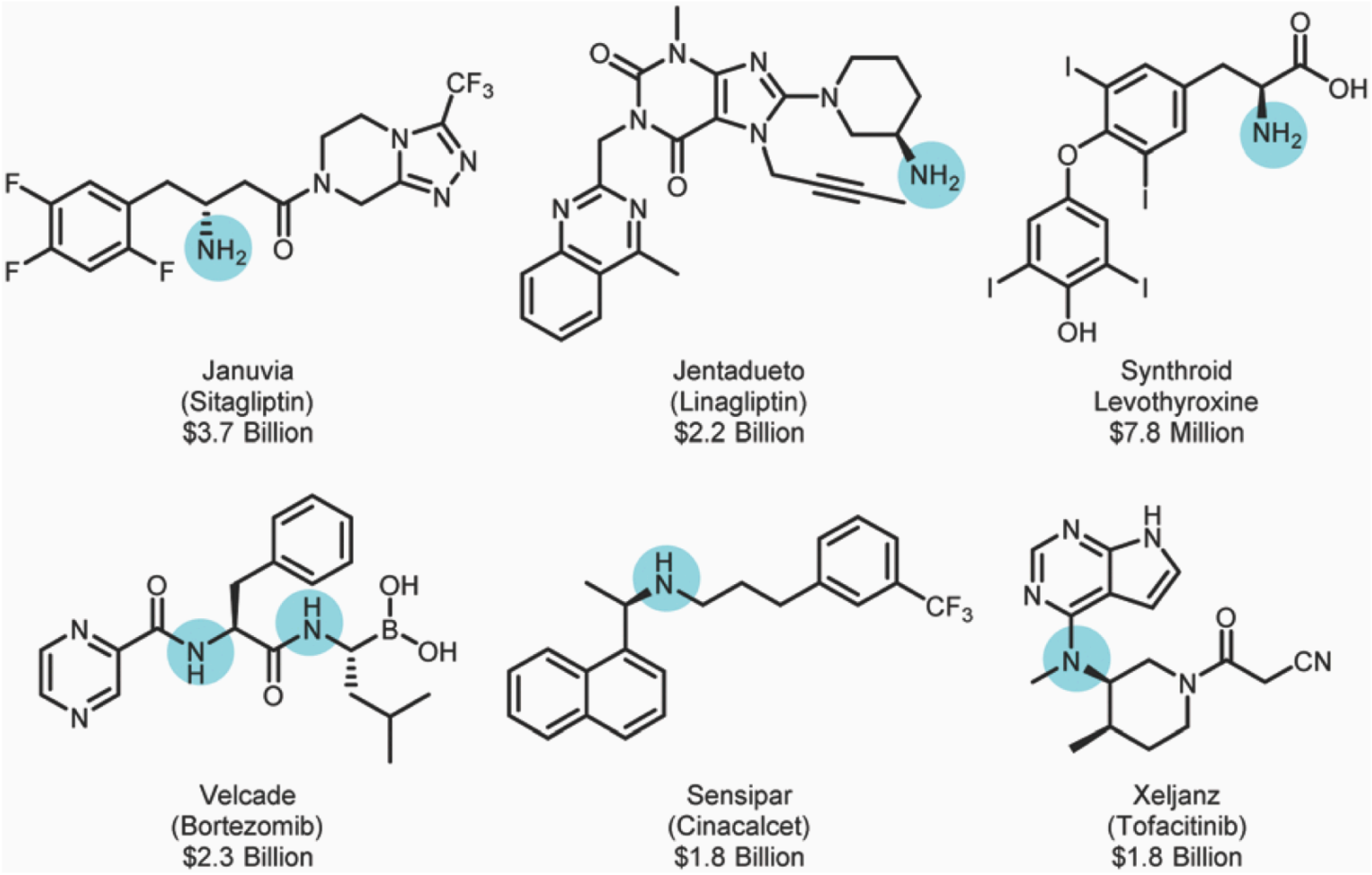
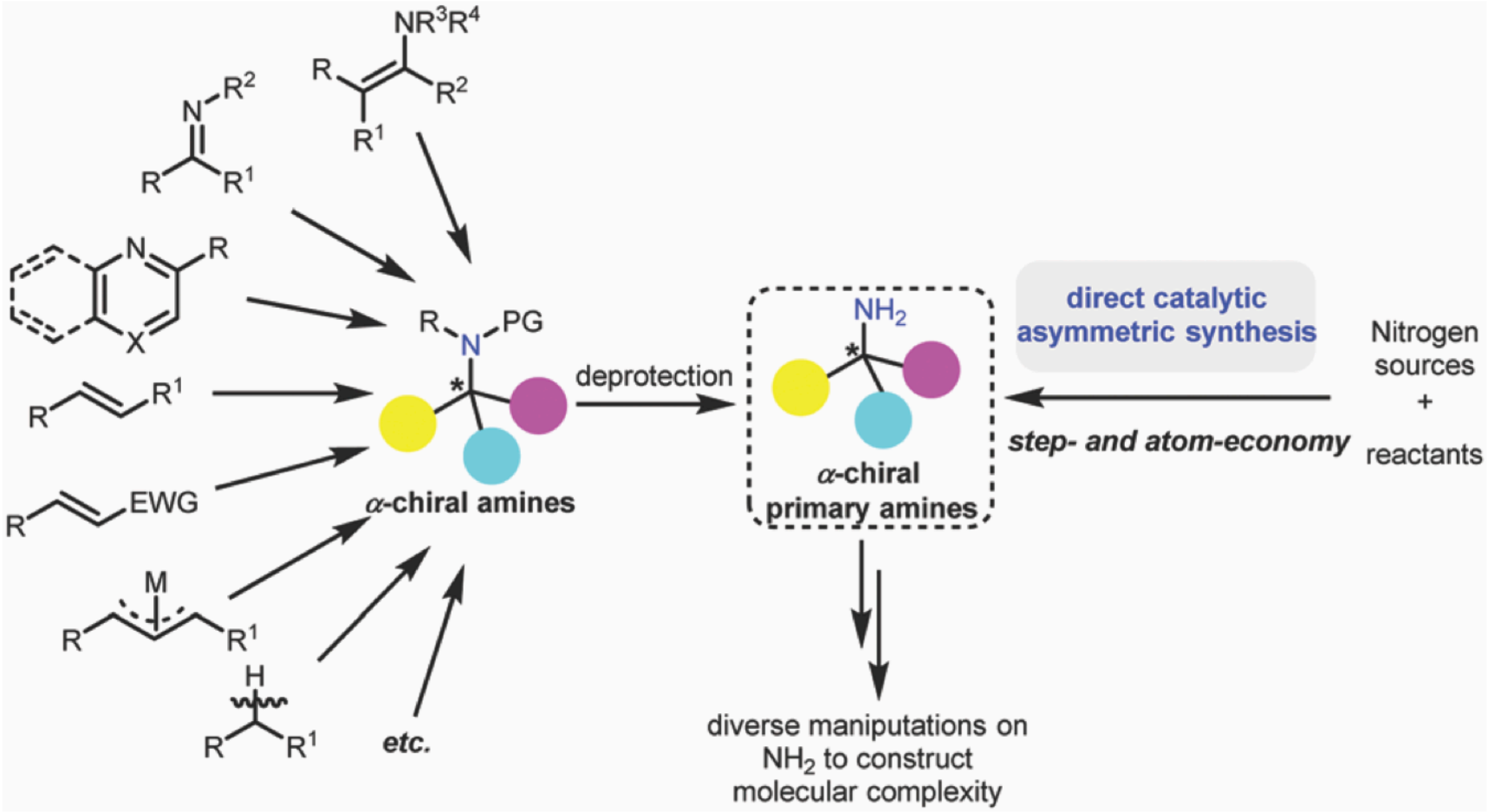
Chiral Amines: A Billion Dollar (Pharmaceutical) Industry



Chem. Soc. Rev. ***2020***, *49*, 6141-6153.

Chiral Amines Preparation: A Tale of Poor Atom Economy



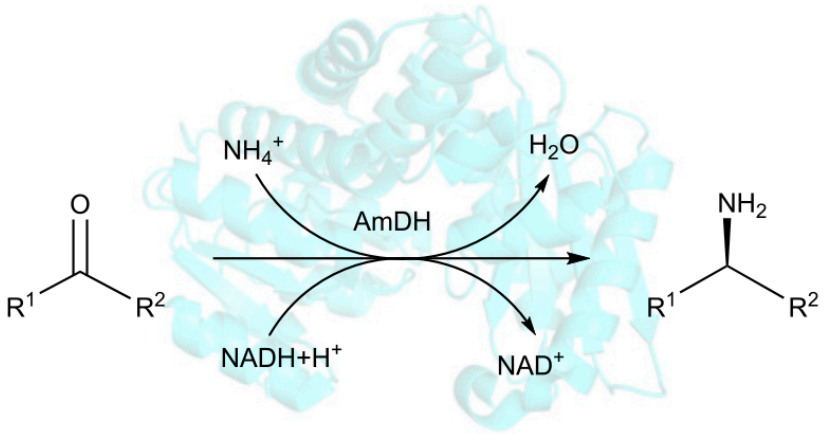
Chem. Soc. Rev. ***2020***, *49*, 6141-6153.

