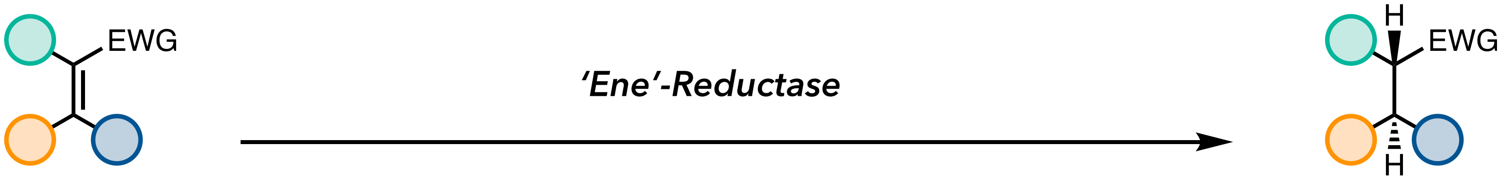
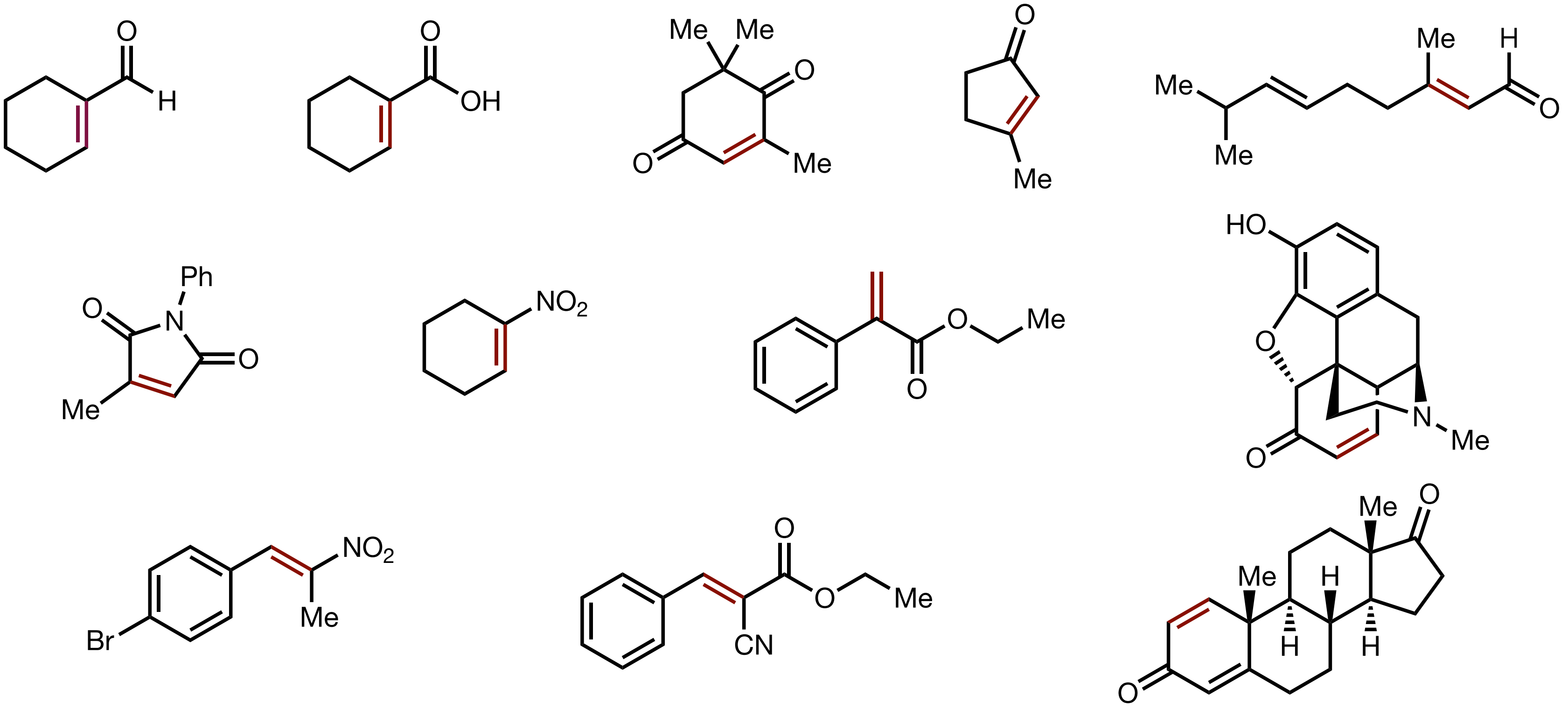
Ene-Reductases for Hydride Delivery

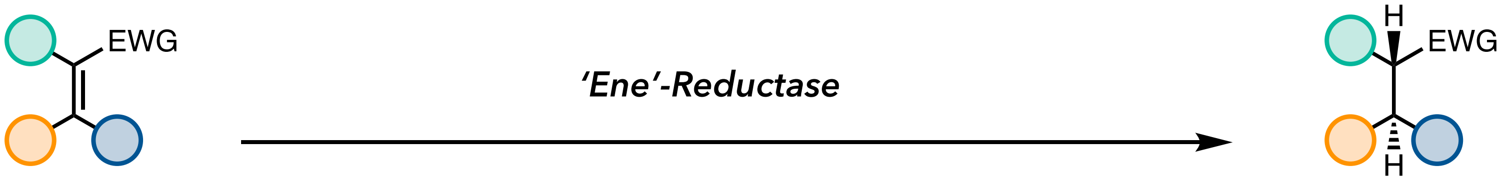


Ene-Reductases (EREDs) catalyze the asymmetric reduction of α,β-unsaturated carbonyl compounds

🟥 One of the most broadly substrate permissive enzyme classes across the family

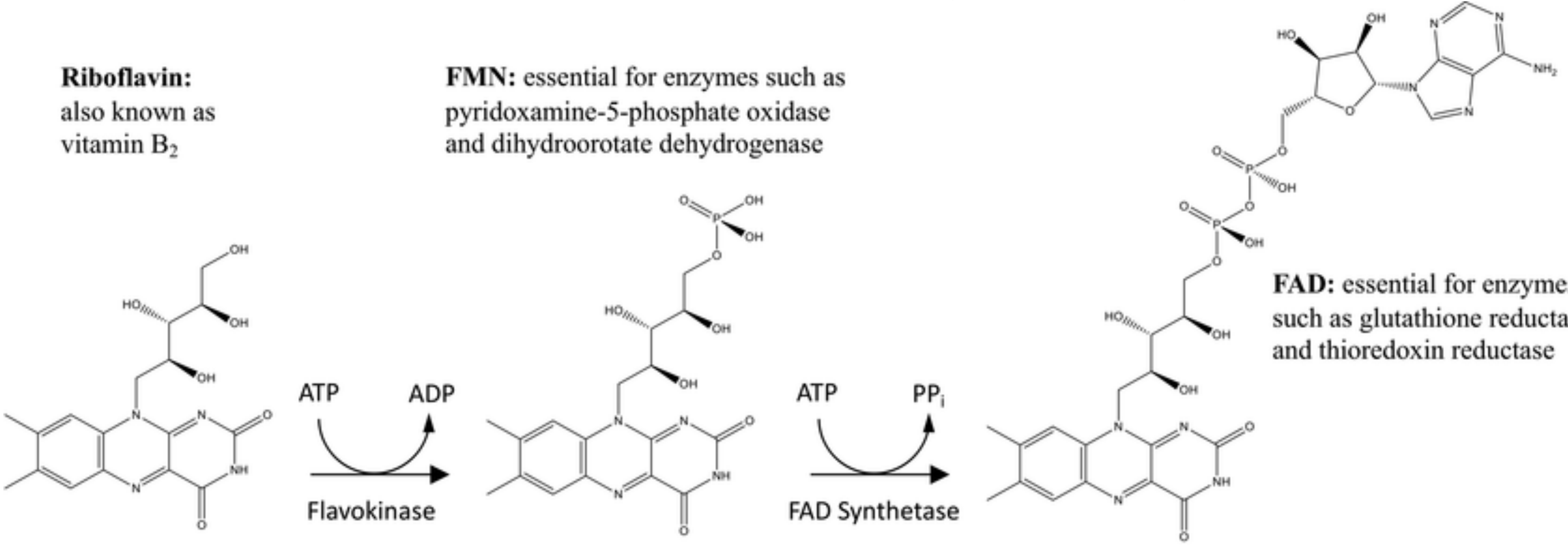


Ene-Reductases for Hydride Delivery



Ene-Reductases (EREDs) catalyze the asymmetric reduction of α,β-unsaturated carbonyl compounds

