2H), 5.21-5.03 (m, 1H), 3.79 (s, 3H), 3.61-3.34 (m, 2H) ppm. $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$): δ_C 168.2 (d, $^2J_{C-F} = 30.0$ Hz), 142.3, 132.5, 128.5, 118.6, 109.7, 87.6 (d, $^1J_{C-F} = 187.5$ Hz), 52.9, 34.7 (d, $^2J_{C-F} = 22.5$ Hz) ppm. HRMS (ESI-TOF) m/z: $[M + OH]^+$ Calcd for $C_{11}H_{11}FNO_3S$ 256.0444; Found 256.0449.

2-fluoro-3-(phenylthio)-2 λ^3 -propanoic acid (4a). The product **4a** was prepared following General Procedure (3.3.3) using **1a** (95 μ L, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude product was obtained as a white powder with a 47% yield (47 mg, 0.23 mmol). 1H NMR (600 MHz, $DMSO-d_6$): δ_H 8.32-8.30 (m, 1H), 7.35-7.27 (m, 4H), 7.17-7.16 (m, 1H), 4.64-4.53 (m, 1H), 3.52-3.46 (m, 1H), 3.22-3.14 (m, 1H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ_C 170.4 (d, $^2J_{C-F} = 20.0$ Hz), 136.6, 129.1, 128.1, 125.7, 89.8 (d, $^1J_{C-F} = 180.0$ Hz), 36.6 (d, $^2J_{C-F} = 20.0$ Hz) ppm. HRMS (ESI-TOF) m/z: $[M - H]^+$ Calcd for $C_9H_8FO_2S$ 199.0229; Found 199.0233. 77:23 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 7/97, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 6.28 min (major), 6.81 min (minor).

2-fluoro-3-(*p*-tolylthio)-2 λ^3 -propanoic acid (4b). The product **4b** was prepared following General Procedure (3.3.3) using **1b** (119 mg, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude product was obtained as a white powder with a 49% yield (52 mg, 0.24 mmol). 1H NMR (400 MHz, $DMSO-d_6$): δ_H 7.25 (dt, $J = 8.4$ Hz, 2.0 Hz, 2H), 7.12 (dt, $J = 8.4$ Hz, 2.8 Hz, 2H), 4.57-

4.41 (m, 1H), 3.48 (dd, $J = 14.4$ Hz, 2.4 Hz, 1H), 3.40 (dd, $J = 14.4$ Hz, 2.4 Hz, 1H), 3.16-3.06 (m, 1H), 2.26 (s, 3H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO- d^6): δ_{C} 170.0 (d, $^2J_{\text{C-F}} = 20.0$ Hz), 135.3, 132.7, 129.7, 128.9, 89.8 (d, $^1J_{\text{C-F}} = 180$ Hz), 37.3 (d, $^2J_{\text{C-F}} = 30.0$ Hz), 20.6 ppm. HRMS (ESI-TOF) m/z : $[\text{M} - \text{H}]^+$ Calcd for $\text{C}_{10}\text{H}_{10}\text{FO}_2\text{S}$ 213.0386; Found: 213.0389. 80:20 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 5/95, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 7.90 min (major), 8.66 min (minor).

2-fluoro-3-((2-methoxyphenyl)thio)-2 λ^3 -propanoic acid (4c). The product **4c** was prepared following General Procedure (3.3.3) using **1c** (116 μL , 1 mmol) and **2a** (45 μL , 0.5 mmol). The crude product was obtained as an off-white powder with a 50% yield (57 mg, 0.25 mmol). ^1H NMR (300 MHz, CDCl_3): δ_{H} 7.72 (d, $J = 15.0$ Hz, 1H), 7.44- 7.35 (m, 1H), 6.99-6.93 (m, 1H), 6.85-6.79 (m, 1H), 5.56- 5.50 (m, 1H), 3.84 (s, 3H), 3.13-3.05 (m, 2H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO- d^6): δ_{C} 170.5 (d, $^2J_{\text{C-F}} = 20.0$ Hz), 156.3, 127.6, 126.6, 124.8, 121.2, 110.9, 89.8 (d, $^1J_{\text{C-F}} = 180.0$ Hz), 55.9, 34.9 (d, $^2J_{\text{C-F}} = 20.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[\text{M} - \text{H}]^+$ Calcd for $\text{C}_{10}\text{H}_{10}\text{FO}_3\text{S}$ 229.0335; Found 229.0339. 72:28 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 10/90, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 8.39 min (major), 9.26 min (minor).

2-fluoro-3-((4-methoxyphenyl)thio)-2 λ^3 -propanoic acid (4d). The product **4d** was prepared following General Procedure (3.3.3) using **1d** (117 μL , 1 mmol) and **2a** (45 μL , 0.5 mmol). The crude product was obtained as an off-white powder with a 49% yield (56 mg, 0.24 mmol). ^1H NMR (400 MHz, DMSO- d^6): δ_{H} 7.35 (dt, $J = 8.8$ Hz, 3.6 Hz, 2H), 6.90 (dt, $J = 8.4$ Hz, 3.2 Hz, 2H), 4.52-4.36 (m, 1H), 3.73 (s, 3H), 3.39-3.28 (m, 1H), 3.09-2.99 (m, 1H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO- d^6): δ_{C} 169.8 (d, $^2J_{\text{C-F}} = 10.0$ Hz), 158.4, 132.3, 126.3, 114.9, 89.8 (d, $^1J_{\text{C-F}} = 190.0$ Hz), 55.2, 38.8 (d, $^2J_{\text{C-F}} = 10.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[\text{M} - \text{H}]^+$ Calcd for $\text{C}_{10}\text{H}_{10}\text{FO}_3\text{S}$ 229.0335; Found 229.0339. 83:17 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 10/90, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 6.87 min (major), 7.42 min (minor).

3-((2-bromophenyl)thio)-2-fluoro-2 λ^3 -propanoic acid (4f). The product **4f** was prepared following General Procedure (3.3.3) using **1f** (116 μL , 1 mmol) and **2a** (45 μL , 0.5 mmol). The crude product was obtained as a white powder with a 42% yield (58 mg, 0.21 mmol). ^1H NMR (400 MHz, DMSO- d^6): δ_{H} 7.63-7.53 (m, 1H), 7.36 (dd, $J = 19.2$ Hz, 7.6 Hz, 2H), 7.04 (t, 7.6 Hz, 1H), 4.59 (dd, $^2J_{\text{H-F}} = 52.2$ Hz, $^2J_{\text{H-H}} = 52.4$ Hz, 9.2 Hz, 1H), 3.25-3.12 (m, 2H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO- d^6): δ_{C} 169.8 (d, $^2J_{\text{C-F}} = 10.0$ Hz), 138.1, 135.4, 133.2, 132.7, 130.3, 128.8, 128.4, 127.1, 126.5, 123.1, 121.4, 89.4 (d, $^1J_{\text{C-F}} = 180.0$ Hz), 35.8 (d, $^2J_{\text{C-F}} = 20.0$

Hz) ppm. HRMS (ESI-TOF) m/z : $[M - H]^+$ Calcd for $C_9H_7BrFO_2S$ 276.9334; Found 276.9338. 74:26 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 1/99, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 12.56 min (major), 13.43 min (minor).

3-((4-bromophenyl)thio)-2-fluoro-2 λ^3 -propanoic acid (4g). The product **4g** was prepared following General Procedure (3.3.3) using **1g** (182 mg, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude product was obtained as a white powder with a 45% yield (62 mg, 0.22 mmol). 1H NMR (400 MHz, DMSO- d^6): δ_H 7.44 (d, $J = 8.8$ Hz, 2H), 7.26 (d, $J = 8.8$ Hz, 2H), 4.57-4.41 (m, 1H), 3.52-3.40 (m, 1H), 3.20-3.10 (m, 1H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, DMSO- d^6): δ_C 169.5 (d, $^2J_{C-F} = 20.0$ Hz), 136.5, 131.8, 129.9, 118.4, 89.8 (d, $^1J_{C-F} = 190.0$ Hz), 36.5 (d, $^2J_{C-F} = 30.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[M - H]^+$ Calcd for $C_9H_7BrFO_2S$ 276.9334; Found 276.9343. 74:26 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 1/99, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 29.09 min (major), 31.40 min (minor).

3-((4-chlorophenyl)thio)-2-fluoro-2 λ^3 -propanoic acid (4h). The product **4h** was prepared following General Procedure (3.3.3) using **1h** (138 mg, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude product was obtained as a white powder with a 44% yield (51 mg, 0.22 mmol). 1H NMR (400 MHz, DMSO- d^6): δ_H 7.39-7.34 (m, 4H), 4.57-4.41 (m, 1H), 3.54-3.43 (m, 1H), 3.23-3.12 (m, 1H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, DMSO- d^6): δ_C 169.2 (d, $^2J_{C-F} = 20.0$ Hz), 135.9, 130.1, 129.6, 128.9, 89.8, (d, $^1J_{C-F} = 190.0$ Hz), 36.8 (d, $^2J_{C-F} = 20.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[M - H]^+$ Calcd for $C_9H_7ClFO_2S$ 232.9839; Found 232.9844. 69:31 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 1/99, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 29.86 min (major), 32.35 min (minor).

3-(benzylthio)-2-fluoro-2 λ^3 -propanoic acid (4j). The product **4j** was prepared following General Procedure (3.3.3) using **1j** (119 μ L, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude product was obtained as an off-white powder with a 21% yield (52 mg, 0.10 mmol). 1H NMR (400 MHz, DMSO- d^6): δ_H 7.31-7.28 (m, 4H), 7.24-7.20 (m, 1H), 4.64-4.48 (m, 1H), 3.77 (s, 2H), 2.95-2.82 (m, 1H), 2.73-2.63 (m, 1H) ppm. $^{13}C\{^1H\}$ NMR (100 MHz, DMSO- d^6): δ_C 170.4 (d, $^2J_{C-F} = 20.0$ Hz), 138.8, 128.9, 128.3, 126.7, 91.2 (d, $^1J_{C-F} = 180.0$ Hz), 36.1, 34.4 (d, $^2J_{C-F} = 30.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[M - H]^+$ Calcd for $C_{10}H_{10}FO_2S$ 213.0386; Found 213.0351. 69:31 *e.r.*, determined by chiral HPLC [Lux i-Cellulose-5 column, isopropanol/hexane (0.1% trifluoroacetic acid) = 10/90, flow rate 1.0 mL/min, $\lambda = 254$ nm], retention time: 5.43 min (major), 5.95 min (minor).

4,4,4-trifluoro-3-(phenylthio)-3 λ^3 -butanoic acid (4l). The product **4l** was prepared following General Procedure (3.3.3) using **1a** (65 μ L, 0.4 mmol) and **2b** (42 μ L, 0.2 mmol). The crude product was obtained as an off-white powder with a 32% yield (24 mg, 0.09 mmol). ^1H NMR (400 MHz, DMSO- d^6): δ_{H} 7.59-7.49 (m, 2H), 7.35-7.23 (m, 3H), 6.47-6.31 (m, 1H), 4.29 (brs, 1H), 2.43 (brs, 1H), 2.21-2.15 (m, 1H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO- d^6): δ_{C} 170.9, 133.8, 131.9, 129.2, 127.8, 124.4, 48.9, 37.3 ppm. HRMS (ESI-TOF $^-$) m/z : $[\text{M} - \text{H}]^+$ Calcd for $\text{C}_{10}\text{H}_8\text{F}_3\text{O}_2\text{S}$ 249.0197; Found 249.0203.

3-((2-aminophenyl)thio)-2-fluoro-2 λ^3 -propanoic acid (4m). The product **4m** was prepared following General Procedure (3.3.3) using **1m** (102 μ L, 1 mmol) and **2a** (45 μ L, 0.5 mmol). The crude product was obtained as yellow powder with a 47% yield (50 mg, 0.23 mmol). ^1H NMR (400 MHz, DMSO- d^6): δ_{H} 7.25 (d, $J = 7.2$ Hz, 1H), 7.02 (t, $J = 6.4$ Hz, 1H), 6.70 (d, $J = 8.4$ Hz, 1H), 6.49 (t, $J = 7.6$ Hz, 1H), 5.38 (s, 2H), 4.51-4.35 (m, 1H), 3.22-3.10 (m, 1H), 2.97-2.87 (m, 1H) ppm. $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, DMSO- d^6): δ_{C} 170.2 (d, $^2J_{\text{C-F}} = 20.0$ Hz) 149.8, 135.4, 129.5, 116.3, 115.7, 114.4, 89.8 (d, $^1J_{\text{C-F}} = 180.0$ Hz), 79.3, 38.0 (d, $^2J_{\text{C-F}} = 20.0$ Hz) ppm. HRMS (ESI-TOF) m/z : $[\text{M} - \text{H}]^+$ Calcd for $\text{C}_9\text{H}_9\text{FNO}_2\text{S}$ 214.0338; Found 214.0331..

4,4,4-trifluoro-3-((4-methoxyphenyl)thio)butanoic acid (4n).⁴ The product **4n** was prepared following General Procedure (3.3.3) using **1d** (73 μ L, 0.6 mmol), **2b** (45 μ L, 0.3 mmol). The crude product was obtained as an off-white powder with a 39% yield (32 mg, 0.11 mmol). ^1H NMR (700 MHz, DMSO- d^6): δ_{H} 7.55-7.43 (m, 2H), 6.91-6.86 (m, 2H), 4.05 (s, 1H), 3.74 (s, 3H), 2.42-2.39 (m, 1H), 2.19-2.15 (m, 1H) ppm. HRMS (ESI-TOF) m/z : $[\text{M} - \text{H}]^+$ Calcd for $\text{C}_{11}\text{H}_{10}\text{F}_3\text{O}_3\text{S}$ 279.0303; Found 279.0310.

3.4: Conclusion

In summary, an asymmetric synthesis of sulfur-based organofluorine compounds has been developed via the integration of organic electrosynthesis with lipase-mediated biocatalysis. Further, a variety of 2-fluoro-3-mercaptopropionic acids have been synthesized in moderate yields and good enantioselectivities by using thiophenols and fluorine-containing α , β -unsaturated alkenes in water as a solvent. To get an insight about the mechanism of the one-pot protocol, molecular docking, and other control experiments were carried out that suggested the role of electricity and enzyme active site in the catalysis. The scalability of the developed protocol was shown by setting up a gram-scale reaction. Besides, the practical efficacy has been illustrated via synthesizing a key intermediate of MMP-3 inhibitor. Notably, this metal, ligand, and additional oxidant-free electro-biocatalyst protocol enable further applications of enzymatic electrosynthesis in asymmetric organic transformations.

