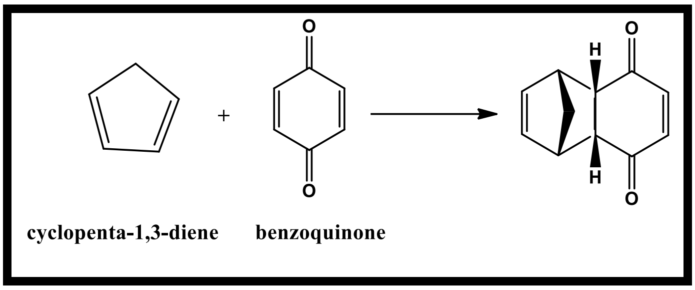
1

Introduction

1.1 DIELS –ALDER REACTION

The most common pathway for the building of six membered rings is Diels-Alder reaction (DA). Otto Diels and Kurt Alder in 1928 discovered this reaction and shared the Nobel Prize for Chemistry in 1950 **(Diels & Kech, 1935).** It is a cycloaddition reaction between a conjugated diene and a substituted alkene to build a substituted cyclohexene system **Scheme 1 (Gujral & Popli, 2013).**

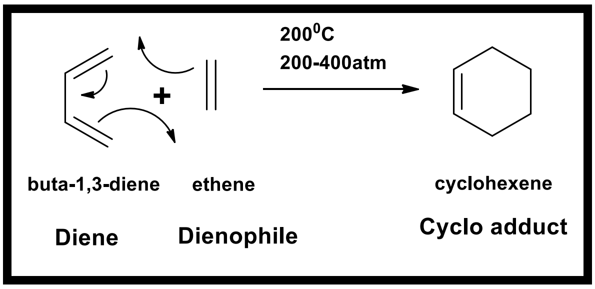


Scheme 1: Diels-Alder reaction

The DA reaction has been successful due to its high efficiency and strong regio- and stereoselectivity, enabling quick synthesis of complex structures while meeting atom-economy constraints. Scientists are now investigating the internal molecular mechanism to better anticipate future reactions.

1.1.1 Mechanism of Diels-Alder Reaction

The DA Reaction is a one-step reaction in which a dienophile provides two electrons and a conjugated diene supplies four electrons, resulting in the formation of six-membered ring **Scheme 2.**



Scheme 2: General outcome of a Diels-Alder Reaction

The suprafacial [π4s + π2s] DA reaction is classified into two types: (i) the normal and (ii) inverse-electron-demand Diels Alder reactions (IEDDA). Both types of reactions are thermally allowed based on Woodward−Hoffmann rules. According to the frontier electron theory [π4s + π2s] cycloaddition involves interaction between highest occupied molecular orbital (HOMO) of the diene and the lowest occupied molecular orbital (LUMO) of the dienophile (**Philosophie & Kessler, 2013).** Therefore the diene will be electron rich and dienophile will be electron poor species **Figure 1**.

On the other hand, in IEDDA reaction LUMO of the diene and HOMO of the dienophile interacts to afford the product. As an outcome, in IEDDA reaction diene is electron poor and dienophile is electron-rich.

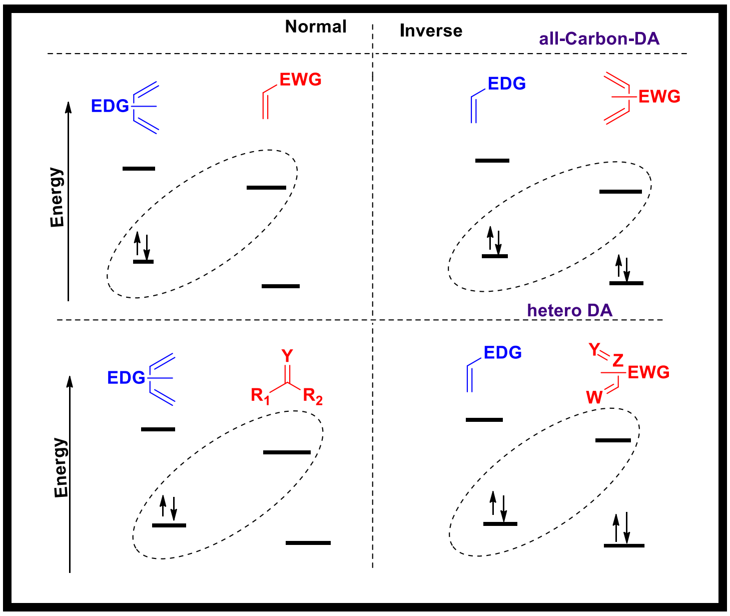
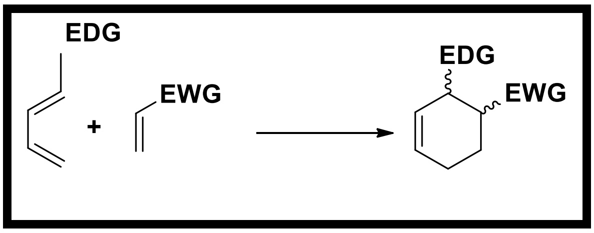


