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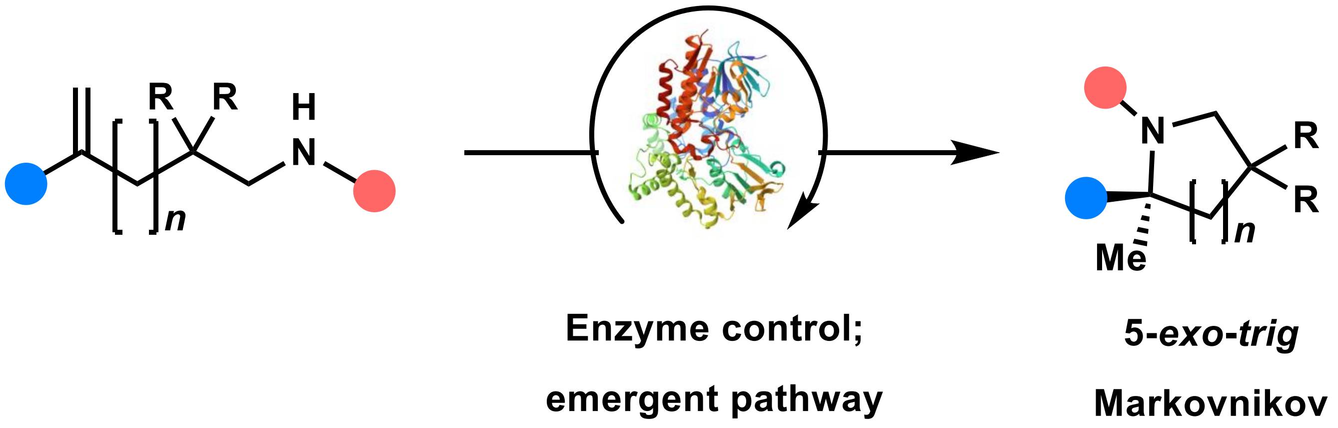
Emergence of a distinct mechanism of C–N bond formation in photoenzymes



Presenter: Fan Linlin

F. C. Raps, A. Rivas-Souchet, C. M. Jones and T. K. Hyster, *Nature*, 2024, 1–3

Author Profile

Todd K. Hyster, Professor of Chemistry at Princeton University

B.S., University of Minnesota, Twin Cities, 2008
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Ph.D., Colorado State University, 2013
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Marie Curie Fellow, University of Basel, 2012
Advisor: Thomas R. Ward

Postdoctoral Fellowship, California Institution of Technology, 2015
Advisor: Frances H. Arnold

Assistant Professor, Princeton University, 2015-2021

Associate Professor, Cornell University, 2021-2023

Professor, Princeton University, 2023-Present

Felix C. Raps

M.S., University of Basel

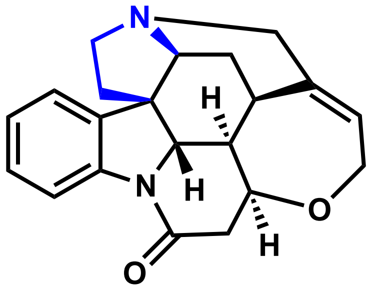
Ph.D., University of Basel
Advisor: Christof Sparr

Research Focus:

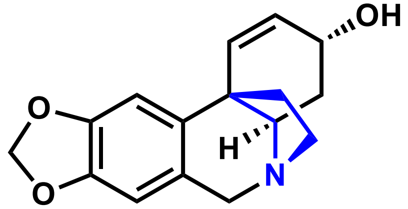
- Discovering versatile **biocatalysts** that can be used to accelerate the synthesis of drugs and agrochemicals.
- Developing strategies that enable **enzymes** to catalyze reactions that are currently unknown in nature.

Saturated Nitrogen-containing Heterocycles

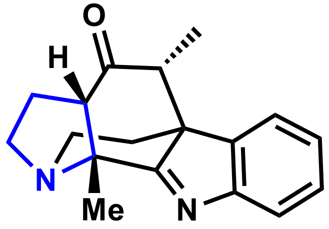
- **Chiral pyrrolidine containing natural products**



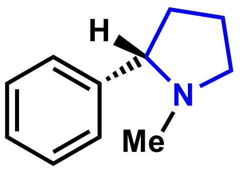
Strychnine



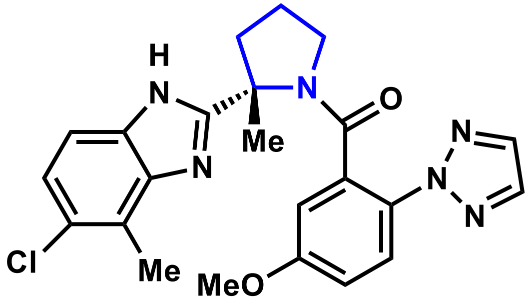
Crinine



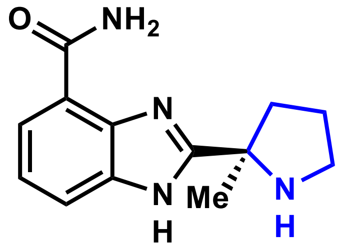
Arborisidine



(*S*)-Nicotine



Daridorexant

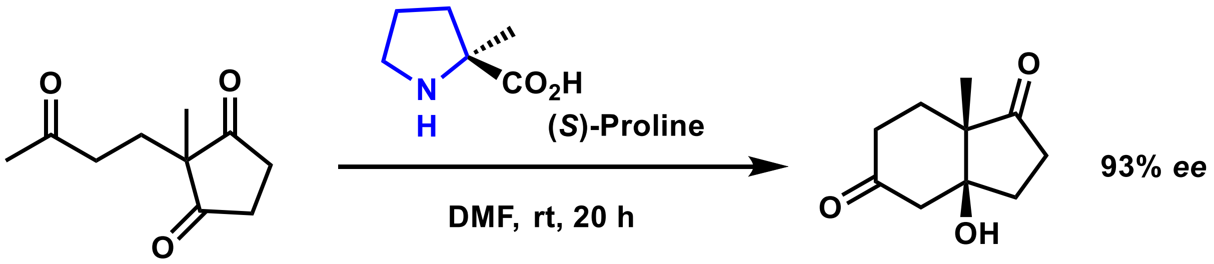


Valiparib

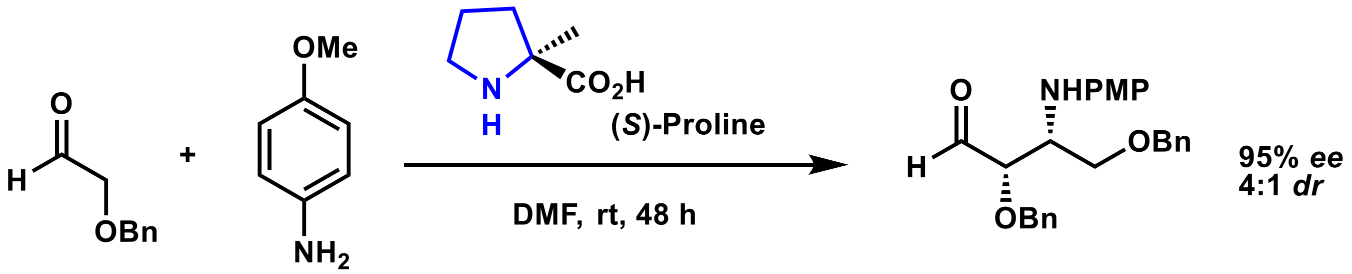
E. Vitaku, D. T. Smith and J. T. Njardarson, *J. Med. Chem.*, 2014, 57, 10257–10274

Saturated Nitrogen-containing Heterocycles

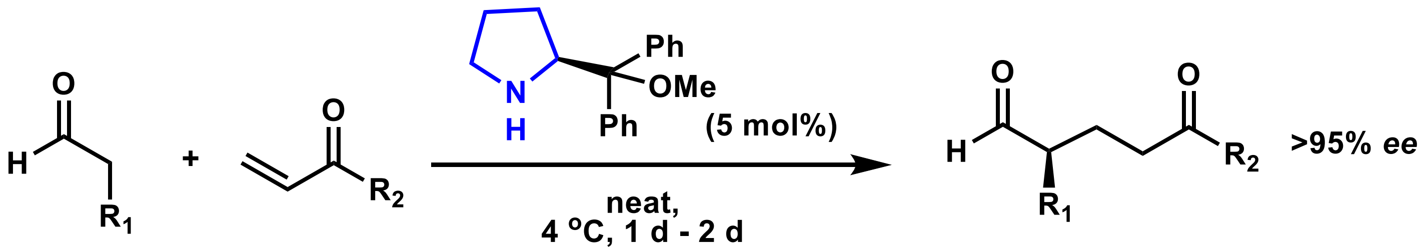
- **Pyrrolidine derivatives as organocatalysts**
  - **Intramolecular Aldolization**



  - **Cross-Mannich Reaction**



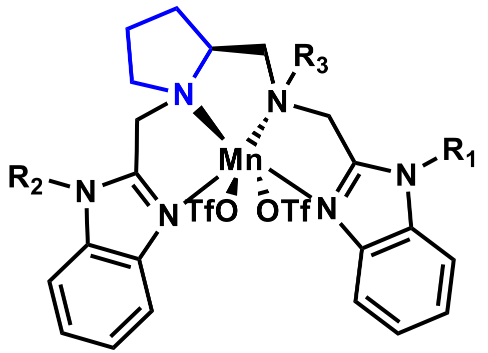
  - **Michael Addition**



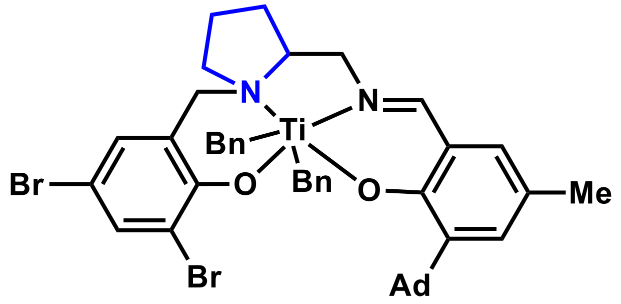
S. Mukherjee, J. W. Yang, S. Hoffmann and B. List, *Chem. Rev.*, 2007, 107, 5471–5569.
G. D. Yadav, P. Chaudhary, B. Pani and S. Singh, *Tetrahedron Lett.*, 2023, 134, 154835–154835.

Saturated Nitrogen-containing Heterocycles

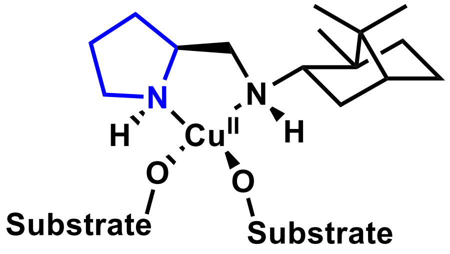
- **Pyrrolidine derivatives as ligands for transition metal catalysis**



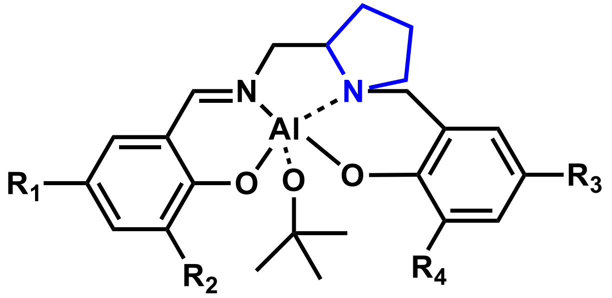
Mn(II) complexes of N_4 ligands
(Asymmetric epoxidation of alkenes)



[LigTiBn$_2$]
(catalyse polymerization reaction)



Cu complex C_1-symmetric diamines
(catalyse enantioselective nitroaldol reaction)



Al complex of aminomethylpyrrolidine ligand
(catalyse polymerization reaction)

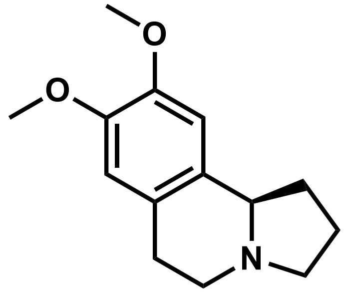
S. Mukherjee, J. W. Yang, S. Hoffmann and B. List, *Chem. Rev.*, 2007, 107, 5471–5569.
G. D. Yadav, P. Chaudhary, B. Pani and S. Singh, *Tetrahedron Lett.*, 2023, 134, 154835–154835.

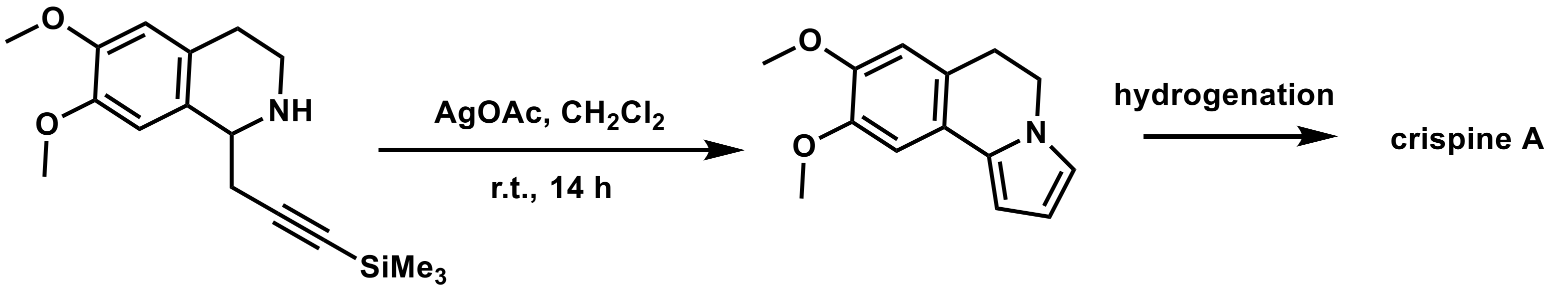
Construction of Saturated Nitrogen-containing Heterocycles

Intramolecular hydroamination of alkenes is an attractive strategy for the synthesis of saturated nitrogen-containing heterocycles proceeding with full atom economy.