- Reliant on one of three flavin analogues for reactivity



Riboflavin: also known as vitamin B_2

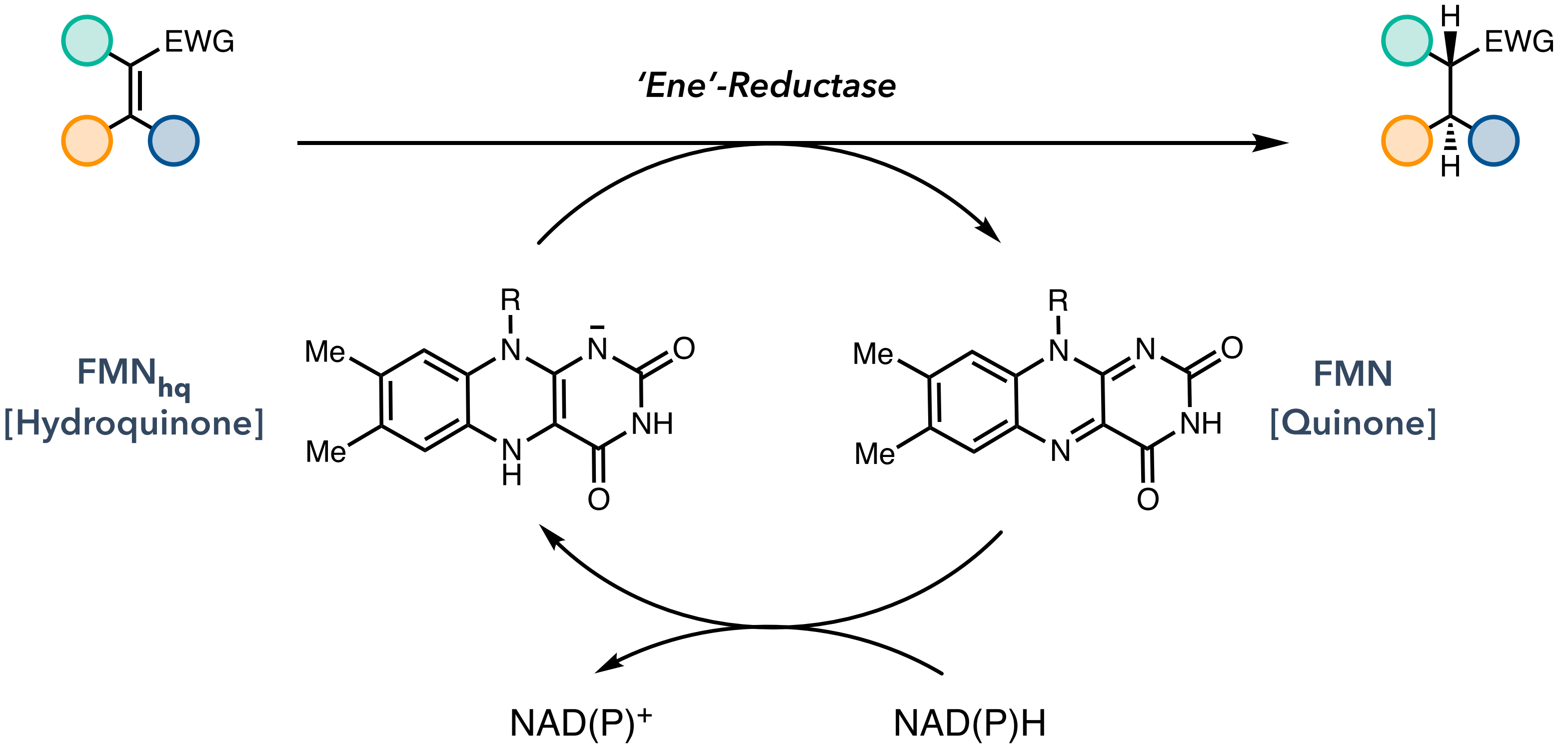
FMN: essential for enzymes such as pyridoxamine-5-phosphate oxidase and dihydroorotate dehydrogenase

FAD: essential for enzymes such as glutathione reductase and thioredoxin reductase

FMN = Flavin Mononucleotide

FAD = Flavin Adenine Dinucleotide

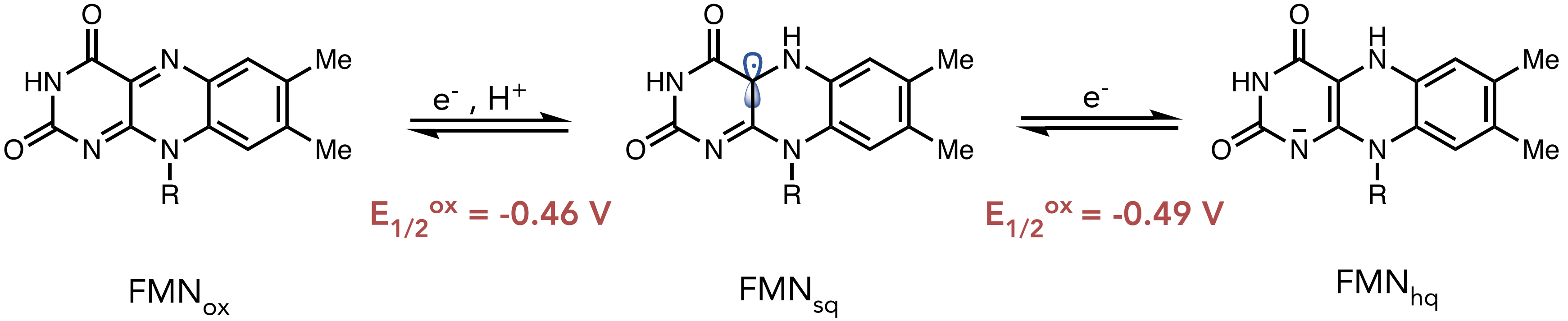
Ene-Reductases for Hydride Delivery



- EREDs primarily toggle between hydroquinone and quinone states in the ERED mechanism
- Flavin is reduced by the NAD(P)/NAD(P)H redox couple
  - For most cases, NAD(P)/NAD(P)H binds to the ERED and performs reduction of flavin
  - Flavin turnover systems based on single electron transfer are also very common (e-chem, photoredox)

Flavin: A Mechanistically Versatile Catalyst

FMN Redox Couples



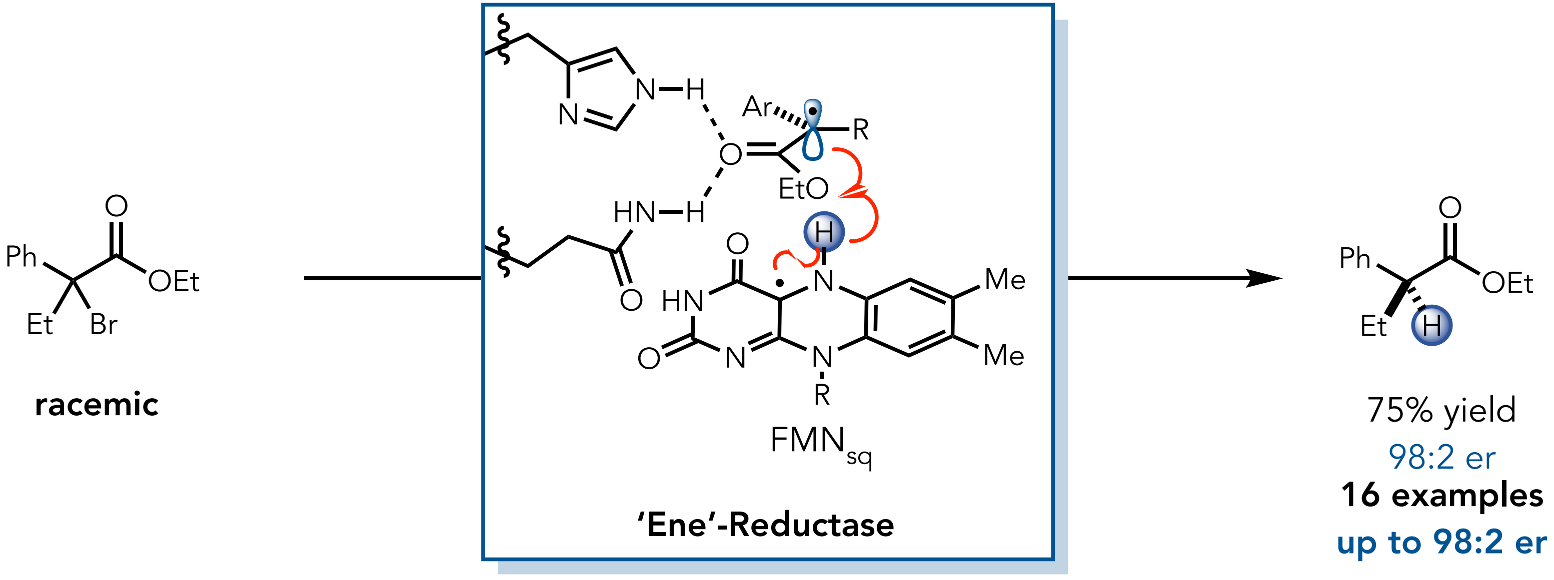
$E_{1/2}^{ox}$ = -0.46 V

$E_{1/2}^{ox}$ = -0.49 V

FMN_{ox}

FMN_{sq}

FMN_{hq}



racemic

'Ene'-Reductase

75% yield

98:2 er

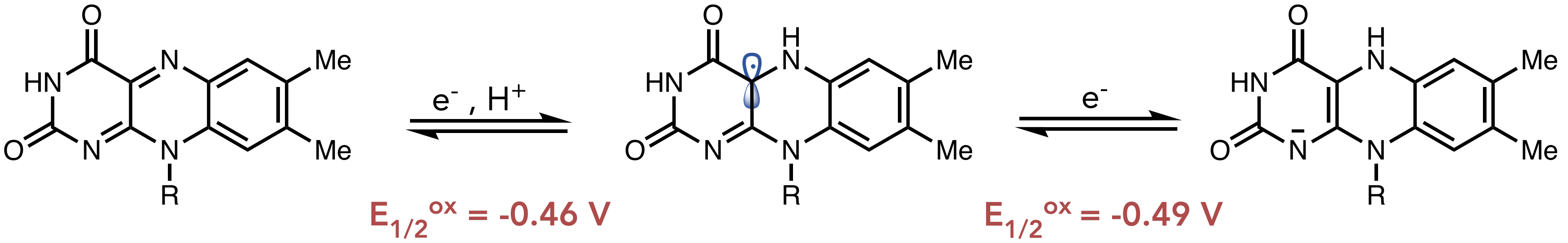
16 examples

up to 98:2 er

Sandoval, B.A.; Meichan, A.J.; Hyster, T.K. *J. Am. Chem. Soc.* **2017**, *139*(33), 11313.