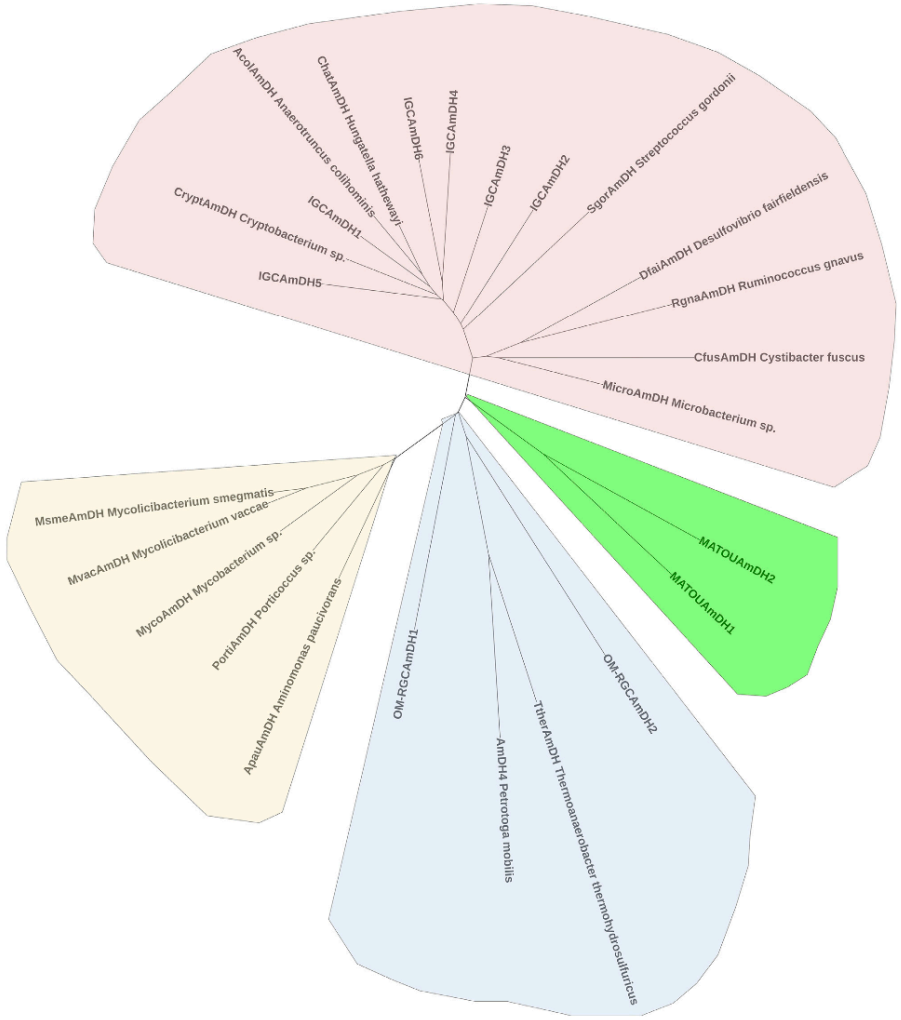
The Reductive Aminase Family: AmDHs, IREDs, and RedAms

Reductive Aminases catalyze selective reductions of imines (pre-formed or generated in situ)

- In analogy to KRED/ADH redox couple, referred to as amine dehydrogenases (AmDHs) in reverse direction
- An oxidoreductase divided formally into three classes
  - **Amine Dehydrogenases (AmDHs)** -reductive amination with ammonia (NH_3)
  - **Imine Reductases (IREDs)** - reductive amination with pre-formed imines or ketone + excess amine
  - **Reductive Amines (RedAms)** - reductive amination with near 1:1 stoichiometry ketone + amine
- Dependent on NAD(P)H cofactor
  - Some reductive aminases use NADH, some use NADPH (difference is 2' ribose phosphate group)
- Catalyzes both imine reduction or amine oxidation, depending on conditions
  - In synthetic applications, often run in reductive direction to generate stereocenters

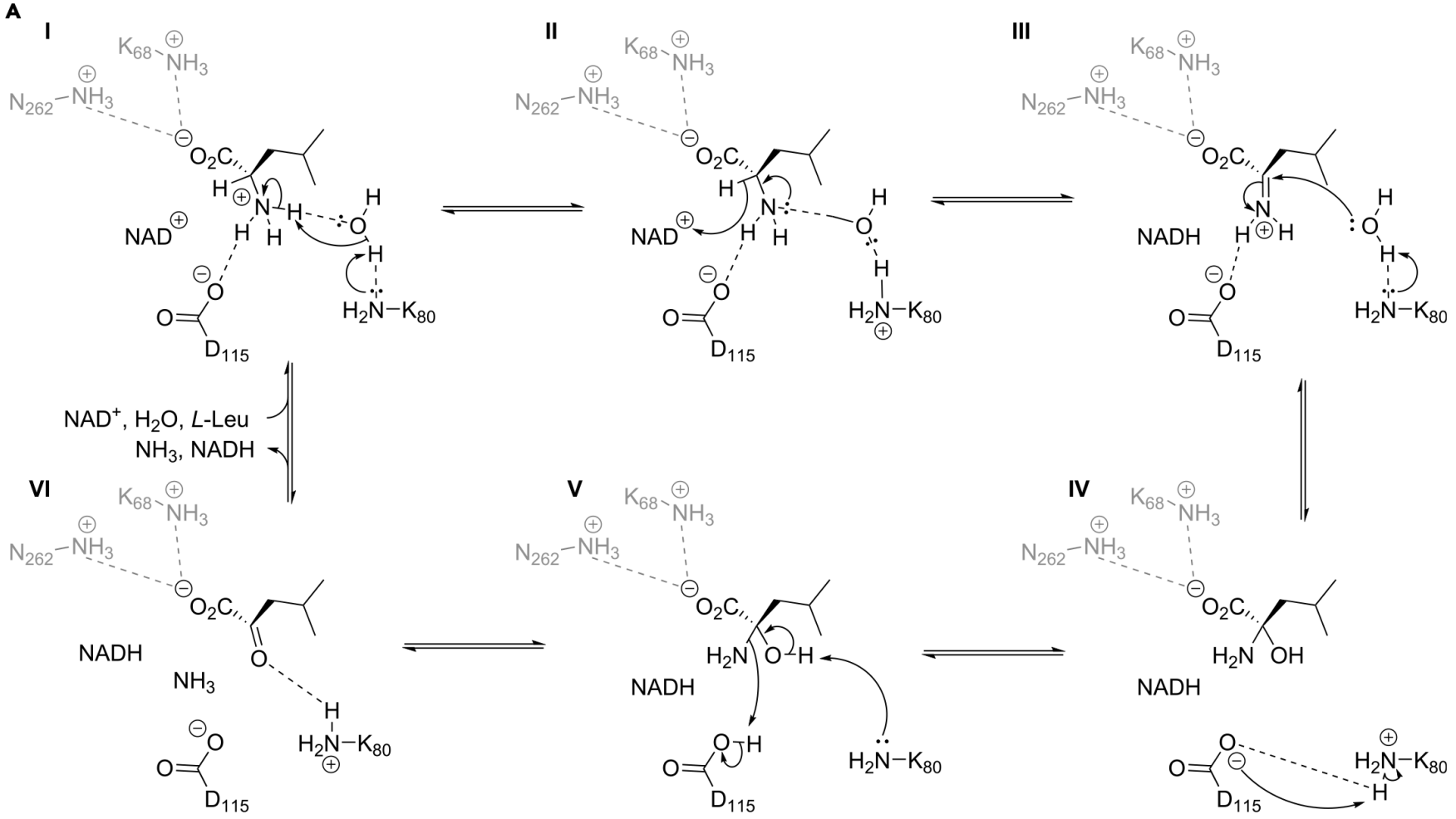
AmDHs: A Small Family (25 Members) of Reductive Aminases





Chem Catal. ***2022***, *2*, 1288-1314.

Representative Mechanism for Amine Dehydrogenase: Leucine Dehydrogease (LeuDH)

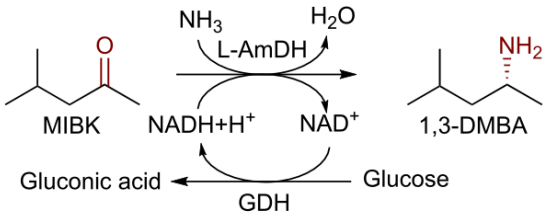


Chem Catal. ***2022***, *2*, 1288-1314.