Figure 1: The normal and inverse-electron-demand Diels-Alder reactions

1.1.2 Normal Diels-Alder reaction

The DA reaction occurs when a diene has electron-donating groups(EDGs) and a dienophile has electron-withdrawing groups (EWGs), (**Scheme 3)** resulting in decreased energy of its frontier molecular orbitals (FMOs) **Figure 2 (Nwagbara, 2015).**



Scheme 3: Normal electron demand Diels -Alder reaction

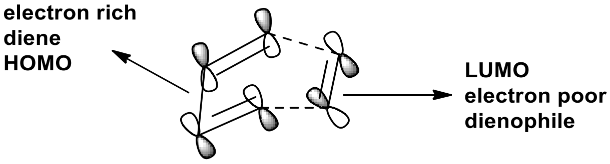
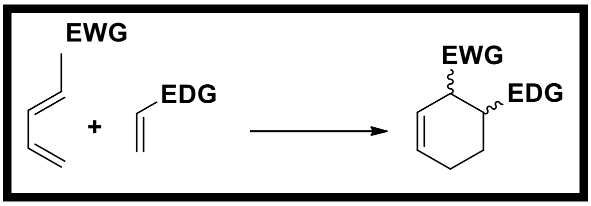


Figure 2: Molecular orbital description of the DA reaction

1.2 INVERSE ELECTRON DEMAND DIELS-ALDER REACTION

IEDDA reactions plays a significant role in the synthesis of heterocyclic compounds. It has grown in popularity over the last 50 years and has a wide range of applications.

When EDGs are attached to the dienophile and EWGs are attached to the diene the IEDDA reaction occurs, and the interaction of the dienophile's HOMO with the diene's LUMO contributes significantly to the reaction energy **Figure 3 (Nwagbara, 2015)**. Therefore the diene is either constituted of extremely electronegative heteroatoms or substituted by EWGs. Similar to the DA reaction, suitable substituted dienes and dienophiles can undergo the reaction in mild circumstances **Scheme 4.**



Scheme 4: Inverse electron demand Diels -Alder reaction

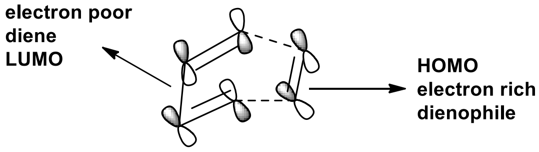
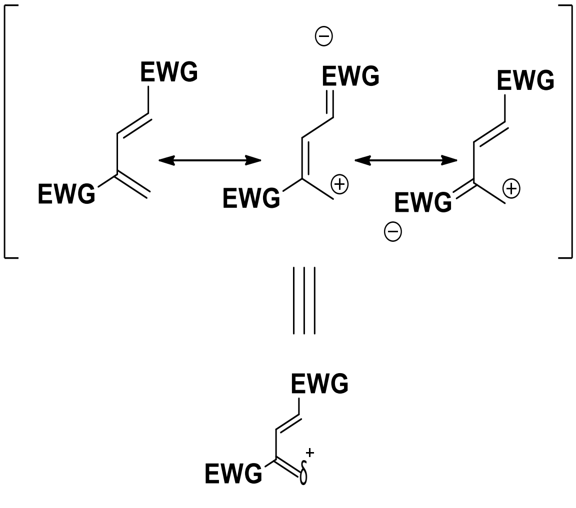


Figure 3: Molecular orbital description of the IEDDA reaction

The EWGs at 1 and 3 positions of a diene work together to electronically bias the diene, resulting in an electronically rich and biased dienophile reacting with its counterpart diene in a regioselective manner **Scheme 5.**



Scheme 5: Resonance consideration of the IEDDA reaction

1.2.1 Dienes and Dienophiles in Inverse Electron Demand Diels Alder reaction

The dienes used in IEDDA are electron-deficient species, with lower molecular orbital energies due to inclusion of either a EWG or electronegative heteroatoms **(Dang *et al.*, 2008).** Dienophiles used in IEDDA reactions are electron rich, resulting in higher orbital energies and orbital overlap with the LUMO of the diene **(Pałasz & Pałasz, 2011). Figure 4** represents some common dienes and dienophiles of IEDDA reaction**.**