Flavin: A Mechanistically Versatile Catalyst

FMN Redox Couples



$E_{1/2}^{ox} = -0.46$ V

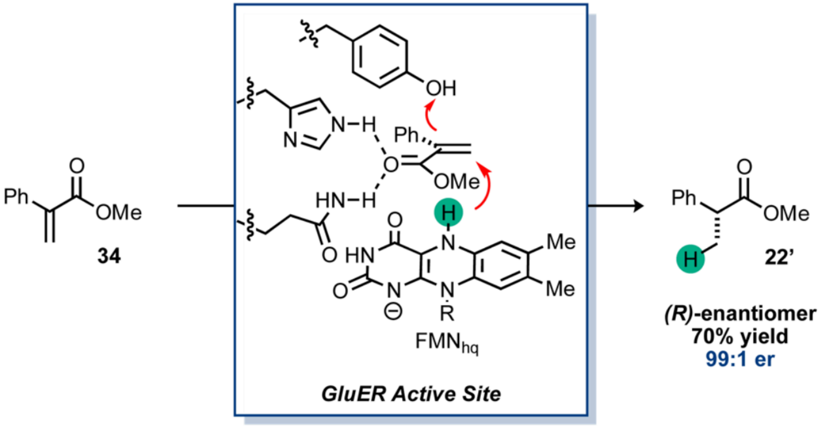
$E_{1/2}^{ox} = -0.49$ V

FMN_{ox}

FMN_{sq}

FMN_{hq}

a. Native Reactivity

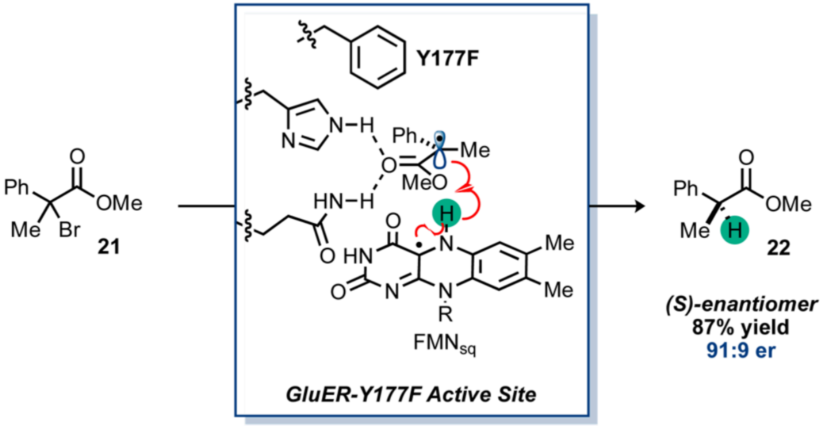


34

22'

***(R)*-enantiomer**
70% yield
99:1 er

b. Non-Natural Reactivity



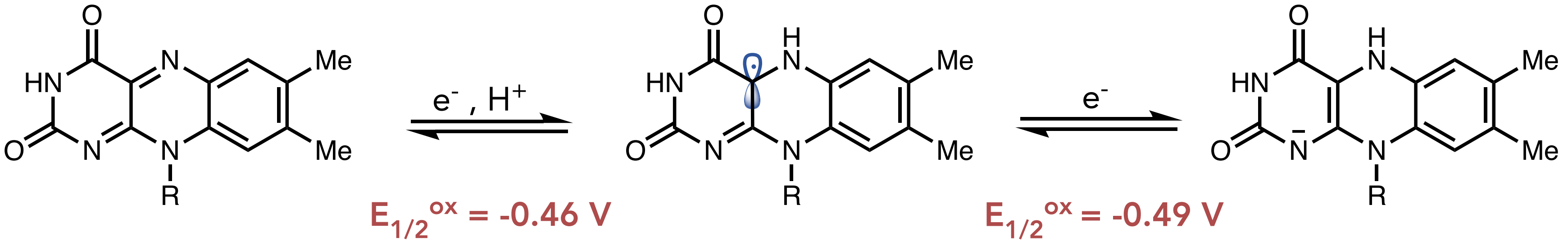
21

22

***(S)*-enantiomer**
87% yield
91:9 er

Flavin: A Mechanistically Versatile Catalyst

FMN Redox Couples



$E_{1/2}^{ox} = -0.46$ V

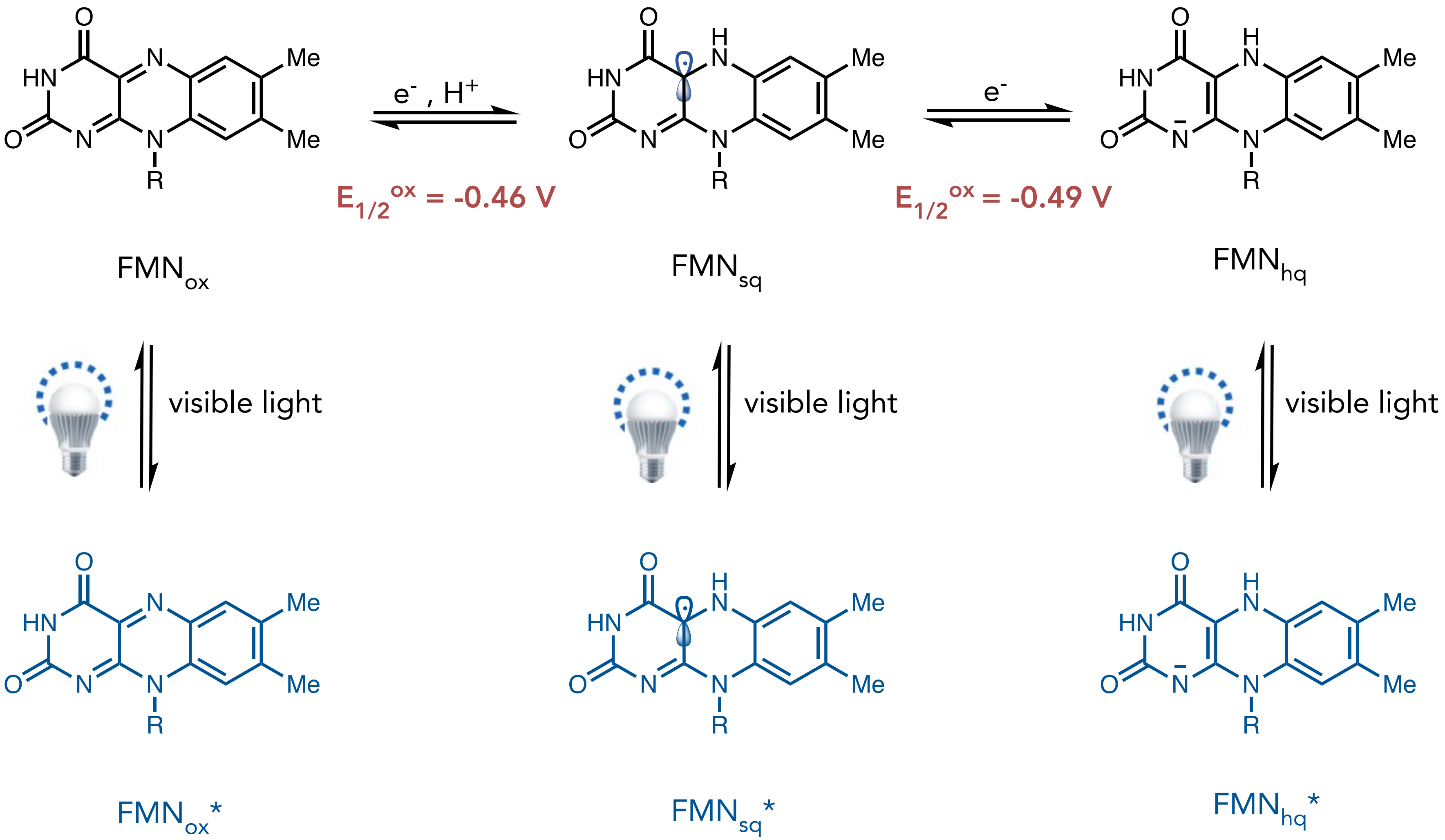
$E_{1/2}^{ox} = -0.49$ V

FMN_{ox} FMN_{sq} FMN_{hq}

How do we expand the redox window of flavin to access substrates that are more challenging to reduce?

Flavin: A Mechanistically Versatile Catalyst

Expanded Redox Versatility



e^- , H^+

$E_{1/2}^{ox}$ = -0.46 V

e^-

$E_{1/2}^{ox}$ = -0.49 V

FMN_{ox}

FMN_{sq}

FMN_{hq}

visible light

visible light

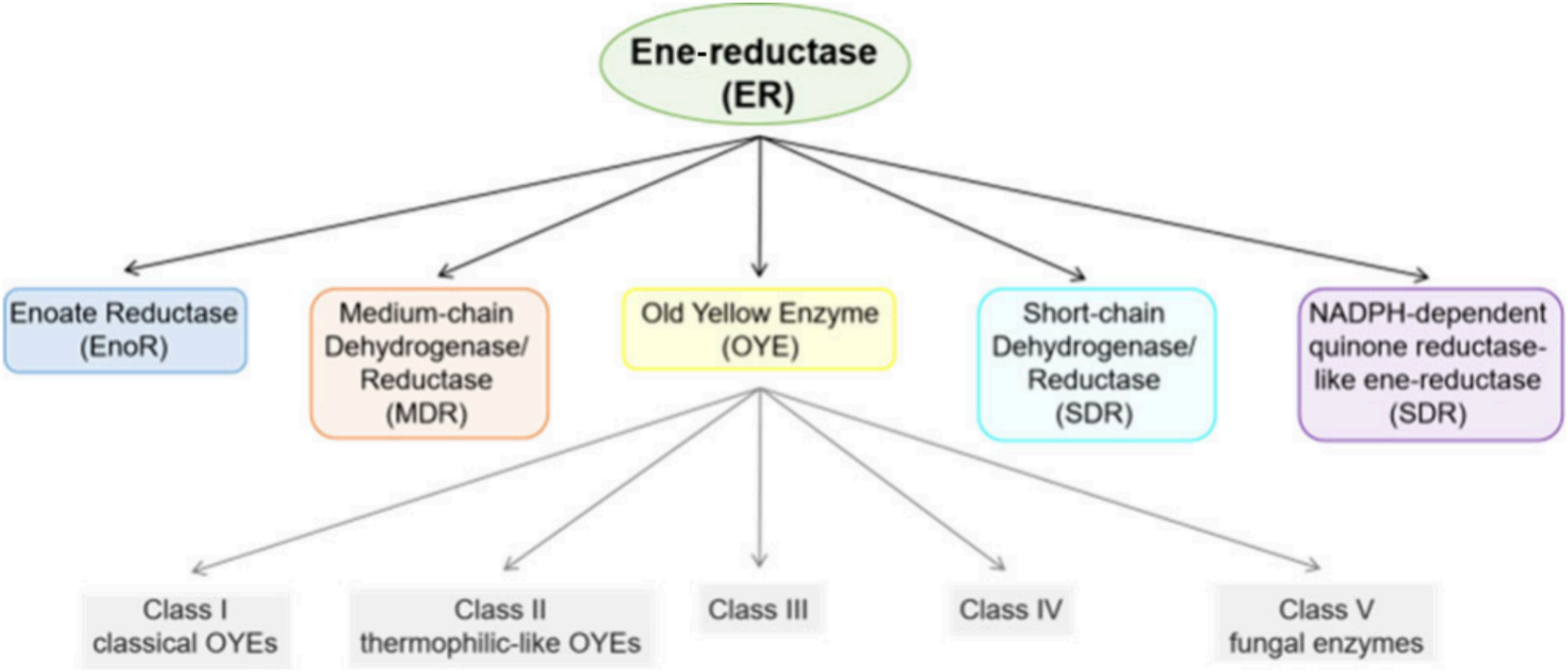
visible light

FMN_{ox}*

FMN_{sq}*

FMN_{hq}*

Classification of EREDs



Ene-reductase (ER)