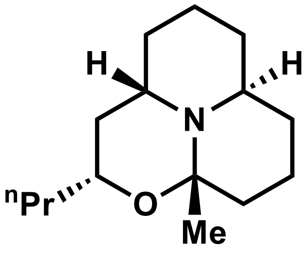
- **Two examples of bioactive natural products accessible via hydroamination**

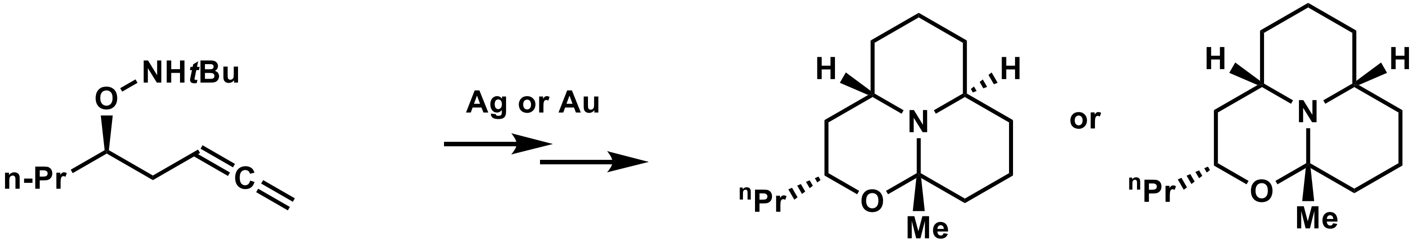
crispine A





porantheridine

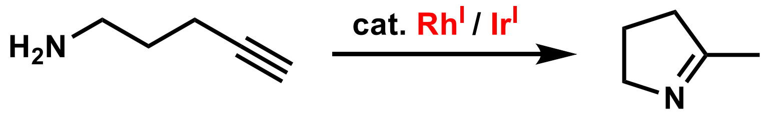


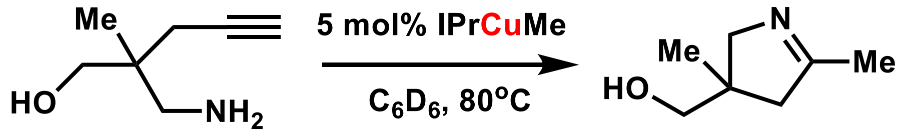


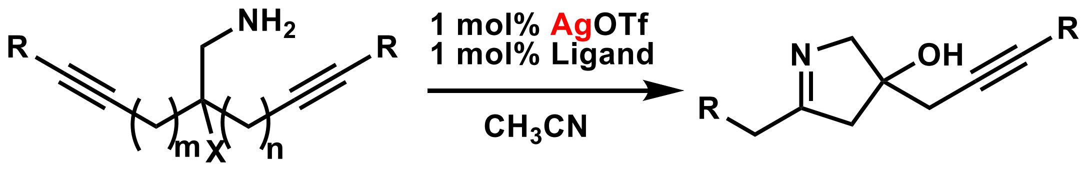
L. Huang, M. Arndt, K. Gooßen, H. Heydt and L. J. Gooßen, *Chem. Rev.*, 2015, 115, 2596–2697

Intramolecular Hydroamination

Transition metal catalysed Intramolecular hydroamination

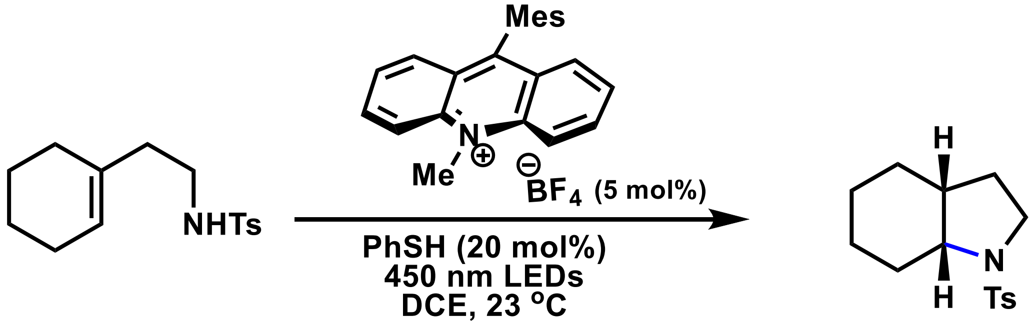






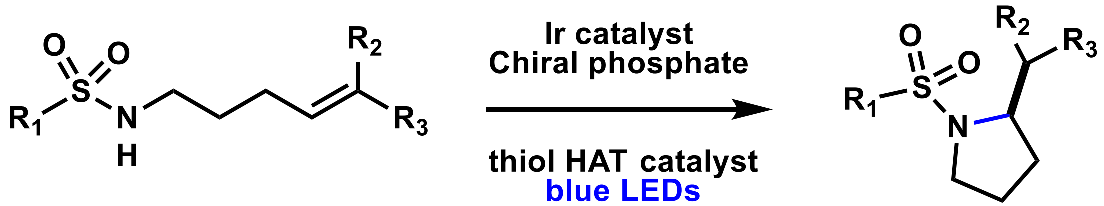
$n = m = 1$, X = OH

Anti-Markovnikov hydroamination via Photoredox catalysis



72% yield
12:1 *d.r.*

Anti-Markovnikov hydroamination via proton-coupled electron transfer (PCET)



R_1 = aryl, heterocycles;
R_2, R_3 = alkyl

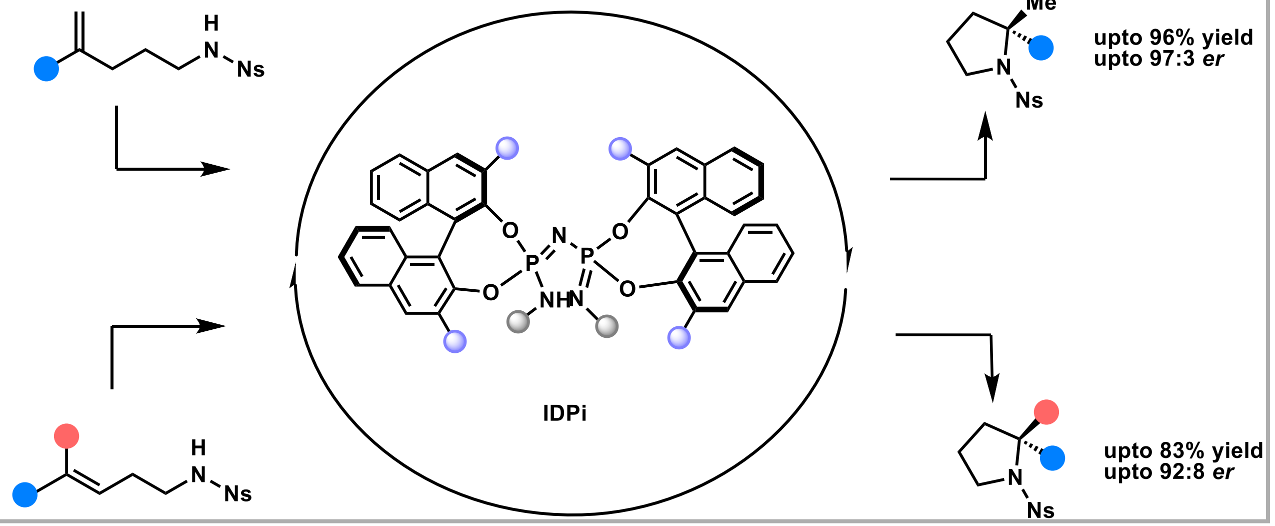
78% - 98% yield
good to excellent enantioselectivity

A. L. Reznichenko and K. C. Hultzsch, *Org. React.*, 2015, 1–554.
T. M. Nguyen and D. A. Nicewicz, *J. Am. Chem. Soc.*, 2013, 135, 9588–9591.
A. J. Musacchio, T. C. Sherwood and R. R. Knowles, *Science*, 2017, 355, 727–730.
C. B. Roos, R. R. Knowles, *J. Am. Chem. Soc.*, 2020, 142, 5974–5979.

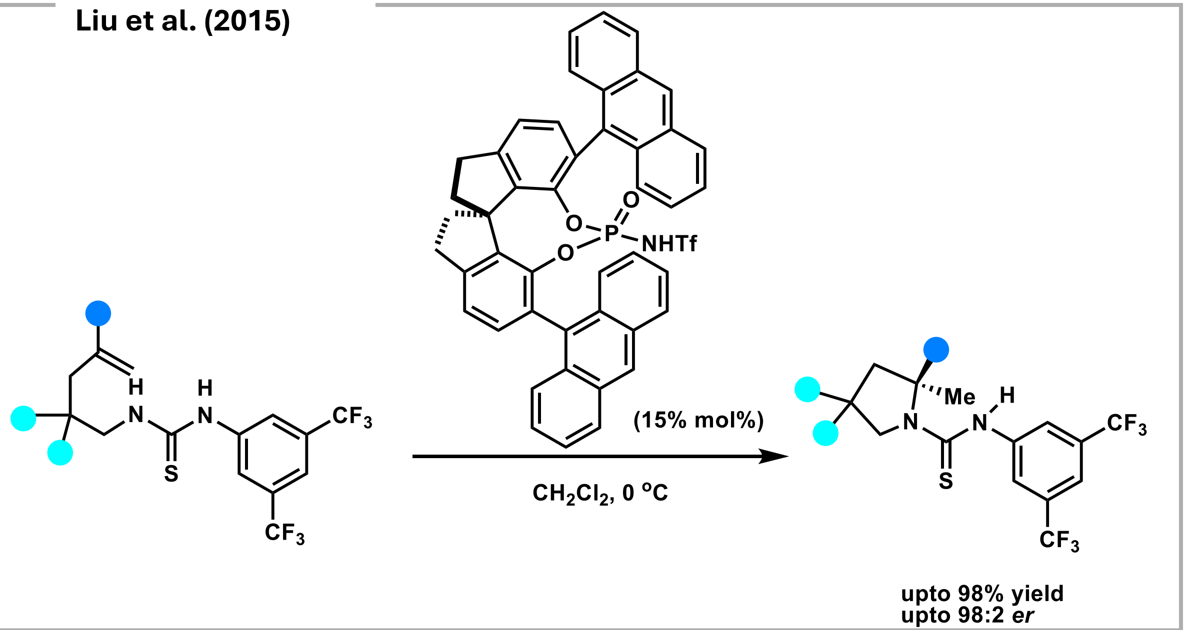
Intramolecular Hydroamination

- Bronsted acid-catalysed intramolecular hydroamination

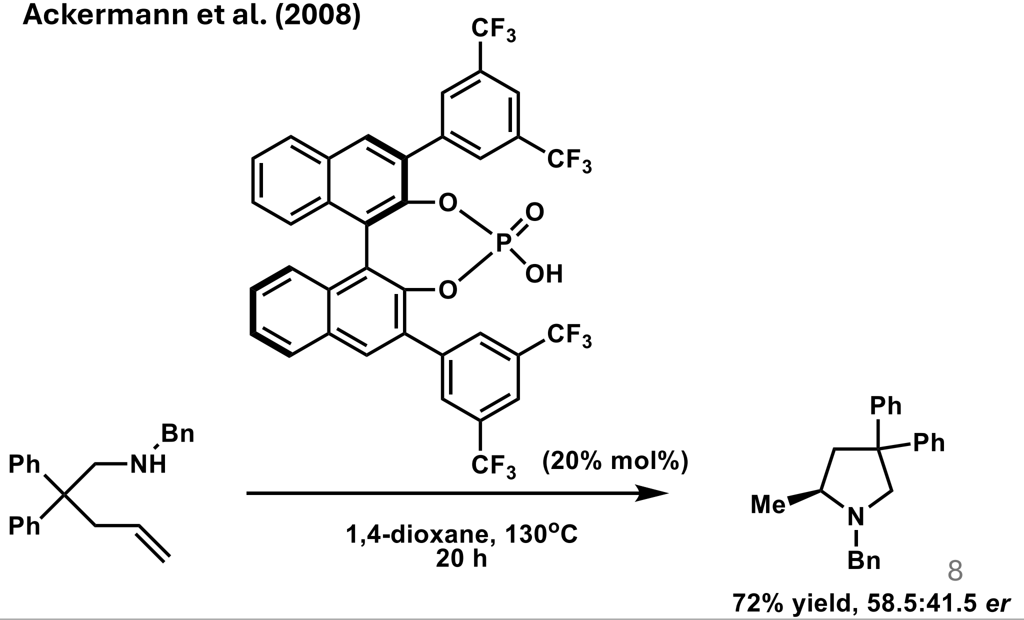
Hennecke et al. (2024)



Liu et al. (2015)