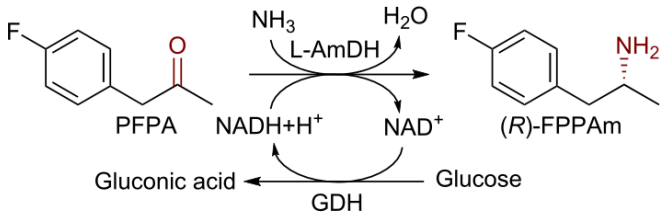
Turnover Strategies for AmDHs

AmDH-GDH

A

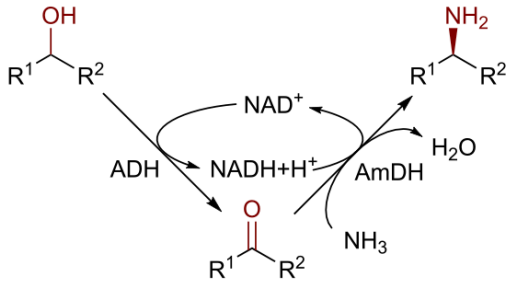


B

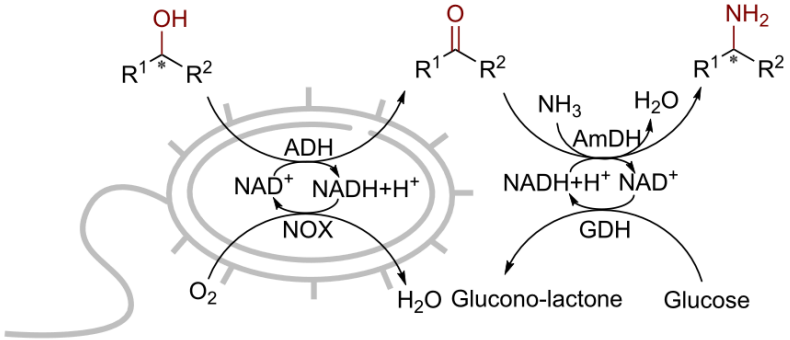


AmDH-ADH

C

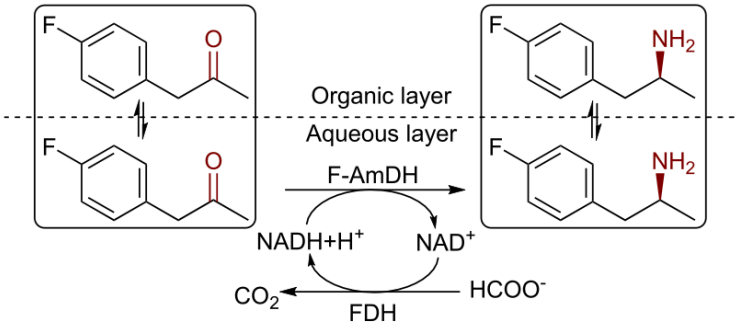


D Cell and cell-free system coupling



AmDH-FDH

E Biphasic reaction system



AmDH: amine dehydrogenase

L-AmDH: leucine dehydrogenase

F-AmDH: phenylalanine dehydrogenase

GDH: glucose dehydrogenase

ADH: alcohol dehydrogenase

FDH: formate dehydrogenase

NOX = NADH Oxidase

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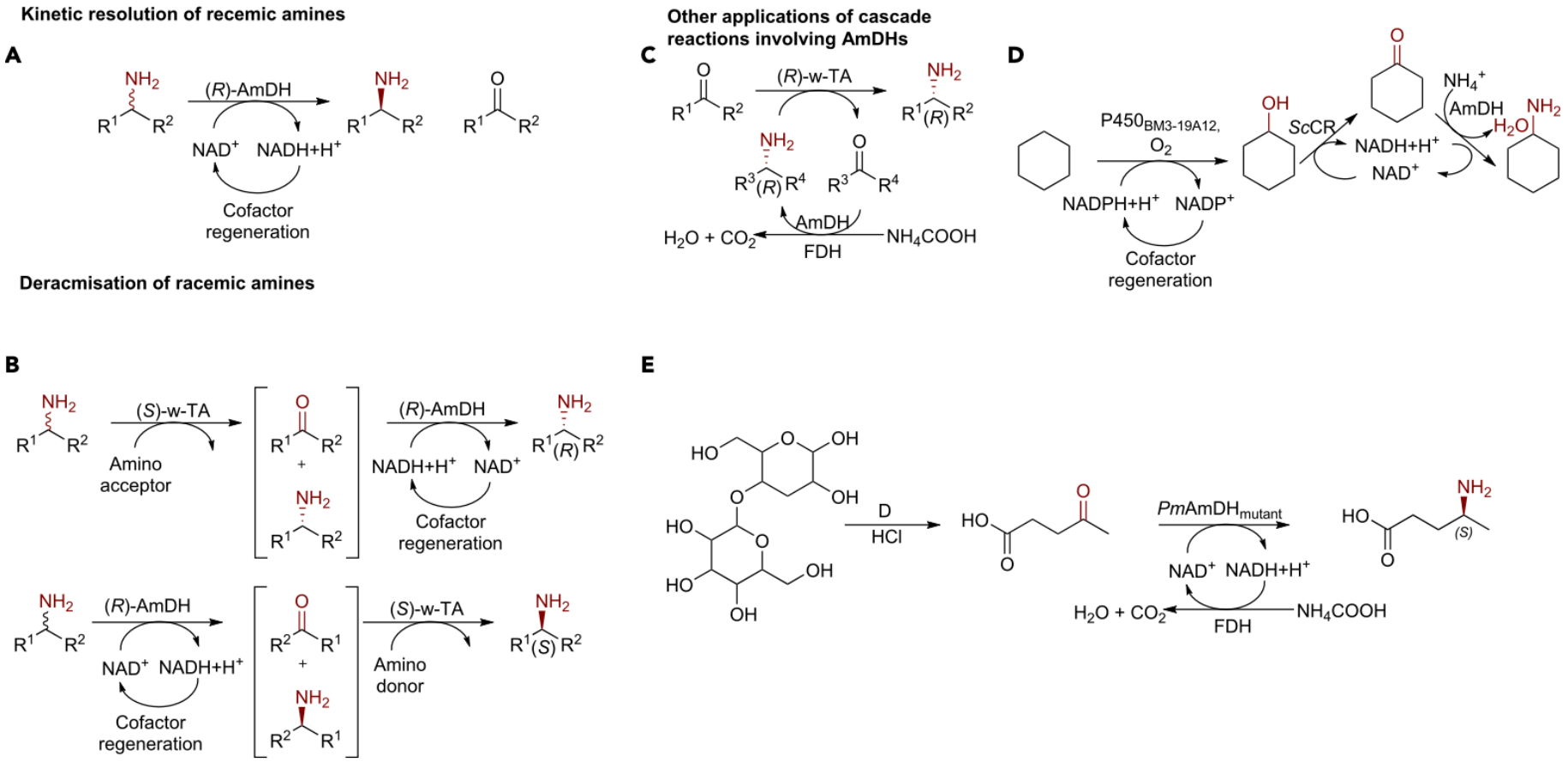
Select Synthetic Applications of AmDHs



Chem Catal. ***2022***, *2*, 1288-1314.

Distinguishing AmDHs from Other Reductive Aminases

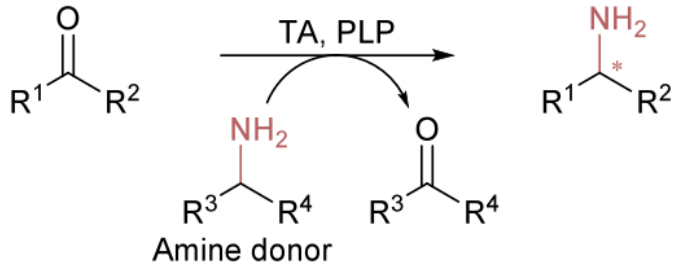
	Enzyme	Mode of action	Cofactor	Will form primary amines	Will form secondary amines	References
A	ω-Transaminase (ω-TA)	Transfer NH_3 from an amine donor *via* PLP/PMP cofactor	PLP/PMP	Yes	No	15 and 16
B	Amine dehydrogenase (AmDH; engineered or native)	Amination of ketones with NH_3 followed by reduction using NAD(P)H	NAD(P)H	Yes	No, but with a few exceptions	19, 20, 29 and 30
C	Opine dehydrogenase (OpDH; engineered)	Amination of ketones with primary amines followed by reduction using NAD(P)H	NAD(P)H	No	Yes	35
D	Imine reductase (IRED)	Reduction of cyclic imines using NAD(P)H; also reduce imines formed in solution from carbonyls and amines	NAD(P)H	Yes, but not preferred	Yes, when amine is provided in excess	36 and 37
E	Reductive aminase (RedAm)	Reductive aminations of carbonyls with amines through catalysis of both imine formation and reduction	NAD(P)H	Yes, but not preferred	Yes, when amine is provided in 1 : 1 ratio with carbonyl	38

Chem Sci. ***2022***, *13*, 4697.

Distinguishing AmDHs from Other Reductive Aminases Schematic Overview

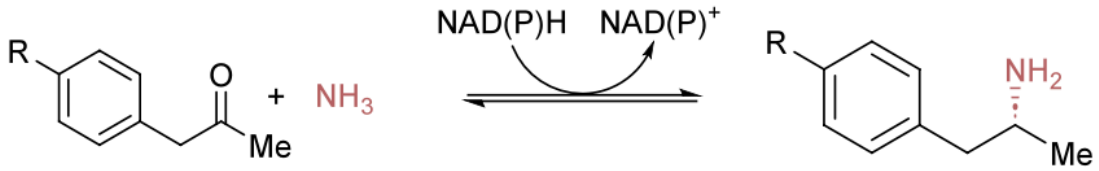
Transaminases (TAs)

Product scope



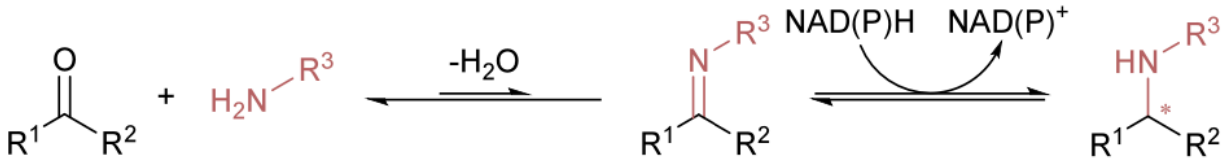
1° amines

Amination/Amine dehydrogenases (AmDHs)



1° amines

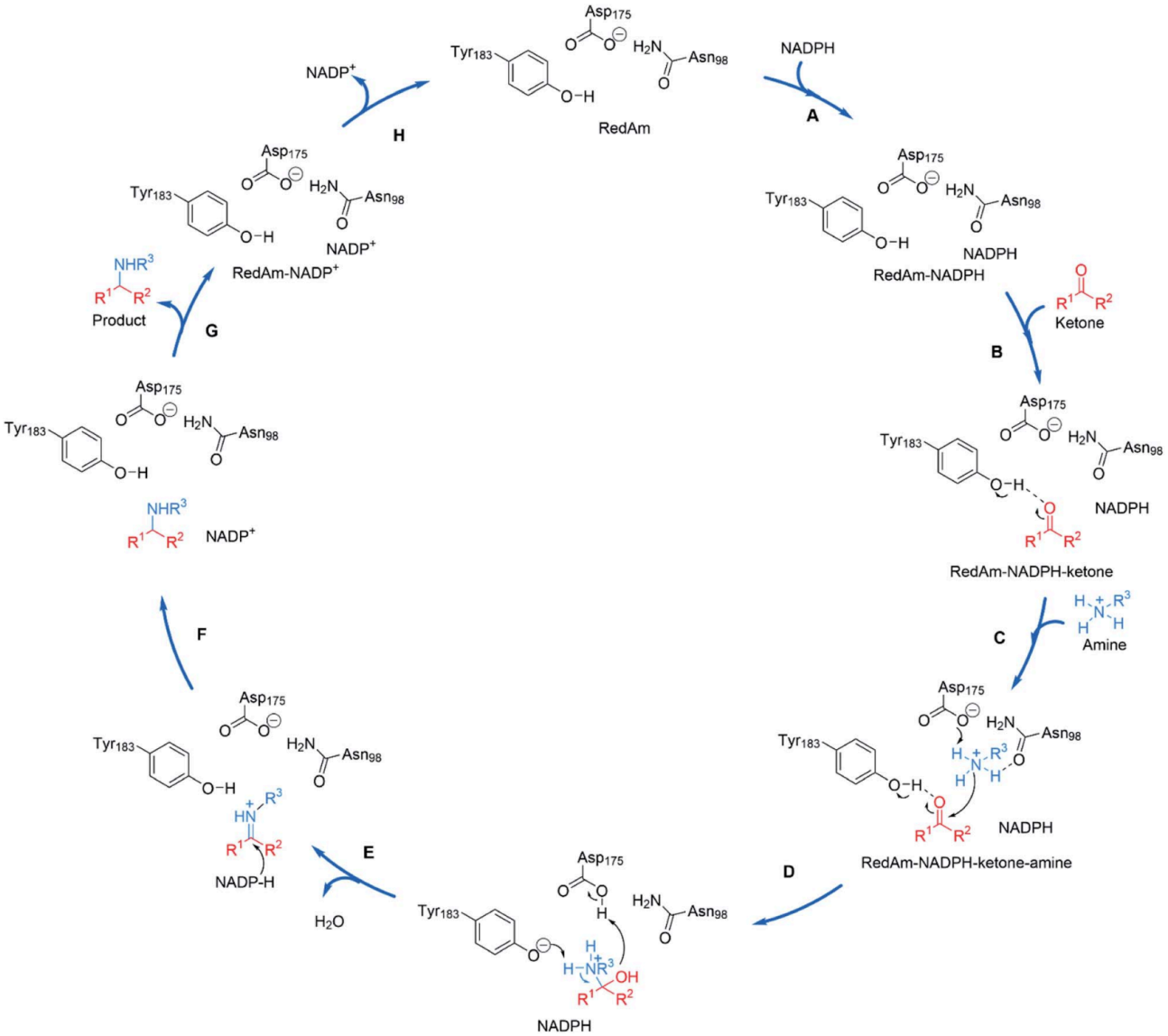
Imine reductases (IREDs) and reductive aminases (RedAms)



1°, 2°, 3° amines

ACS Catal. ***2024***, *14*, 14308-14329.