3.5: References

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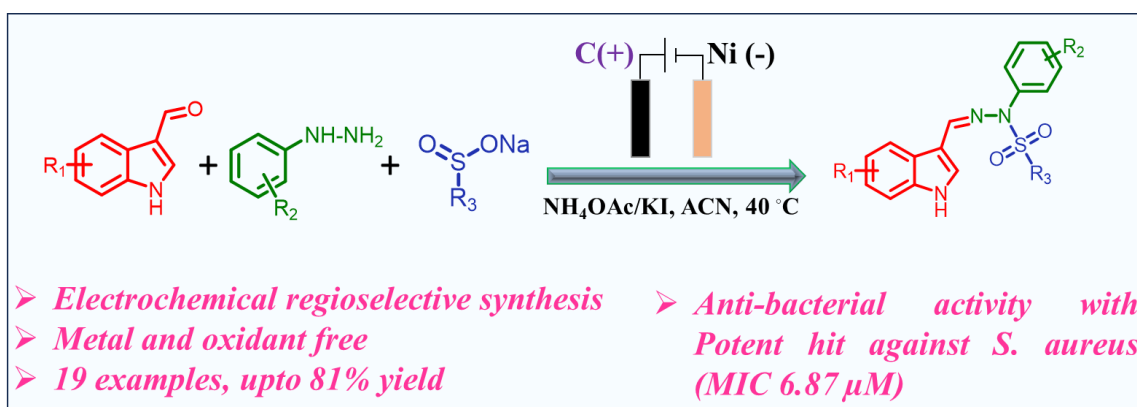
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CHAPTER 4

Electrochemical N-sulfonylation of in-situ Generated Indole-based Hydrazones and Antimicrobial Evaluation



4.1: Introduction

Hydrazone formation is a powerful strategy for functionalizing carbonyl compounds, enabling the introduction of diverse functional groups into organic molecules, biomolecules, and polymers.¹ Further, hydrazones also represent a key class of biologically active compounds with a wide range of medicinal properties, drawing considerable interest from pharmaceutical chemists.² In particular, numerous indole-based hydrazone derivatives have been developed and systematically studied for their therapeutic potential, including antibacterial, antimalarial, antitumor, antitubercular, anti-inflammatory, anti-breast cancer and MIF-inhibitory activities (**Figure 4.1**).³ Their broad pharmacological profiles make them promising drug candidates with the potential for enhanced efficacy and reduced toxicity.⁴ Hence, synthetic strategies for the synthesis and functionalization of hydrazones have garnered significant attention over the past years.⁵

On the other hand, organosulfones constitute an important class of compounds with broad utility in pharmaceuticals, advanced materials, and synthetic chemistry.⁶ They display a wide range of biological activities, including antibacterial and anticancer properties, and are frequently incorporated as key structural units in therapeutic agents (**Figure 4.1**).⁷ Furthermore, organosulfones can be synthesized through various methods using sulfonylating agents such as sulfonyl chlorides,⁸ sulfonyl hydrazides⁹ and sodium sulfinates.¹⁰ Traditional approaches, including the oxidation of sulfides¹¹ and electrophilic aromatic substitution¹² often involve strong oxidants,¹³ odorous thiols¹⁴ or harsh acidic conditions, limiting functional group compatibility.¹⁵ Modern strategies such as transition-metal-catalyzed couplings,¹⁶ C-H activation,¹⁷ and multi-component reactions with SO₂ surrogates¹⁸ have broadened synthetic scope but still face challenges like high temperatures, toxic by-products, and limited substrate tolerance. In this context, sodium sulfinates have emerged as highly effective sulfonyl donors owing to their bench stability, ease of synthesis, and compatibility with mild reaction conditions, making them a preferred choice for constructing sulfones.¹⁹ Interestingly, the integration of sulfone scaffolds with hydrazones within a single structural unit holds significant promise for the search for new bioactive compounds. The combination of sulfone and hydrazone functionalities in the same structural unit not only offers potential for innovative drug development but also opens new avenues in the design of advanced organic optoelectronic materials.²⁰ As a result, the sulfonylation of hydrazones has received considerable attention in recent years. In this context, Liu and co-workers reported an oxidative sulfonylation approach to synthesize β -ketosulfones using a dual-metal co-catalytic system involving copper and silver

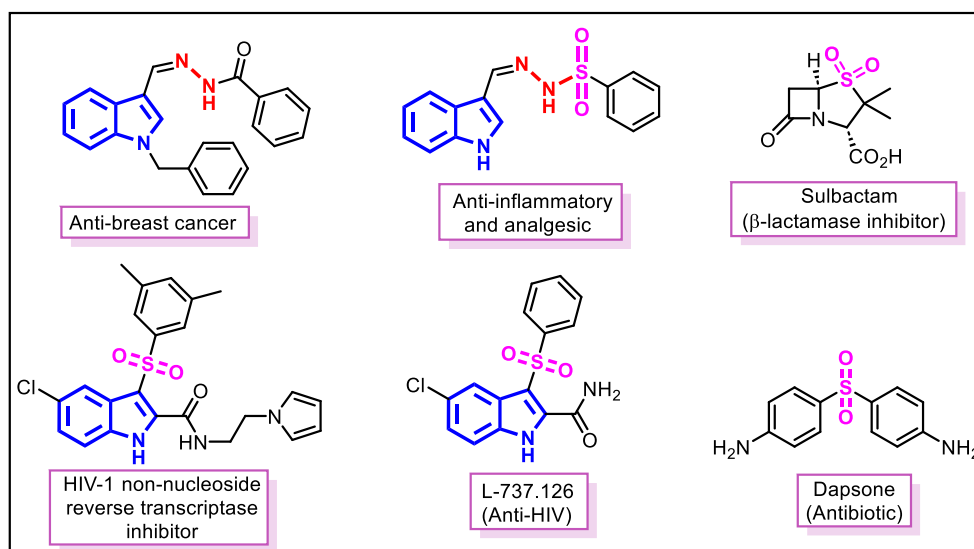
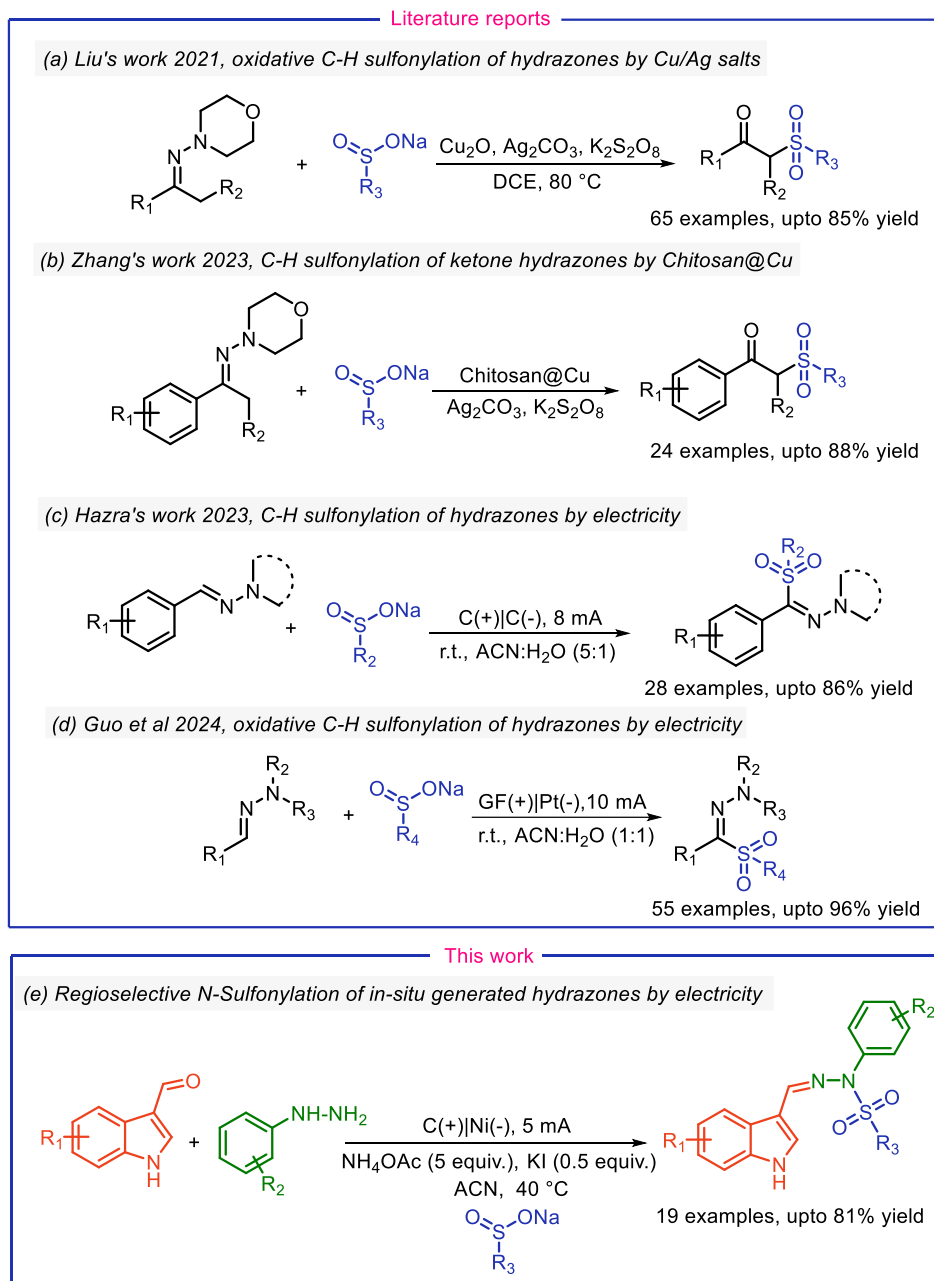


Figure 4.1: Pharmacologically relevant indole-based hydrazones and organosulfones

salts (**Scheme 4.1a**).²¹ Further, Zhang and colleagues introduced a heterogeneous chitosan@Cu catalyst for C-H sulfonylation of hydrazones by employing sodium sulfonates as sulfonyl donors to access β -ketosulfones (**Scheme 4.1b**).²²

Next, electrochemical synthesis has emerged as a green and sustainable alternative in organic chemistry.²³ It utilizes electricity as a clean reagent, minimizes waste generation, reduces reliance on hazardous chemicals, and typically proceeds under mild conditions, making it an eco-friendly and energy-efficient approach.²⁴ In this context, Hazra et al. developed an electrochemical method for the direct C-H sulfonylation of aldehyde hydrazones using sodium sulfonates as sulfonylating agents, leading to the synthesis of various (*E*)-sulfonylated hydrazones (**Scheme 4.1c**).^{7c} Subsequently, Guo and co-workers reported a related C-H sulfonylation approach employing a range of sodium sulfonates to obtain both alkylated and arylated sulfonylated hydrazones (**Scheme 4.1d**).²⁵ In contrast to these approaches (**Scheme 4.1a-d**), which predominantly rely on metal salts, heterogeneous metal catalysts, or C-H functionalization pathways, the present work focuses on a regioselective *N*-sulfonylation of in-situ generated indole-based hydrazones to access a broad range of clinically important *N*-sulfonylated hydrazones under electrochemical conditions (**Scheme 4.1e**). This strategy avoids the use of external oxidants and noble-metal co-catalysts, operates under mild and environmentally benign conditions, and provides complementary site selectivity relative to reported C-H sulfonylation methods.



Scheme 4.1: Recent methods for sulfonylation of hydrazones using sodium sulfonates

4.2: Results and Discussion

4.2.1: Optimization of electrochemical reaction conditions

To establish the optimal reaction conditions for the one-pot synthesis of compound (**5a**), we initiated our study using indole-3-carboxaldehyde (**1a**), phenylhydrazine (**2a**), and sodium *p*-toluenesulfonate (**3a**) as model substrates. The reaction was initially performed in an undivided electrochemical cell using a carbon anode and a nickel cathode under a constant current of 5 mA (**entry 1, Table 4.1**). A mixture of ammonium acetate (NH₄OAc) and potassium iodide (KI) served as the supporting electrolyte, with acetonitrile (ACN) as the solvent. Under this

condition, compound (**5a**) was achieved in 82% conversion (**entry 1, Table 4.1**). Subsequent screening of various electrode combinations (**entries 2-6, Table 4.1**), while keeping all other reaction parameters unchanged, did not lead to improved yields. When iron and copper were used as cathodes, the product was formed but starting material (**1a**) was also recovered (**entries 5-6, Table 4.1**). Further, the reaction provided almost similar yield, when platinum or nickel electrodes were employed as a cathode along with carbon as anode (**entries 1 & 3, Table 4.1**), however, nickel was preferred over the platinum owing to its cost-effectiveness, and selected for further optimization. Additionally, when the reaction was carried out in the absence of electricity, only trace amounts of product (**5a**) were detected after 1 hour (**entry 7, Table 4.1**). Next, we examined the impact of the electrolyte on the reaction. In this context, replacing the $\text{NH}_4\text{OAc}/\text{KI}$ combination with single salts such as tetrabutylammonium iodide (TBAI), tetrabutylammonium acetate (TBAOAc), or lithium perchlorate (LiClO_4) resulted in no product formation (**entries 8-10, Table 4.1**), suggesting that a synergistic effect of both ammonium acetate and iodide salts is essential. Further investigations showed that when NH_4OAc or KI were used individually, no product (**5a**) was formed (**entries 11-12, Table 4.1**). However, combining TBAOAc with various halide salts revealed that the reaction proceeded efficiently only with KI (**entry 13, Table 4.1**), while the use of KBr or MgBr_2 led to significantly lower conversions (**entries 14-15, Table 4.1**). These observations highlights the important role of iodide and acetate ions in this reaction. Additionally, reactions using TBAI in combination with NH_4OAc also led to successful product formation, further confirming the necessity of both iodide and acetate ions (**entries 16, Table 4.1**).