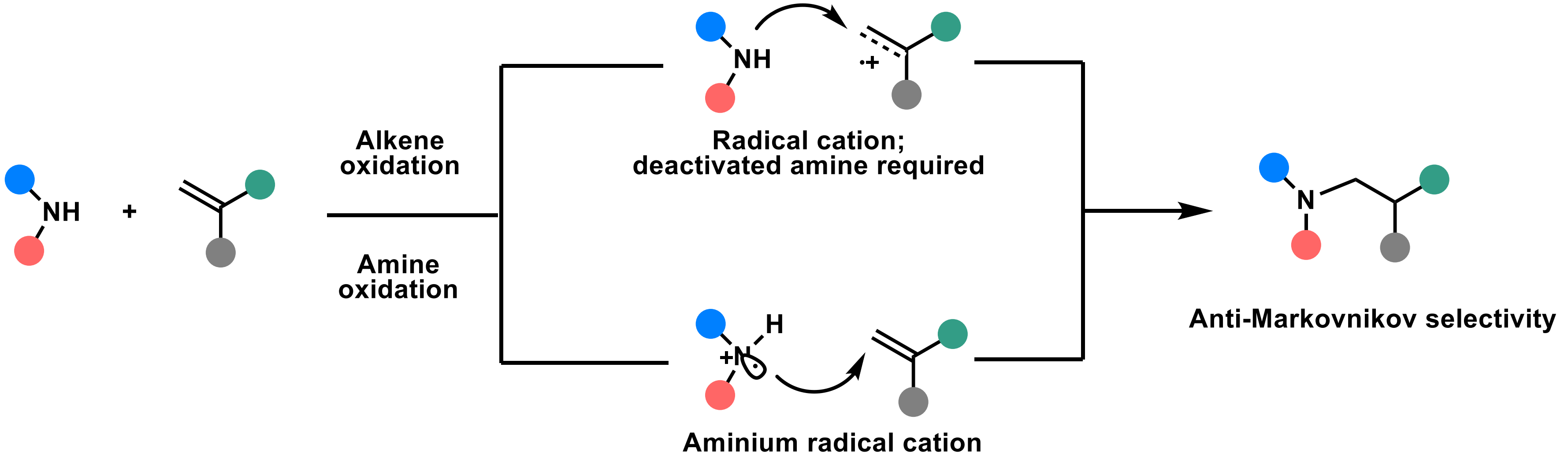


Ackermann et al. (2008)

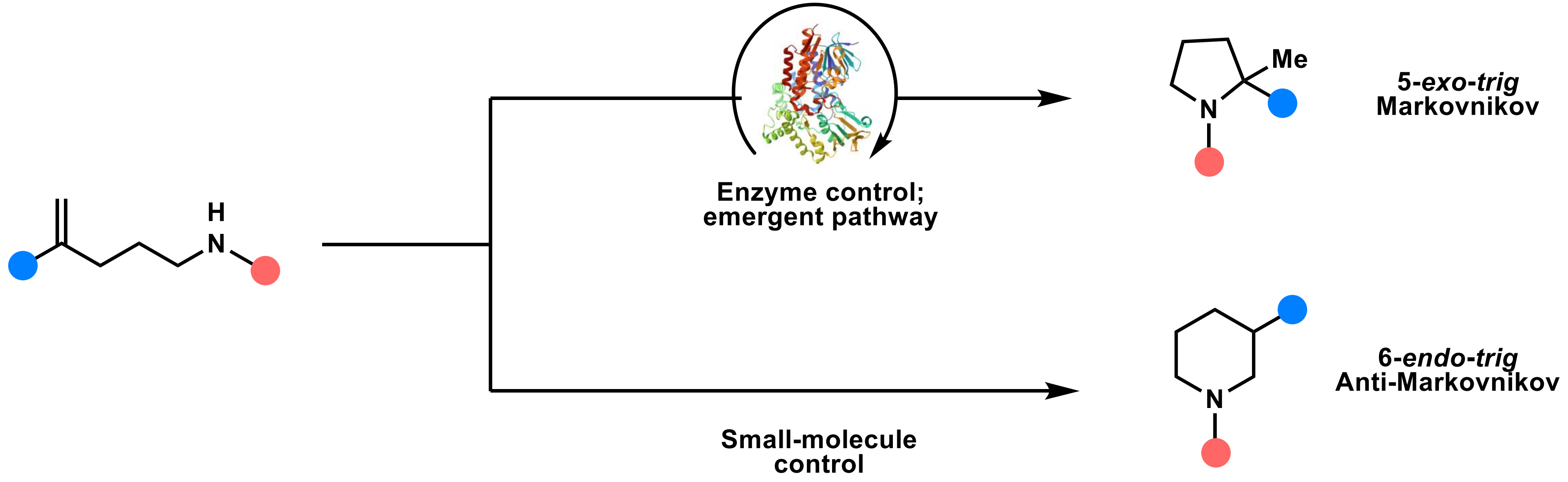


Construction of saturated nitrogen-containing heterocycles

- **Small molecule catalysed radical hydroamination strategies and limitations**



- **Enzyme control can address outstanding challenges in radical hydroamination**



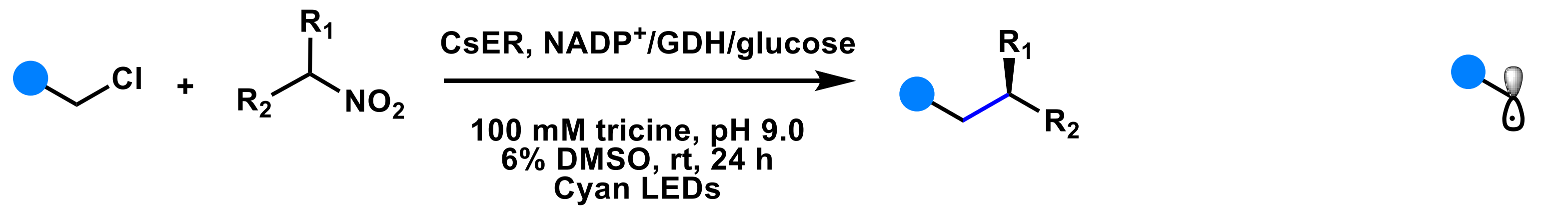
Expected to achieve:

1) Regioselectivity of cyclization
2) Stereoselectivity of α-tertiary amine centre
3) Unfunctionalized amine transformation by photoenzymes

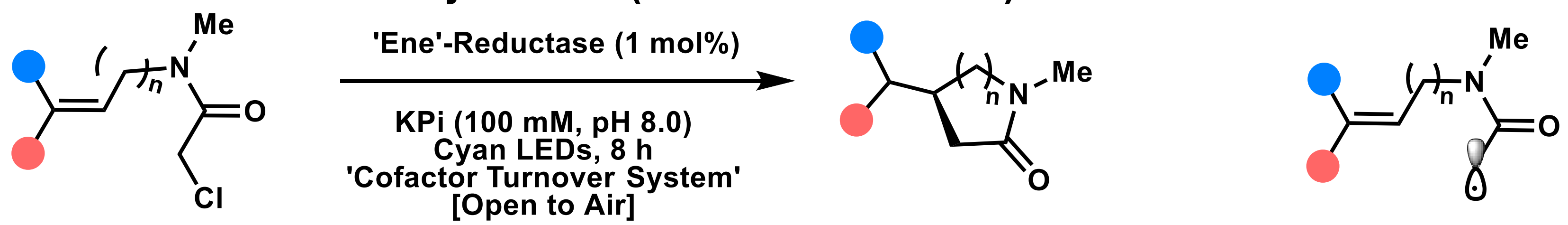
F. C. Raps, A. Rivas-Souchet, C. M. Jones and T. K. Hyster, *Nature*, 2024, 1–3

Previous reported bond-forming reactions using ‘ene’-reductases

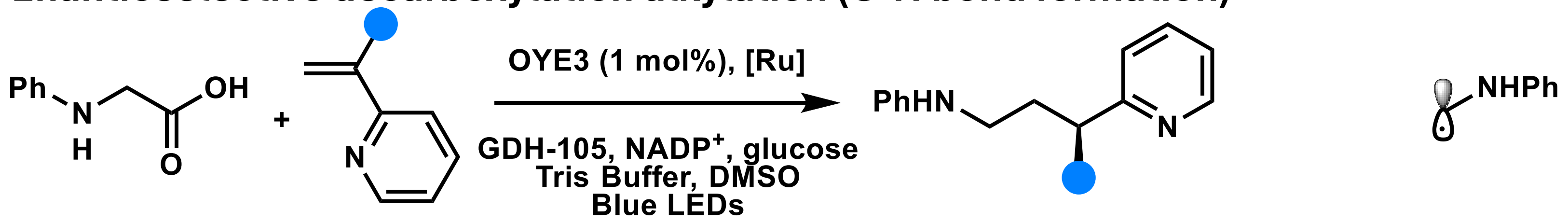
- **Asymmetric *sp*3-*sp*3 cross-electrophile coupling (C-C bond formation)**



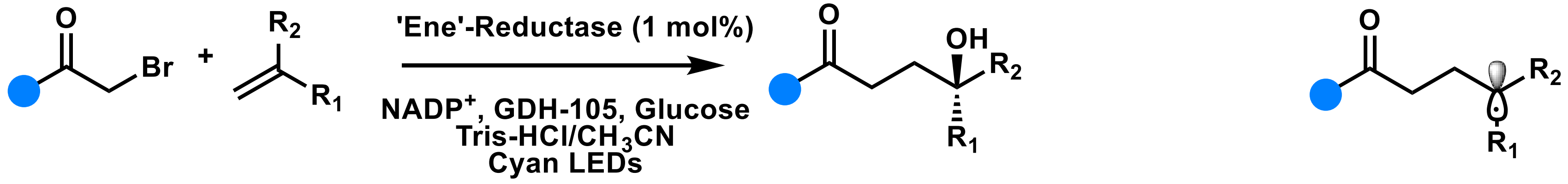
- **Stereoselective radical cyclization (C-C bond formation)**



- **Enantioselective decarboxylation alkylation (C-N bond formation)**



- **Asymmetric Carbohydroxylation of alkenes (C-O bond formation)**

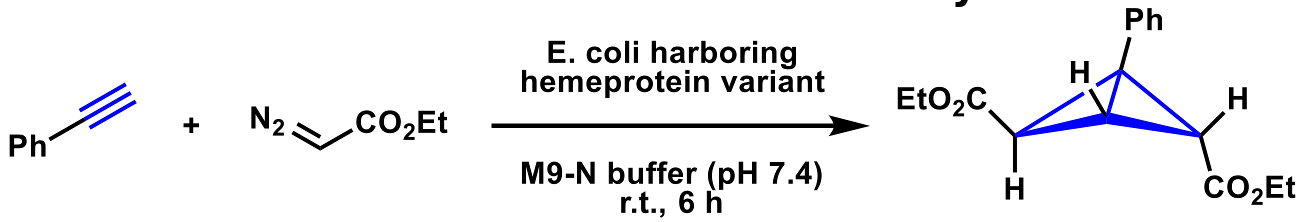


H. Fu, J. Cao,and T. K. Hyster, *Nature*, 2022, 1–2.
K. F. Biegasiewicz,and T. K. Hyster, *Science*, 2019, **364**, 1166–1169.
Y. Ye, J. Cao,and T. K. Hyster, *Nat. Chem.*, 2023, **15**, 206–212.
Y. Ouyang, J. Turek-Herman, T. Qiao and T. K. Hyster, *J. Am. Chem. Soc.*, 2023, **145**, 17018–17022.

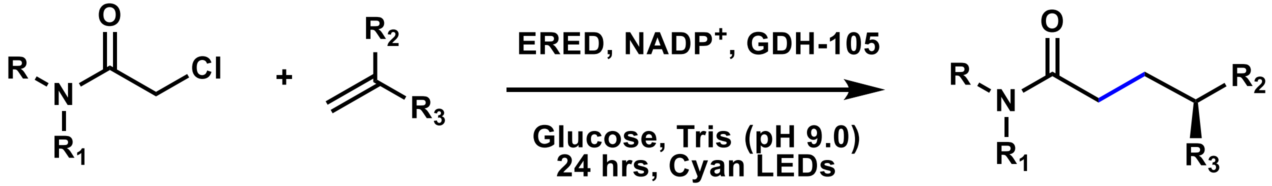
Emerging Mechanisms in Previous Enzymatic Reactions

A complex system with access to mechanisms in which individual parts do not display a property is known as emergence.

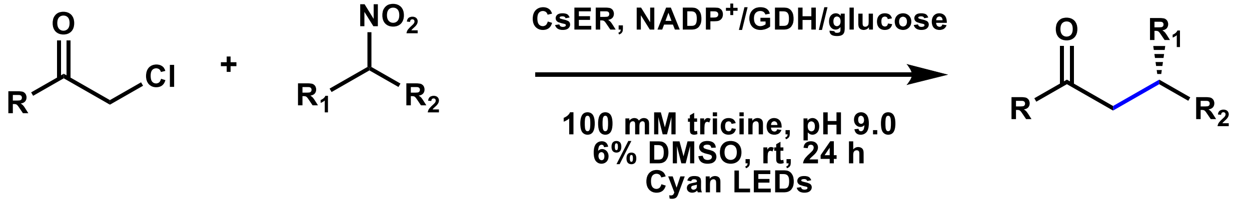
- **P411-catalysed nitrene insertion into C-H bonds and bicyclobutane formation**



- **Quaternary charge transfer complex with flavin-dependent oxidoreductases**

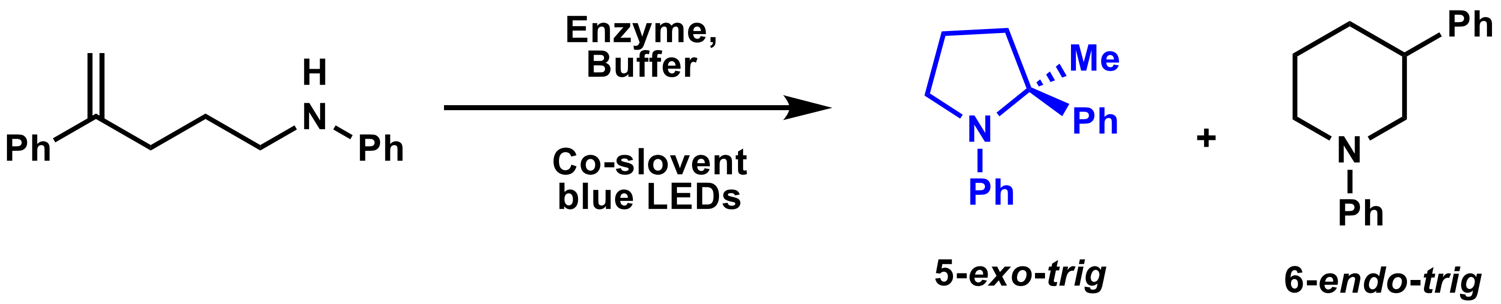


- **Mesolytic cleavage of nitroradical anions in reductive coupling using 'ene' reductases**



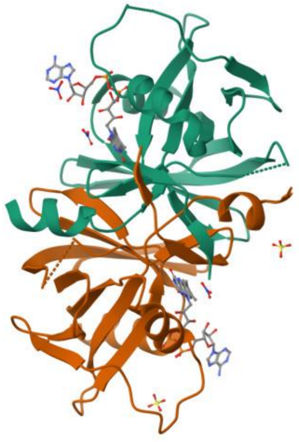
K. Chen, X. Huang, S. B. J. Kan, R. K. Zhang and F. H. Arnold, *Science*, 2018, 360, 71–75.
C. G. Page, S. J. Cooper, and T. K. Hyster, *J. Am. Chem. Soc.*, 2020, 143, 97–102.
H. Fu, J. Cao, T. Qiao, Y. Qi, S. J. Charnock, S. Garfinkle and T. K. Hyster, *Nature*, 2022, 1–2.