Mechanism for Imine Reductases (IREDs) and Reductive Aminases (RedAms)



Chem Sci. ***2022***, *13*, 4697.

Characterized Imine Reductase (IREDs) Panels

S/N	trivial name	panel size (no. of IREDs)	characterized by	Clade origin	refs
1	Turner-Prozomix IREDs	384	Turner's group and Prozomix	bacterial, fungal, and metagenomic	54,55
2	Turner Group's IREDs	95	Turner's group	bacterial, fungal, plants	55
3	Zhu group's IREDs	154	Zhu's group and collaborators	bacterial, fungal, and metazoans	53,56,57
4	GSK's IREDs	85	GSK Biocatalysis group and academic collaborators	bacterial and fungal	24,50
5	Pfizer's IREDs	80	Pfizer Biocatalysis group and academic collaborators	Predominantly bacterial	23,58
6	Roche's IREDs	28	Roche Biocatalysis group and academic collaborators	Bacterial	48,49
7	Kroutil group's IREDs	14	Kroutil's group	Bacterial	52
8	JM's IRED	>90	Johnson Matthey	bacterial and fungal	46
9	Almac's IREDs	>50	Almac	-	
10	Novartis' IREDs	26	Novartis	bacterial and fungal	47
11	Ward group's IREDs	29	Ward's group	bacterial (predominantly actinobacterial)	51
12	Grogan's group solved the first IRED/RedAm structures and provided mechanistic insights into IRED/RedAm catalysis; Grogan's group has solved most of the characterized structures of IREDs/RedAms to date. Selected examples of IRED/RedAm complexes include Q1EQE0 (PDB: 3ZGY), *Ao*IRED (PDB: 5fwn), *Asp*RedAm (5G6S), *At*RedAm (i03), *Aa*RedAm (PDB: 8BJ5 and 8BK1).				22,38,39,41−43

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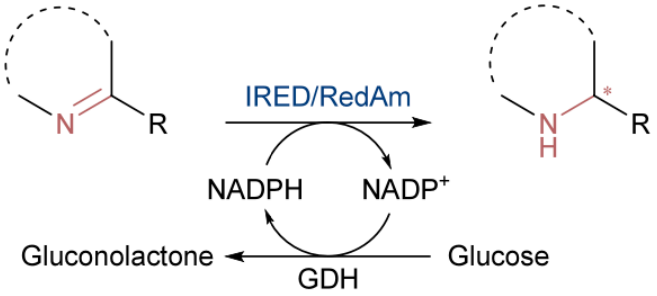
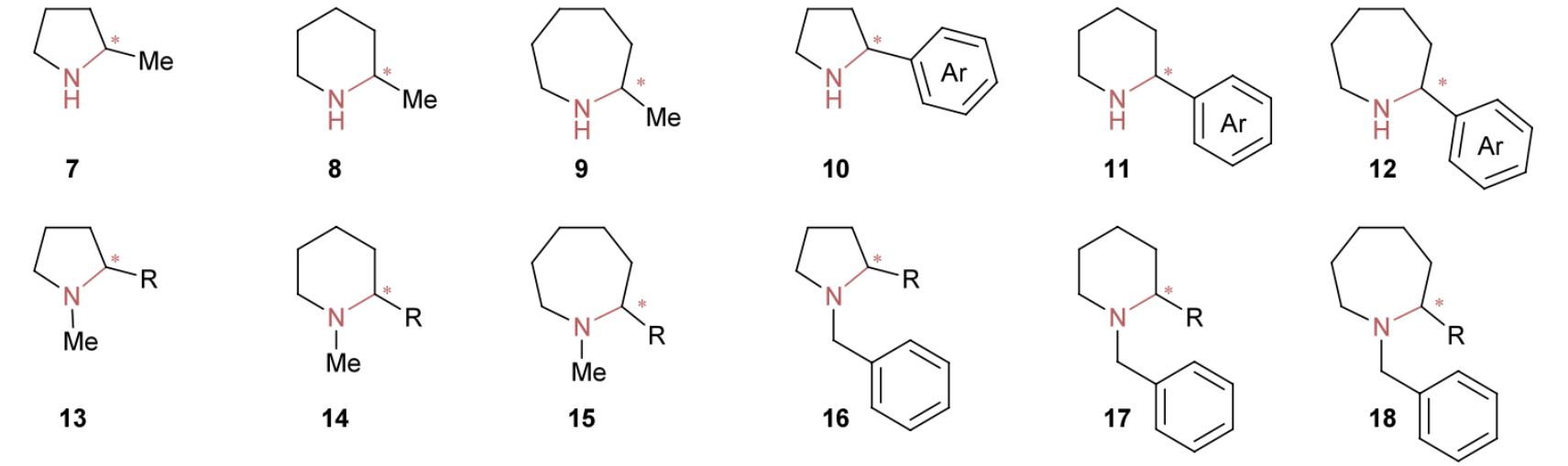
IRED Catalyzed Reduction of Cyclic Amines: All Carbon





R= alkyl, aryl, cyclohexyl

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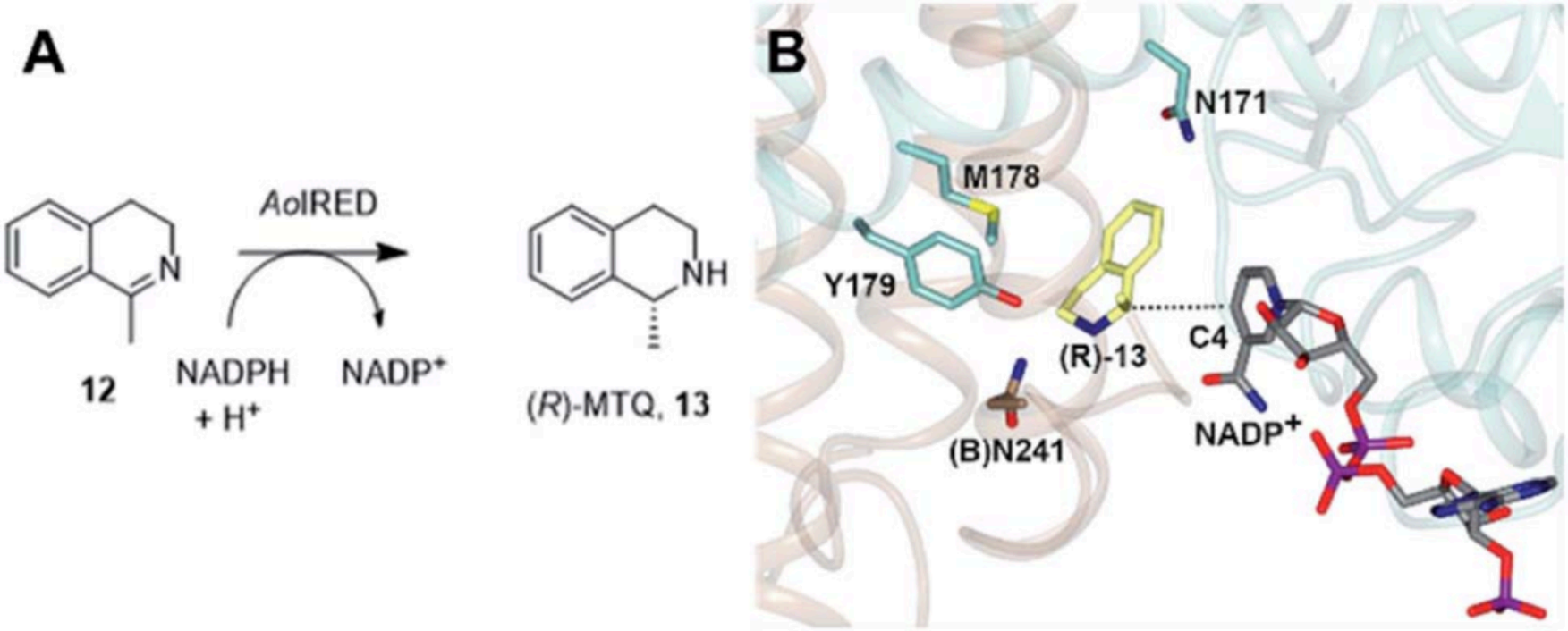
A Peak Inside an IRED Reduction