Table 4.1: Optimization of Reaction conditions^a

| entry | electrode | current | salts | equiv. of salts | solvent | (Z)-conversion (%) ^b |
|-------|-------------|---------|-----------------------------------|-----------------|---------|---------------------------------|
| 1 | C(+) Ni(-) | 5 mA | $\text{NH}_4\text{OAc}/\text{KI}$ | 5:0.5 | ACN | 82 |
| 2 | Pt(+) Pt(-) | 5 mA | $\text{NH}_4\text{OAc}/\text{KI}$ | 5:0.5 | ACN | 77 |
| 3 | C(+) Pt(-) | 5 mA | $\text{NH}_4\text{OAc}/\text{KI}$ | 5:0.5 | ACN | 79 |
| 4 | C(+) C(-) | 5 mA | $\text{NH}_4\text{OAc}/\text{KI}$ | 5:0.5 | ACN | 70 |
| 5 | C(+) Fe(-) | 5 mA | $\text{NH}_4\text{OAc}/\text{KI}$ | 5:0.5 | ACN | 68 |
| 6 | C(+) Cu(-) | 5 mA | $\text{NH}_4\text{OAc}/\text{KI}$ | 5:0.5 | ACN | 66 |
| 7 | - | - | $\text{NH}_4\text{OAc}/\text{KI}$ | 5:0.5 | ACN | 4 |
| 8 | C(+) Ni(-) | 5 mA | TBAI | 5.0 | ACN | 0 |
| 9 | C(+) Ni(-) | 5 mA | TBAOAc | 5.0 | ACN | 0 |

| | | | | | | |
|----|------------|-------|--------------------------|-------|------------------------------------|-----------------|
| 10 | C(+) Ni(-) | 5 mA | LiClO ₄ | 5.0 | ACN | 0 |
| 11 | C(+) Ni(-) | 5 mA | NH ₄ OAc | 5.0 | ACN | 0 |
| 12 | C(+) Ni(-) | 5 mA | KI | 5.0 | ACN | 0 |
| 13 | C(+) Ni(-) | 5 mA | TBAOAc/KI | 5:0.5 | ACN | 72 |
| 14 | C(+) Ni(-) | 5 mA | TBAOAc/KBr | 5:0.5 | ACN | 40 |
| 15 | C(+) Ni(-) | 5 mA | TBAOAc/MgBr ₂ | 5:0.5 | ACN | 36 |
| 16 | C(+) Ni(-) | 5 mA | TBAI/NH ₄ OAc | 5:0.5 | ACN | 73 |
| 17 | C(+) Ni(-) | 5 mA | LiClO ₄ /KI | 5:0.5 | ACN | 0 |
| 18 | C(+) Ni(-) | 5 mA | I ₂ | 5.0 | ACN | 0 |
| 19 | C(+) Ni(-) | 5 mA | NH ₄ OAc/ KI | 5:0.5 | ACN:H ₂ O (1:1, v/v) | 22 |
| 20 | C(+) Ni(-) | 5 mA | NH ₄ OAc/KI | 5:0.5 | DCE | 5 |
| 21 | C(+) Ni(-) | 5 mA | NH ₄ OAc/KI | 5:0.5 | DMSO | 0 |
| 22 | C(+) Ni(-) | 1 mA | NH ₄ OAc/KI | 5:0.5 | ACN | 70 |
| 23 | C(+) Ni(-) | 10 mA | NH ₄ OAc/KI | 5:0.5 | ACN | 77 |
| 24 | C(+) Ni(-) | 5 mA | NH ₄ OAc/KI | 5:0.5 | ACN | 54 ^c |
| 25 | C(+) Ni(-) | 5 mA | NH ₄ OAc/KI | 5:0.5 | ACN | 65 ^d |
| 26 | C(+) Ni(-) | 5 mA | NH ₄ OAc/KI | 5:1 | ACN | 79 |
| 27 | C(+) Ni(-) | 5 mA | NH ₄ OAc/KI | 3:0.5 | ACN | 63 |

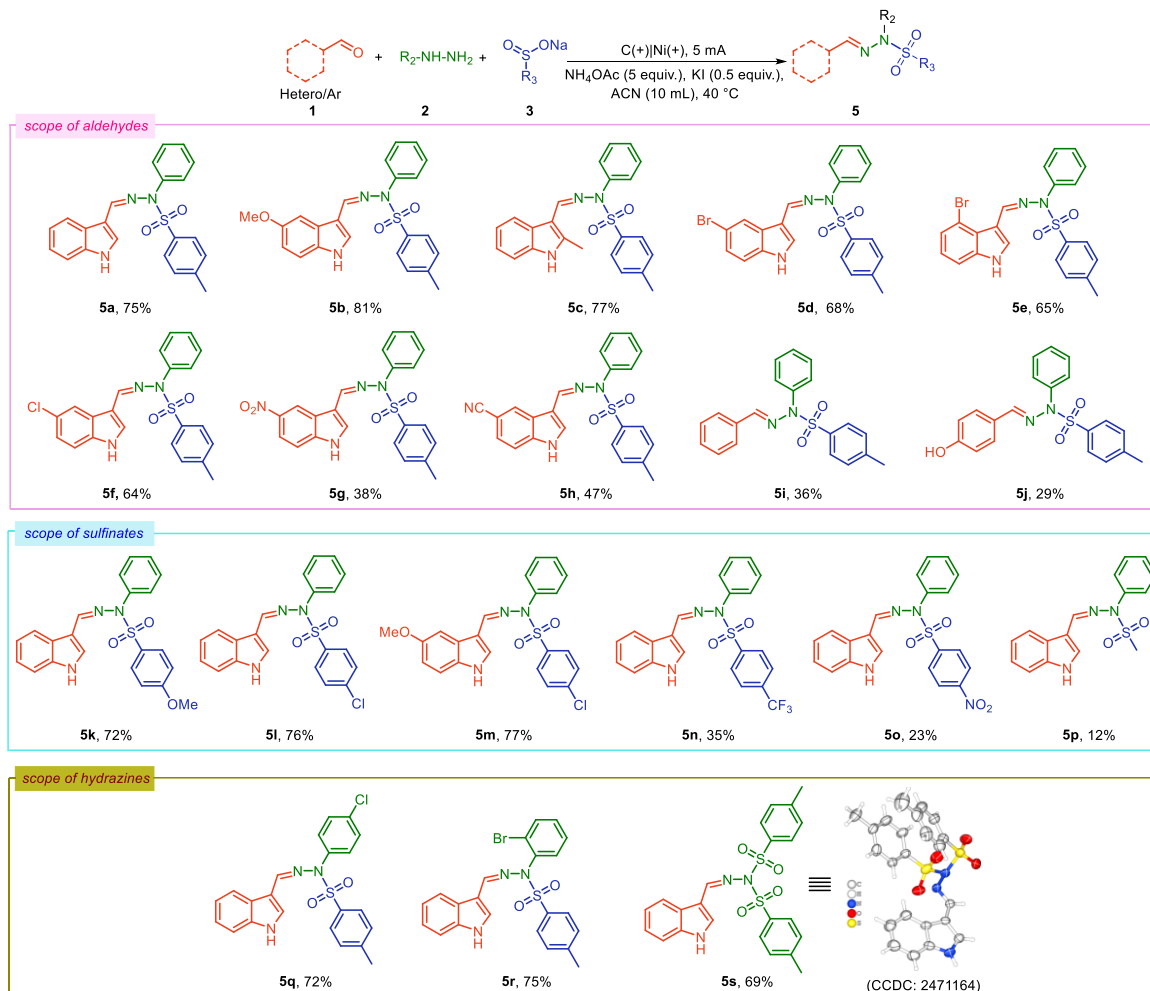
^aReaction conditions: **1a** (10 mg, 0.07 mmol), **2a** (8 μ L, 0.08 mmol), **3a** (24 mg, 0.13 mmol), solvent (2 mL) under a constant current flowed in an undivided cell with electrodes at 40 °C ^bdetermined by HPLC, ^ctemperature 30 °C, ^dtemperature 50 °C.

The use of LiClO₄ with KI again failed to yield the product, reinforcing the specificity of the required electrolyte system (**entry 17, Table 4.1**). Importantly, the use of elemental iodine (I₂) instead of iodide salts resulted in no product formation, further emphasizing the crucial role of the iodide ion (**entry 18, Table 4.1**).

Next, solvent screening showed that replacing acetonitrile (ACN) with ACN:H₂O (1:1, v/v), 1,2-dichloroethane (DCE), or DMSO resulted in significantly reduced conversions (**entries 19-21, Table 4.1**). In the case of ACN:H₂O (1:1, v/v), 22% conversion of (**5a**) was observed, with the recovery of starting material (**1a**). Similarly, the reaction in DCE led to 5% conversion only. In contrast, no conversion was observed in DMSO, and (**1a**) was recovered unchanged. These findings confirmed that acetonitrile is the optimal solvent for achieving higher product yields. Variation of the applied current also affected the reaction outcome. Reducing the current to 1 mA resulted in a substantial drop in yield, likely due to insufficient conductivity, which prevented the reaction from proceeding efficiently (**entry 22, Table 4.1**). Conversely, increasing the current to 10 mA led to partial decomposition of the reactants; however, a moderate 77% conversion to product (**5a**) was still attained (**entry 23, Table 4.1**). Finally, changing the reaction temperature (**entries 24-25, Table 4.1**) did not lead to any notable

improvement in yield. Next, the ratios of NH₄OAc and KI were varied; however, the reaction gave an inferior yield (**entries 26-27, Table 4.1**). Moreover, a change in the substrate's molar ratio does not improve the reaction outcome.

4.2.2: Substrate scope



Scheme 4.2: Substrate scope of electrochemical *N*-sulfonylation of *in-situ* generated hydrazones^a

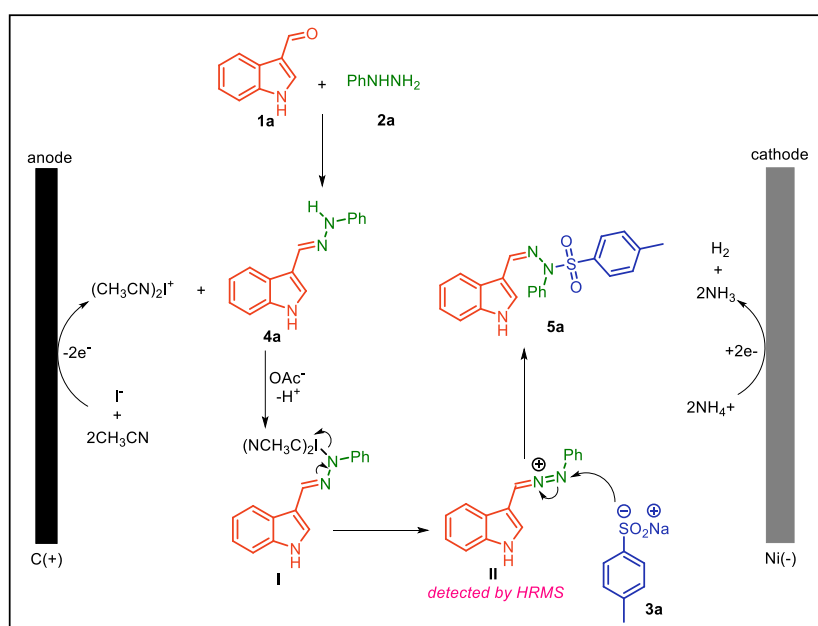
^a**Reaction conditions:** C anode, Ni cathode, aldehydes **1** (100 mg, 0.68 mmol, 1 equiv.), hydrazines **2** (81 μ L, 0.82 mmol, 1.2 equiv), sodium sulfonates **3** (245 mg, 1.37 mmol, 2 equiv.), NH₄OAc (265 mg, 3.4 mmol, 5 equiv.), KI (57 mg, 0.34 mmol, 0.5 equiv.), ACN (10 mL) under a constant current of 5 mA flowed in an undivided cell with electrodes at 40 °C, isolated yields of (*Z*)-**5** compounds.

Having the best reaction conditions, we explored the substrate scope by employing a range of electronically and structurally diverse aldehydes, sulfinate salts, and hydrazines. Further, indole-3-carboxaldehydes bearing electron-donating groups afforded higher yields compared to the unsubstituted indole-3-carboxaldehydes (**5a-5c, Scheme 4.2**). Moreover, indole-3-carboxaldehydes containing halide substituents on the C-4 and C-5 position gave moderate yields of the desired products (**5d-5f, Scheme 4.2**). However, a significant drop in yield was

observed when aldehydes containing strong electron-withdrawing groups such as NO_2 and CN were used under the same electrochemical conditions with phenyl hydrazine and sodium tosyl sulfinate (**5g** and **5h**, **Scheme 4.2**). Further extending the methodology to benzaldehydes, we observed that these compounds showed a marked decline in product yields than the indole-3-carboxaldehydes (**5i-5j**, **Scheme 4.2**). Further, phenyl sulfinate salts bearing electron-donating group or halide substituents gave satisfactory results (**5k-5m**, **Scheme 4.2**). In contrast, phenyl sulfinate salts with strong electron-withdrawing groups such as CF_3 and NO_2 led to a considerable decrease in yield, likely due to reduced nucleophilicity of the PhSO_2^- anion (**5n** and **5o**, **Scheme 4.2**).²⁶ When methyl sodium sulfinate (MeSO_2Na) was used in place of aryl sulfinate salts, a noticeable decline in product yield was observed, which may be attributed to the absence of conjugation and reduced nucleophilicity of the sulfonyl sulfur (**5p**, **Scheme 4.2**).²⁷ Next, we examined hydrazines bearing halide substituents, which also resulted in decreased yields (**5q** and **5r**, **Scheme 4.2**). Interestingly, reaction gave corresponding product in 69% isolated yield when tosyl hydrazine was employed instead of phenyl hydrazine in the reaction (**5s**, **Scheme 4.2**).