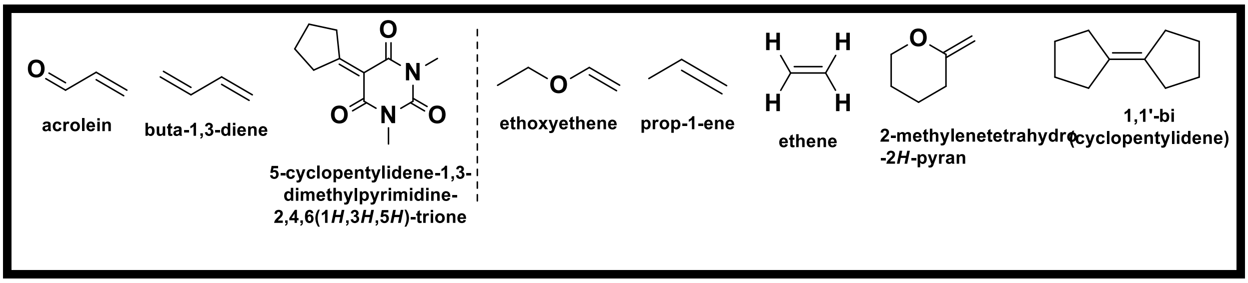
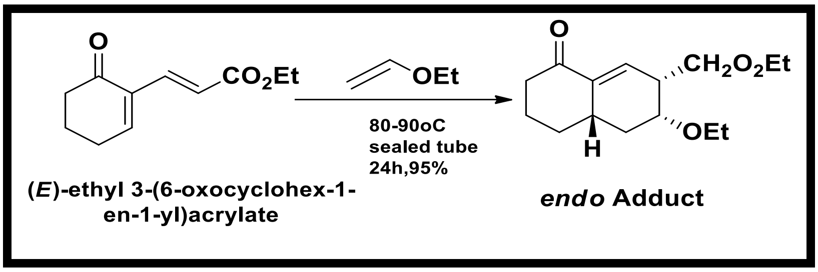


Figure 4: Common dienes and dienophiles in the Inverse electron Diels-Alder reaction

1.2.2 Stereo selectivity

The IEDDA reaction follows a general *endo* selection rule, in which EWGs on the dienophile approach *endo* with respect to the diene. Example the diene (E)-ethyl 3-(-6oxocyclohex-1-en-1-yl) acrylate interacted with ethyl vinyl ether to form adducts with perfect regioselectivity and *endo*-selectivity as a single diastereomer **Scheme 6 (Nwagbara, 2015).**

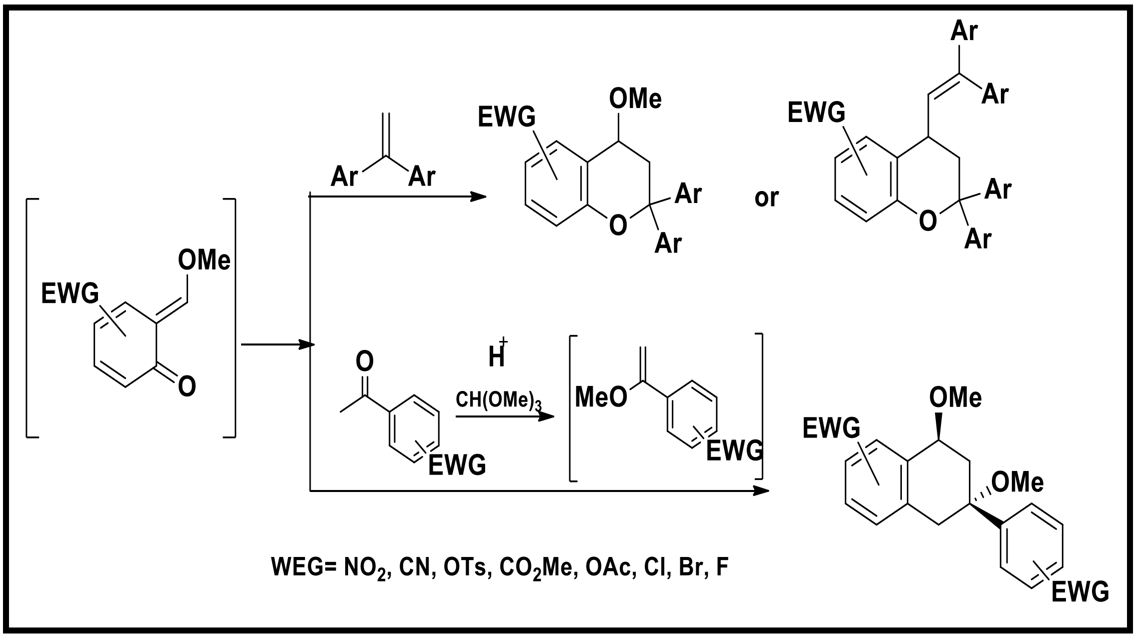


Scheme 6: Reaction of diene with dienophile to give an IEDDA adduct

1.2.3 Application of IEDDA reaction

Total synthesis of seventy natural products including isochrysohermidin, prodigiosin, anhydrolycorinone, hippadine and minovine by IEDDA reaction of heterocyclic aza dienes are reported by **Zhang *et al.*, 2019**.