Photoenzymatic Alkene Hydroamination — Enzyme Selection



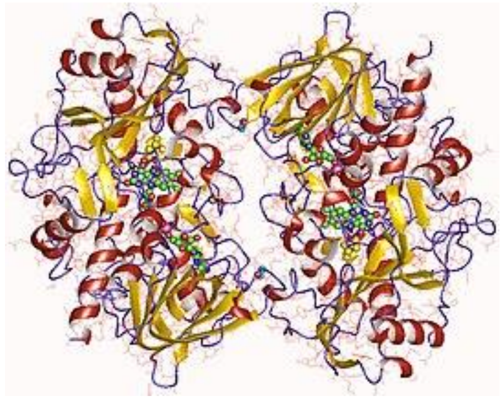
"An enzyme that naturally catalyse an oxidative transformation would best tolerate the anticipated radicals generated in the active site"

Monoamine oxidase (MAO-N)

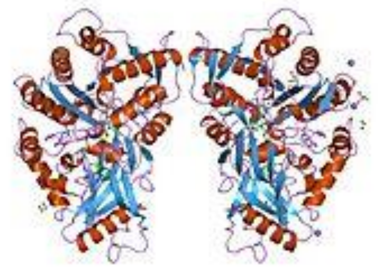


Styrene mono-oxygenase (StyA1)

D-amino acid oxidase (*Ac*DAAO)

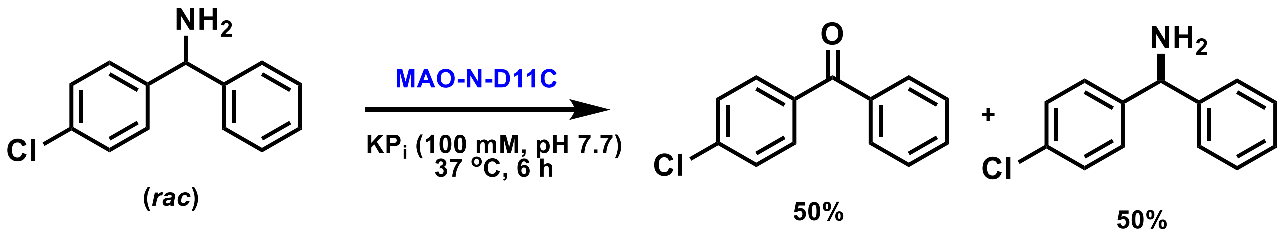


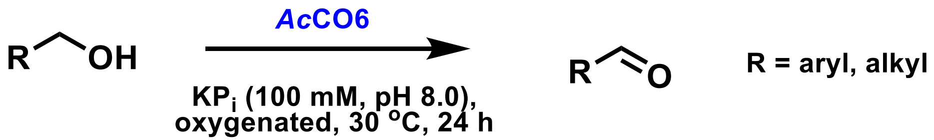
Choline oxidase (*Ac*CO6)

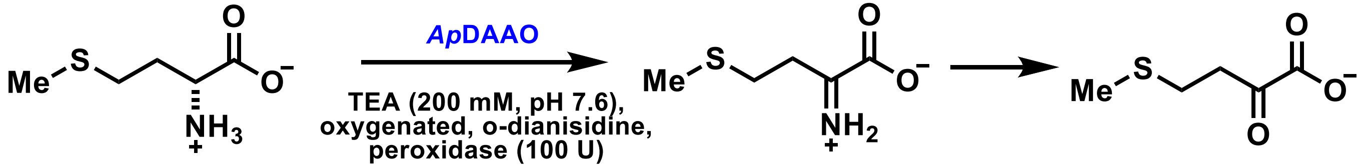


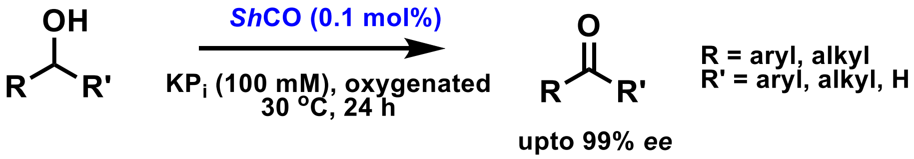
Cholesterol oxidase (*Sh*CO)

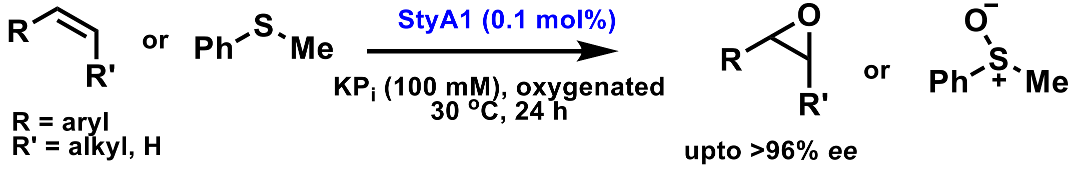
Photoenzymatic Alkene Hydroamination — Enzyme Selection









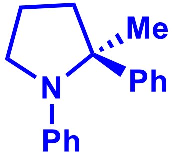


V. F. Batista, J. L. Galman, and N. J. Turner, *ACS Catal.*, 2018, **8**, 11889–11907.
D. Tischler, René Kermer, M. Schlömann, *J. Bacteriol*, 2010, **192**, 5220–5227.
L. Pollegioni and G. Molla, *Trends Biotechnol*, 2011, **29**, 276–283.

R. S. Heath, W. R. Birmingham, and N. J. Turner, *ChemBioChem 20*, 2019, 276-281
R. S. Heath, J. J. Sangster and N. J. Turner, *ChemBioChem 23.*

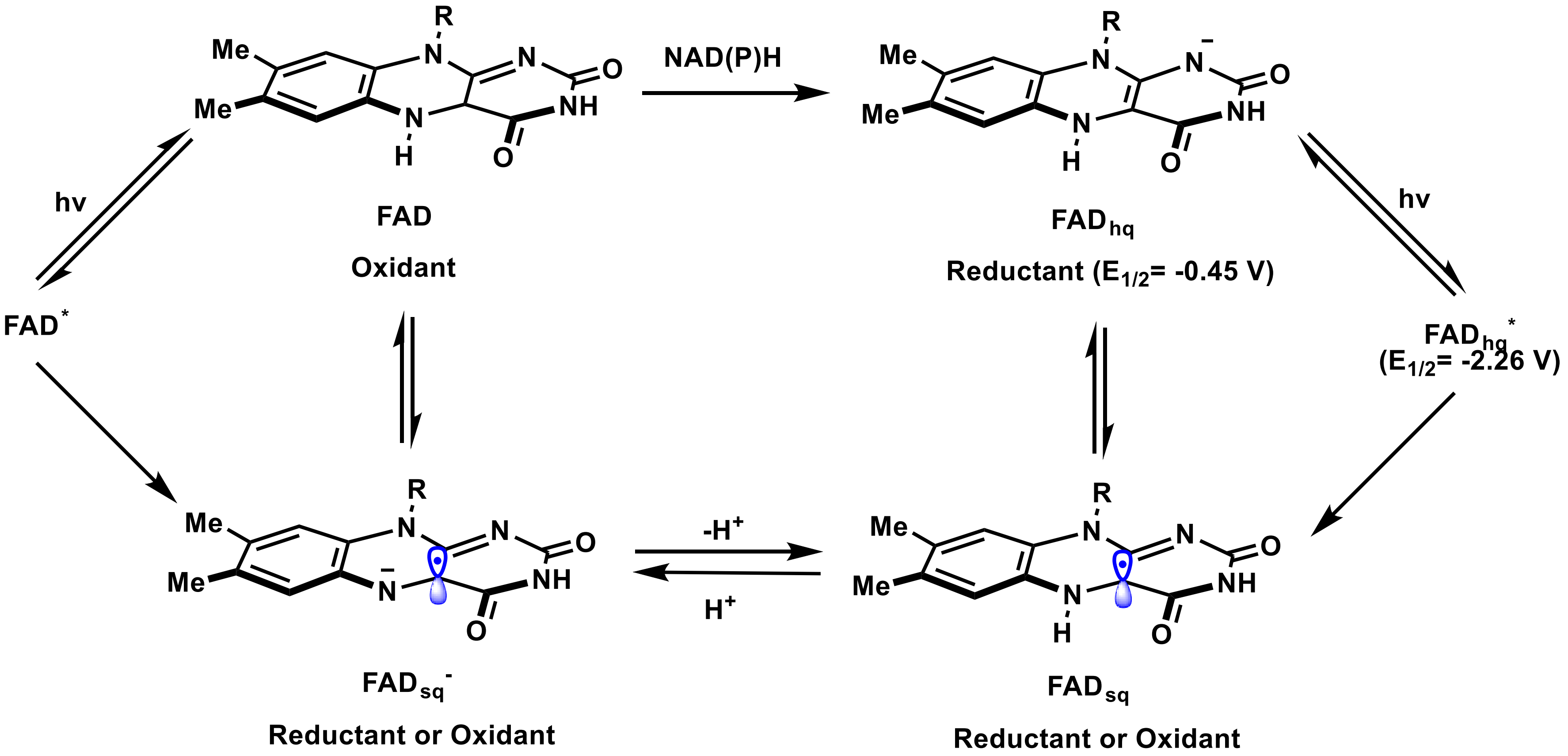
Photoenzymatic Alkene Hydroamination — Enzyme Selection

Cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* (*Ac*CO6)



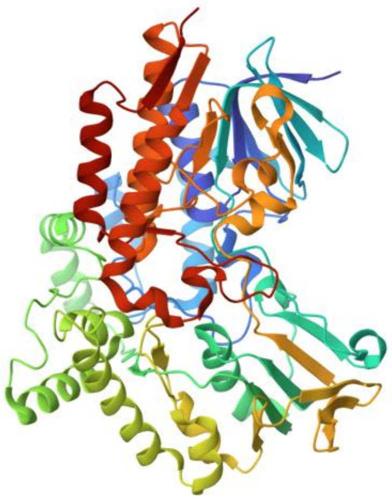
5-*exo-trig*

1% yield, 55:45 *er*

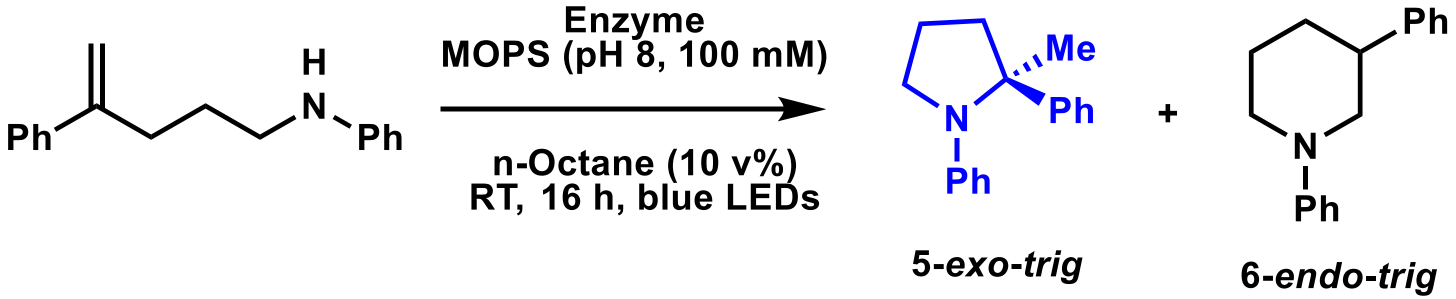


D. Grosheva and T. K. Hyster, 2021, 291-313

Photoenzymatic Alkene Hydroamination — Enzyme Selection



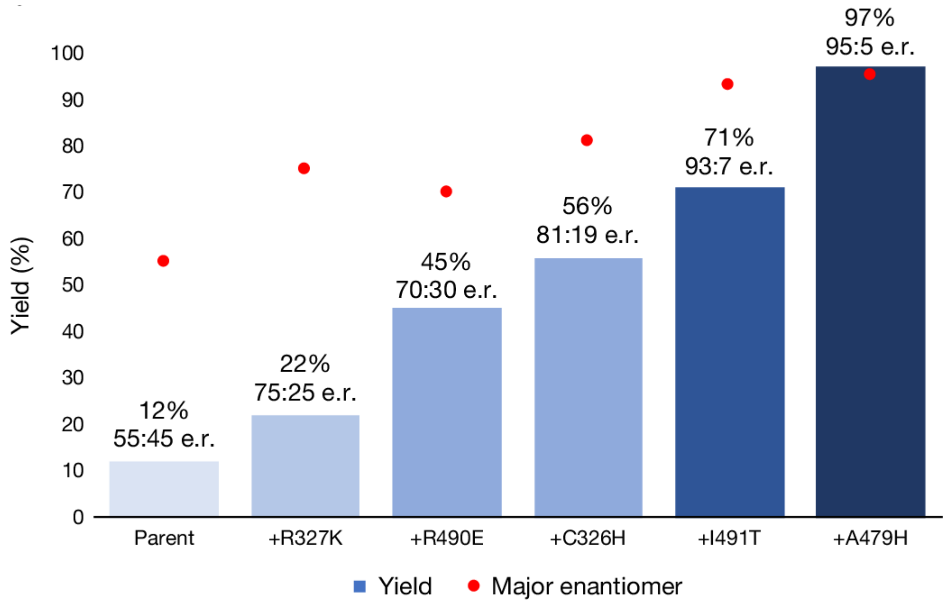
Cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* (*Ac*CO6)