4.2.3: Plausible mechanism

A proposed reaction mechanism for the regioselective synthesis of compound (**5a**) is depicted in **Scheme 4.3**. Initially, KI reacts with ACN and undergoes anodic oxidation to generate the iodonium species $[(\text{CH}_3\text{CN})_2\text{I}^+]$.²⁸ This electrophilic iodonium species subsequently reacts with the *in situ* generated hydrazone (**4a**), which is formed from the condensation of (**1a**) and (**2a**). Further, the abstraction of a proton by the acetate ion facilitates the formation of the indole



Scheme 4.3: Plausible mechanism

hydrazone iodinium intermediate (**I**), which undergoes a rearrangement to form intermediate (**II**) (confirmed using HRMS data). Thereafter, nucleophilic attack by the sulfinate anion on intermediate (**II**) leads to the formation of the final product (**5a**). The use of NH_4OAc in excess is crucial, as it serves not only as a proton abstractor but also undergoes reduction at the cathode during the electrochemical process.²⁸

4.2.4: Control experiments

Further, to get more insight into the proposed reaction pathway, a series of control experiments were performed. First, the reaction was conducted in the presence of radical scavengers such as 2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl (TEMPO) (4 equiv.) and butylated hydroxytoluene (BHT) (4 equiv.), the formation of product **5a** was still observed in good yield under these conditions, thereby ruling out a radical pathway and suggesting that no sulfinate radical intermediate is involved as reported previously by various groups.^{25,29}

4.2.5: Cyclic voltammetry experiment

Next, cyclic voltammetry studies were carried out to get more insight about the proposed electrochemical reaction mechanism. In this context, hydrazone (**4a**) exhibited two distinct oxidation potentials at 0.43 V and 1.78 V (**Figure 4.2**). Separately, KI in acetonitrile showed oxidation waves at 1.00 V and 1.50 V. Interestingly, upon addition of KI to the reaction mixture, the first oxidation potential of hydrazone shifted to a more positive value (0.53 V), indicating the formation of the indole hydrazone iodinium intermediate (**I**).²⁸

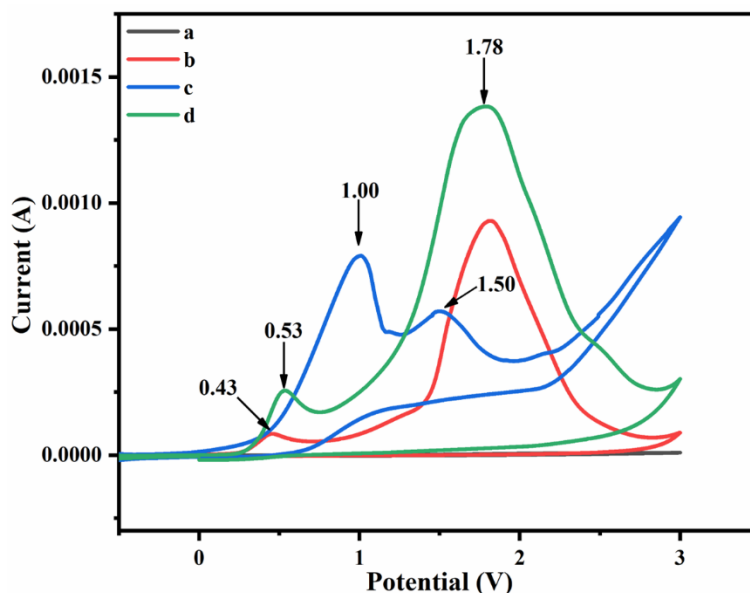


Figure 4.2: Cyclic voltammograms (IUPAC convention) recorded in 0.1 M $t\text{Bu}_4\text{NPF}_6/\text{ACN}$ at room temperature using a Pt working electrode, Pt wire counter electrode, and Ag/AgCl reference electrode. Scan rate: 0.05 V/s; initial potential: 0 V; scan direction: 0 \rightarrow +3 V (oxidative). (a) ACN, (b) **4a** (0.001 M), (c) KI (0.024 M), (d) KI (0.024 M) and **4a** (0.001 M).

4.2.6: Antibacterial susceptibility analysis of the synthesized derivatives (Done by our collaborator Dr. Kuljit Singh, CSIR-IIIM, Jammu)

Primarily, the whole-cell screening of the (5a-5s) synthesized derivatives was conducted at a single concentration of 50 μM against representative Gram-positive and Gram-negative pathogens. This led to the identification of four potent hits, 5d, 5e, 5l, and 5q, that exhibited a growth inhibition of >85% against the *Staphylococcus aureus* pathogen. On the other hand, the synthesized derivatives showed a moderate inhibitory effect against Gram-negative pathogens. This experimental finding highlights the selective antibacterial potential of the identified

Table 4.2: Percentage viability of *S. aureus*, *K. pneumoniae*, *A. baumannii*, and *E. coli* pathogens in the presence of synthesized derivatives tested at 50 $\mu\text{g/mL}$. The results are expressed as mean \pm standard deviation of three independent experiments.

| Code | <i>S. aureus</i> | <i>K. pneumoniae</i> | <i>A. baumannii</i> | <i>E. coli</i> |
|------|------------------|----------------------|---------------------|------------------|
| 5d | 0.24 \pm 0.12 | 84.17 \pm 2.31 | 80.52 \pm 1.68 | 78.59 \pm 1.77 |
| 5e | 10.26 \pm 0.87 | 94.93 \pm 2.03 | >100 | 82.79 \pm 2.85 |
| 5l | 0.24 \pm 0.11 | >100 | >100 | 81.78 \pm 2.59 |
| 5q | 1.73 \pm 0.13 | 73.27 \pm 1.46 | 85.54 \pm 2.24 | 98.15 \pm 1.25 |

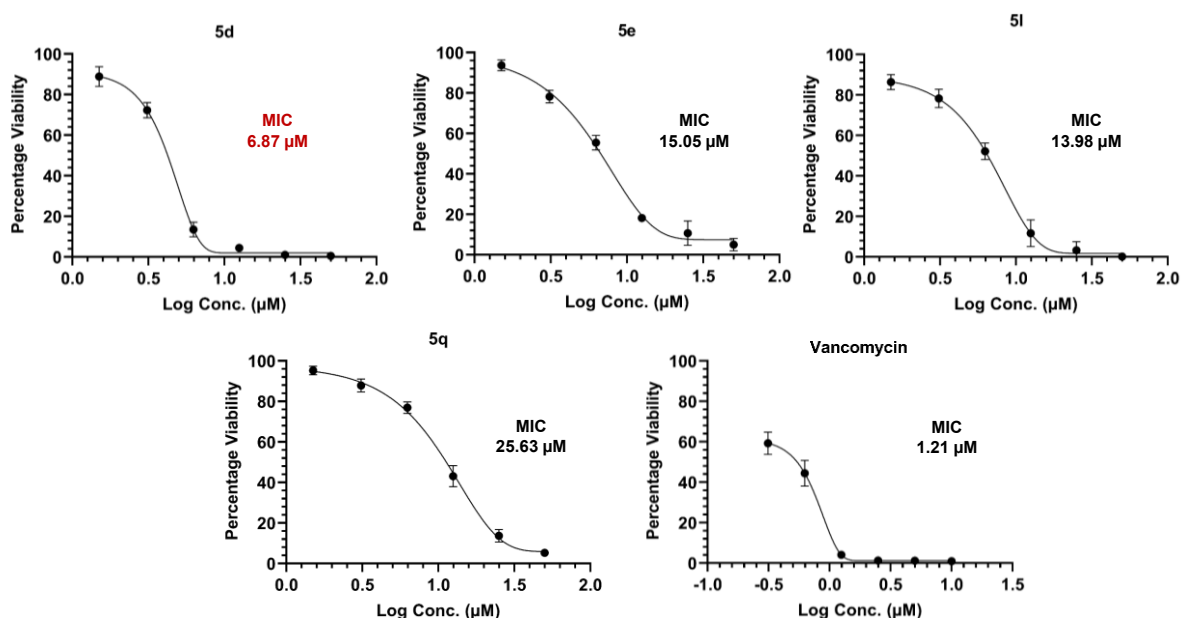


Figure 4.3: Dose-dependent curves of the four shortlisted hits against the *S. aureus* pathogen. Vancomycin was used as a standard drug control in the experiment. All the results are presented as the means \pm standard deviations of three independent experiments.

molecules toward the Gram-positive pathogen *S. aureus*. The average percentage viability of the pathogens in the presence of the tested compounds is summarized in **Table 4.2**. Next, Minimum inhibitory concentration (MIC) of shortlisted hits was determined against *S. aureus* by broth micro-dilution assay to further assess their antibacterial potency. The active hits were tested at varied concentrations with vancomycin used as a positive control in the experimentation. The compounds **5d**, **5e**, **5l**, and **5q** exhibited effective antimicrobial activity against *S. aureus*. Among all the tested derivatives, the compound **5d** emerged as the most potent hit with an MIC value of 6.87 μM . The other three active hits (**5e**, **5l**, and **5q**) displayed MIC values in the range of 10 to 26 μM . The dose-response curves of the four shortlisted hits and the standard drug (vancomycin) are represented in **Figure 4.3**.

4.2.7: Scanning electron microscopy (SEM) analysis

The SEM technique can be used to visualize the ultrastructural changes in the bacterial cell after treatment with biocidal molecules. Thus, in the present study, we have treated *S. aureus* cells with 1X and 2X MIC concentrations of the potent hit (**5d**) to investigate any structural

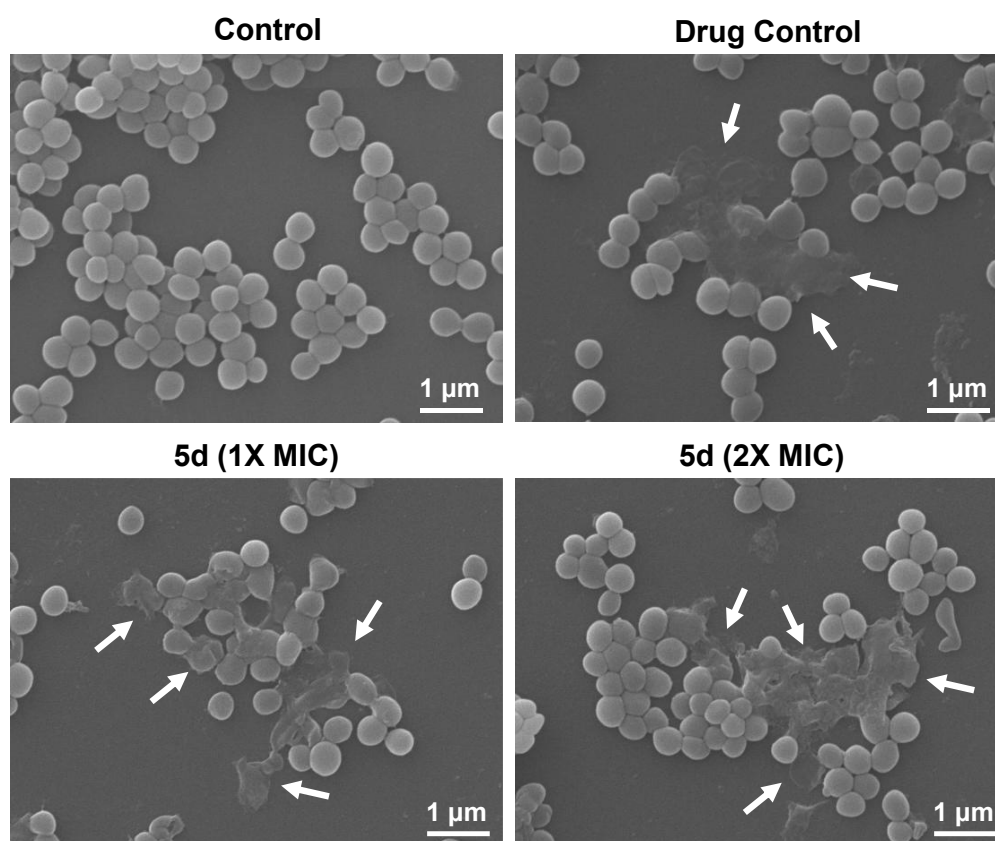


Figure 4.4: Scanning electron microscopy (SEM) analysis of *S. aureus* cells. Untreated control and drug control (vancomycin) at 1X MIC concentration. *S. aureus* pathogen exposed to potent hit (**5d**) at 1X and 2X MIC concentrations, respectively. White arrows in the figure depict the damage to the bacterial pathogen after treatment. (Scale bar = 1 μm).

irregularities and morphological alterations in the bacterial pathogen (**Figure 4.4**). SEM analysis revealed that untreated cells had smooth surfaces with round shapes and cells treated with drug control at 1X MIC concentration showed considerable damage. Upon treatment, with the potent hit (**5d**) at 1X and 2X MIC concentrations, substantial morphological changes, including surface depression, wrinkled and irregular appearance, are visible, leading to bacterial cell death.

4.3: Experimental Section

4.3.1: General information

All chemicals and solvents were obtained from commercial vendors and used without any further purification. All the glass and plasticware utilized in experimentation were sourced from Borosil Scientific (India) and Tarsons (India). Electrochemical reactions were carried out using an OWON (P4305) instrument in an undivided electrochemical cell equipped with a magnetic stirrer. Reaction progress was monitored by thin-layer chromatography (TLC), using silica gel-coated glass plates. Column chromatography was performed using silica gel (60-120 mesh) as the stationary phase, with a solvent system of diethyl ether and hexane serving as the mobile phase. ^1H and ^{13}C NMR spectra of the synthesized compounds were recorded on a Bruker spectrometer operating at 700/500/400 MHz and 176/126/101 MHz respectively. Tetramethylsilane (TMS) was used as an internal reference in CDCl_3 . Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are reported in hertz (Hz). Residual solvent signals were used for calibration: CDCl_3 at 7.26 ppm (^1H) and 77.16 ppm (^{13}C). The NMR data were annotated using the following abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), and m (multiplet). High-resolution mass spectrometry (HRMS) was performed on a Water QTOF (XEVO G2 XS) mass spectrometer using electrospray ionization in positive mode (ESI^+). Reaction conversions were quantified using high-performance liquid chromatography (HPLC) with 1,3-benzodioxole as an internal standard. The HPLC system consisted of a Shimadzu LC-20AD pump, a CTO-10AS column oven, and a photodiode array (PDA) detector. Cyclic voltammetry was conducted using a DY2300 potentiostat to study the redox behaviour relevant to the electrochemical process. Essential reagents like standard antibiotics and resazurin dye were received from Hi-Media (India). The bacterial strains, *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (ATCC 15380), *Acinetobacter baumannii* (ATCC 19606), and *Escherichia coli* (ATCC 25922) were procured from the institutional microbial repository. For culturing bacterial strains, Luria Bertani (LB) broth and LB agar were procured from Hi-Media (India). Bacterial stocks were preserved in cryovials at $-80\text{ }^\circ\text{C}$ in 30% glycerol to maintain

viability. Fluorescence readings were taken using a Tecan Infinite M200-pro multimode plate reader. Centrifuge (Thermo Fisher- 5830R) and shaker incubator (REMI CIS-24-Plus) were utilized during the experimental procedure. All the biological assays were performed in a biosafety cabinet (ESCO AC2-4S1). The SEM study involved the utilization of GelBond film obtained from Lonza (Rockland, USA).