Regioselective IEDDA reaction of ortho-Quinone Methides with 1,1 disubstituted ethylenes in one-pot synthesis afforded 2,2-disubstituted chromanes with electron-withdrawing substituents **Scheme 7.**

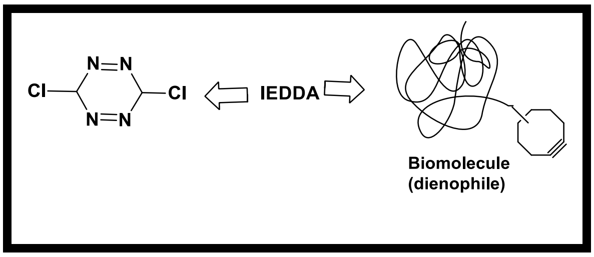


Scheme 7: Synthesis of Chromanes with Various Substituents

This allows for flexible approach for the functionalized electron-deficient chromanes and chromenes, making it a viable method for the generation of biologically and photochemically active compounds **(Tanaka *et al.*, 2019).**

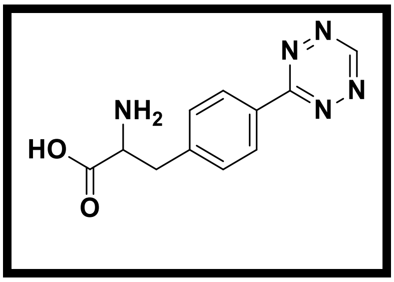
Bio-labeling is vital for the study of biological processes. A key technology for enabling this is the progress of bioorthogonal chemistry. Bioorthogonal reactions will not interfere with biological processes and is stable under physiological conditions. IEDDA reactions are widely used in bioorthogonal reactions. Highly selective IEDDA reaction of tetrazines for cancer cell labelling and cell surface imaging are reported by various authors **(Devaraj *et al.*, 2008 & Wittmann *et al.*, 2014)** IEDDA reaction of tetrazine with norbornene modified oligonucleotides is used to chemoselectively tag DNA strands **(Schoch *et al.*, 2010).**

To assemble imaging probes, dichlorotetrazine can be used as a modular platform which then can be conjugated to proteins in a biorthogonal IEDDA reaction. A series of difunctionalized tetrazine compounds containing various chelating agents and fluorescent dyes is tested for fluorescence imaging of tumor tissues **(Canovas *et al.*, 2021).**



Two intracellular targets are labelled with orthogonal enzymatic reactions using the strain-promoted azide-alkyne cycloaddition (SPAAC) and IEDDA reaction. By adorning biological materials with functions other than fluorophores, this two-step "orthogonal-bioorthogonal" labelling procedure will boost the use of SNAP/CLIP tags **(Macias-Contreras *et al.*, 2020).**

Yang & Kwon, 2021 used site-specific insertion of a non-natural amino acid with a bioorthogonal functional group to create well-defined Human serum albumin (HSA)-conjugated urate oxidase variants that kept their enzymatic activity. IEDDA is used to enhance the number of HSA molecules conjugated to one urate oxidase. This may be utilised to improve the pharmacokinetic profile of therapeutic proteins as well as their therapeutic efficacy *in-vivo* studies.



Fast-reacting tetrazine group

1.3 LEWIS ACID CATALYST

Although DA reaction can occur without the need of catalysts, they occasionally require physical acceleration techniques like high pressure, high temperature, or ultrasonic irradiation. Catalysis, which accelerates reactions without subjecting them to severe heat or pressure, is more attractive. Lewis acids are the most effective catalysts in DA reactions, acting as electron-pair acceptors when coupled with a Lewis base to form a Lewis adduct**.** Lewis acids accelerate DA reactions by reducing LUMO of the dienophile, while for IEDDA reaction it reduces the LUMO of diene **(Figure 5).** Thus it reduces the energy gap between LUMO and HOMO of dienophile and diene respectively. **(Mubofu & Engberts, 2004).**