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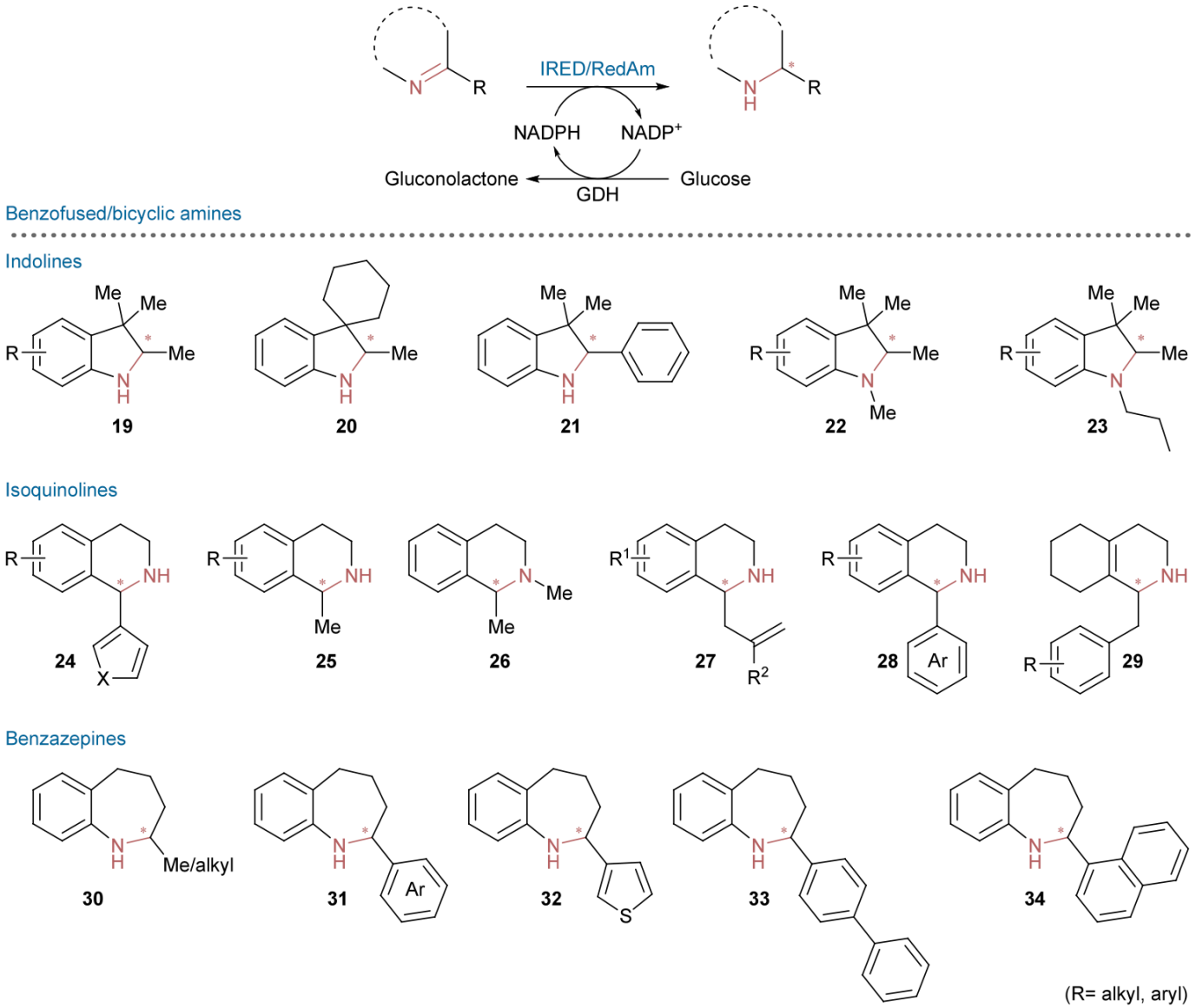
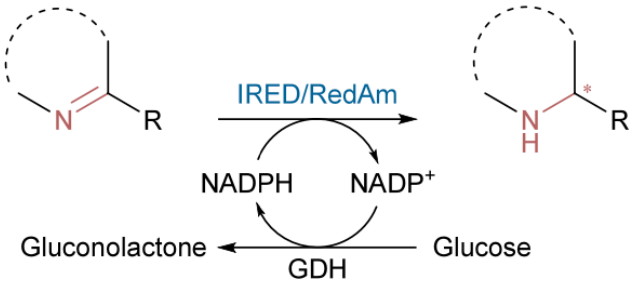
IRED Catalyzed Reduction of Benzofused/Bicyclic Imines

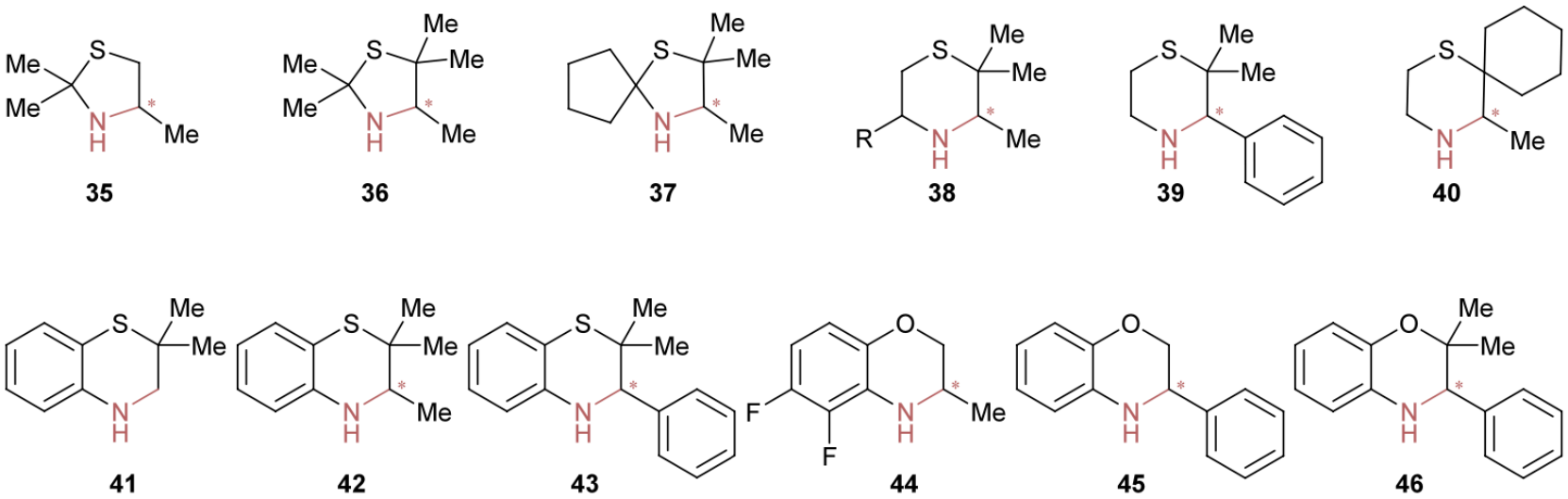


ACS Catal. ***2024***, *14*, 14308-14329.

IRED Catalyzed Reduction of Sulfur and Oxygen-Containing Monocylic Amines

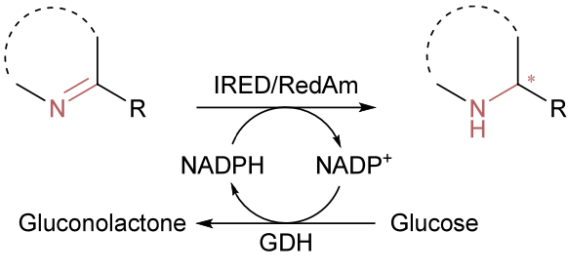


Sulphur and oxygen-containing cyclic amines

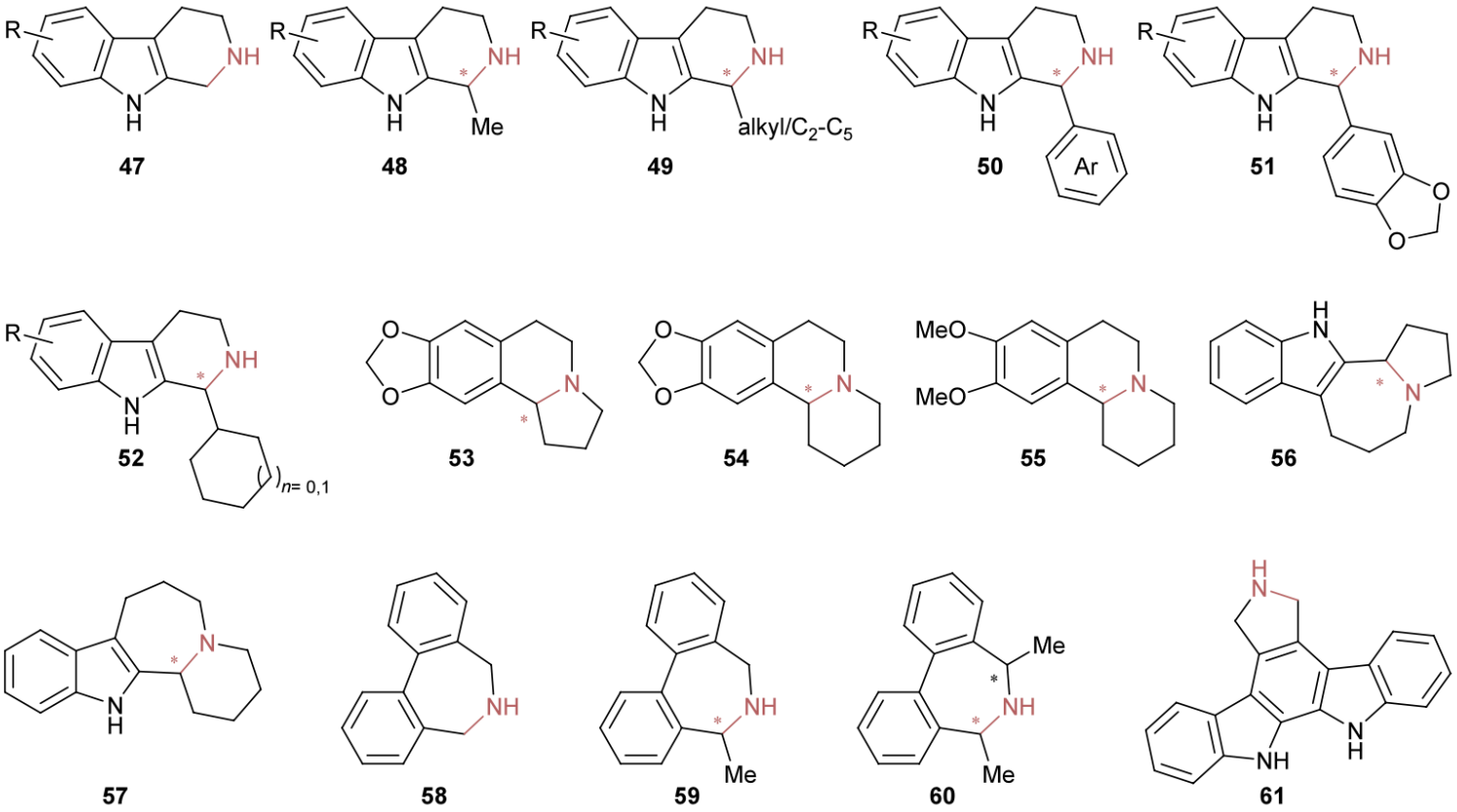


ACS Catal. ***2024***, *14*, 14308-14329.