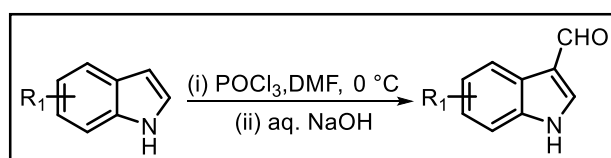
4.3.2: General procedure for the synthesis of compounds (5a-5s)

A mixture of indole-3-carboxaldehyde (100 mg, 0.68 mmol, 1.0 equiv.), phenylhydrazine (81 μ L, 0.82 mmol, 1.0 equiv.), and sodium *p*-toluenesulfinate (245 mg, 1.37 mmol, 2.0 equiv.) was dissolved in 10 mL of ACN in a reaction vial. Thereafter, NH_4OAc (265 mg, 3.4 mmol, 5.0 equiv.) and KI (57 mg, 0.34 mmol, 0.5 equiv.) added to the reaction mixture. Electrolysis was then carried out by applying a constant current of 5 mA using a carbon anode and a nickel cathode, with the reaction mixture maintained at 40 °C in an oil bath. The mixture was stirred under these conditions for 12 hours. Upon consumption of starting materials, as confirmed by TLC, the electric current was turned off, and the solvent was removed under reduced pressure using a rotary evaporator. The resulting crude product was purified by column chromatography to afford the desired compounds.

4.3.3: General procedure for synthesis of starting materials (1 and 3)

4.3.3a: Procedure for synthesis of 1*H*-indole-3-carbaldehydes (1)³⁰

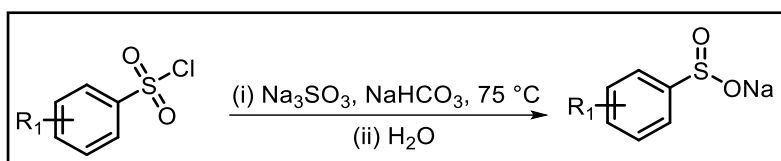
In an oven-dried round-bottom flask equipped with a magnetic stir bar, dimethylformamide (DMF) and phosphorus oxychloride (POCl_3) are mixed in a 5:1 molar ratio by slowly adding POCl_3 (0.6 mL, 8.3 mmol, 1.5 equiv.) to DMF (1.6 mL, 21.2 mmol, 5 equiv.) under continuous stirring. The flask is kept in an ice bath throughout the addition while maintaining a low temperature. After 30 minutes, indole (500 mg, 4.2 mmol, 1 equiv.) is added to the cooled reaction mixture, and the solution is stirred for an additional hour to allow the reaction to proceed. Upon completion, the reaction is quenched by the slow addition of ice-cold water, followed by the dropwise addition of an aqueous sodium hydroxide solution (11 equiv.). This neutralization results in the formation of yellow precipitates. The solid product (1) is collected by filtration and thoroughly washed with cold water to remove any remaining impurities.



Scheme 4.4: General synthetic route for indole aldehydes

4.3.3b: Procedure for synthesis of sodium sulfinates (3)^{7c}

Sodium sulfinates (**3a**, **3m**, **3p** and **3q**) were prepared by heating a mixture of sodium sulfite (600 mg, 4.8 mmol, 1.8 equiv.) and sodium carbonate (420 mg, 5 mmol, 2 equiv.) in 5 mL of water at 75 °C. After 15 minutes of stirring, sulfonyl chloride (484 mg, 2.5 mmol, 1 equiv.) was added slowly to the reaction mixture over a period of approximately 30 minutes. The reaction was then allowed to proceed for 3-4 hours. Upon cooling the mixture to room temperature, white precipitates began to form. The crude white solid was collected by filtration and used without further purification.



Scheme 4.5: General synthetic route for sodium sulfinates

4.3.4: Method for crystallization

Crystallization of compound (**5s**) was achieved using the vapor diffusion method. A solution of the compound (10 mg in 1 mL of dichloromethane) was placed in a small inner glass vial. This vial was then placed inside a larger, tightly sealed container holding 4 mL of hexane. As the hexane vapor slowly diffused into the DCM solution, the solvent environment gradually changed, promoting the slow formation of single crystals over time.³¹

4.3.5: Antibacterial susceptibility test (single point assay)

The antibacterial susceptibility of synthesized compounds was determined against Gram-positive (*S. aureus*) and Gram-negative (*K. pneumoniae*, *A. baumannii*, and *E. coli*) pathogens at a consistent test concentration of 50 μM. Briefly, the parent stocks of 20 mM concentration of the compounds were prepared by dissolving them in DMSO. The logarithmic phase of overnight-grown culture in LB medium was adjusted to 0.5 McFarland and then diluted 1:100 to obtain a final cell density of 1x10⁶ CFU/mL.^{32,33} Subsequently, 100 μL of this freshly prepared bacterial suspension was dispensed into a 96-well flat-bottom plate containing 50 μM of synthesized compounds in triplicate and kept for incubation at 37 °C for 24 hrs. After incubation, 10 μL of freshly prepared resazurin dye (0.04% dissolved in 1X PBS) was added to the culture plate, followed by another hour of incubation at 37 °C. Finally, the Tecan Infinite M200-pro multimode reader was utilized to measure the fluorescence intensity with excitation and emission wavelengths set at 530 nm and 600 nm, respectively, to calculate the cell viability. The percentage viability of bacterial pathogens was calculated for each compound, and results

are represented as mean \pm standard deviation of three independent measurements performed in triplicate.

4.3.6: Minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC), defined as the lowest compound concentration with no visible bacterial growth, was determined for the identified hits using a broth microdilution assay in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI).^{34,35} Briefly, the compounds were serially diluted at a gradient concentration ranging from 50 μ M to 1.56 μ M in 96-well flat-bottom plates. Then, 100 μ L of bacterial suspension with a cell density of 1×10^6 CFU/mL was added to each well. Following incubation for 24 hrs, resazurin dye was added, and fluorescence was measured using a plate reader (as described above). Finally, the Gompertz equation was used to determine the MIC, and the data were plotted using GraphPad Prism 9 (Version 9.3.1). The experiment for MIC determination was performed in biological triplicate.

4.3.7: Scanning electron microscopy analysis

Scanning electron microscopy (SEM) was performed as reported previously.^{33,36} Bacterial cultures of *S. aureus* in the exponential phase were treated with 1X and 2X MIC of potent hit (**5d**) and incubated at 37 °C for 4 hours. Following incubation, bacterial cultures were centrifuged at 5000 rpm for 5 minutes, and the pellet was washed thrice with 1X PBS. After this, the pellet was placed onto SEM sheets and left to air dry. Furthermore, the slides were fixed with 4% paraformaldehyde and 2.5% glutaraldehyde and air-dried overnight. After fixation, the slides were immersed in a graded ethanol series (30%, 50%, 70%, 90%, and 100%). The dried samples were coated with colloidal gold using a sputter coater, and morphological changes were examined under a JEOL JSM 6010 PLUS-LA (Akishima, Tokyo, Japan) scanning electron microscope.

4.3.8: Characterization of compounds

(Z)-N'-((1H-indol-3-yl)methylene)-4-methyl-N-phenylbenzenesulfonohydrazide (5a). off-white solid, ¹H NMR (400 MHz, CDCl₃): δ 9.26 (brs, 1H), 8.09 (s, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.71 (s, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.32 (s, 1H), 7.30 (s, 1H), 7.25 (s, 1H), 7.23 (s, 1H), 7.21 (s, 1H), 7.19 (s, 1H), 7.12 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 6.91 (d, J = 7.6 Hz, 2H), 2.45 (s, 3H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ 144.2, 142.7, 138.8, 136.4, 136.0, 129.6, 129.3, 128.8, 128.4, 124.6, 123.3, 122.1, 121.5, 119.7, 113.9, 112.5, 21.7 ppm. HRMS (ESI-TOF) m/z : [M + H]⁺ calcd for C₂₂H₂₀N₃O₂S, 390.1276; found, 390.1263.

(Z)-N'-((5-methoxy-1H-indol-3-yl)methylene)-4-methyl-N-phenylbenzenesulfonohydrazide (5b). white solid, ¹H NMR (500 MHz, CDCl₃): δ 9.02 (brs,

1H), 8.02 (s, 1H), 7.83 (d, $J = 8.5$ Hz, 2H), 7.60 (d, $J = 2.5$ Hz, 1H), 7.33 (d, $J = 9.0$ Hz, 1H), 7.29-7.26 (m, 2H), 7.21-7.18 (m, 2H), 6.92-6.85 (m, 4H), 6.52 (d, $J = 2.0$ Hz, 1H), 3.63 (s, 3H), 2.42 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 155.4, 144.2, 142.8, 139.1, 136.4, 129.5, 129.4, 129.0, 128.6, 125.2, 122.1, 113.9, 113.8, 113.3, 101.0, 100.1, 55.8, 21.7 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{22}\text{N}_3\text{O}_3\text{S}$, 420.1381; found, 420.1372.

(Z)-4-methyl-N'-((2-methyl-1H-indol-3-yl)methylene)-N-phenylbenzenesulfonohydrazide (5c). white solid, ^1H NMR (500 MHz, CDCl_3): δ 8.70 (brs, 1H), 7.90 (s, 1H), 7.79-7.76 (m, 2H), 7.33 (d, $J = 8.0$ Hz, 1H), 7.26 (s, 1H), 7.24 (s, 1H), 7.20 (t, $J = 8.0$ Hz, 2H), 7.15-7.12 (m, 1H), 6.99-6.98 (m, 2H), 6.93-6.90 (m, 3H), 2.41 (s, 3H), 2.32 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 144.0, 142.6, 139.7, 138.5, 137.1, 135.8, 129.5, 129.3, 128.8, 126.4, 122.5, 122.2, 121.1, 118.9, 114.0, 111.2, 97.8, 21.7, 13.2 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{22}\text{N}_3\text{O}_2\text{S}$, 404.1432; found, 404.1422.

(Z)-N'-((5-bromo-1H-indol-3-yl)methylene)-4-methyl-N-phenylbenzenesulfonohydrazide (5d). off-white solid, ^1H NMR (500 MHz, CDCl_3): δ 9.43 (brs, 1H), 7.98 (s, 1H), 7.88 (d, $J = 8.5$ Hz, 2H), 7.62 (d, $J = 3.0$ Hz, 1H), 7.37 (d, $J = 8.5$ Hz, 2H), 7.30 (d, $J = 3.0$ Hz, 2H), 7.26-7.23 (m, 3H), 6.97 (t, $J = 7.5$ Hz, 1H), 6.89 (d, $J = 8.0$ Hz, 2H), 2.51 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 144.6, 142.5, 138.3, 135.8, 134.7, 129.7, 129.4, 129.3, 129.1, 126.4, 126.3, 122.4, 121.9, 114.8, 114.0, 99.4, 21.8 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{19}\text{BrN}_3\text{O}_2\text{S}$, 468.0381; found, 468.0374.

(Z)-N'-((4-bromo-1H-indol-3-yl)methylene)-4-methyl-N-phenylbenzenesulfonohydrazide (5e). off-white solid, ^1H NMR (500 MHz, CDCl_3): δ 9.62 (brs, 1H), 7.89 (d, $J = 6.5$ Hz, 2H), 7.80 (s, 1H), 7.36 (d, $J = 2.5$ Hz, 1H), 7.33 (dd, $J = 7.5, 4.5$ Hz, 3H), 7.20-7.15 (m, 3H), 6.96 (t, $J = 8.5$ Hz, 1H), 6.89 (t, $J = 7.5$ Hz, 1H), 6.82 (d, $J = 8.0$ Hz, 2H), 2.45 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 144.4, 142.6, 138.4, 137.1, 135.8, 129.5, 129.4, 129.3, 129.0, 125.3, 125.1, 124.2, 122.2, 114.1, 113.2, 111.7, 99.0, 21.8 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{19}\text{BrN}_3\text{O}_2\text{S}$, 468.0381; found, 468.0377.

(Z)-N'-((5-chloro-1H-indol-3-yl)methylene)-4-methyl-N-phenylbenzenesulfonohydrazide (5f). off-white solid, ^1H NMR (500 MHz, CDCl_3): δ 9.14 (brs, 1H), 7.92 (s, 1H), 7.82 (d, $J = 8.5$ Hz, 2H), 7.66 (d, $J = 3.0$ Hz, 1H), 7.36-7.30 (m, 3H), 7.22-7.15 (m, 3H), 7.08 (d, $J = 2.0$ Hz, 1H), 6.93 (t, $J = 7.5$ Hz, 1H), 6.86 (d, $J = 8.0$ Hz, 2H), 2.44 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 144.5, 142.6, 138.2, 136.0, 134.3, 129.6, 129.5, 129.4, 129.0, 127.4, 125.8, 123.9, 122.4, 119.0, 114.0, 113.4, 21.8 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{19}\text{ClN}_3\text{O}_2\text{S}$, 424.0886; found, 424.0883.

(Z)-4-methyl-N'-((5-nitro-1H-indol-3-yl)methylene)-N-phenylbenzenesulfonylhydrazide (5g). yellow solid, ^1H NMR (500 MHz, CDCl_3): δ 9.69 (brs, 1H), 8.07-8.04 (m, 2H), 7.91 (s, 1H), 7.84 (d, $J = 8.0$ Hz, 2H), 7.76 (d, $J = 2.5$ Hz, 1H), 7.47 (d, $J = 9.0$ Hz, 1H), 7.34 (d, $J = 8.0$ Hz, 2H), 7.21-7.18 (m, 2H), 6.97-6.92 (m, 1H), 6.83 (d, $J = 7.5$ Hz, 2H), 2.45 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 138.9, 135.4, 131.4, 129.8, 129.5, 129.4, 129.1, 124.5, 122.8, 118.9, 116.5, 114.1, 112.6, 102.4, 21.8 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{19}\text{N}_4\text{O}_4\text{S}$, 435.1127; found, 435.1119.