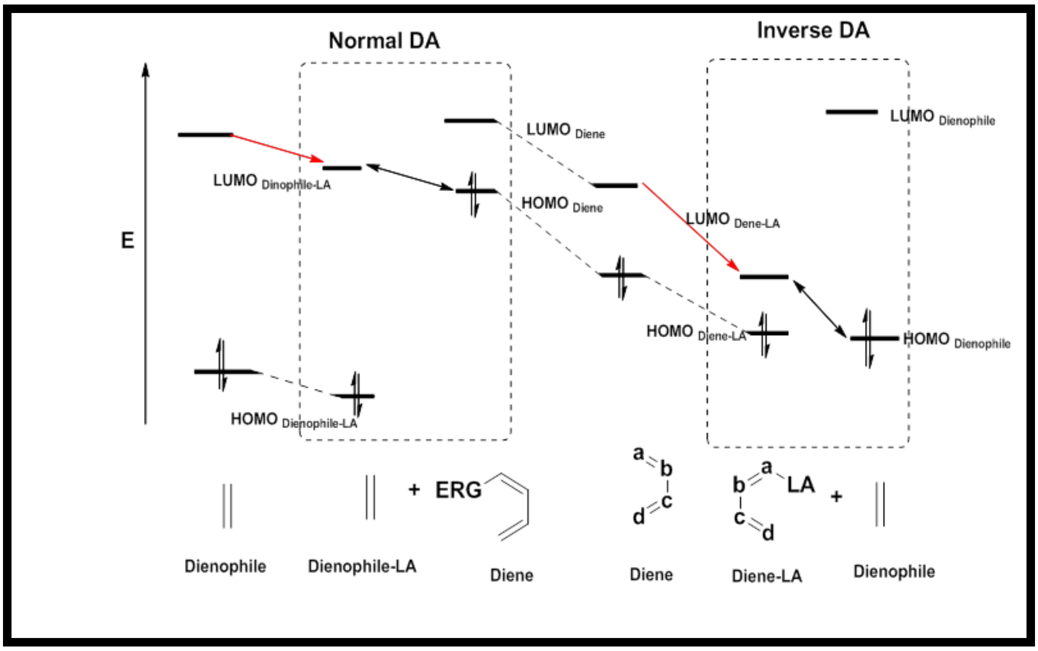
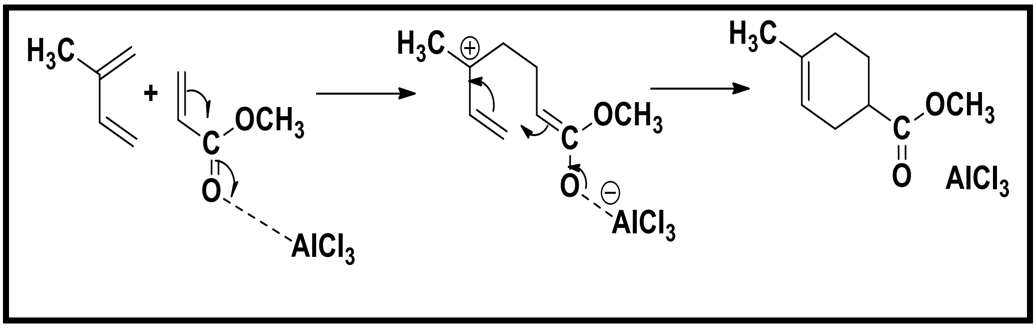


Figure 5: Effect of Lewis acid on the dienophile or diene in the normal and IEDDA reaction

Peter Yates and Philip Eaton, 1960 reported the first study on rate acceleration of the DA reactions catalysed by Lewis acid, aluminium chloride **(Scheme 8). Inukai & Kojima, 1967** extended the study to include aluminium chloride for the reaction between methyl acrylate and butadiene derivatives and reported rate acceleration of cycloadditions due to the complex produced between the carbonyl oxygen and the aluminium chloride lewis acid.



Scheme 8: Catalytic Actions of Aluminium Chloride in DA reaction

K. N. Houk and R. W. Strozier, 1973 established that the Lewis acid decreases the energy gap between diene and dienophile which increases the reaction rate, regioselectivity, and stereoselectivity. Molecular orbital studies of acrolein versus protonated acrolein revealed a considerable increase in the orbital coefficient on the carbonyl carbon, resulting in increased secondary orbital interaction between the reacting species **Figure 6.**