Enoate Reductase (EnoR)

Medium-chain Dehydrogenase/ Reductase (MDR)

Old Yellow Enzyme (OYE)

Short-chain Dehydrogenase/ Reductase (SDR)

NADPH-dependent quinone reductase-like ene-reductase (SDR)

Class I classical OYEs

Class II thermophilic-like OYEs

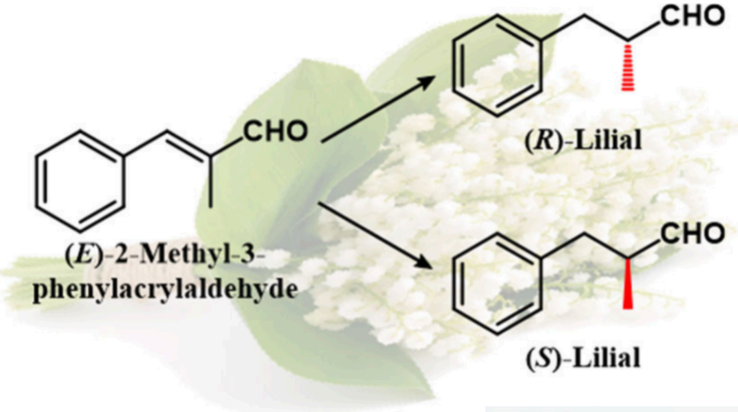
Class III

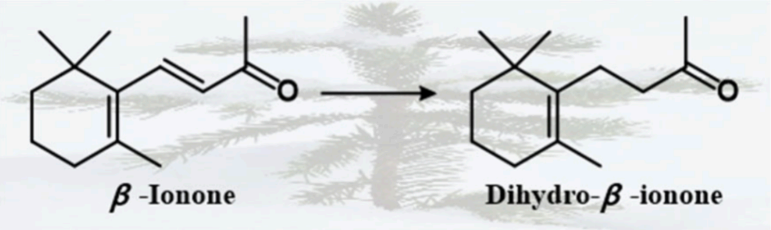
Class IV

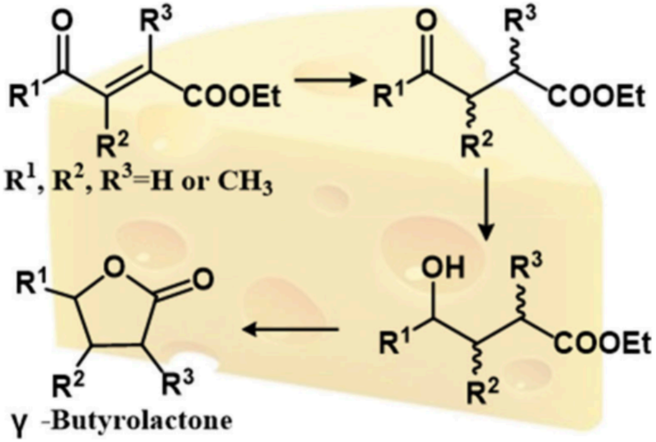
Class V fungal enzymes

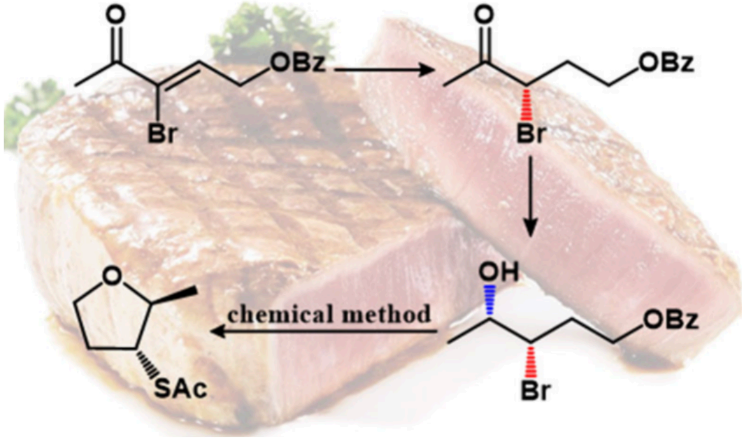
J. Agric. Food Chem **2024**, *72*, 18305-18320.

EREDs in Flavors and Frangrances



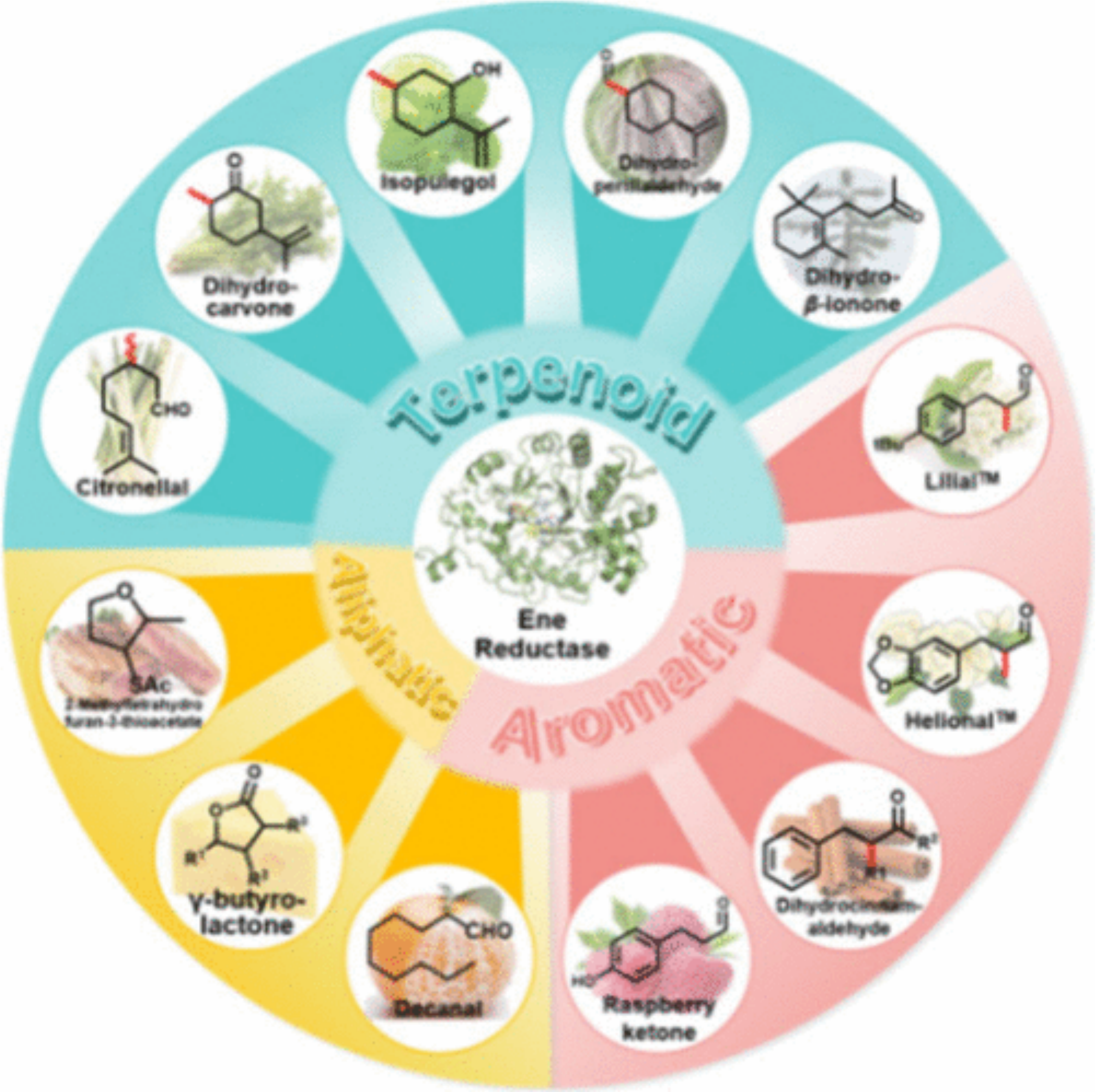






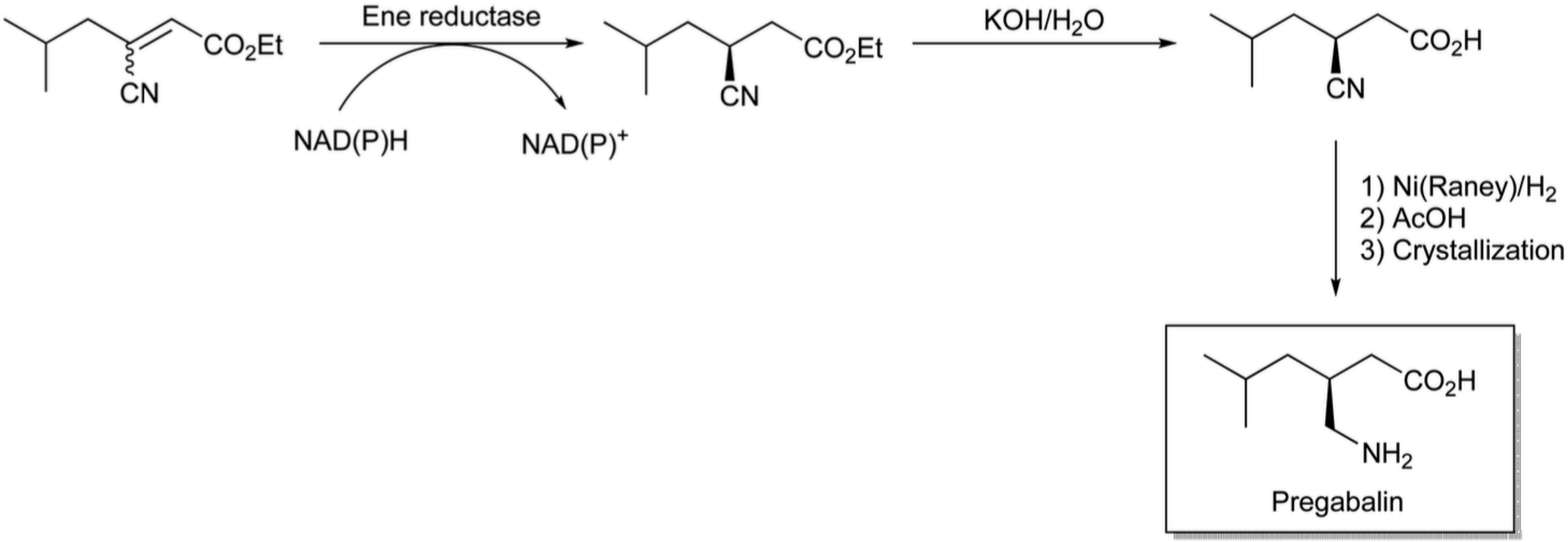
J. Agric. Food Chem **2024**, *72*, 18305-18320.