(Z)-N'-((5-cyano-1H-indol-3-yl)methylene)-4-methyl-N-phenylbenzenesulfonylhydrazide (5h). yellow solid, ^1H NMR (500 MHz, CDCl_3): δ 9.56 (brs, 1H), 7.87 (s, 1H), 7.82 (d, $J = 8.0$ Hz, 2H), 7.73 (s, 1H), 7.49-7.46 (m, 2H), 7.42 (d, $J = 8.5$ Hz, 1H), 7.34 (d, $J = 8.0$ Hz, 2H), 7.22-7.18 (m, 2H), 6.95 (t, $J = 7.5$ Hz, 1H), 6.83 (d, $J = 8.0$ Hz, 2H), 2.47 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 144.9, 142.3, 137.4, 135.6, 130.5, 129.8, 129.5, 129.1, 126.3, 124.9, 122.8, 119.7, 114.0, 113.5, 105.0, 101.0, 21.8 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{19}\text{N}_4\text{O}_2\text{S}$, 415.1228; found, 415.1223.

(E)-N'-benzylidene-4-methyl-N-phenylbenzenesulfonylhydrazide (5i). yellow viscous, ^1H NMR (500 MHz, CDCl_3): δ 7.50 (d, $J = 2.0$ Hz, 1H), 7.48 (t, $J = 2.0$ Hz, 1H), 7.24 (s, 1H), 7.20 (d, $J = 2.0$ Hz, 1H), 7.19 (d, $J = 2.0$ Hz, 1H), 7.15 (d, $J = 1.0$ Hz, 1H), 7.14 (d, $J = 2.0$ Hz, 1H), 7.00-6.99 (m, 1H), 6.97 (s, 1H), 6.93 (t, $J = 7.5$ Hz, 1H), 6.83 (s, 1H), 6.82 (s, 1H), 6.80 (s, 1H), 6.78 (s, 1H), 6.74 (q, $J = 4.0$ Hz, 4.5 Hz, 1H), 2.24 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 145.4, 144.8, 135.9, 134.0, 132.8, 132.4, 129.3, 129.1, 129.0, 128.6, 128.5, 128.3, 128.0, 127.9, 127.7, 126.7, 123.8, 122.7, 120.7, 112.8, 70.3, 21.6 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{NaO}_2\text{S}$, 373.0986; found, 373.0978.

(E)-N'-(4-hydroxybenzylidene)-4-methyl-N-phenylbenzenesulfonylhydrazide (5j). yellow viscous, ^1H NMR (500 MHz, CDCl_3): δ 7.84 (s, 1H), 7.79 (d, $J = 8.0$ Hz, 2H), 7.33 (d, $J = 7.5$ Hz, 2H), 7.26 (s, 1H), 7.22-7.18 (m, 3H), 6.94-6.91 (m, 3H), 6.88 (d, $J = 9.0$ Hz, 2H), 2.46 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 158.3, 144.5, 142.9, 142.5, 136.0, 131.7, 129.7, 129.4, 129.1, 122.4, 117.4, 117.0, 113.9, 21.8 ppm. HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}_3\text{S}$, 367.1116; found, 367.1111.

(Z)-N'-((1H-indol-3-yl)methylene)-4-methoxy-N-phenylbenzenesulfonylhydrazide (5k). white solid, ^1H NMR (500 MHz, CDCl_3): δ 9.22 (brs, 1H), 8.03 (s, 1H), 7.86 (d, $J = 9.0$ Hz, 2H), 7.66 (d, $J = 3.0$ Hz, 1H), 7.45 (d, $J = 8.0$ Hz, 1H), 7.22-7.16 (m, 4H), 7.08 (t, $J = 8.0$ Hz, 1H), 6.93 (d, $J = 9.0$ Hz, 2H), 6.90 (s, 1H), 6.87 (d, $J = 7.5$ Hz, 2H), 3.84 (s, 3H) ppm. ^{13}C NMR (126 MHz, CDCl_3): δ 163.6, 142.8, 139.1, 136.0, 131.0, 130.7, 129.3, 128.4, 124.6,

123.3, 122.1, 121.5, 119.7, 114.2, 113.8, 112.5, 100.3, 55.7 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{22}H_{20}N_3O_3S$, 406.1225; found, 406.1219.

(Z)-N'-((1H-indol-3-yl)methylene)-4-chloro-N-phenylbenzenesulfonohydrazide (5l). off-white solid, 1H NMR (500 MHz, $CDCl_3$): δ 9.15 (brs, 1H), 8.10 (s, 1H), 7.88 (d, $J = 8.5$ Hz, 2H), 7.70 (s, 1H), 7.49-7.45 (m, 3H), 7.27-7.20 (m, 3H), 7.17-7.10 (m, 2H), 6.94 (t, $J = 7.5$ Hz, 1H), 6.88 (d, $J = 7.5$ Hz, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 142.5, 140.0, 138.2, 138.0, 135.9, 130.2, 129.4, 129.2, 128.5, 124.5, 123.5, 122.4, 121.7, 119.6, 113.9, 112.6, 100.0 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{21}H_{17}ClN_3O_2S$, 410.0730; found, 410.0720.

(Z)-4-chloro-N'-((5-methoxy-1H-indol-3-yl)methylene)-N-phenylbenzenesulfonohydrazide (5m). brown solid, 1H NMR (500 MHz, $CDCl_3$): δ 8.98 (brs, 1H), 8.07 (s, 1H), 7.88 (d, $J = 9.0$ Hz, 2H), 7.64 (d, $J = 2.5$ Hz, 1H), 7.46 (d, $J = 8.5$ Hz, 2H), 7.35 (d, $J = 8.5$ Hz, 1H), 7.23-7.20 (m, 2H), 6.93 (t, $J = 7.0$ Hz, 1H), 6.88 (d, $J = 7.5$ Hz, 3H), 6.51 (s, 1H), 3.65 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 155.5, 142.6, 140.0, 138.5, 138.0, 130.8, 130.4, 129.4, 129.2, 128.6, 125.1, 122.4, 114.0, 113.8, 113.3, 100.9, 99.8, 55.9 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{22}H_{19}ClN_3O_3S$, 440.0835; found, 440.0826.

(Z)-N'-((1H-indol-3-yl)methylene)-N-phenyl-4-(trifluoromethyl)benzenesulfonohydrazide (5n). light brown solid, 1H NMR (500 MHz, $CDCl_3$): δ 9.02 (s, 1H), 8.12 (s, 1H), 8.07 (d, $J = 7.5$ Hz, 2H), 7.75 (d, $^3J_{H-F} = 3.0$ Hz, 2H), 7.73 (s, 1H), 7.49 (d, $J = 8.0$ Hz, 1H), 7.20 (t, $J = 7.7$ Hz, 3H), 7.15-7.12 (m, 2H), 6.94 (t, $J = 7.5$ Hz, 1H), 6.84 (d, $J = 7.5$ Hz, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 143.3, 142.4, 137.8, 135.9, 129.5, 129.4, 128.5, 126.0, 124.4, 123.7, 122.6, 121.9, 119.6, 113.9, 112.5, 100.0 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{22}H_{17}F_3N_3O_2S$, 444.0993; found, 444.0972.

(Z)-N'-((1H-indol-3-yl)methylene)-4-nitro-N-phenylbenzenesulfonohydrazide (5o). yellow solid, 1H NMR (500 MHz, $CDCl_3$): δ 8.93 (brs, 1H), 8.28 (d, $J = 8.5$ Hz, 2H), 8.18 (s, 1H), 8.10 (d, $J = 9.0$ Hz, 2H), 7.79 (d, $J = 3.0$ Hz, 1H), 7.51 (d, $J = 8.5$ Hz, 1H), 7.31-7.27 (m, 1H), 7.24-7.21 (m, 2H), 7.14-7.10 (m, 2H), 6.96 (t, $J = 7.5$ Hz, 1H), 6.90 (d, $J = 7.5$ Hz, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 150.4, 145.8, 142.2, 137.2, 135.9, 130.0, 129.5, 128.6, 124.2, 124.0, 123.8, 122.9, 122.1, 119.5, 114.0, 112.6, 100.1 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{21}H_{17}N_4O_4S$, 421.0970; found, 421.0959 ppm.

(Z)-N'-((1H-indol-3-yl)methylene)-N-phenylmethanesulfonohydrazide (5p). white solid, 1H NMR (500 MHz, $CDCl_3$): δ 9.10 (brs, 1H), 8.06 (s, 1H), 7.65 (d, $J = 2.5$ Hz, 1H), 7.42 (d, $J = 8.0$ Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 1H), 7.26-7.22 (m, 3H), 7.15 (t, $J = 8.0$ Hz, 1H), 7.00 (d, $J = 8.0$ Hz, 2H), 6.93 (t, $J = 7.5$ Hz, 1H), 3.24 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 142.7, 138.9, 136.1, 129.5, 128.4, 124.5, 123.6, 122.3, 121.8, 119.8, 113.8, 112.6, 98.9, 40.0

ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{16}H_{16}N_3O_2S$, 314.0963; found, 314.0959 ppm.

(Z)-N'-((1H-indol-3-yl)methylene)-N-(4-chlorophenyl)-4-

methylbenzenesulfonohydrazide (5q). Brown solid, 1H NMR (500 MHz, $CDCl_3$): δ 9.23 (brs, 1H), 7.99 (s, 1H), 7.91 (d, $J = 8.5$ Hz, 1H), 7.78 (d, $J = 8.0$ Hz, 2H), 7.65 (s, 1H), 7.43 (d, $J = 8.0$ Hz, 1H), 7.24 (s, 1H), 7.19 (t, $J = 7.0$ Hz, 2H), 7.12 (s, 1H), 7.10 (s, 1H), 7.07 (d, $J = 7.5$ Hz, 1H), 6.76 (d, $J = 9.0$ Hz, 2H), 2.39 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 144.4, 141.4, 139.7, 136.1, 136.0, 130.1, 129.6, 129.3, 128.9, 128.6, 126.9, 124.5, 123.4, 121.6, 119.6, 115.0, 112.6, 100.0, 21.7 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{22}H_{19}ClN_3O_2S$, 424.0886; found, 424.0860 ppm.

(Z)-N'-((1H-indol-3-yl)methylene)-N-(2-bromophenyl)-4-

methylbenzenesulfonohydrazide (5r). Brown solid, 1H NMR (500 MHz, $CDCl_3$): δ 9.02 (s, 1H), 8.51 (s, 1H), 7.83 (s, 1H), 7.82 (s, 1H), 7.80 (d, $J = 2.0$ Hz, 2H), 7.48 (d, $J = 8.0$ Hz, 1H), 7.33 (d, $J = 1.5$ Hz, 1H), 7.32-7.31 (m, 2H), 7.30 (s, 1H), 7.28 (s, 1H), 7.21 (s, 1H), 7.13 (t, $J = 7.5$ Hz, 1H), 6.78 (t, $J = 7.5$ Hz, 1H), 2.41 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 144.3, 143.7, 141.3, 140.0, 139.2, 136.2, 132.4, 129.8, 129.6, 128.9, 128.6, 128.4, 126.5, 123.5, 122.5, 121.5, 120.6, 115.3, 112.3, 108.2, 100.4, 21.7 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{22}H_{19}BrN_3O_2S$, 468.0381; found, 468.0367 ppm.

(Z)-N'-((1H-indol-3-yl)methylene)-4-methyl-N-tosylbenzenesulfonohydrazide (5s). Light yellow solid, 1H NMR (700 MHz, $CDCl_3$): δ 9.06 (brs, 1H), 8.62 (s, 1H), 7.99 (d, $J = 7.7$ Hz, 1H), 7.62 (d, $J = 8.4$ Hz, 4H), 7.47 (d, $J = 9.1$ Hz, 2H), 7.32 (t, $J = 7.0$ Hz, 1H), 7.23 (t, $J = 7.7$ Hz, 1H), 7.13 (d, $J = 8.4$ Hz, 4H), 2.42 (s, 6H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$): δ 168.9, 144.9, 137.0, 134.0, 133.7, 129.4, 129.1, 124.6, 124.3, 122.7, 122.4, 112.1, 21.8 ppm. HRMS (ESI-TOF) m/z : $[M + H]^+$ calcd for $C_{23}H_{22}N_3O_4S_2$, 468.1051; found, 468.1051 ppm.

4.4: Conclusion

In summary, we have developed a novel electrochemical methodology for the regioselective *N*-sulfonylation of *in-situ* generated indole-based hydrazones. The scope and robustness of this eco-friendly protocol were demonstrated through the successful transformation of a wide variety of aldehydes, hydrazines, and sodium sulfonates, yielding the corresponding *N*-sulfonylated products in good yields. To confirm the essential role of electrical input in this transformation, a series of control experiments were carried out. Furthermore, cyclic voltammetry analyses were conducted to gain mechanistic insights and support the proposed reaction pathway. Next, synthesized compounds were tested at 50 μ M to evaluate their antibacterial activity. Four compounds- **5d**, **5e**, **5l**, and **5q** stood out by showing strong

inhibition (over 85%) against *S. aureus*, a common Gram-positive bacterium. However, they were only moderately effective against Gram-negative bacteria. Further testing confirmed that these hits, especially **5d** with an MIC of 6.87 μM , have promising and selective activity against *S. aureus*. These findings suggest that the identified compounds could serve as potential leads for developing targeted treatments against Gram-positive infections. Other advanced antimicrobial biological studies are in progress in our lab and will be reported in due course. This study not only underscores the utility of electrochemical synthesis in modern organic chemistry but also opens new avenues for the sustainable development of medically important sulfonamide derivatives as antibacterial compounds.

4.5: References

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Conclusion of the thesis

Sustainability in organic synthesis refers to the development of chemical methodologies that are efficient, environmentally friendly, cost-effective, and make use of renewable resources to ensure long-term ecological balance. It is deeply rooted in the twelve principles of green chemistry, which guide the sustainable production of pharmaceuticals and their precursors. Traditionally, organic synthesis has relied on resource-demanding and often hazardous practices that prioritize high yields and product purity, which often overlooking environmental and societal considerations. One promising strategy to enhance sustainability in organic synthesis is through catalyst-driven transformations. In addition, green catalysis includes approaches such as biocatalysis, electrocatalysis, and photocatalysis, each offering unique merits and demerits. Integrating these catalytic disciplines into one-pot systems can enable bi- or multicatalytic cascade reactions, which significantly reduce toxic waste generation, minimize solvent use, simplify work-up procedures, and lower energy consumption. However, combining these catalytic methods presents challenges. For example, enzymes typically require mild reaction conditions, making their integration with other catalytic approaches complex. Nevertheless, by carefully optimizing reaction parameters, such integration can be successfully achieved through sequential or concurrent addition of reagents. This combined strategy holds great promise for sustainable synthesis of structurally diverse and complex molecules. The first chapter of this thesis highlights such integrative approaches, focusing on the use of hydrolase enzymes in combination with chemical catalysis, electrocatalysis, and photocatalysis. It also provides a concise literature review on electricity-driven organic reactions and their applications in the synthesis of bioactive molecules.