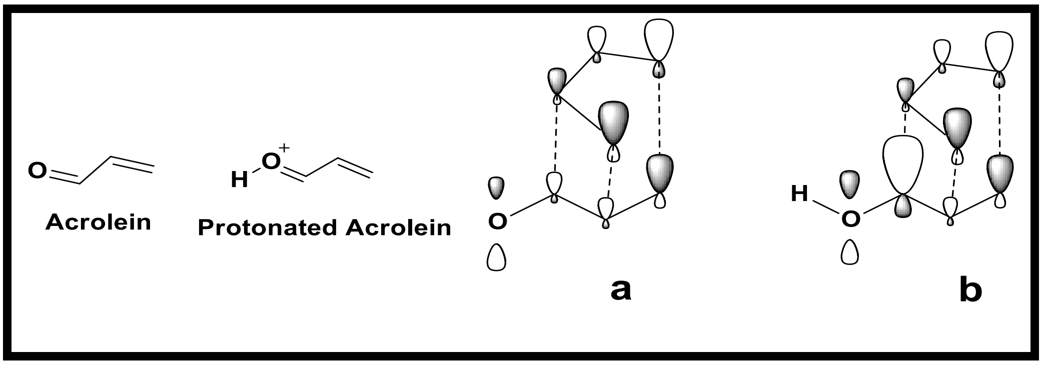
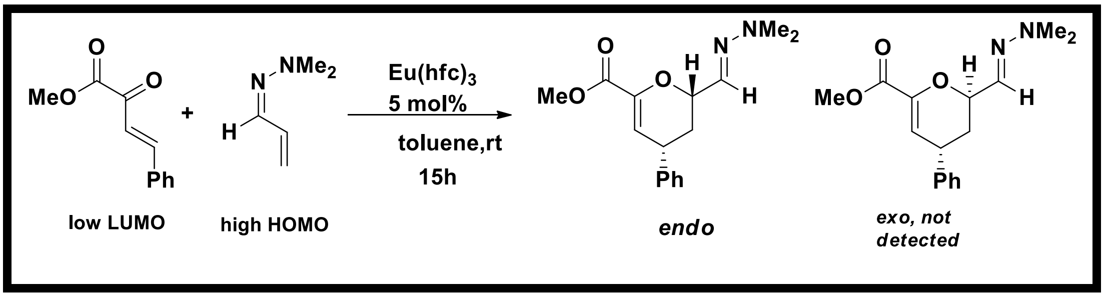


Figure 6: Diene HOMO-dienophile LUMO interactions in *endo* transitions states with (a) acrolein and (b) protonated acrolein.

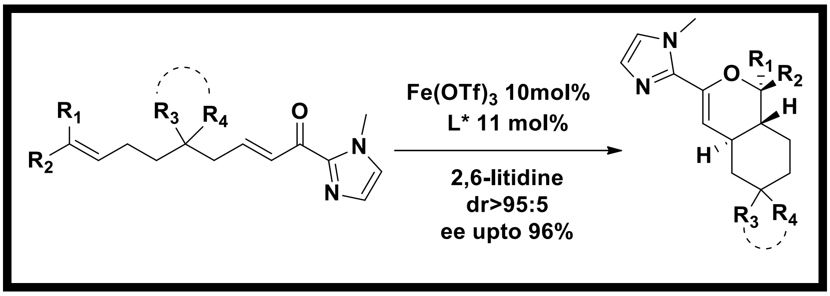
Several Lewis acids have been employed in DA reactions. Some of the more common catalysts include $TiCl_4$, $ZnCl_2$, BF_3, $SnCl_4$, Mg $(ClO_4)_2$, $Sc(OTf)_3$,$FeCl_3$, and $Cu(OTf)_2$. The choice of the Lewis acid also influences the stereoselectivity of cycloadditions, because the catalyst is involved in an *endo* or an *exo*-transition state structure.

Inverse-electron-demand oxa-Diels−Alder reaction of α-keto-β,γ-unsaturated esters with α,β-unsaturated hydrazones as electron-rich olefins catalyzed by $Eu(hfc)_3$ provides a variety of *endo*-selective substituted dihydropyrans in high yields **Scheme 9 (Hashimoto *et al.,* 2019).**



Scheme 9: IEDDA reaction of α-keto-β,γ-unsaturated ester

A highly enantioselective iron-catalyzed Intramolecular Hetero- IEDDA reaction of chiral semicorrin ligand and 2,6-lutidine, resulting in highly valuable bicyclic dihydropyran derivatives in greater yields **Scheme 10** is reported by **Lauberteaux *et al.,* (2019).**



Scheme 10: Iron-Catalyzed Enantioselective IEDHDA Reaction

1.3.1 Scope of Indium based Lewis acid catalyst in IEDDAR

Indium (III) complexes such as $InCl_3$, $InBr_3$, $In(OTf)_3$, and $In(OAc)_3$ have been reported as successful Lewis acid catalysts for the hetero-diels alder reaction in recent years, because of the following reasons,

- high coordination and fast coordination dissociation tendency
- non-toxic and readily available
- It can be recovered for reuse after the reaction is completed
- Excellent yield with high chemo- and regioselectivity
- Excellent moisture compatibility