EREDs in Flavors and Frangrances



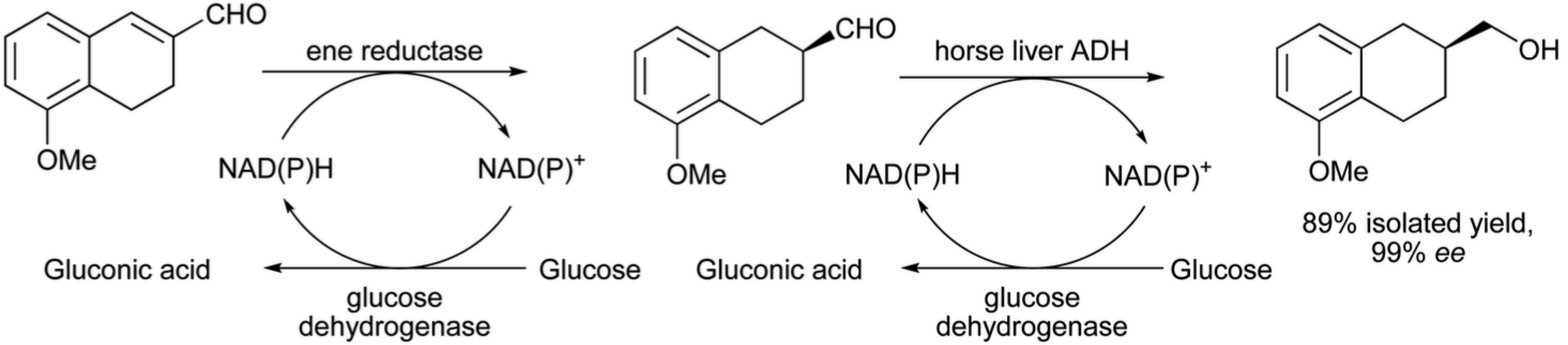
J. Agric. Food Chem **2024**, *72*, 18305-18320.

ERED Applications in Industry: Toward Pregabalin (Lyrica: Nerve Pain Medication)



Almac Industrial Perspective **2024**

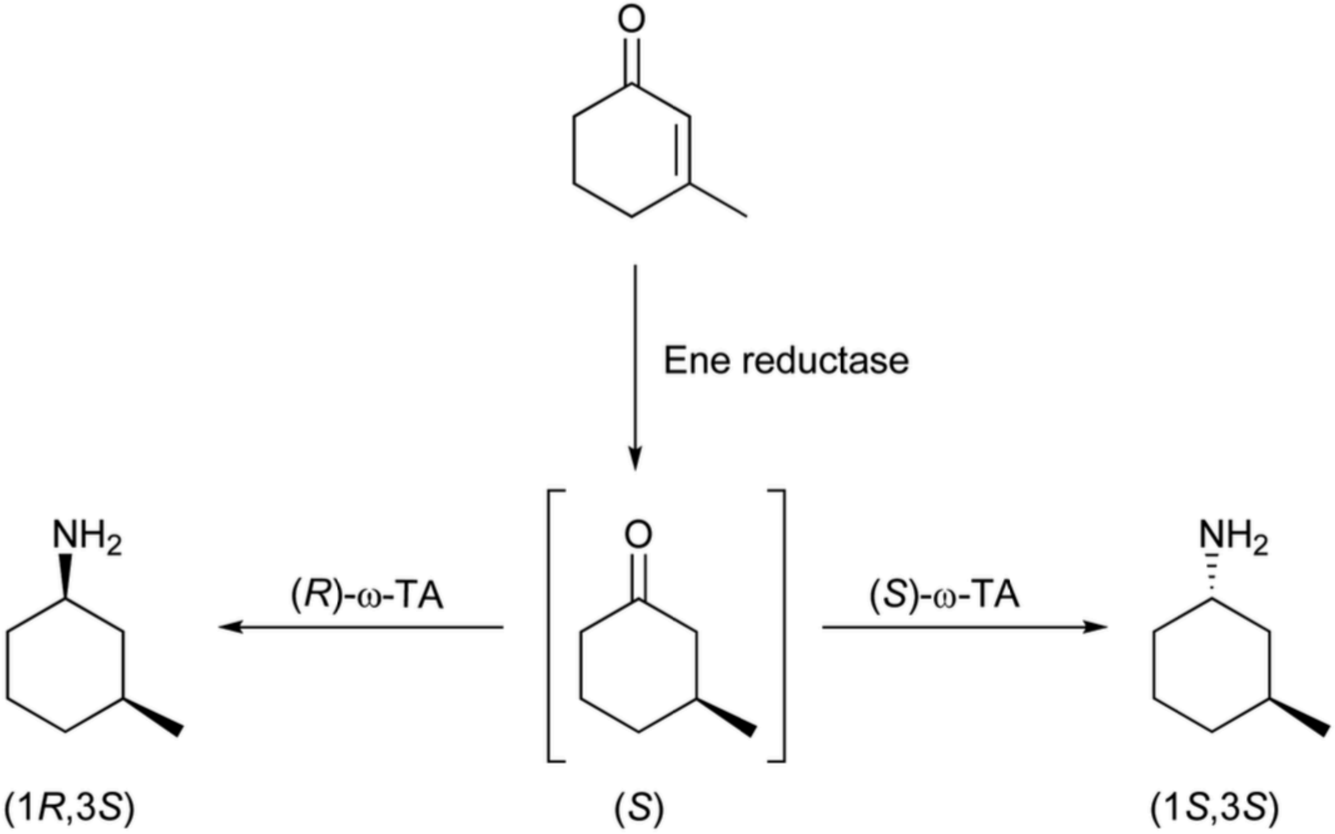
ERED Applications in Industry: Gluconic Acid to CNS Drugs