In **Chapter 2**, we report the development of a bifunctional nanobiohybrid catalyst composed of palladium nanoparticles and the α -amylase enzyme from *A. oryzae*. This metal–enzyme hybrid catalyst was successfully applied in the chemoenzymatic synthesis of functionalized biphenyls and bis(indolyl)methanes with moderate to good yields. The reactions involved Pd-catalyzed Suzuki–Miyaura couplings and α -amylase-mediated aza-Michael additions or electrophilic substitution reactions, respectively. Notably, the developed catalyst demonstrated excellent reusability, enhanced thermostability, and a prolonged shelf life, making it highly effective for chemoenzymatic transformations.

Chapter 3 describes the sustainable synthesis of chiral sulfur-based organofluorine compounds through an integrative approach combining electrosynthesis and biocatalysis. A variety of thiophenols and fluorine-containing α,β -unsaturated alkenes were employed to generate a diverse range of 2-fluoro-3-mercaptopropionic acids in good isolated yields. Mechanistic

insights were gained through cyclic voltammetry studies, control experiments, and molecular docking analyses, which revealed the formation of radical species during the electrochemical reaction and highlighted the crucial role of the lipase's active site in enabling enantioselective synthesis. Furthermore, the practical applicability of the developed protocol was demonstrated by conducting a gram-scale reaction and synthesizing a key intermediate of an MMP-3 inhibitor.

In **Chapter 4**, we present a metal-free electrochemical methodology for regioselective *N*-sulfonylation of in situ generated indole-based hydrazones. The versatility of this protocol was demonstrated using a range of indole aldehydes, sodium sulfinates, and phenylhydrazines to afford *N*-sulfonylated products in good yields. Mechanistic insights were obtained through cyclic voltammetry studies and control experiments. The synthesized compounds were evaluated for their antibacterial activity against both Gram-positive and Gram-negative pathogens. Notably, compound (**5d**) exhibited promising antibacterial potential against *S. aureus* with a MIC value of 6.87 μM . Furthermore, SEM analysis revealed that compound (**5d**) induces significant morphological alterations in bacterial cells, exerting its effects by causing substantial cellular damage.

Outlook

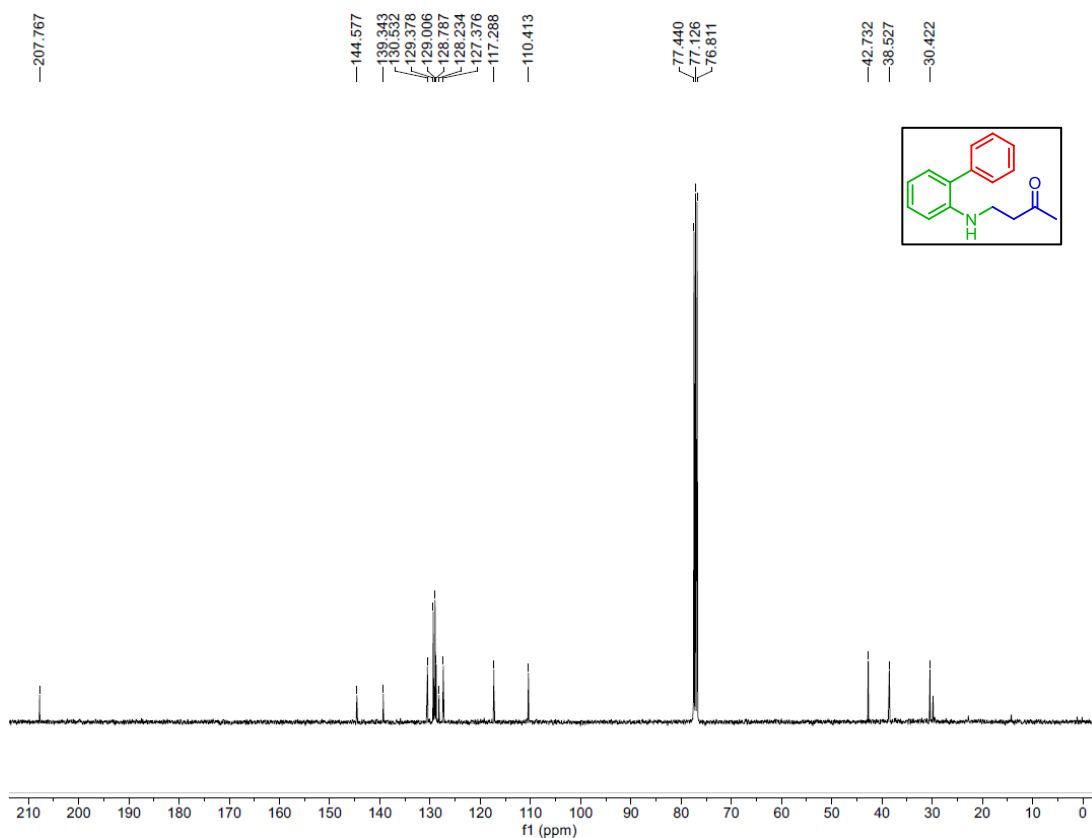
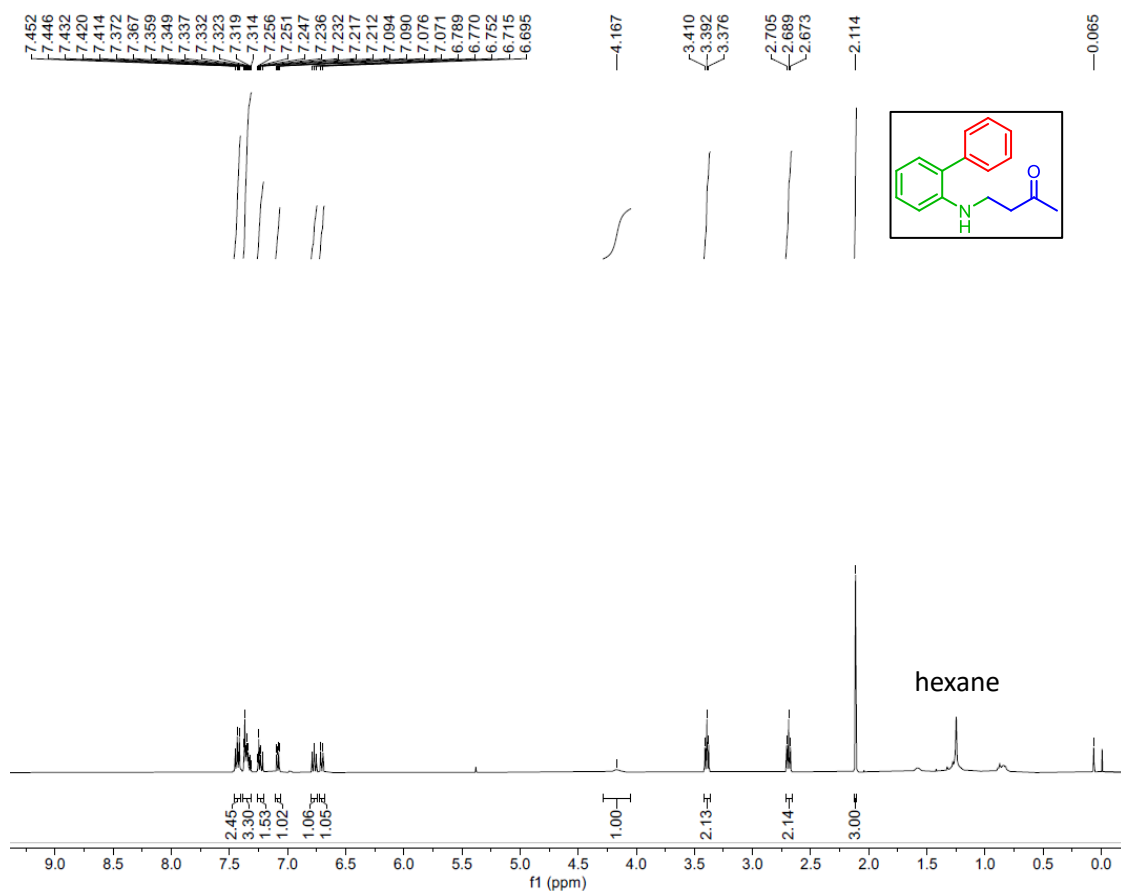
- ❖ We developed a nanobiohybrid catalyst to overcome the challenges typically encountered in performing one-pot chemoenzymatic reactions involving Pd-catalyzed Suzuki-Miyaura coupling and α -amylase-mediated aza-Michael addition or electrophilic substitution. However, this nanobiohybrid catalyst exhibited certain substrate scope limitations, preventing it from efficiently facilitating diverse one-pot reactions. These constraints could potentially be addressed by designing hybrid catalysts that incorporate multienzyme systems or metal alloys immobilized on heterogeneous supports, enabling the execution of diverse multicatalytic reactions in a one-pot manner.
- ❖ We have also developed an integrative protocol combining electricity and lipase-mediated hydrolysis for the synthesis of chiral sulfur-based organofluorine compounds. This represents a sustainable approach, although so far it has been applied only to a limited set of electron-deficient fluorine-containing α,β -unsaturated alkenes. The methodology holds potential for expansion to a broader range of Michael acceptor alkenes. Additionally, we demonstrated its utility by synthesizing a key intermediate of an MMP-3 inhibitor. This integrative green protocol could be further applied to the synthesis of other biologically relevant sulfur-containing organofluorine compounds. Although moderate

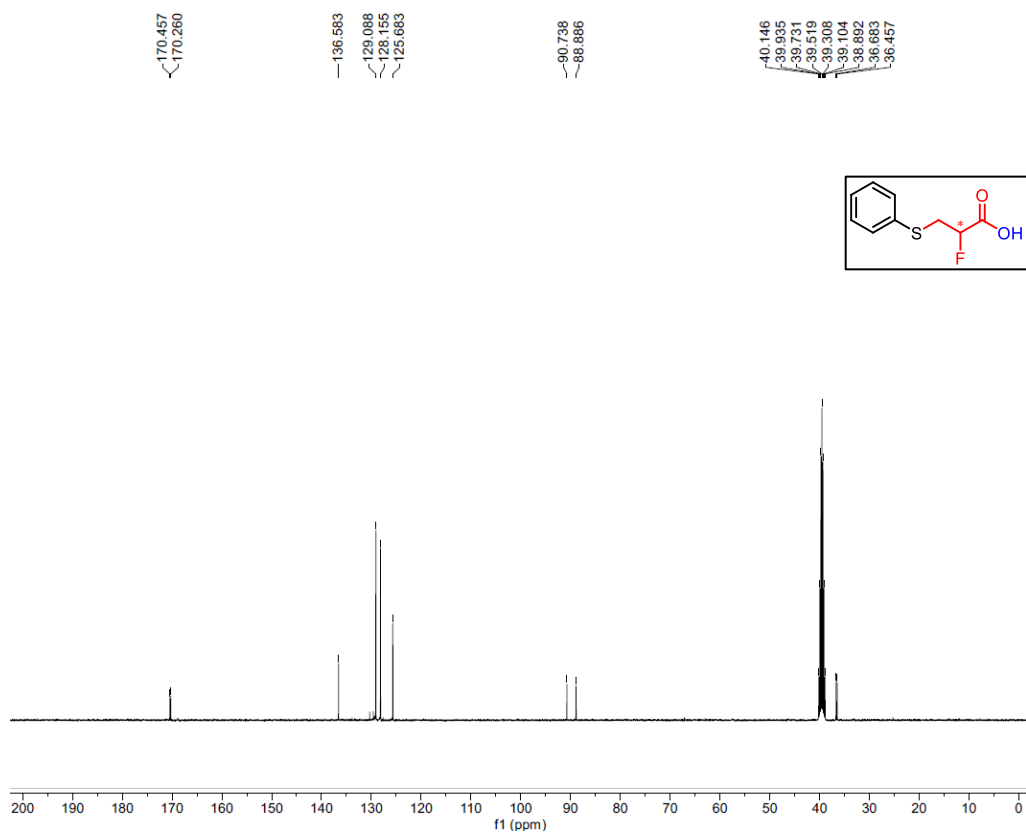
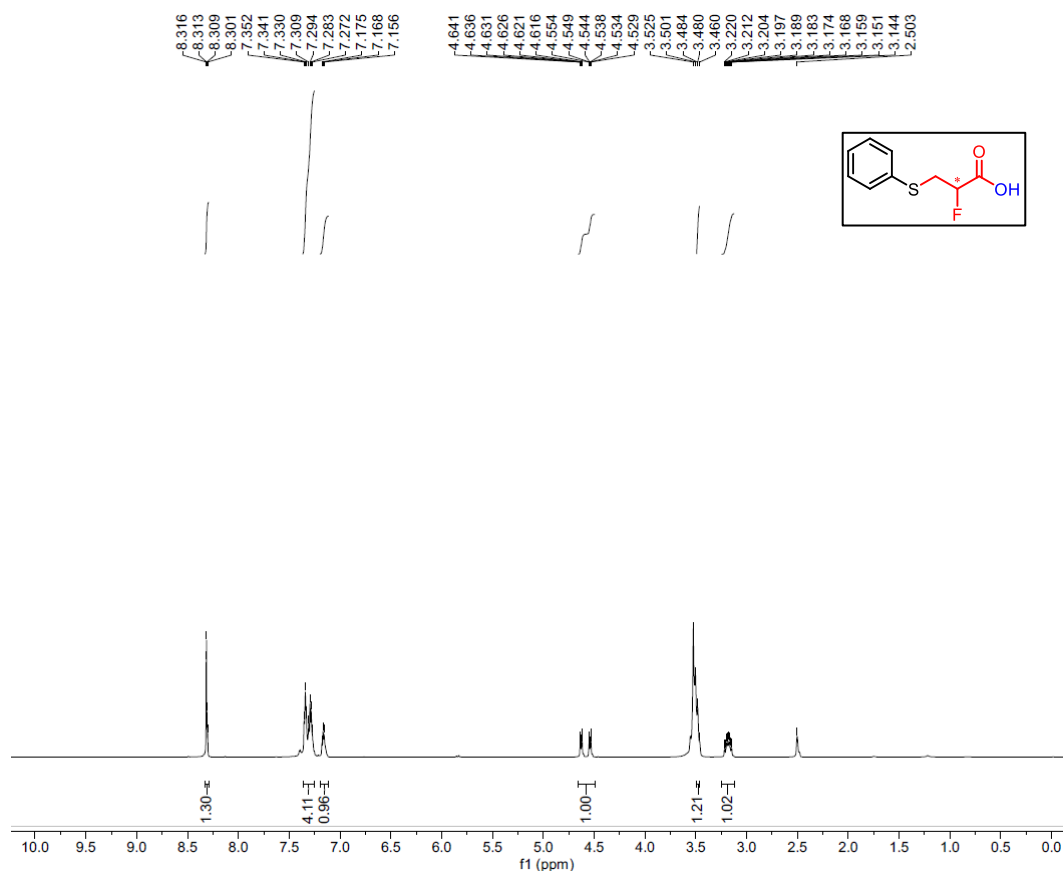
CONCLUSION AND OUTLOOK

enantioselectivity was achieved in these transformations, further optimization or enzyme engineering could enhance its efficiency and enable its application at an industrial scale.