The Indium (III) chloride -catalyzed hetero-IEDDA reaction of 1-oxo-1,3-butadiene with 2,3-dihydrofuran afforded *cis*-fused furopyrano [2,3-*d*] pyrimidines in excellent yields **(Prajapati & Gohain, 2006).**

Indium (III) chloride efficiently catalysed the intramolecular aza-DA cyclization process of aldimines produced from aromatic amines and N-prenyl/cinnamyl derivatives of pyrrolo[2,3-*d*]pyrimidine to produce tetrahydroquinoline derivatives in high yields **(Ramesh *et al.*, 2008).**

The imino-DA reaction of 3-aminocarbazoles and substituted benzaldehydes with electron-rich alkenes catalysed by Indium (III) chloride results in the formation of novel isomeric ellipticine derivatives with high diastereoselectivity and high yields **(Gaddam & Nagarajan, 2009)**

Although Indium (III) chloride catalysts have several advantages, their reactivity in IEDDA reactions is largely unknown. As a result, the current research concerns the synthesis of pyrido[1,2-*a*]pyrimidine derivatives employing an Indium (III) chloride as Lewis acid catalyst.

1.4 PYRIDOPYRIMIDINE DERIVATIVES

Nitrogen heterocyclic compounds are more prevalent in nature than other types of heterocyclic ring systems because they are used as structural components in several key bioactive substances, including antibiotics, vitamins, hormones, and others. Purines, pteridines, quinazolines, pyridopyrimidines, triazolopyrimidines, pyrazolopyrimidines, pyrimidoazepines, furopyrimidines, and pyrrolopyrimidines etc., are examples of such systems**(Balaban *et al.*, 2004).**

The history of pyrimidine compounds extends from the days of their discovery as important constituents of nucleic acid to their current use in the chemotherapy. Hetero fused pyrimidines have potential antimicrobial, anti-AIDS, and antinociceptive properties. They are widely utilised in neurology, notably in the treatment of neurodegenerative illnesses including Parkinson's disease, anxiety disorders, and depression (MDR) **(Toche *et al.*, 2008).**

Pyridopyrimidines are a well-known heterocyclic compound that has captivated organic chemists for last few decades due to its pharmacological and chemotherapeutic significance. As a prominent pharmacophore in drug development, the pyridopyrimidine molecule **Figure 7** is associated with a wide spectrum of pharmacological characteristics **(Campos *et al.*, 2022).**

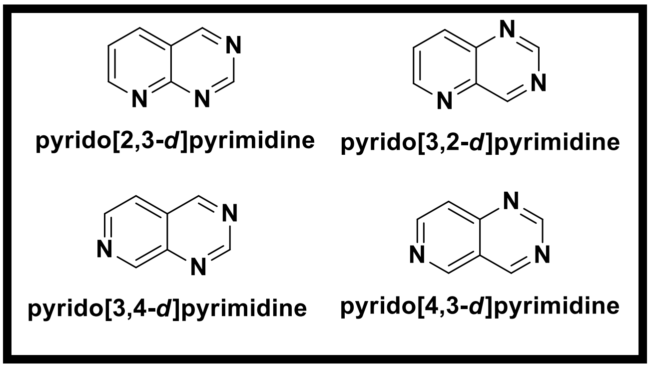
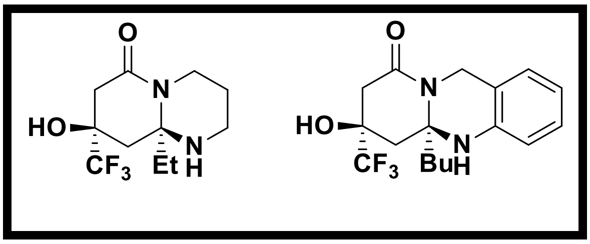


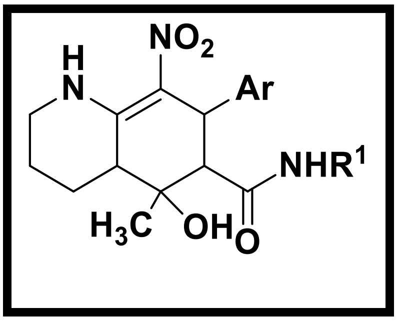
Figure 7: Pyridopyrimidine scaffolds

Pyrido [1, 2-*a*]pyrimidines are one of the N-heterocyclic compounds with a 2-aminopyridine moiety that have piqued the interest of medicinal chemists due to their beneficial biological effects **(Harutyunyan, 2016).** Pyrido [1,2-*a*]pyrimidine core is found in medicines such as antipsychotic Risperidone, tranquilizer Pirenperone **(Meltzer *et al.*, 1983**), Romestin (anti-allergic) **(Awouters *et al.*, 1986)**, anti-HIV-1 agent **(Donghi *et al.*, 2009)**, drug S345(anti-ulcer) and Pemirolast(anti-asthmatic) **(Yanagihara *et al.*, 1988).**