IRED Catalyzed Reduction of Tricyclic/Polycyclic Amines

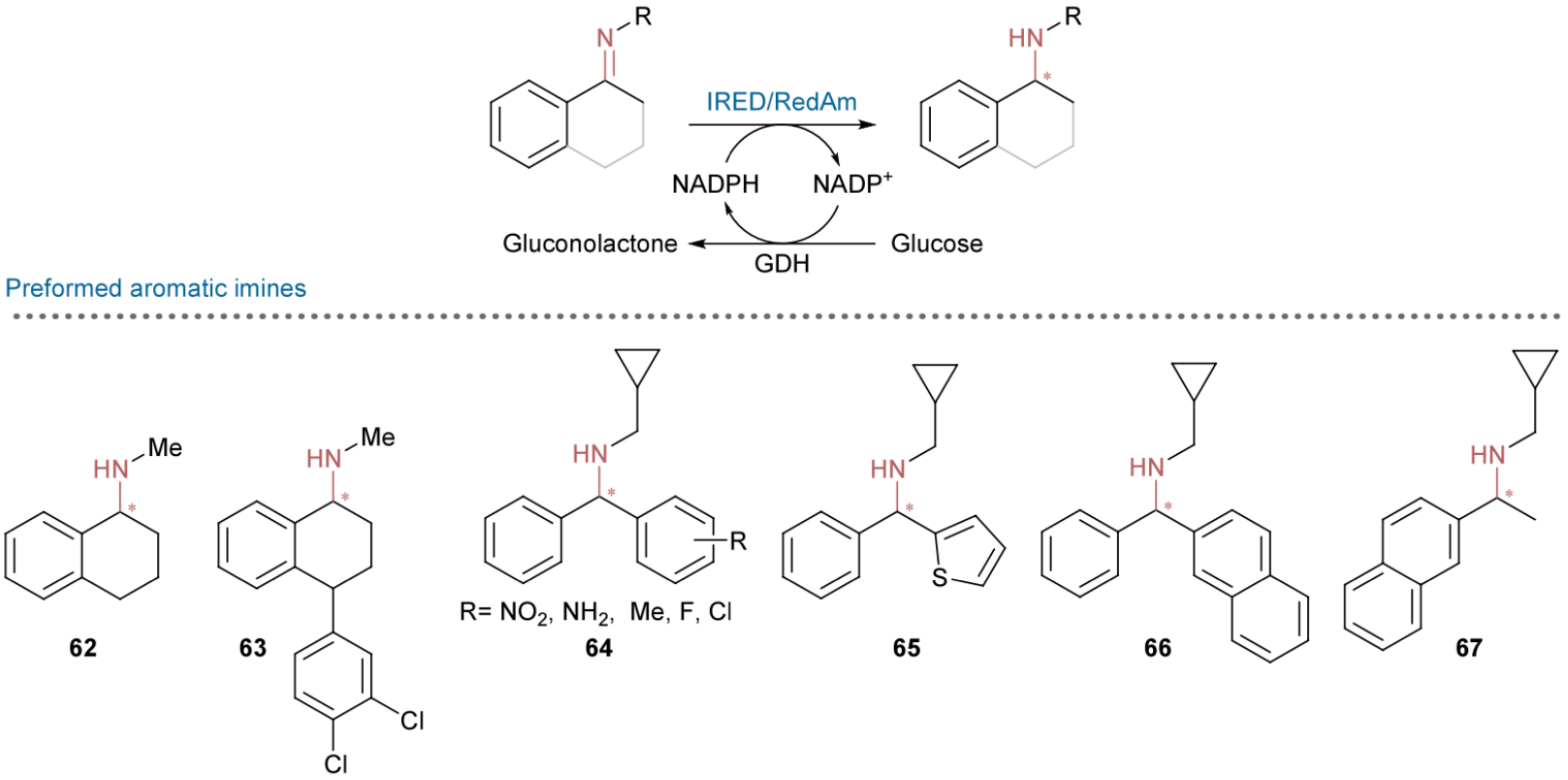


Tricyclic and other polycyclic amines



ACS Catal. ***2024***, *14*, 14308-14329.

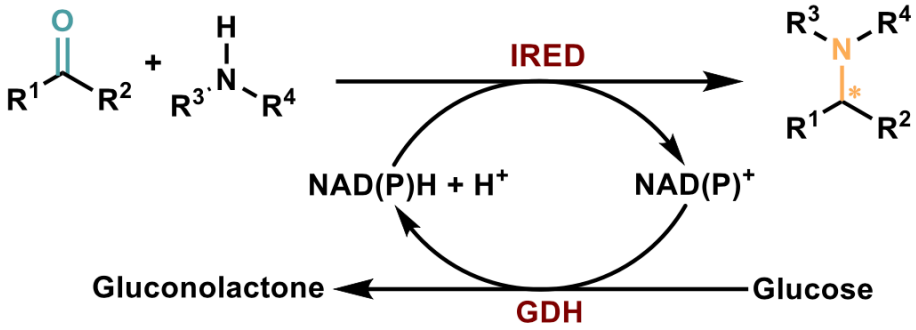
IRED Catalyzed Reduction of Exocyclic Aromatic Amines



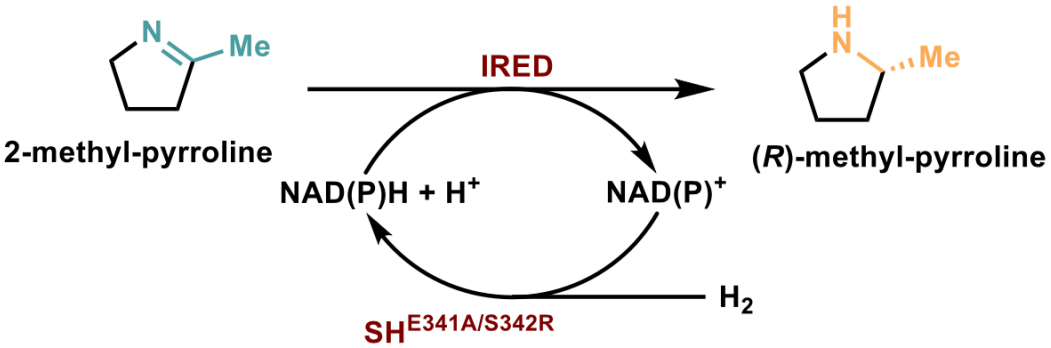
ACS Catal. ***2024***, *14*, 14308-14329.

Cofactor Recovery Strategies for IREDs

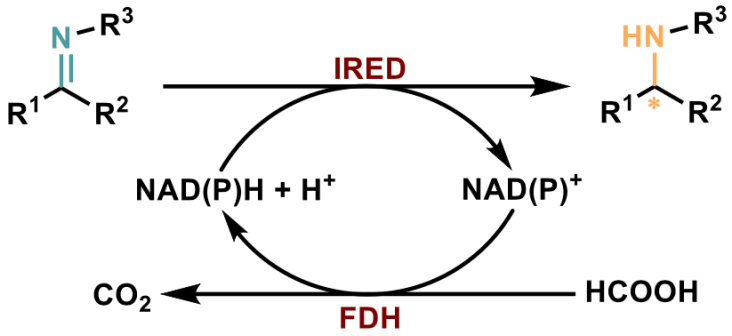
(a) GDH participates in the catalytic circulation system



(b) SH participates in the catalytic circulation system



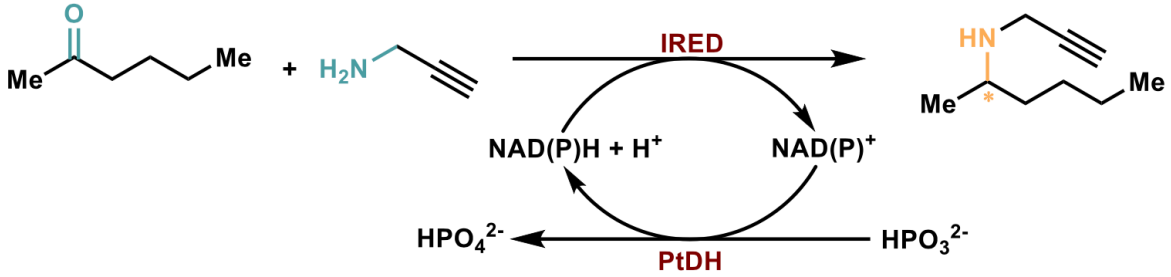
(c) FDH participates in the catalytic circulation system



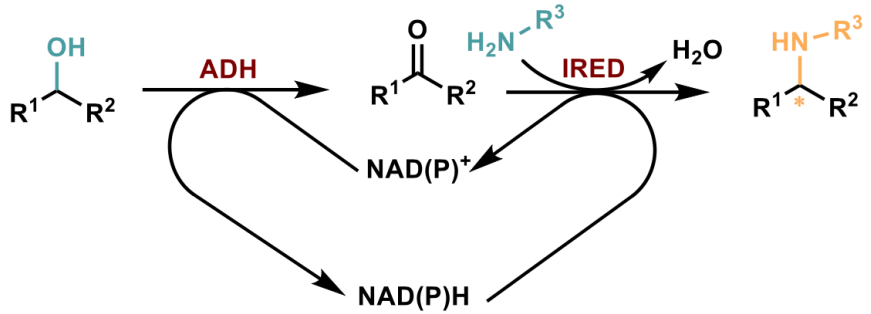
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