- ❖ In our study, we elucidated a metal-free, oxidant-free, and reductant-free electrochemical approach for the functionalization of *in-situ* generated hydrazones. This strategy enables the regioselective synthesis of clinically important sulfonamides. Future research could further explore the potential of electricity-driven processes to facilitate diverse organic transformations with high selectivity. Moreover, integrating this electrochemical methodology with other catalytic disciplines holds promise for the sustainable synthesis of novel and complex molecules.

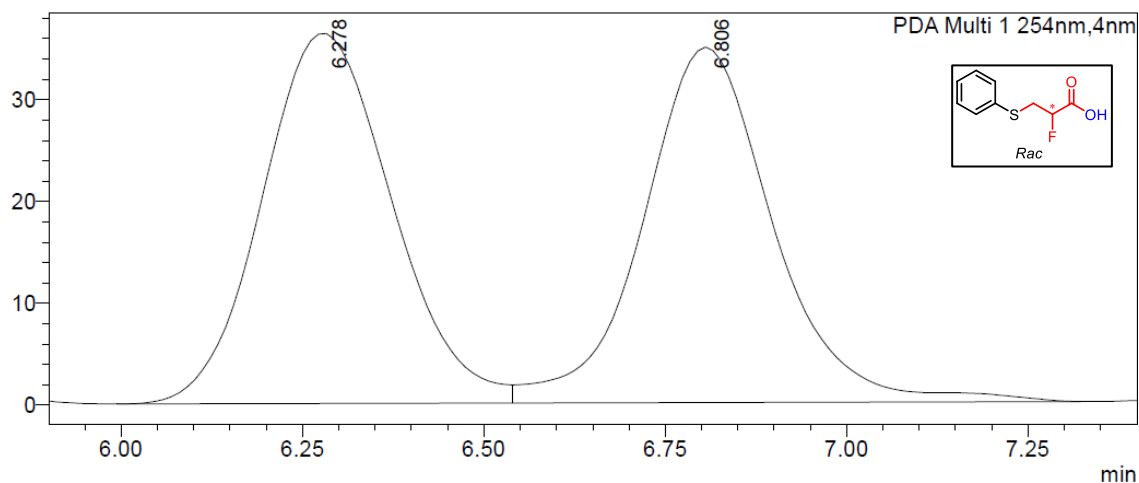
Representative ^1H and ^{13}C -NMR spectra of Compound (5a) of Chapter 2



Representative ^1H and ^{13}C -NMR spectra of Compound (4a) of Chapter 3

Representative HPLC chromatograms of Racemic and Enantioselective Compound (4a) of Chapter 3

mAU



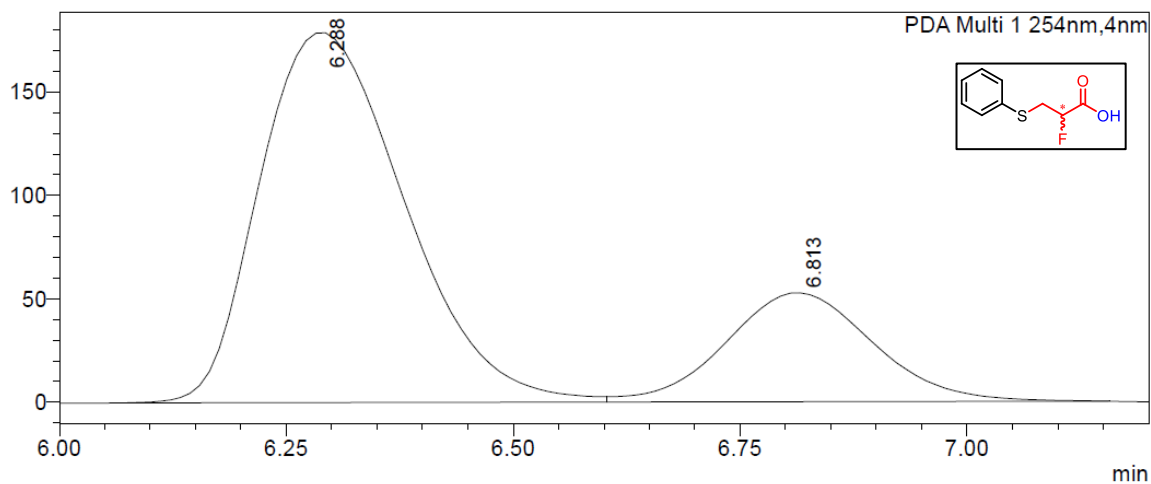
<Peak Table>

PM729 7 97 rac ref.lcd

PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Area% |
|-------|-----------|--------|--------|---------|
| 1 | 6.278 | 451445 | 36360 | 50.901 |
| 2 | 6.806 | 435458 | 34926 | 49.099 |
| Total | | 886903 | 71286 | 100.000 |

mAU



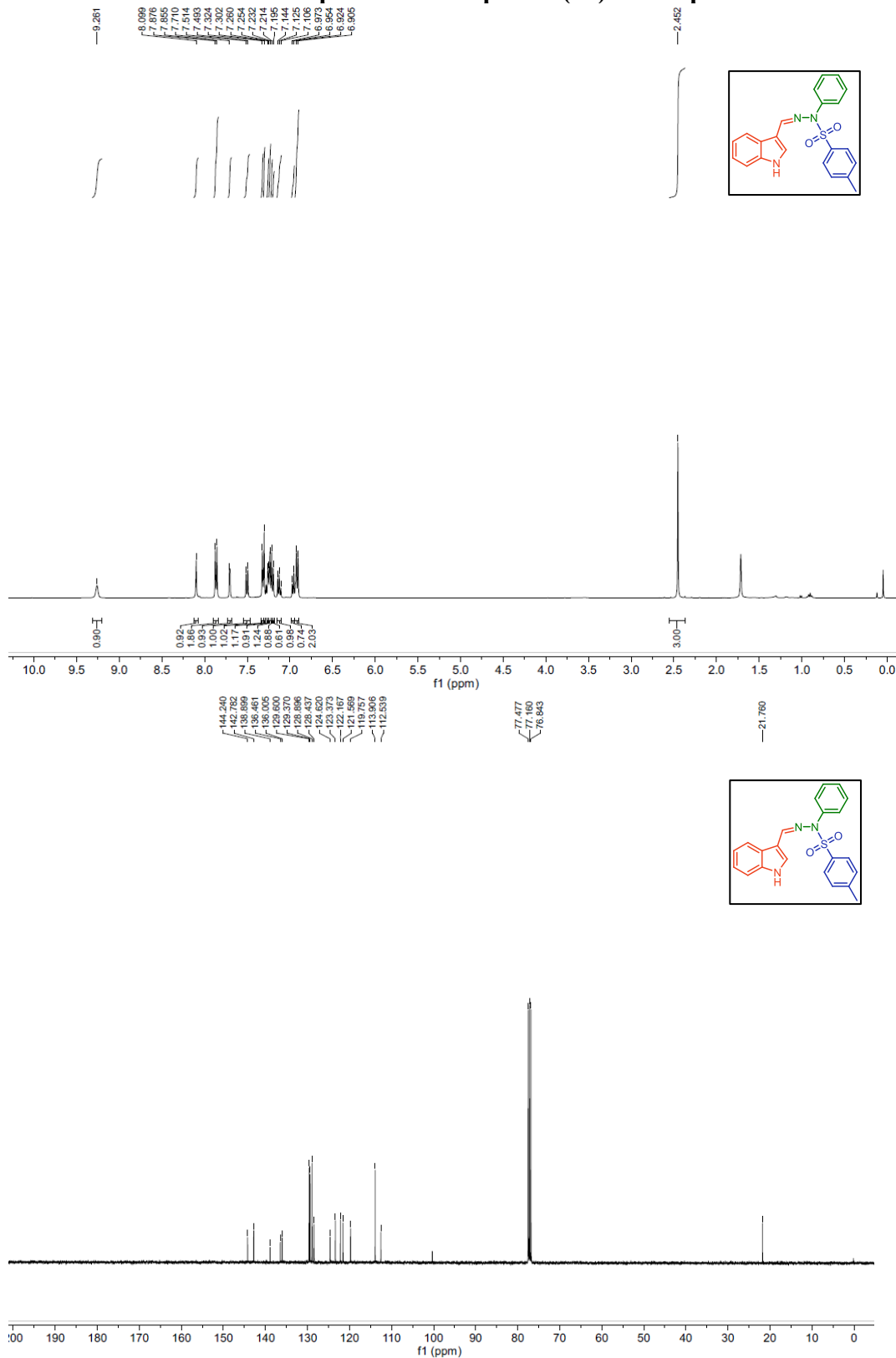
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rep simple lip .lcd

PDA Ch1 254nm

| Peak# | Ret. Time | Area | Height | Area% |
|-------|-----------|---------|--------|---------|
| 1 | 6.288 | 1987545 | 178819 | 76.942 |
| 2 | 6.813 | 595624 | 52720 | 23.058 |
| Total | | 2583169 | 231540 | 100.000 |

Representative ¹H and ¹³C-NMR spectra of Compound (5a) of Chapter 4



Representative Crystallographic Data of Compound (5s) of Chapter 4

| | |
|---|--|
| Identification code | Ex-PM908 |
| Empirical formula | C ₂₃ H ₂₁ N ₃ O ₄ S ₂ |
| Formula weight | 467.55 |
| Temperature/K | 293(2) |
| Crystal system | triclinic |
| Space group | P-1 |
| a/Å | 7.5075(5) |
| b/Å | 10.2822(5) |
| c/Å | 15.6878(9) |
| α /° | 72.906(5) |
| β /° | 88.731(5) |
| γ /° | 80.529(5) |
| Volume/Å ³ | 1141.28(12) |
| Z | 2 |
| ρ calcg/cm ³ | 1.361 |
| μ /mm ⁻¹ | 0.268 |
| F(000) | 488.0 |
| Crystal size/mm ³ | 0.02 × 0.02 × 0.02 |
| Radiation | Mo K α (λ = 0.71073) |
| 2 Θ range for data collection/° | 6.466 to 54.158 |
| Index ranges | -9 ≤ h ≤ 9, -12 ≤ k ≤ 13, -19 ≤ l ≤ 18 |
| Reflections collected | 14961 |
| Independent reflections | 4595 [R _{int} = 0.1534, R _{sigma} = 0.1333] |
| Data/restraints/parameters | 4595/0/291 |
| Goodness-of-fit on F ² | 0.957 |
| Final R indexes [I ≥ 2 σ (I)] | R ₁ = 0.0676, wR ₂ = 0.1555 |
| Final R indexes [all data] | R ₁ = 0.1364, wR ₂ = 0.1952 |
| Largest diff. peak/hole / e Å ⁻³ | 0.22/-0.39 |

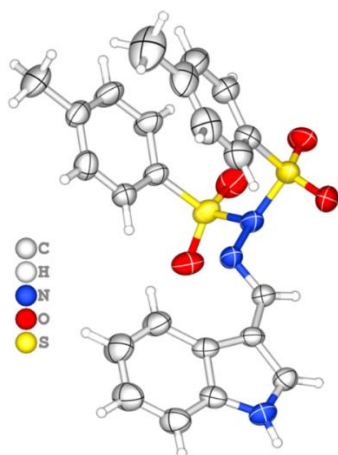


Figure (i): X-ray crystal structure of **5s** (CCDC 2471164)

Thesis Publications

- ❖ **Kaur, P.;** Tyagi, V. Recent advances in iron-catalyzed chemical and enzymatic carbene-transfer reactions. *Adv. Synth. Catal.* **2021**, *363*, 877-905.
- ❖ **Kaur, P.;** Tyagi, V. Merging electrocatalysis and biocatalysis to access sulfur-based chiral α -fluorinated carboxylic acids. *J. Org. Chem.* **2025**, *90*, 5378-5392.
- ❖ **Kaur, P.;** Tyagi, V. Design and preparation of a bifunctional nanobiohybrid catalyst by combining palladium and α -amylase enzyme: application in one-pot chemoenzymatic catalysis. *ChemNanoMat* **2024**, *10*, e202300441.
- ❖ **Kaur, P.;** Kumar, A.; Palmo, T.; Singh, K.; Tyagi, V. Electrochemical *N*-sulfonylation of *in situ* generated indole-based hydrazones and antimicrobial evaluation. *Org. Biomol. Chem.* **2025**, *23*, 8948-8960.
- ❖ **Kaur, P.;** Tyagi, V. Hydrolase Biocatalysis Integrated with Chemo-, Electro-, and Photocatalysis for Sustainable Organic Synthesis. *Org. Biomol. Chem.* **2026**, DOI:10.1039/d5ob01698c (*accepted*).

Other Publications

- ❖ Soam, P.; Singh, K.; **Kaur, P.;** Tyagi, V. Synthesis of unsymmetrical urea derivatives via domino ring-opening/Pd-catalyzed decarboxylative cross-coupling reaction of isatin-3-oxime and isocyanide. *Tetrahedron* **2025**, *185*, 134809.
- ❖ Kamboj, P.; Bhardwaj, M.; **Kaur, P.;** Bajaj, P.; Tyagi, V. Chemoenzymatic synthesis of *N*-phenyl glycine derivatives via integration of Au/TiO₂ and enzyme as catalysts in water. *Biocatal. Biotransform.* **2024**, *43*, 158-165.

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31/7/25*