89% isolated yield, 99% *ee*

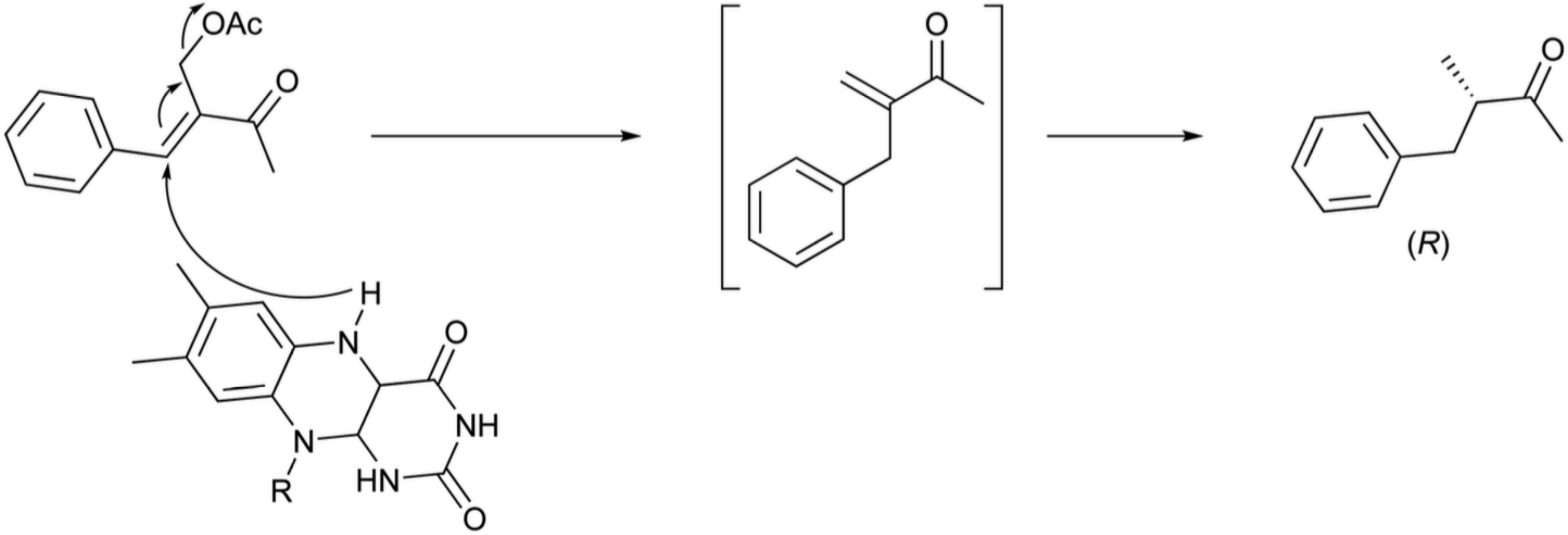
Almac Industrial Perspective **2024**

ERED Applications in Industry: Cascade Reactions with ATAs



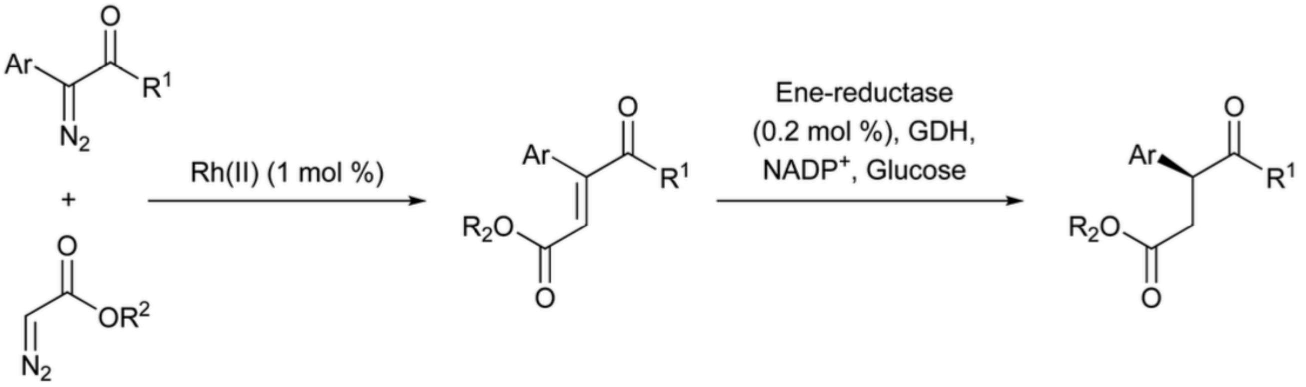
Almac Industrial Perspective **2024**

ERED Applications in Industry: Tandem S_N2' and Stereocenter Formation



Almac Industrial Perspective **2024**

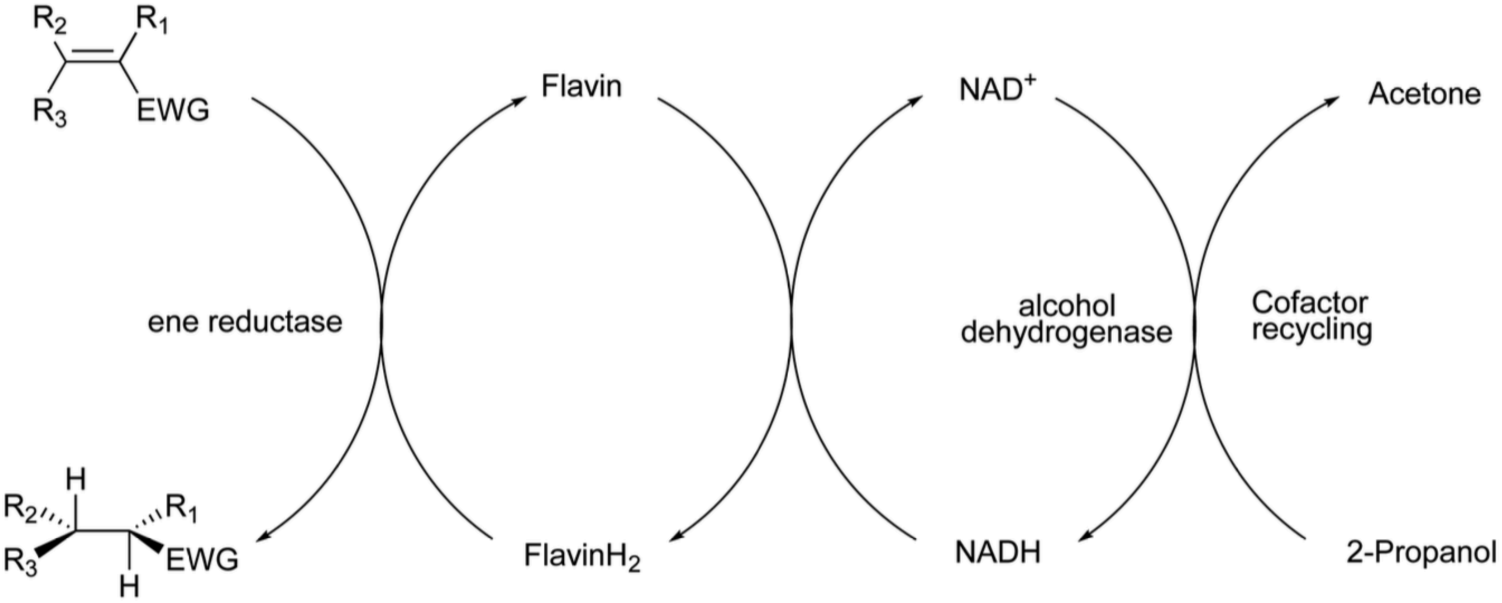
ERED Applications in Industry: Diazo Coupling and ERED Reduction



R_1: OMe, Me
R_2: OEt, O^tBu, OBn, 4-F-Ph
up to 88 % yield,
99 % *ee*,
>7000 turnover

Almac Industrial Perspective **2024**

Turnover Systems for EREDs on Industrial Scale



β-D-Glucose + NAD$^+$ $\xrightarrow[\text{dehydrogenase}]{\text{glucose}}$ D-Glucono-1,5-lactone + NADH

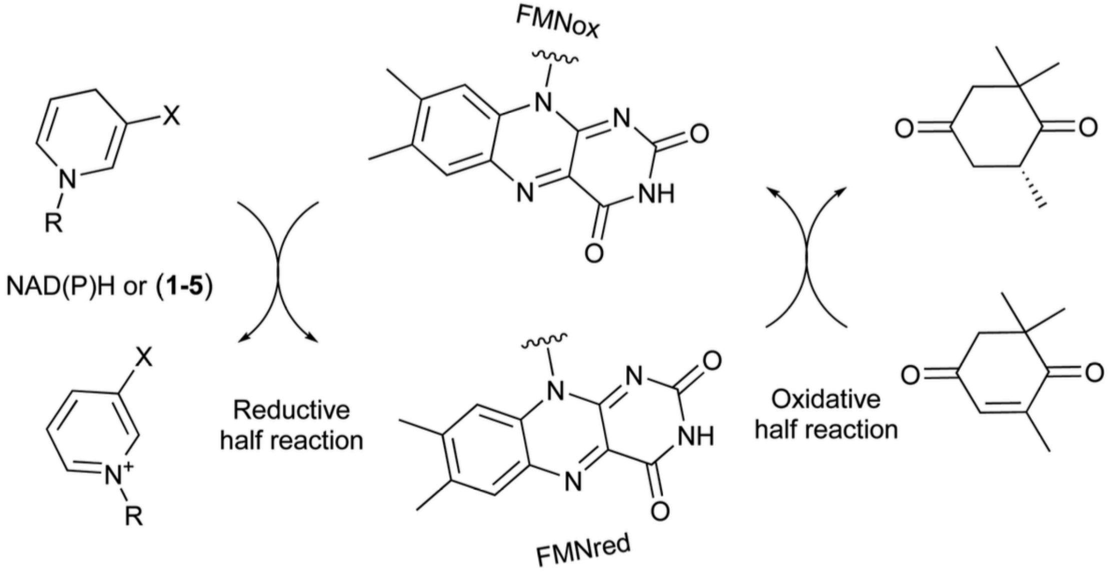
β-D-Glucose-6-phosphate + NADP$^+$ $\xrightarrow[\text{dehydrogenase}]{\text{glucose-6-phosphate}}$ D-Glucono-1,5-lactone-6-phosphate + NADPH

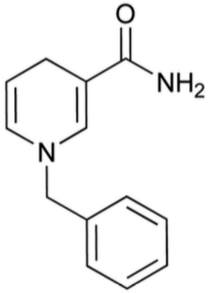
Formate + NAD$^+$ $\xrightarrow[\text{dehydrogenase}]{\text{formate}}$ CO_2 + NADH

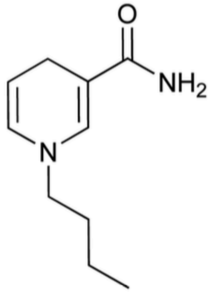
Phosphite + NAD$^+$ $\xrightarrow[\text{dehydrogenase}]{\text{phosphite}}$ Phosphate + NADH

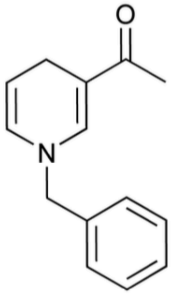
Almac Industrial Perspective **2024**

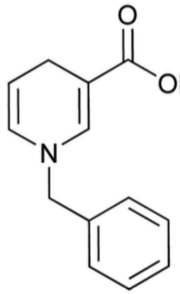
Common Turnover Systems to Avoid Nicotinamide: Biomimetics

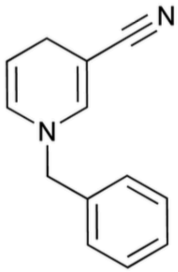



mNH$_2$ (**1**)


mBu (**2**)

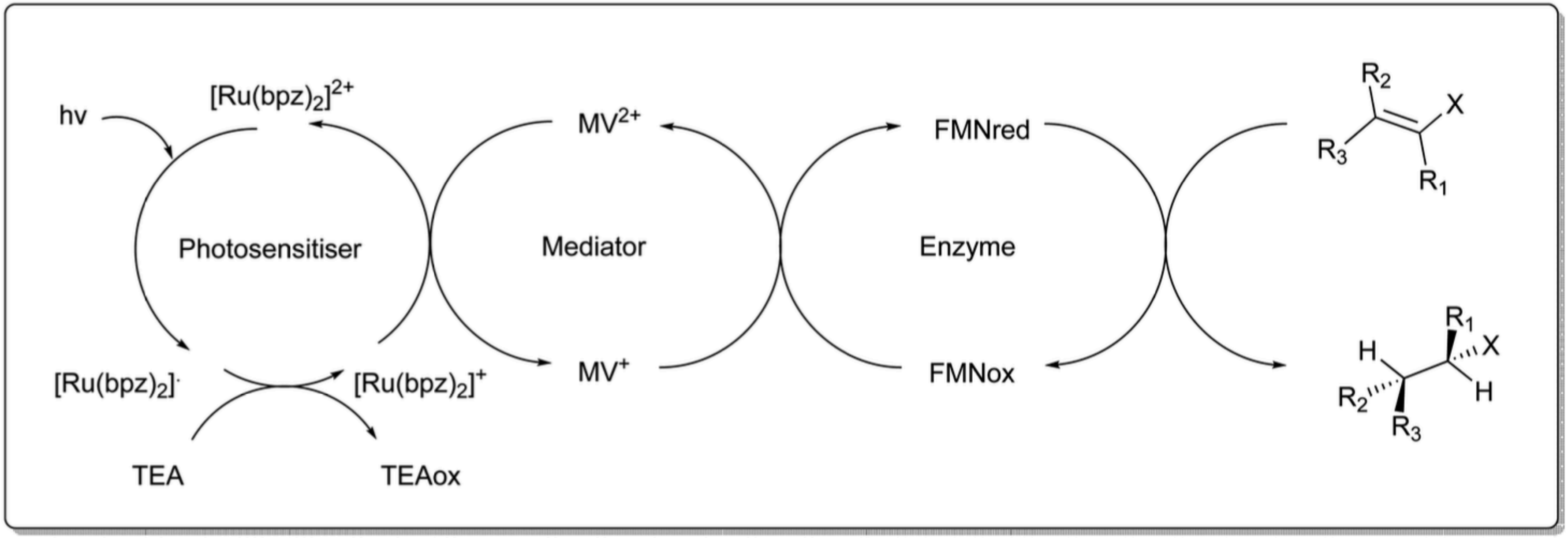

mAc (**3**)


mCOOH (**4**)


mCN (**5**)