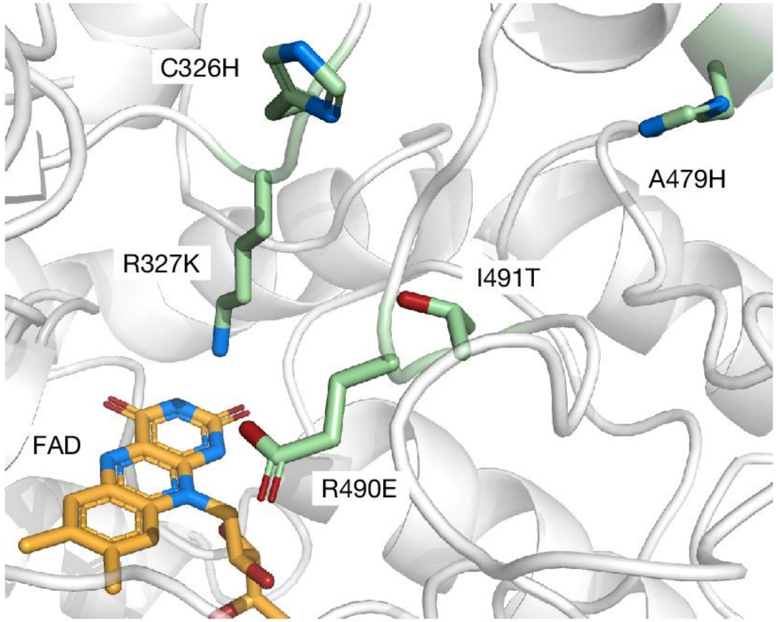


Entry	Deviation	*5-exo-trig*	*e.r.*
1	*Ac*CHMO	1%	55:45
2	*Ac*CHMO-M10	12%	55:45
3	Lysate (0.04 mol%)	15%	95:5
4	FAD	-	-
5	No light	-	-
6	No enzyme	-	-

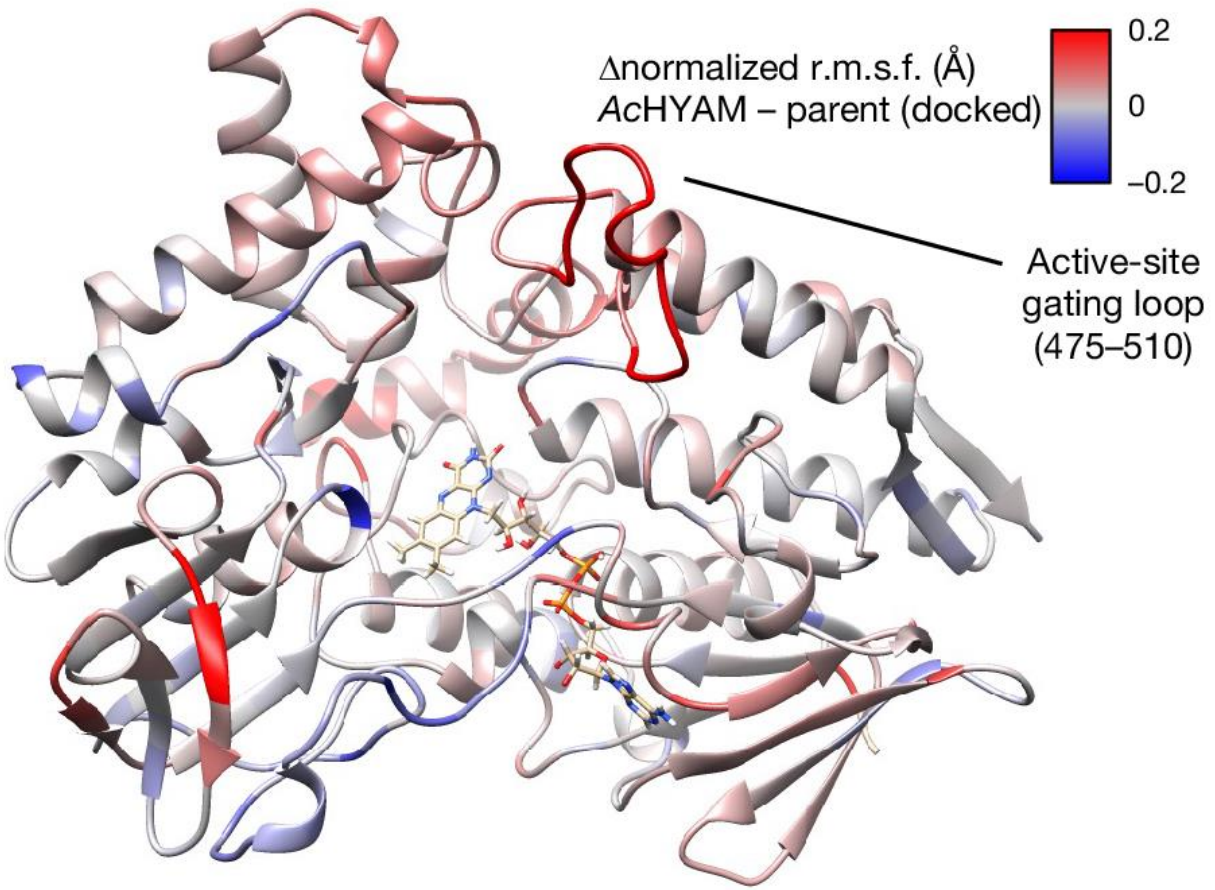
Photoenzymatic Alkene Hydroamination—Protein Engineering

Entry	Starting point	Mutants Screened	Best Mutant	Yield	*e.r.*
1	*Ac*CHMO-M10	140	-R327K	22%	75:25
2	Round 1	260	-R327K-R490E	45%	70:30
3	Round 2	200	-R327K-R490E-C326H	56%	81:19
4	Round 3	180	-R327K-R490E-C326H-I491T	71%	93:7
5	Round 4	100	-R327K-R490E-C326H-I491T-A479H	97%	95:5



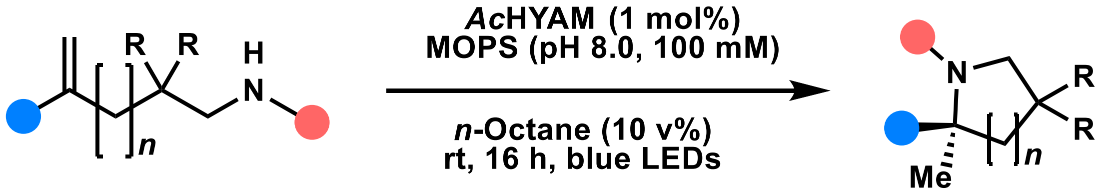


Photoenzymatic Alkene Hydroamination—Protein Engineering

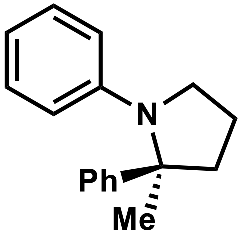


The introduction of these distal substitutions appears to disrupt interactions between the active-site gating loop and other regions of the enzyme, which prevents the loop from shielding the active site.

Scope of C-N-forming reactions

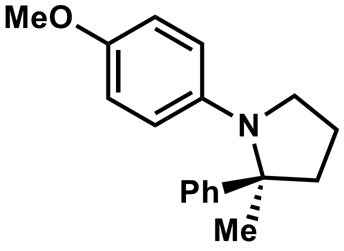


Aniline scope (n = 1)



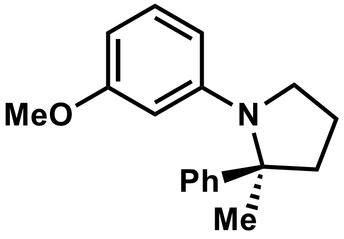
1

97% yield, 95:5 *e.r.*



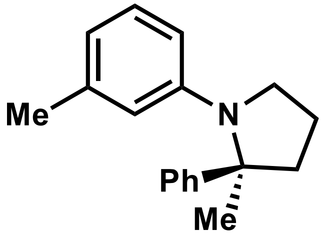
2

99% yield, 97:3 *e.r.*



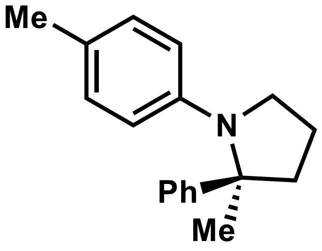
3

97% yield, 90:10 *e.r.*



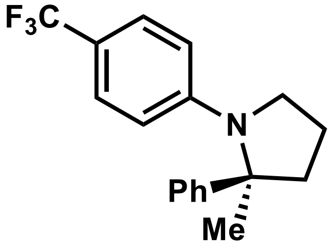
4

98% yield, 94:6 *e.r.*



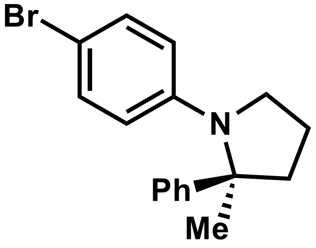
5

79% yield, 94:6 *e.r.*



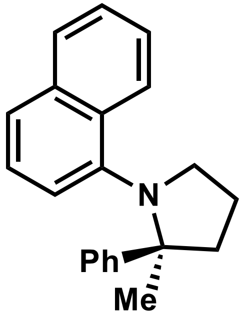
6

70% yield, 96:4 *e.r.*



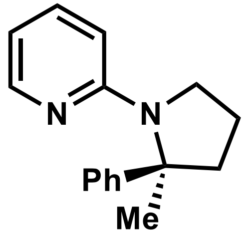
7

27% yield, 92:8 *e.r.*



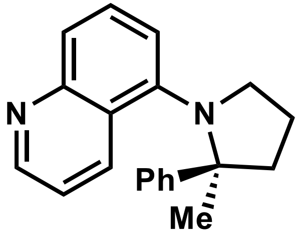
8

98% yield, 95:5 *e.r.*



9

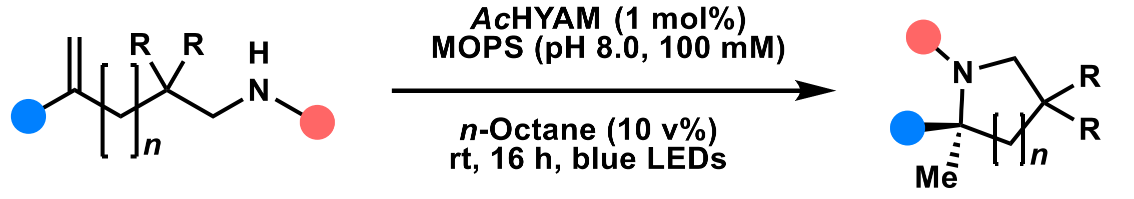
78% yield, 79:21 *e.r.*



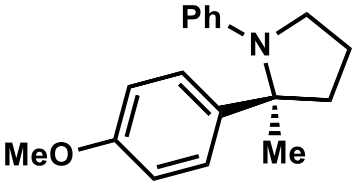
10

62% yield, 98:2 *e.r.*
6-*exo-trig* (n = 2)

Scope of C-N-forming reactions

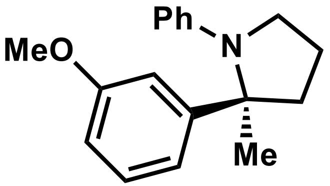


Styryl scope (n = 1)



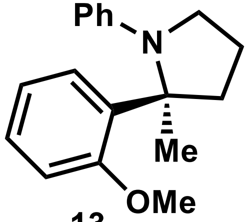
11

60% yield, 94:6 *e.r.*



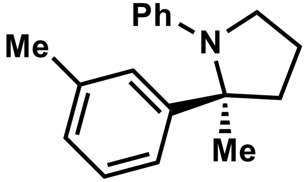
12

99% yield, 80:20 *e.r.*



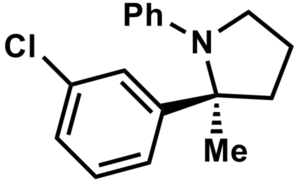
13

61% yield, 60:40 *e.r.*



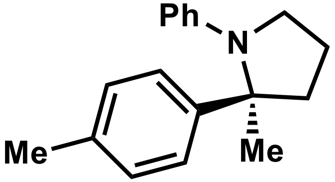
14

98% yield, 81:19 *e.r.*



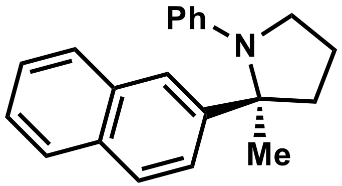
15

19% yield, 89:11 *e.r.*



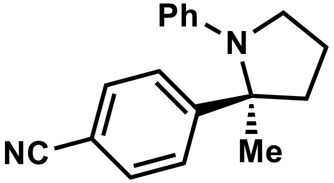
16

76% yield, 97:3 *e.r.*



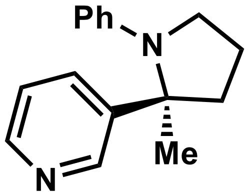
17

74% yield, 94:6 *e.r.*



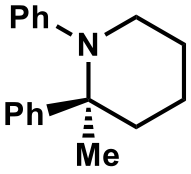
18

14% yield, 67:33 *e.r.*



19

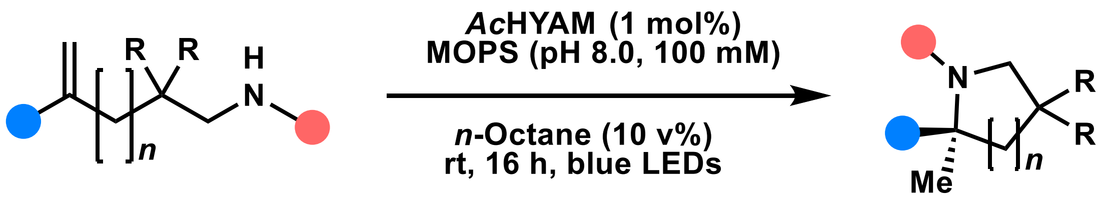
59% yield, 82:18 *e.r.*



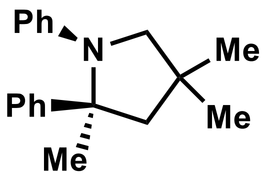
20

13% yield, 78:22 *e.r.*

Scope of C-N-forming reactions



Backbone modifications (n = 1)