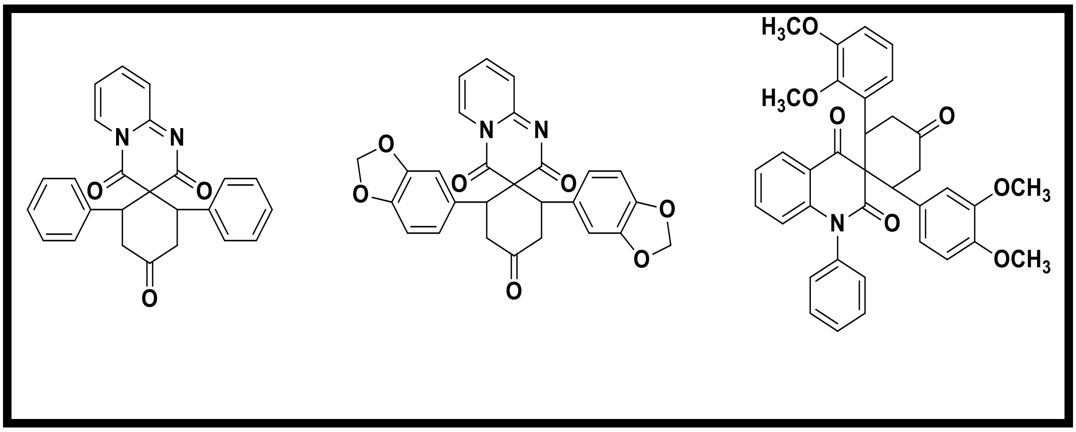
8-hydroxy-9*a*-alkyl(phenyl)-8-(polyfluoroalkyl)octahydro-6*H*-pyrido[1,2-*a*]pyrimidin-6-ones synthesized from 1,3-diaminopropane and 3-oxo esters and methyl ketones, showed antiviral activity against Coxsackie B3 and influenza A/Puerto Rico/8/34 (H1N1) **(Goryaeva *et al.*, 2019).**



A five-component reaction comprising of primary amines, diketene, differentially substituted aromatic aldehydes, nitro ketenedithioacetal, and propanediamine resulted in an effective heteroannulation of N-fused pyrido[1,2-*a*]pyrimidines. It offers appealing properties such as optimum convergence, high bond-forming efficiency, ease of work-up/purification, low waste generation and atom economy **(Rezvanian *et al.,* 2018).**



Verma *et al.,* 2017 described a simple synthesis and biological screening of some new Spiro derivatives incorporating pyrido[1,2-*a*] pyrimidine and quinoline moieties exhibiting high antimicrobial efficacy.



In contrast to pyrido[1,2-*a*] pyrimidin-4-ones, the regioisomeric pyrido[1,2-*a*] pyrimidin-2-ones are considerably less studied, because of lack of versatile synthetic method for their construction. Therefore strategies for the synthesis of this heterocycle, is need of an hour, which will lead to further investigation of the biological properties of these scaffolds.

Hence the present work has sought to develop a fused pyridopyrimidines by IEDDA reaction of electron poor diene, 2-oxo-2*H*-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid and electron rich dienophiles such as butyl vinyl ether, 1-phenyl-3-methyl-pyrazolone, 1-methyl-1-cyclohexene, 3, 4-dihydro-2*H*-pyran, 1-morpholinocyclohexene.

1.5 OBJECTIVE OF THE STUDY

To synthesis fused 2-oxo-2*H*-pyrido [1, 2-*a*] pyrimidin-3(4*H*)-ylidiene acetic acid by IEDDA reaction with different dienophiles such as butyl vinyl ether, 1-phenyl-3-methyl-pyrazolone,1-methyl-1-cyclohexene, 3, 4-Dihydro-2*H*-pyran, 1-morpholinocyclohexene using Lewis acid catalyst Indium (III) Chloride

- ✓ To characterize the compounds by
  - FT-IR,
  - ^{1}H NMR ,
  - ^{13}C NMR,
  - ESI-MS
- ✓ To study the mechanistic details of the IEDDA reaction by computational method
- ✓ To study the antibacterial activity of the synthesized compound against the gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli.*
- ✓ To carry out *in-silico* studies for the synthesized compounds with the target receptors *Staphylococcus aureus* (PDB-2W9S) and *Escherichia coli* (PDB-1MBT).
- ✓ To correlate the *in-vitro* studies with the *in-silico* studies.