Cofactor Recovery Strategies for IREDs

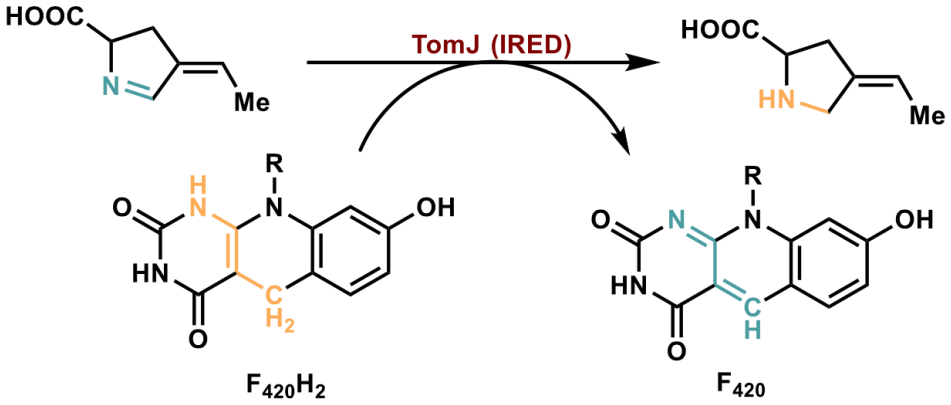
(d) PtDH participates in the catalytic circulation system



(e) Enzymatic hydrogen borrowing reaction catalyzed by ADH and IRED

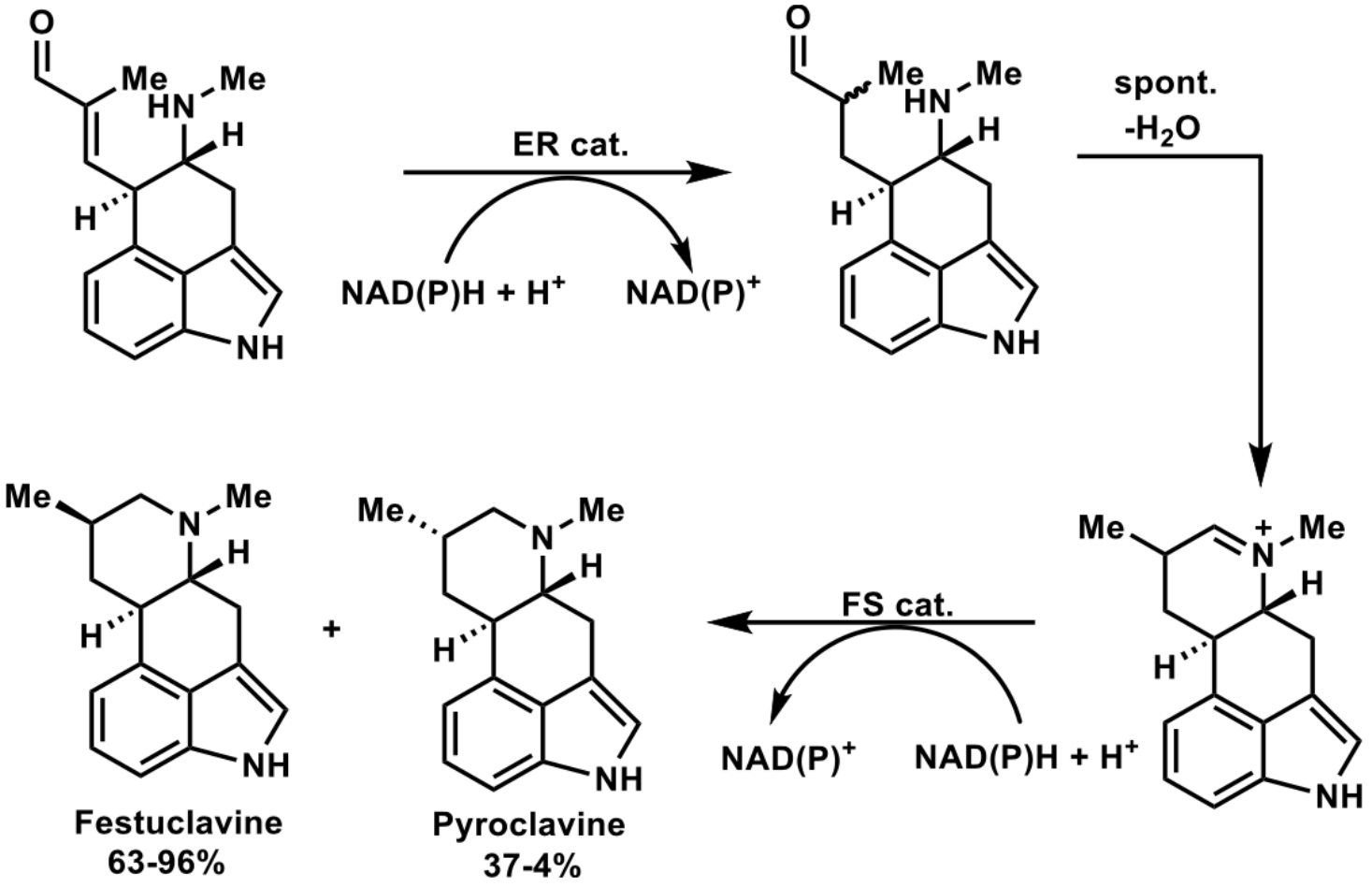


(f) Biocatalytic reaction of F_{420}-dependent imine reductase



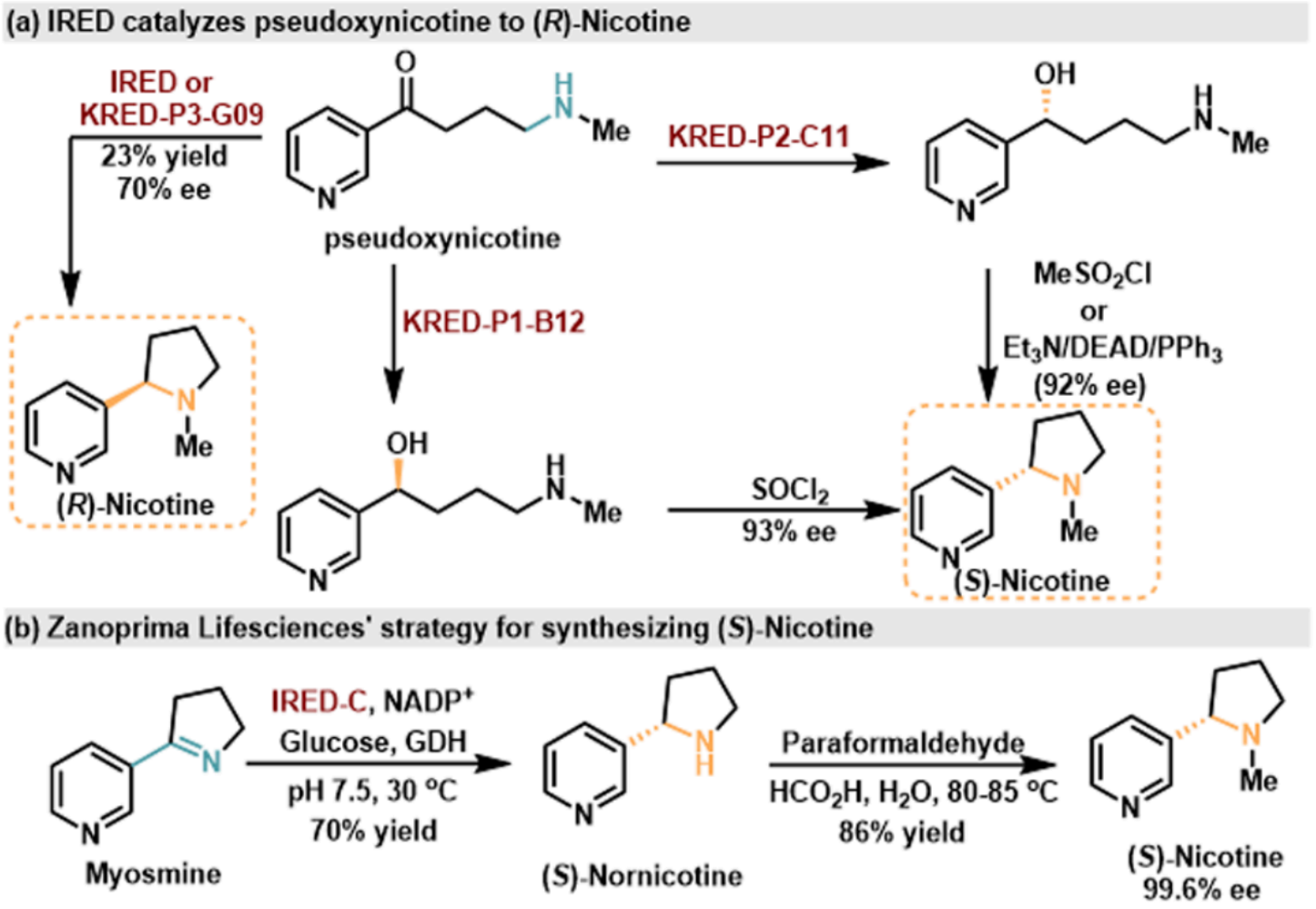
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