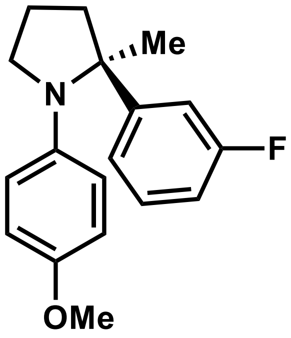


21

43% yield, 27:73 *e.r.*

API fragments (n = 1)

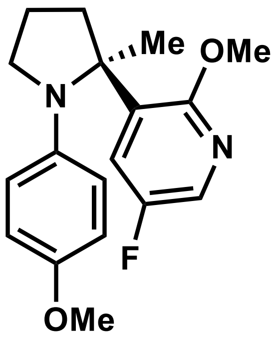
GNF-8625
alpha-methyl analogue



22

85% yield, 94:6 *e.r.*

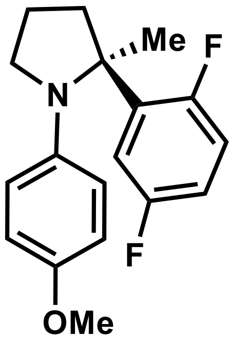
gp140trk inhibitor
alpha-methyl analogue



23

25% yield, 73:27 *e.r.*

Larotretinib
alpha-methyl analogue



24

77% yield, 75:25 *e.r.*

Investigations on Mechanism of the Reaction

? **Initiate the reaction through substrate oxidation**

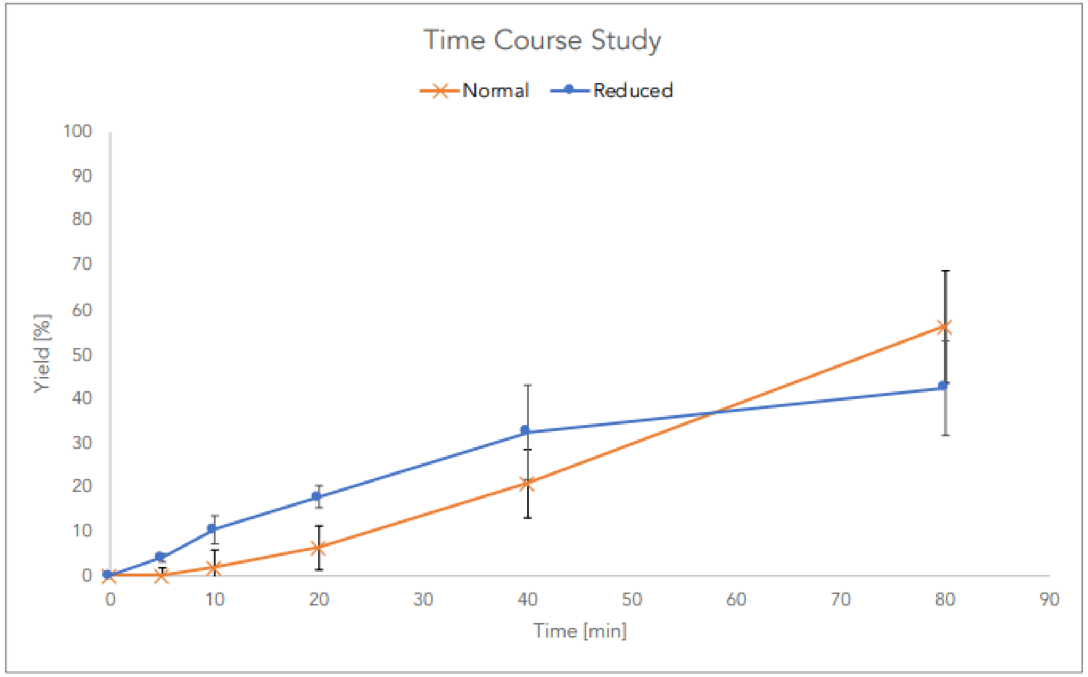
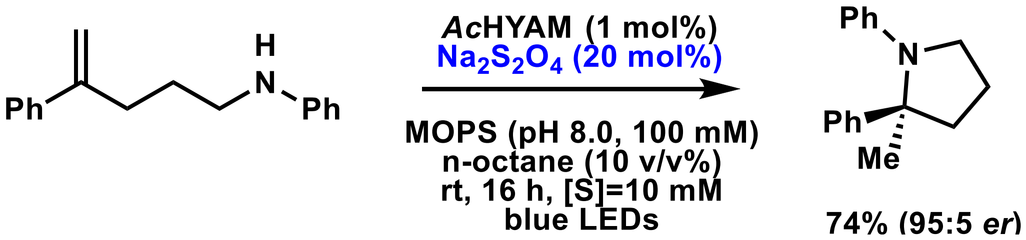


Fig. Time course study of standard reaction conditions versus the chemically reduced system



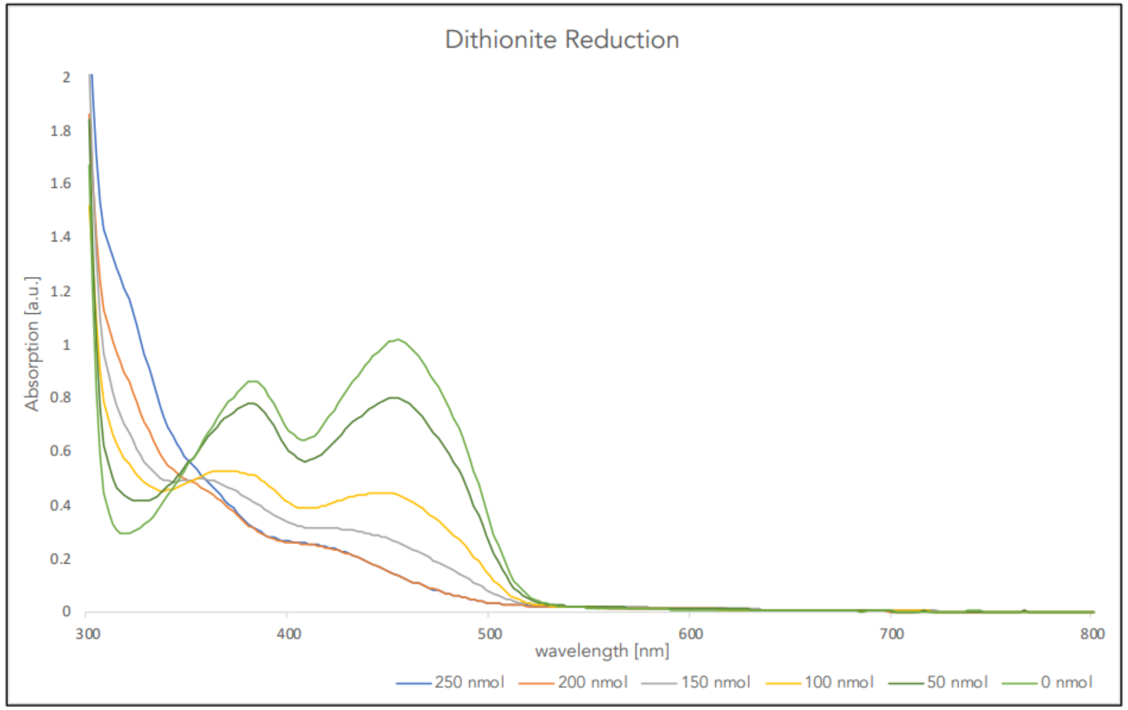
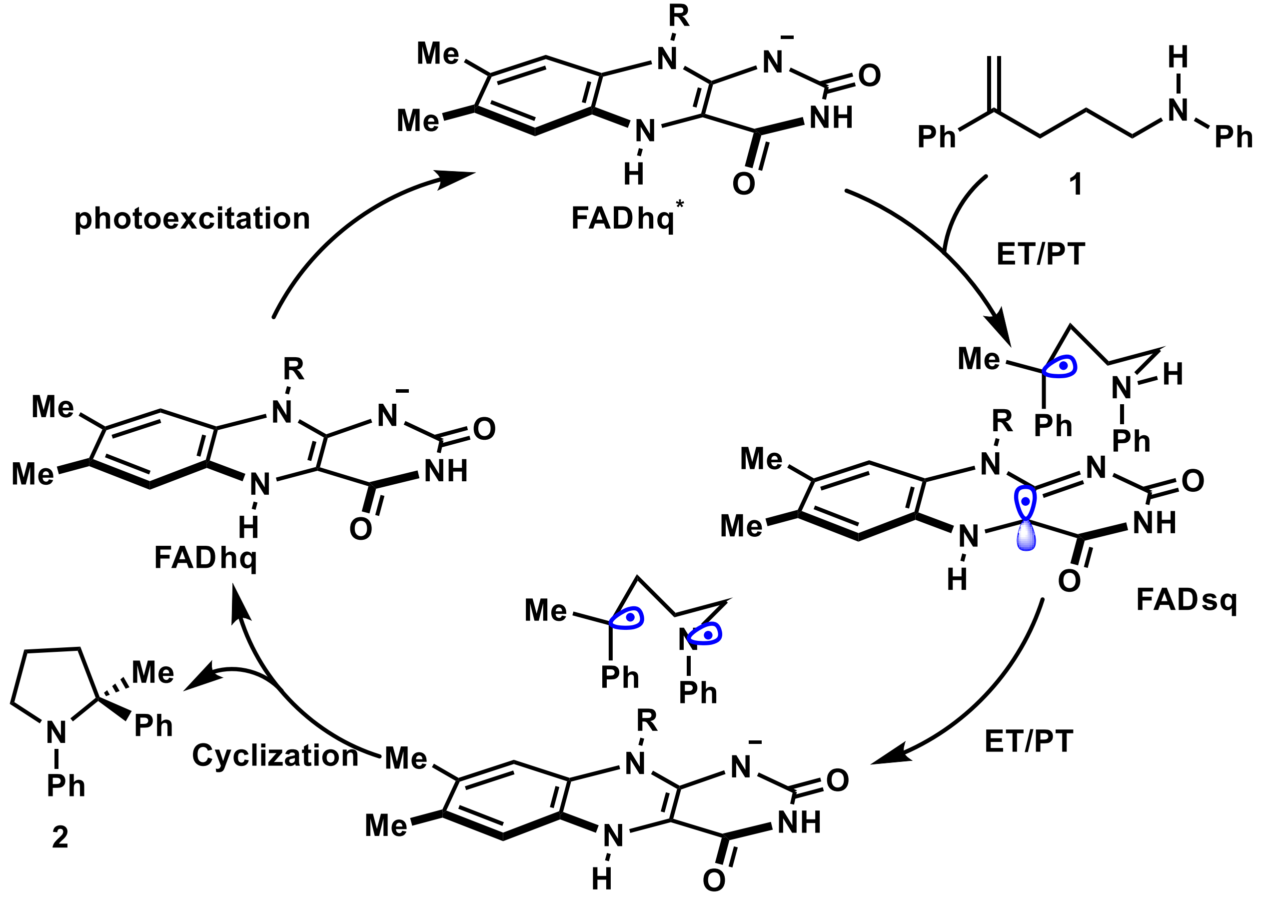


Fig. Dithionite reduced spectrum of *Ac*HYAM

- **The reaction initiates from the photoreduction of FAD to FADhq**
- **Exclude a nitrogen-centred radical as the reactive intermediate**

Investigations on Mechanism of the Reaction

? **Reduced flavin serving as an energy transfer catalyst (A diradical mechanism)**

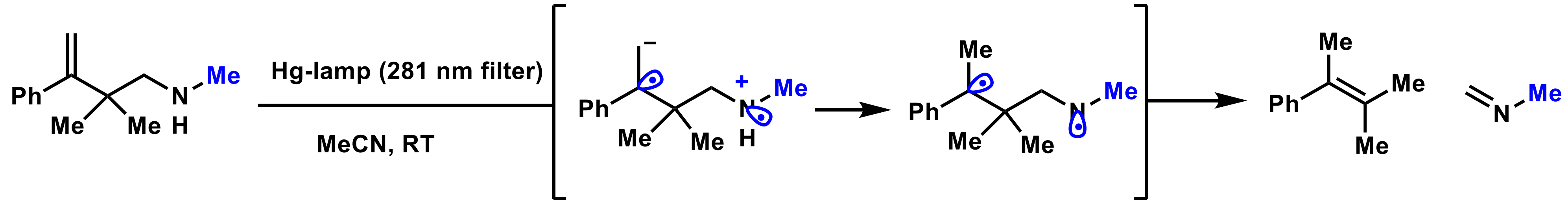


Investigations on Mechanism of the Reaction

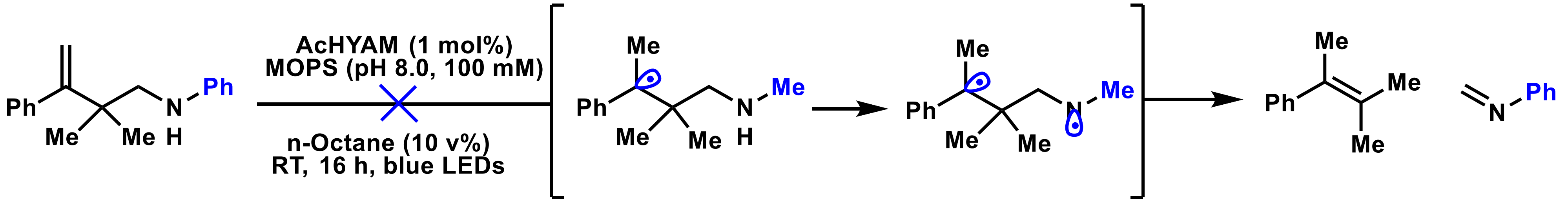
? A diradical mechanism

Probe of Diradical Character

Lewis et al.