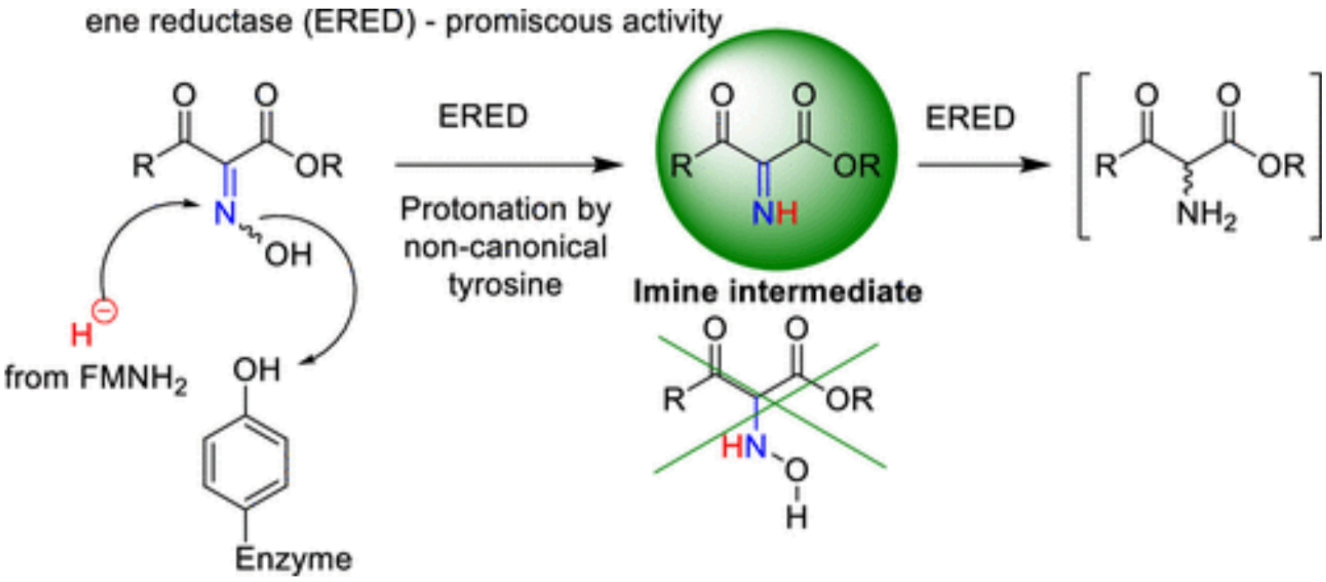
Almac Industrial Perspective **2024**

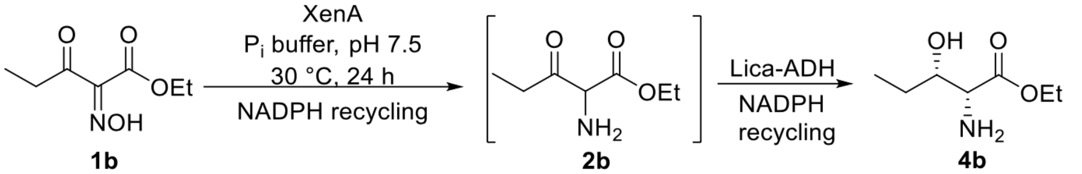
Common Turnover Systems to Avoid Nicotinamide: Reduction Through Photoredox



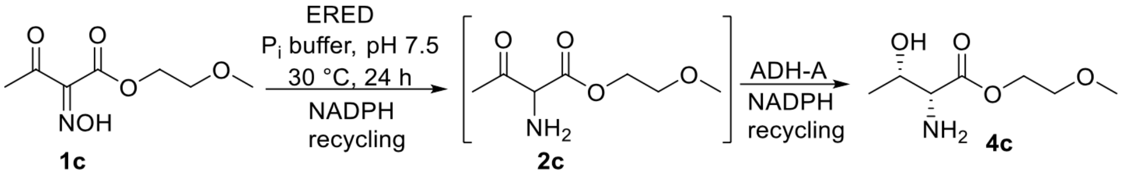
Almac Industrial Perspective **2024**

Emerging Applications of EREDs: Oxime Reduction





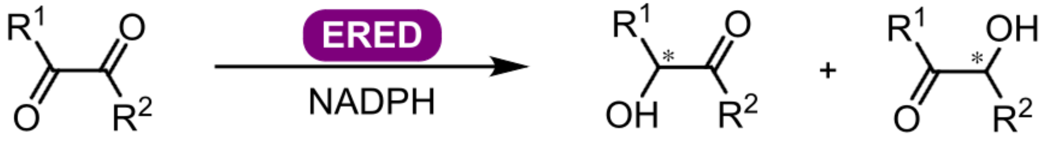
75:25 (2*R*,3*S*):(2*S*,3*S*)
68% product formation
57% isolated yield, 305 mg



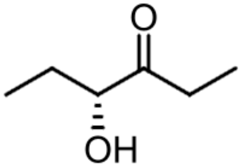
98% ee, 93% de for (2*R*,3*S*)
44% product formation (analytical scale, OPR3)
27% isolated yield, 154 mg (preparative scale, OYE3)

ACS Catal. **2023**, 13, 2610-2618.

Emerging Applications of EREDs: ERED Becomes a KRED

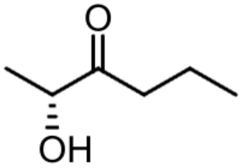


1a-10a **1b-10b** **1c-10c**



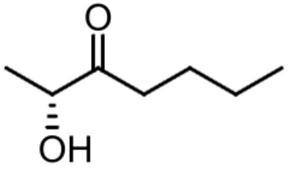
1b

GluER 8% *ee*: 92%



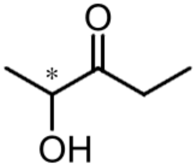
2b

GluER 19% *ee*: 83%



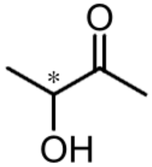
3b

GluER 17% *ee*: 67%



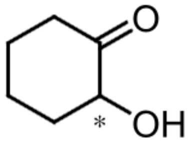
4b

GluER <1%



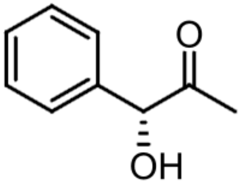
5b

GluER <1%



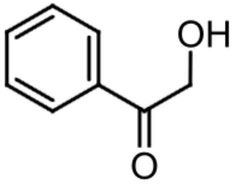
6b

OYE2 2%
*Ts*OYE 2%



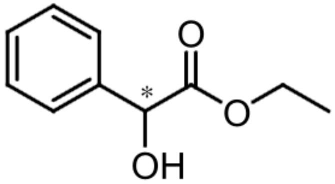
7b

GluER 37% *ee*: 79%
OYE3 91% *ee*: >99.9%
OYE2 79% *ee*: >99%
YqjM 2%
*Ts*OYE 14% *ee*: 74%
*Nt*DBR 6% *ee*: 21%



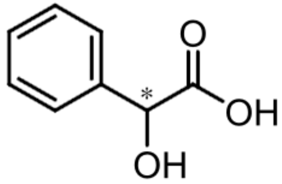
8c

GluER 93%
OYE3 86%
OYE2 62%
YqjM 55%
*Ts*OYE 47%
*Nt*DBR 30%



9b

0%

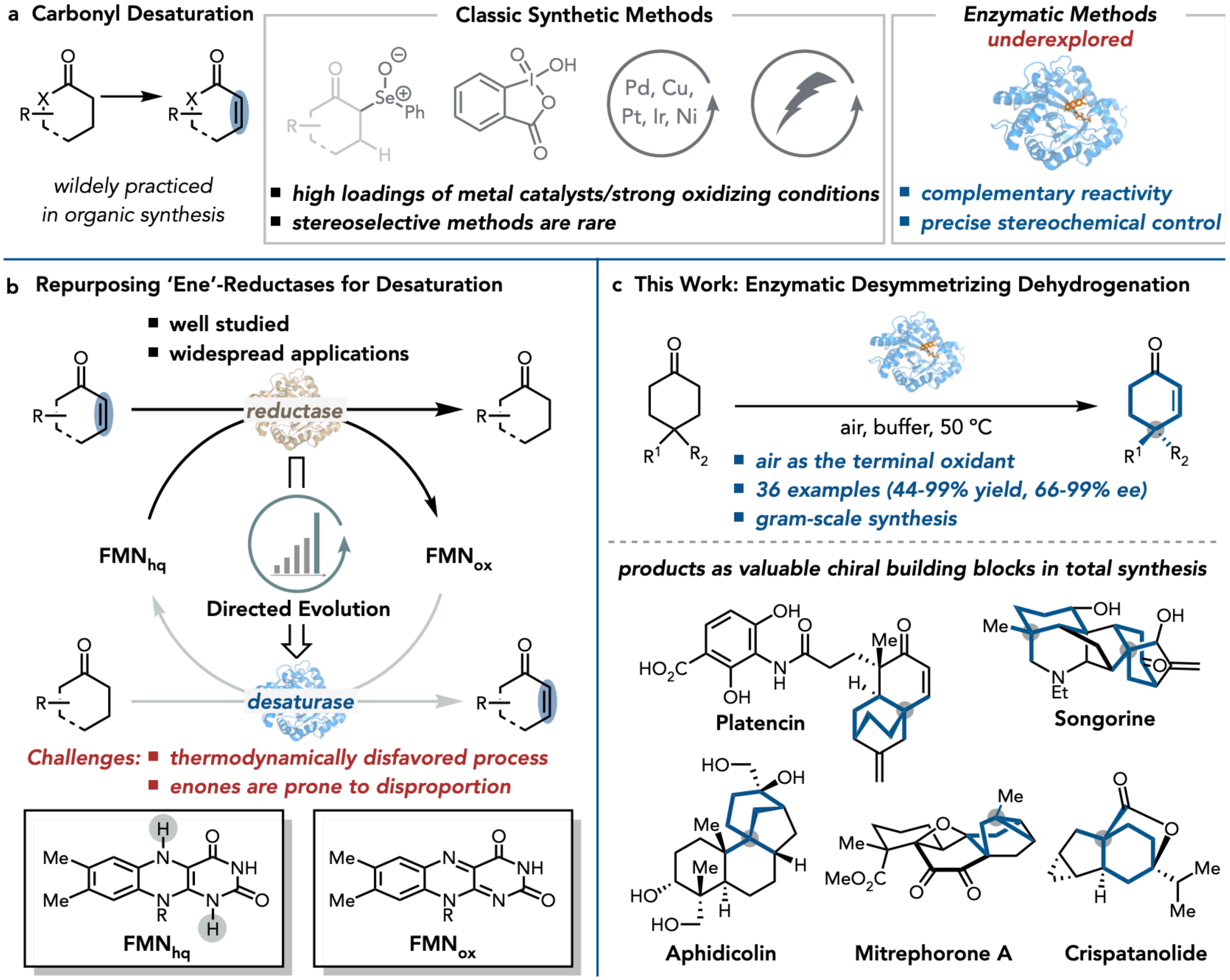


10b

0%

ACS Catal. **2024**, *14*, 15713-15720.