Enzymatic Test

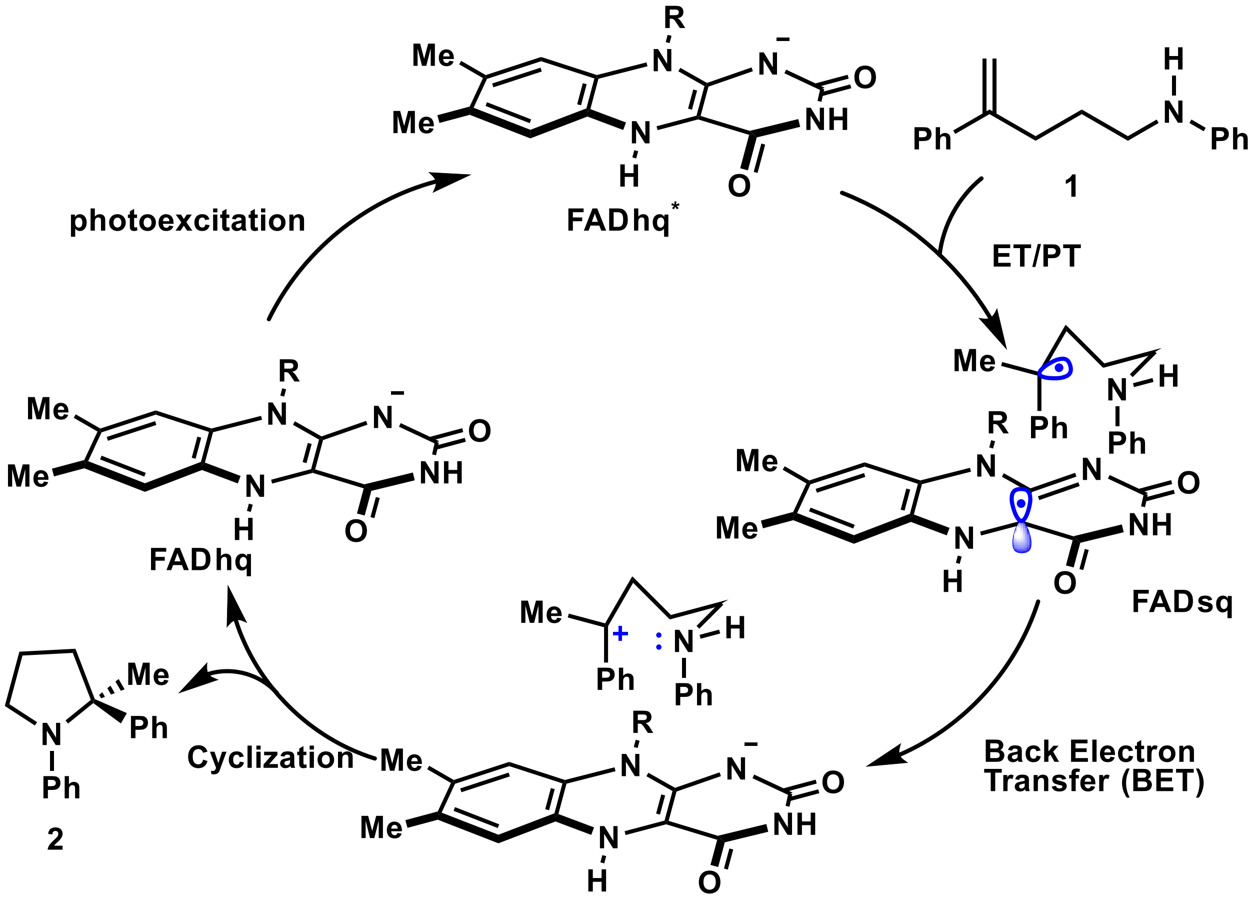


Proved to be incorrect. No fragmentation is observed

F. D. Lewis, D. M. Bassani and G. D. Reddy, *J. Org. Chem.*, 1993, **58**, 6390–6393.

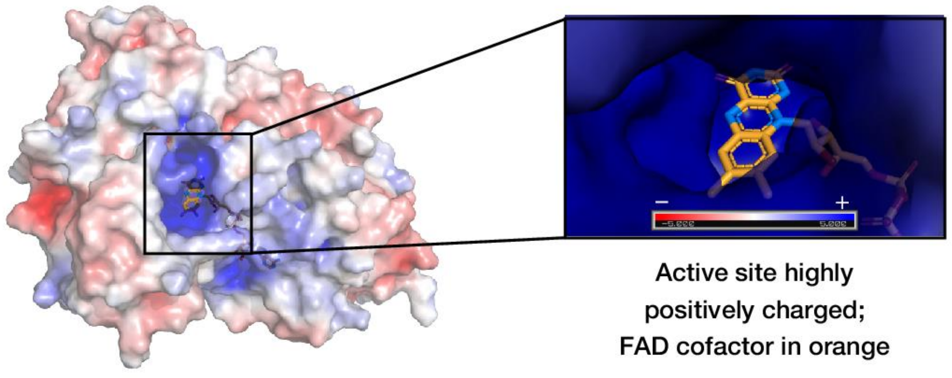
Investigations on Mechanism of the Reaction

? Alkene reduction by FAD_{hq*} is responsible for radical initiation (A radical polar cross-over mechanism)



FAD_{hq*}: E_o = -2.26 V versus saturated calomel electrode

Substrate (α-methyl styrene): E_{red} = -2.6 V versus SCE



Electrostatic potential map of *Ac*HYAM

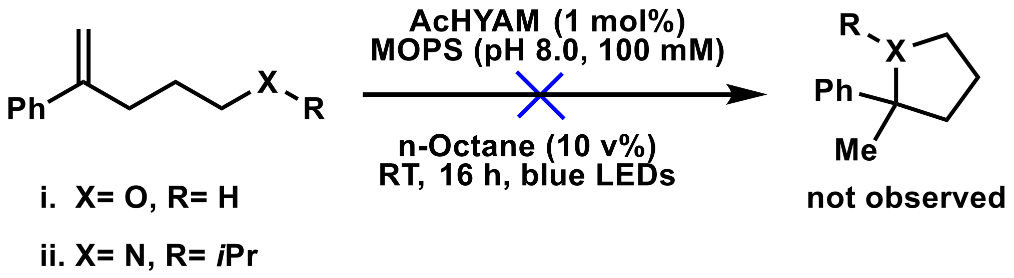
Active site highly positively charged; FAD cofactor in orange

- Attenuate the potential required to reduce the alkene
- Stabilize the resulting anionic flavin semiquinone (FAD_{sq}-)

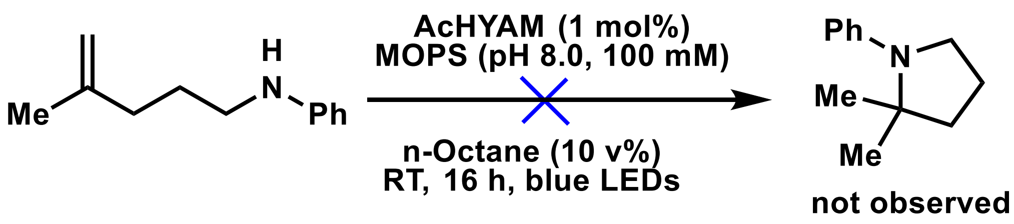
Investigations on Mechanism of the Reaction

? Alkene reduction by FADhq* is responsible for radical initiation (A radical polar cross-over mechanism)

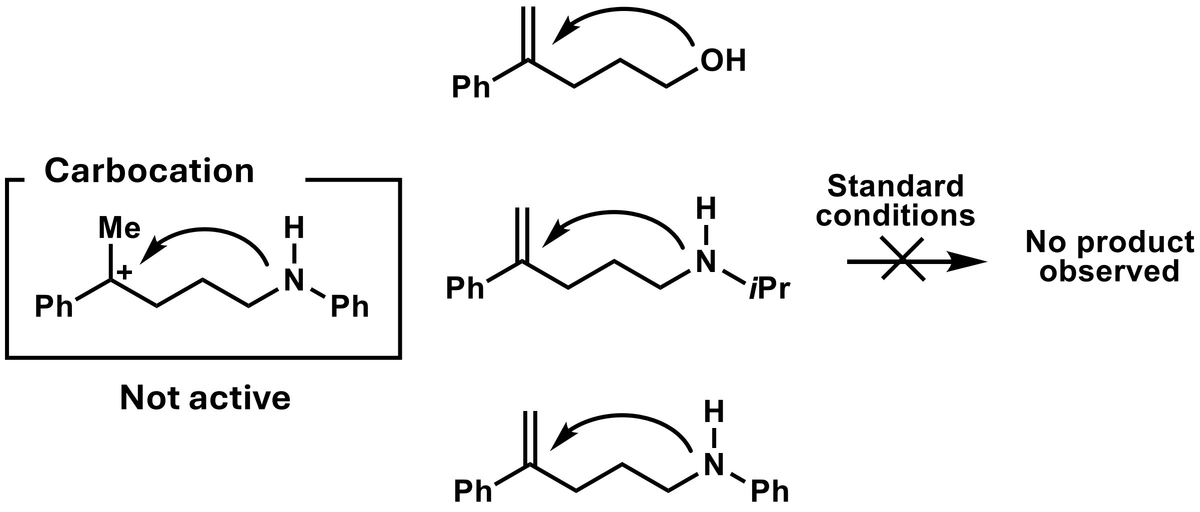
Cation probes



A carbocationic intermediate less likely to be involved



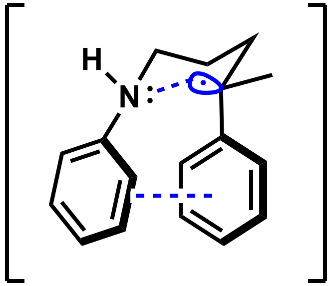
An ionic activation via proton transferless likely to be involved



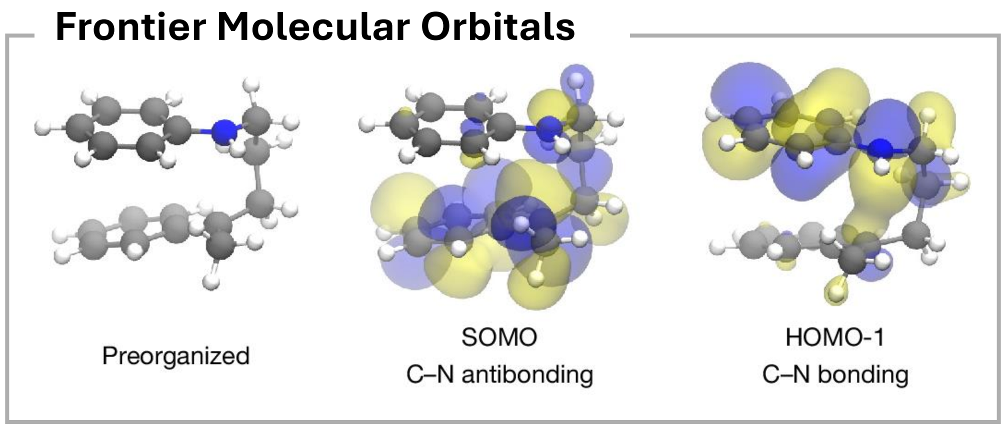
Proved to be incorrect. No product formation with the use of dialkyl substituted amines and alcohol substrates

Investigations on Mechanism of the Reaction

? **An interaction between the aniline and benzylic radical could alter the redox potential of the system**



Transannular n-*pi* interactions

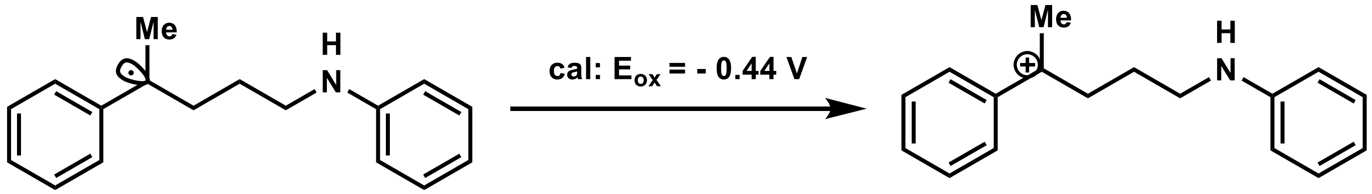


- **Hyperconjugative interactions between lone pairs and radicals will increase the energy of SOMO, making the radical easier to oxidise**
- **SOMO populates an antibonding configuration with the orbitals out of phase for C-N bond formation with strong character on the styrenyl arene**
- **HOMO has a bonding character between the nitrogen and benzylic radical with marked orbital coefficients on the aniline arene**

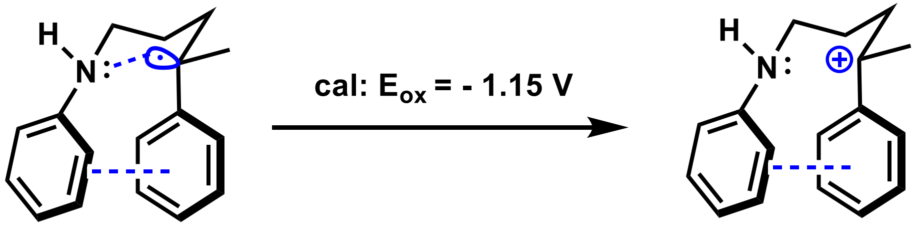
Investigations on Mechanism of the Reaction

? An interaction between the aniline and benzylic radical could alter the redox potential of the system

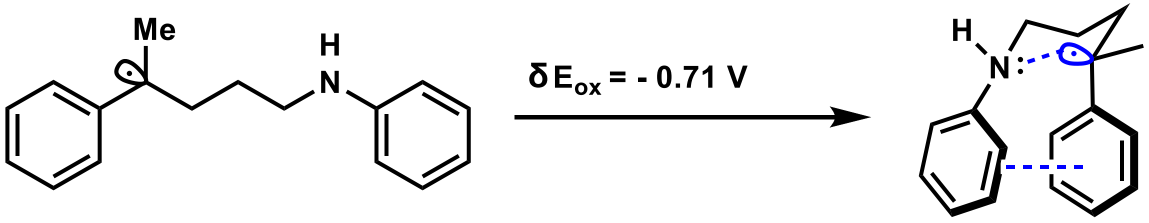
Open-form:



Aligned form:



Attenuation of oxidation potential

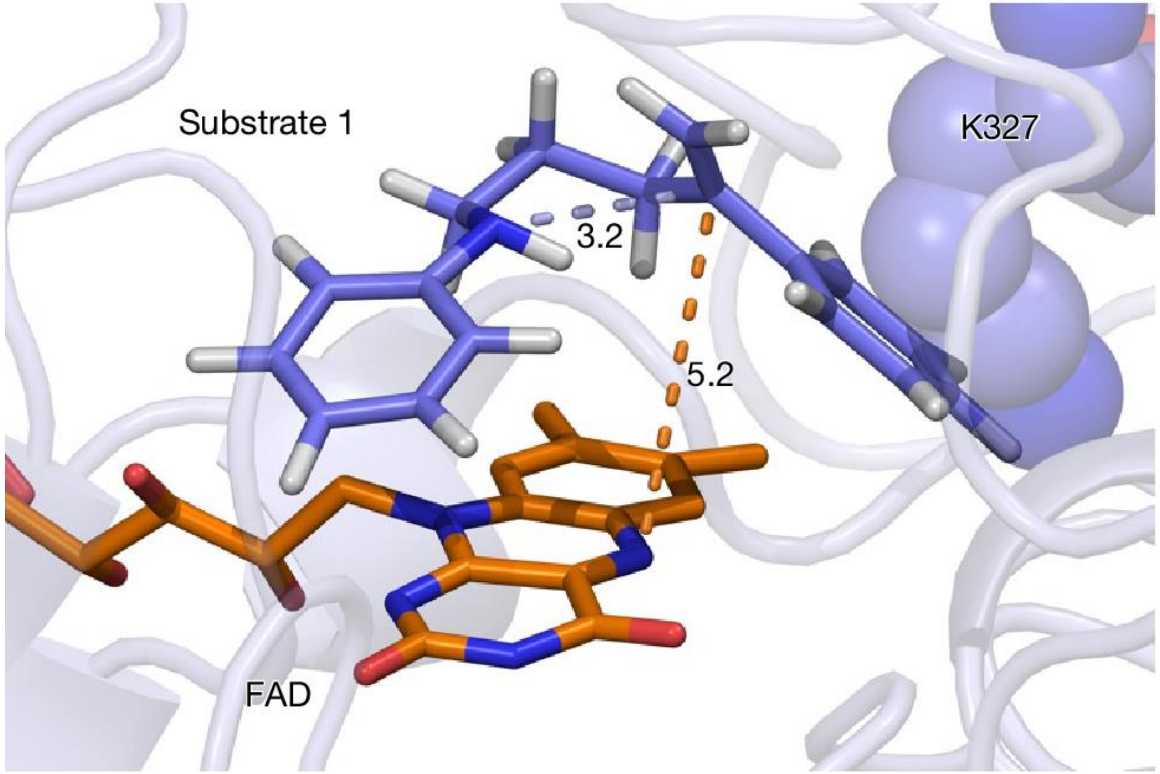


Open chain

Preorganized

Investigations on Mechanism of the Reaction

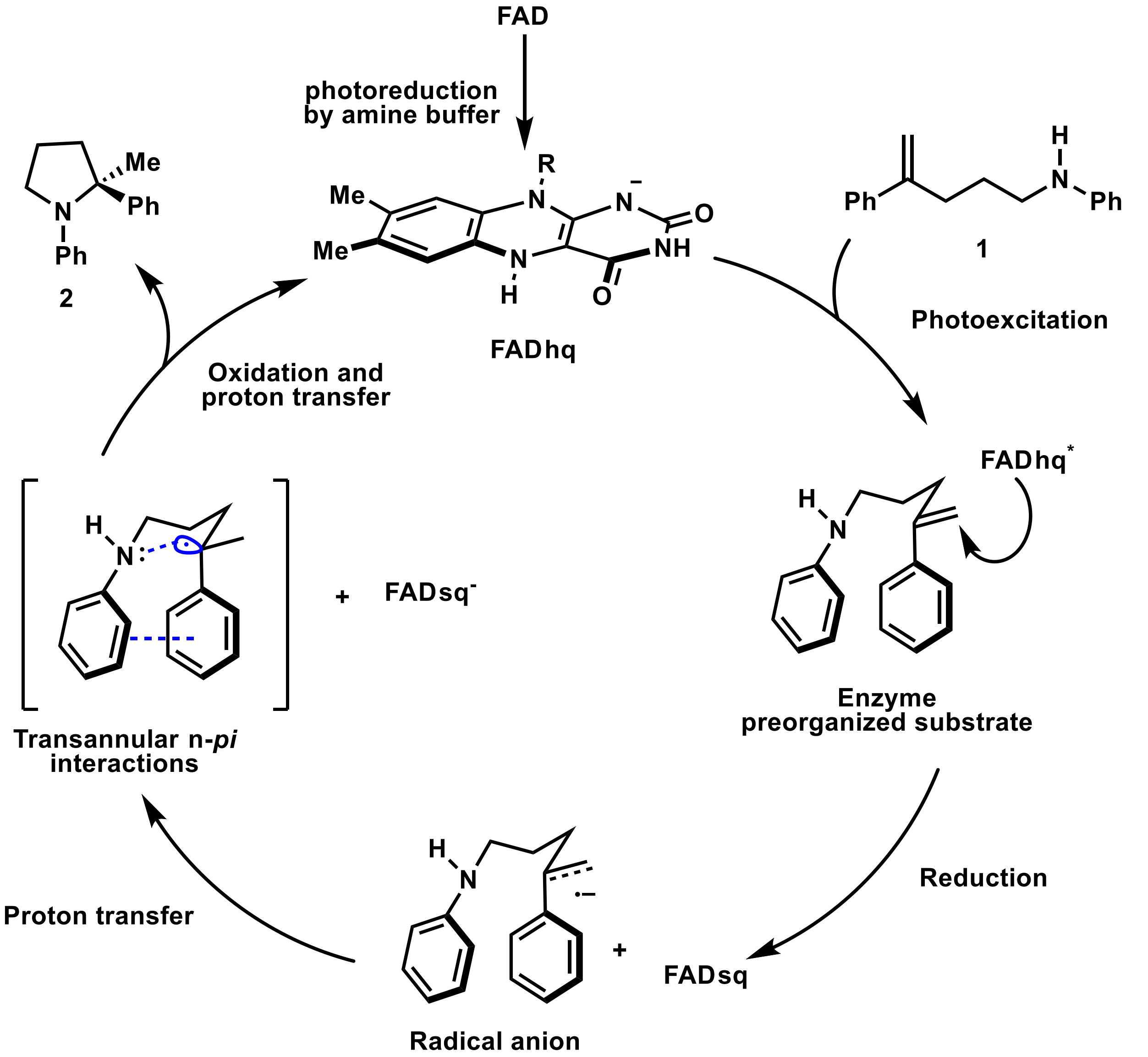
Snapshot of a model substrate binding conformation



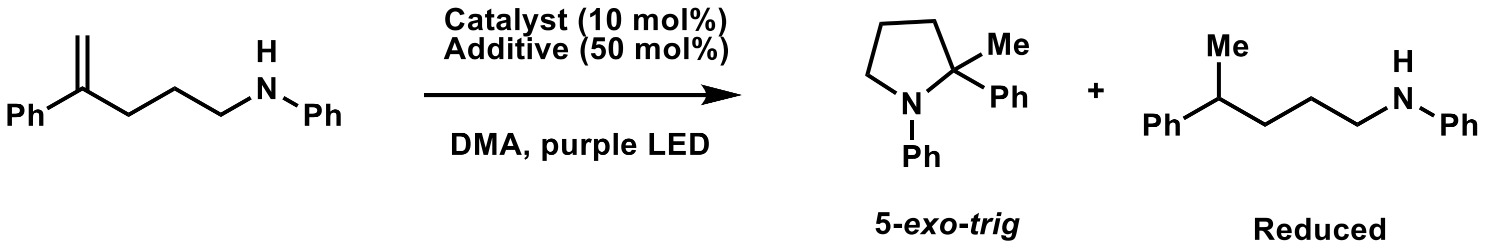
- **Preorganization provided by the protein**
- ***Ac*HYAM environment provides the substrate with more rotational freedom**
- **Amino acid substitutions (R327K and C326H) are probably responsible for the enhanced conformational flexibility**

Investigations on Mechanism of the Reaction

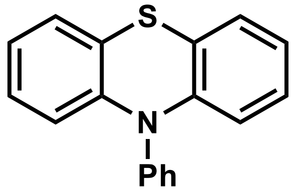
Relies on repulsive interaction from a two-centre three-electron antibonding intermediate to attenuate the oxidation potential of the radical in the active site.



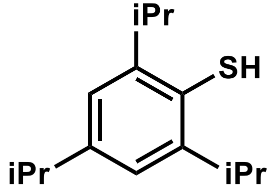
Further Investigations on Ingenuity — Small Molecule Tests



Entry	Catalyst	Additive	5-exo-trig	Reduced
1	10-Phenylphenothiazine	-	-	-
2	10-Phenylphenothiazine	TripSH	-	34%



10-Phenylphenothiazine
-2.10 V versus SCE

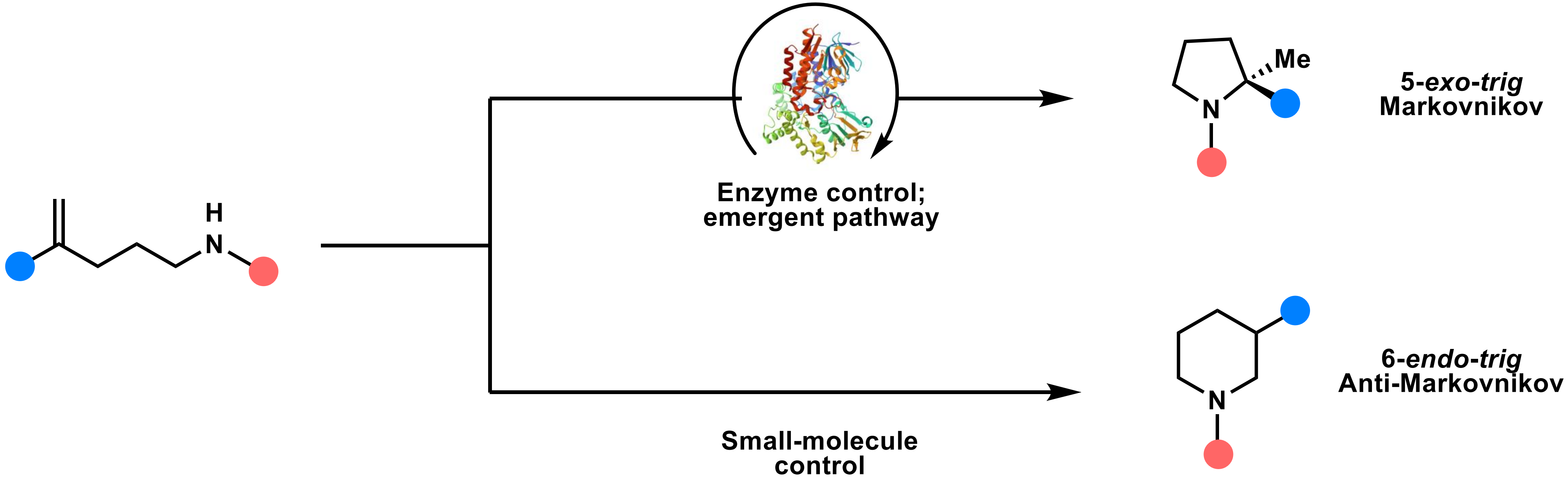


TripSH

Zhang, B. et al., *J. Am. Chem. Soc.*, 146, 1410–1422 (2024)

Conclusions

- **This work:**



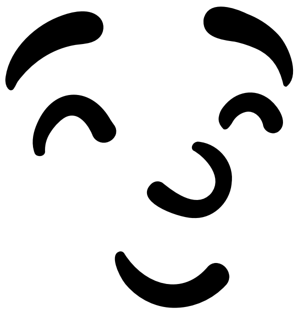
Addressed challenges:

1) Regioselectivity of cyclization

2) Stereoselectivity of α-tertiary amine centre

3) Unfunctionalized amine transformation by photoenzymes

F. C. Raps, A. Rivas-Souchet, C. M. Jones and T. K. Hyster, *Nature*, 2024, 1–3



Thanks for Your Listening
&
Happy Lunar New Year!