Emerging Applications of EREDs: Run it In Reverse for Desaturation



a Carbonyl Desaturation

wildely practiced in organic synthesis

Classic Synthetic Methods

- *high loadings of metal catalysts/strong oxidizing conditions*
- *stereoselective methods are rare*

Enzymatic Methods *underexplored*

- *complementary reactivity*
- *precise stereochemical control*

b Repurposing 'Ene'-Reductases for Desaturation

- well studied
- widespread applications

Directed Evolution

Challenges:
- *thermodynamically disfavored process*
- *enones are prone to disproportion*

c This Work: Enzymatic Desymmetrizing Dehydrogenation

air, buffer, 50 °C

- *air as the terminal oxidant*
- *36 examples (44-99% yield, 66-99% ee)*
- *gram-scale synthesis*

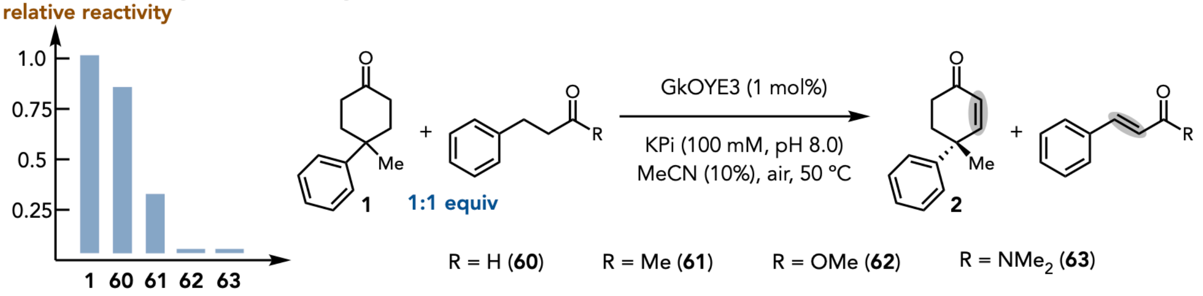
products as valuable chiral building blocks in total synthesis

Platencin **Songorine** **Aphidicolin** **Mitrephorone A** **Crispatanolide**

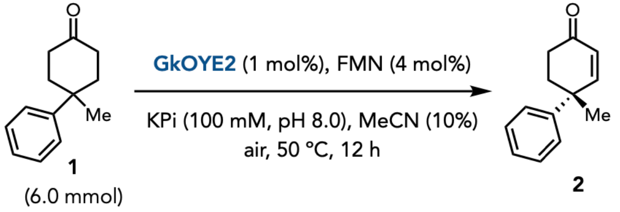
Ye, *ChemRxiv* **2024**

Emerging Applications of EREDs: Run it In Reverse for Desaturation

a Chemoselectivity between aldehyde, ketone, ester, and amide.

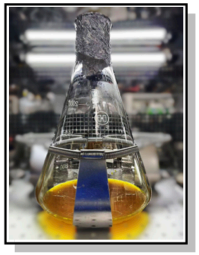


b Gram-Scale Preparation of 2 using Enzyme Wet Lysate

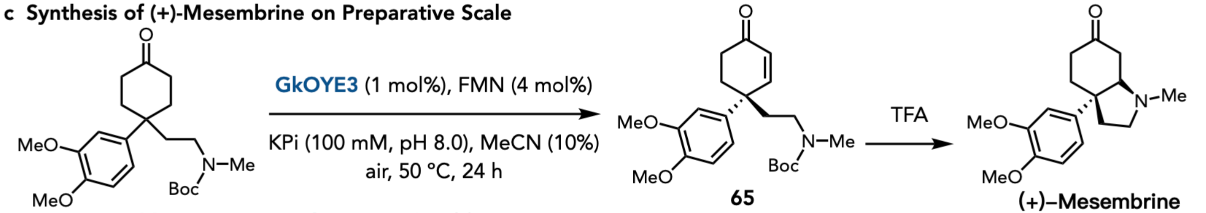


wet lysate:
88% yield, >99:1 er (0.99 g)

lyophilized lysate (447 wt% to **1**):
81% yield, >99:1 er (0.91 g)



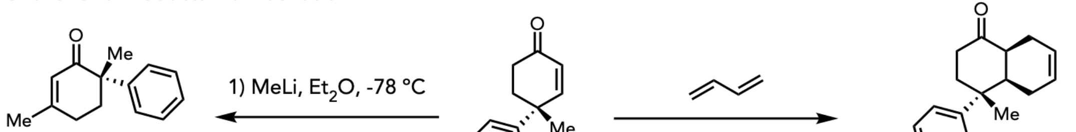
c Synthesis of (+)-Mesembrine on Preparative Scale



wet lysate: 86% yield, >99:1 er (0.97 g)
lyophilized lysate (187 wt% to **60**): 83% yield, >99:1 er (0.94 g)

(+)–Mesembrine
62% yield (two steps)
>99:1 er, >20:1 dr

d Cyclohexenone Products Derivatization



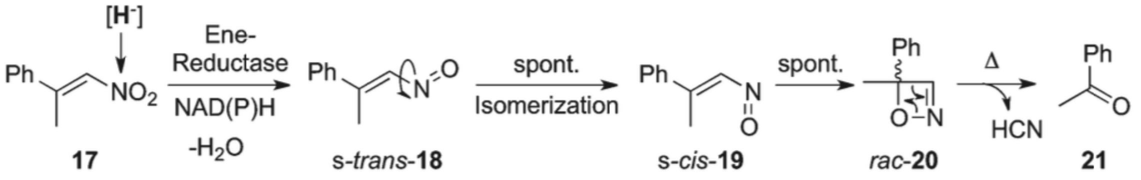
66
67% yield
>99:1 er, >99% es

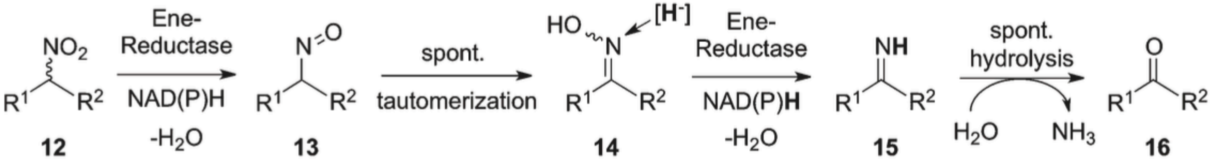
2
(>99:1 er)

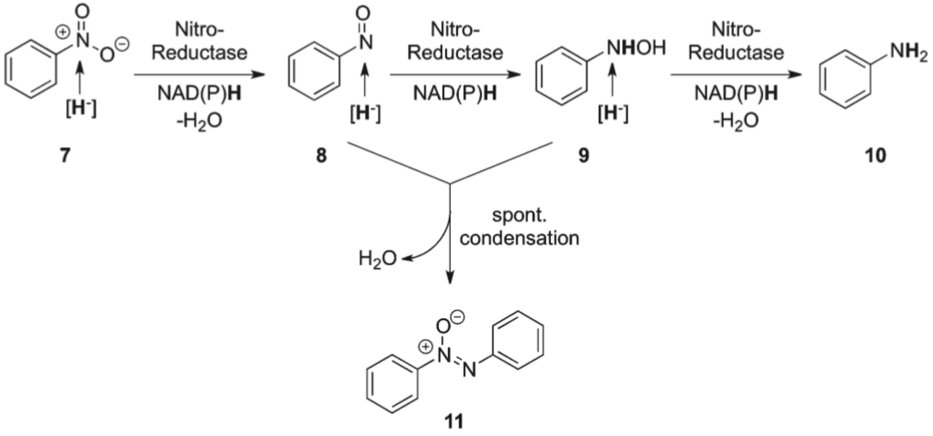
67
71% yield
>99:1 er, 7:1 dr, >99% es

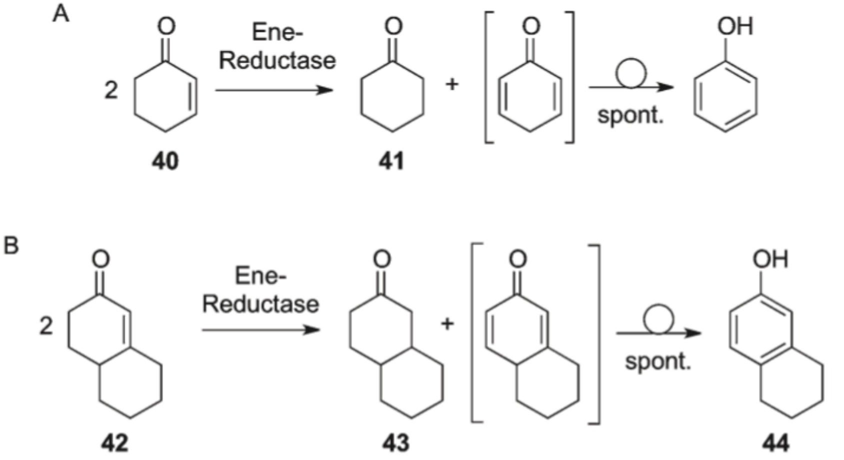
Ye, *ChemRxiv* **2024**

Other Synthetic Applications of EREDs









Green Chem **2013**, *15*, 1764-1772.