Ergot Alkaloid Synthesis with IREDs



Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

Nicotine Synthesis with IREDs



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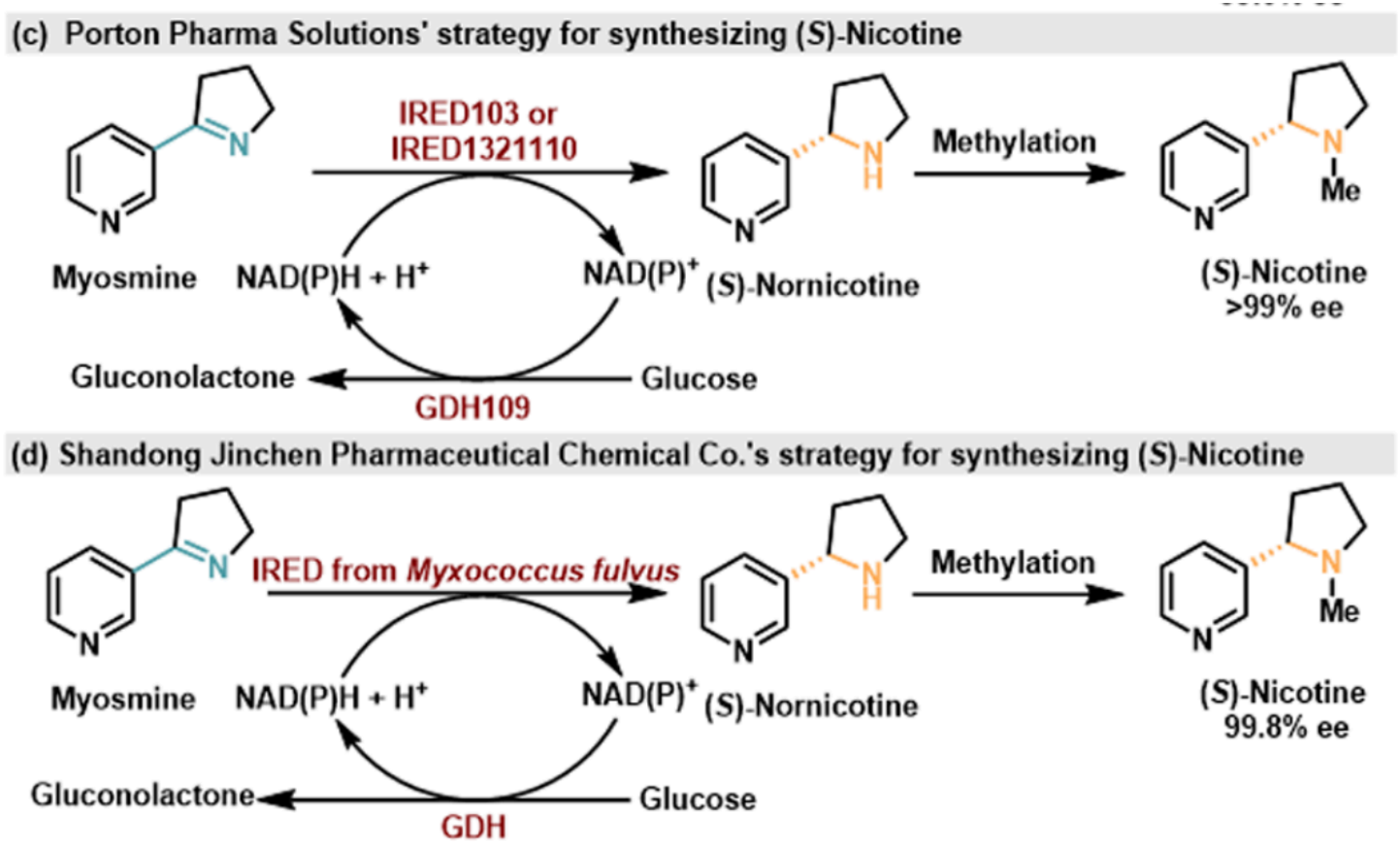
Nicotine Synthesis with IREDs



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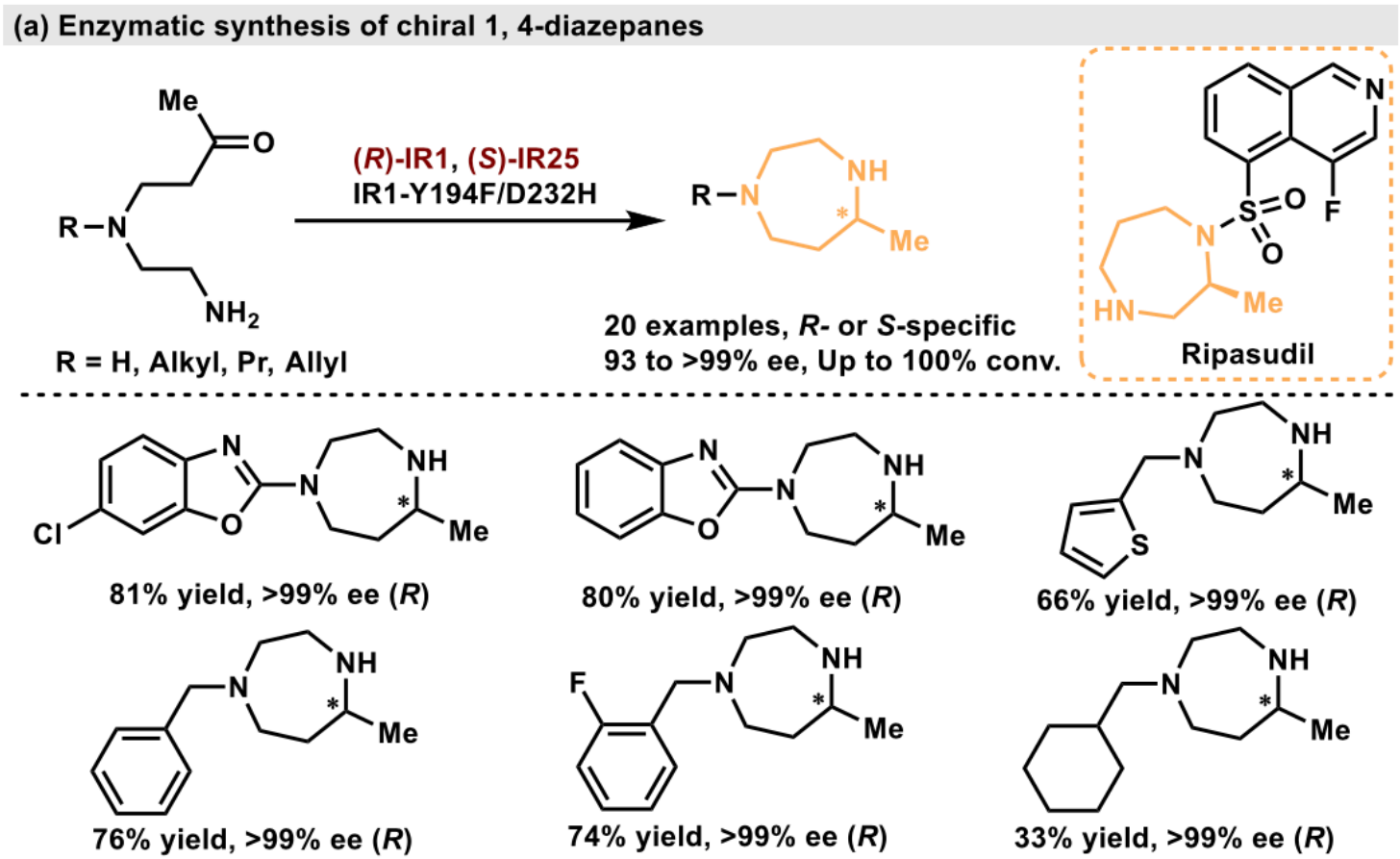
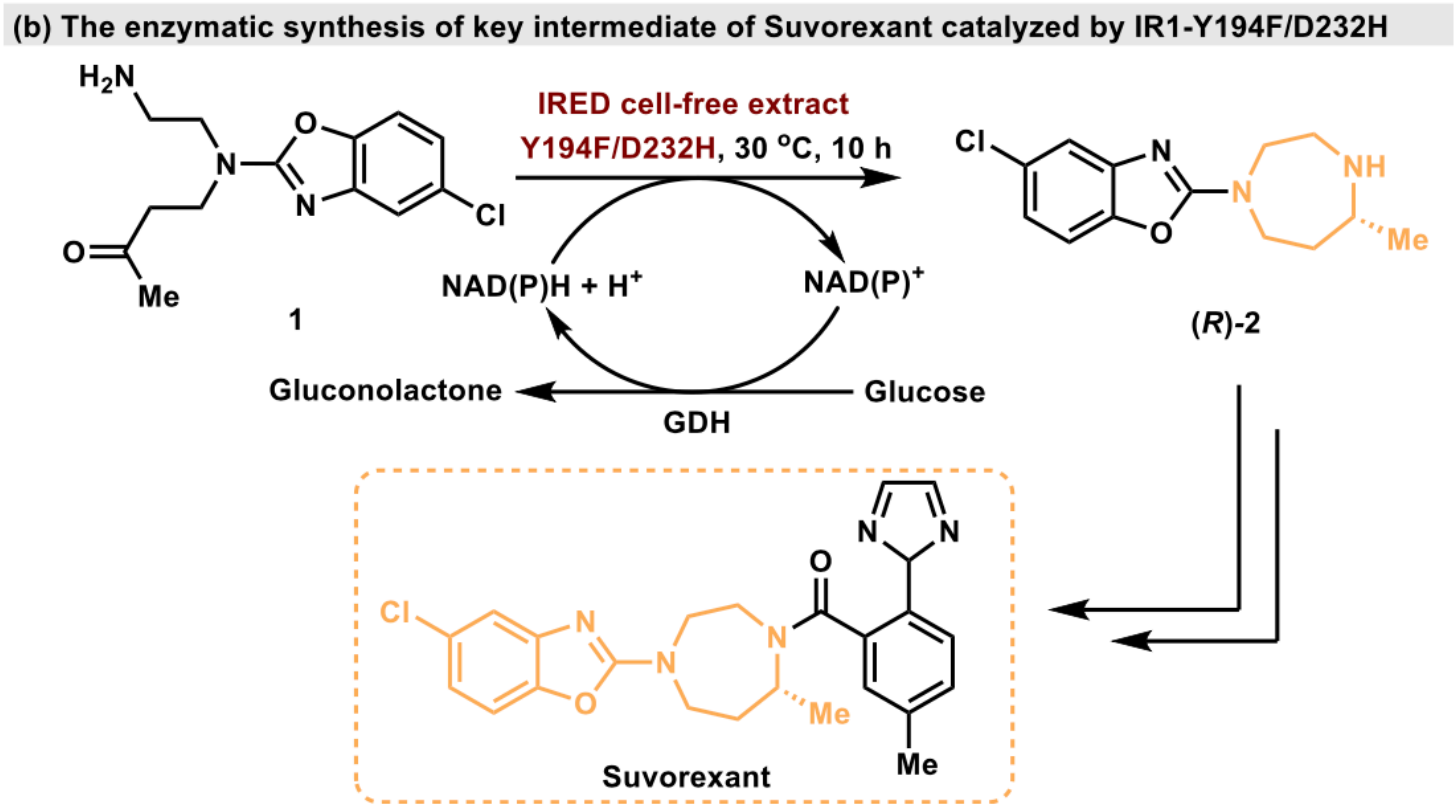
Synthesis of 1,4-Diazapanes with IREDs



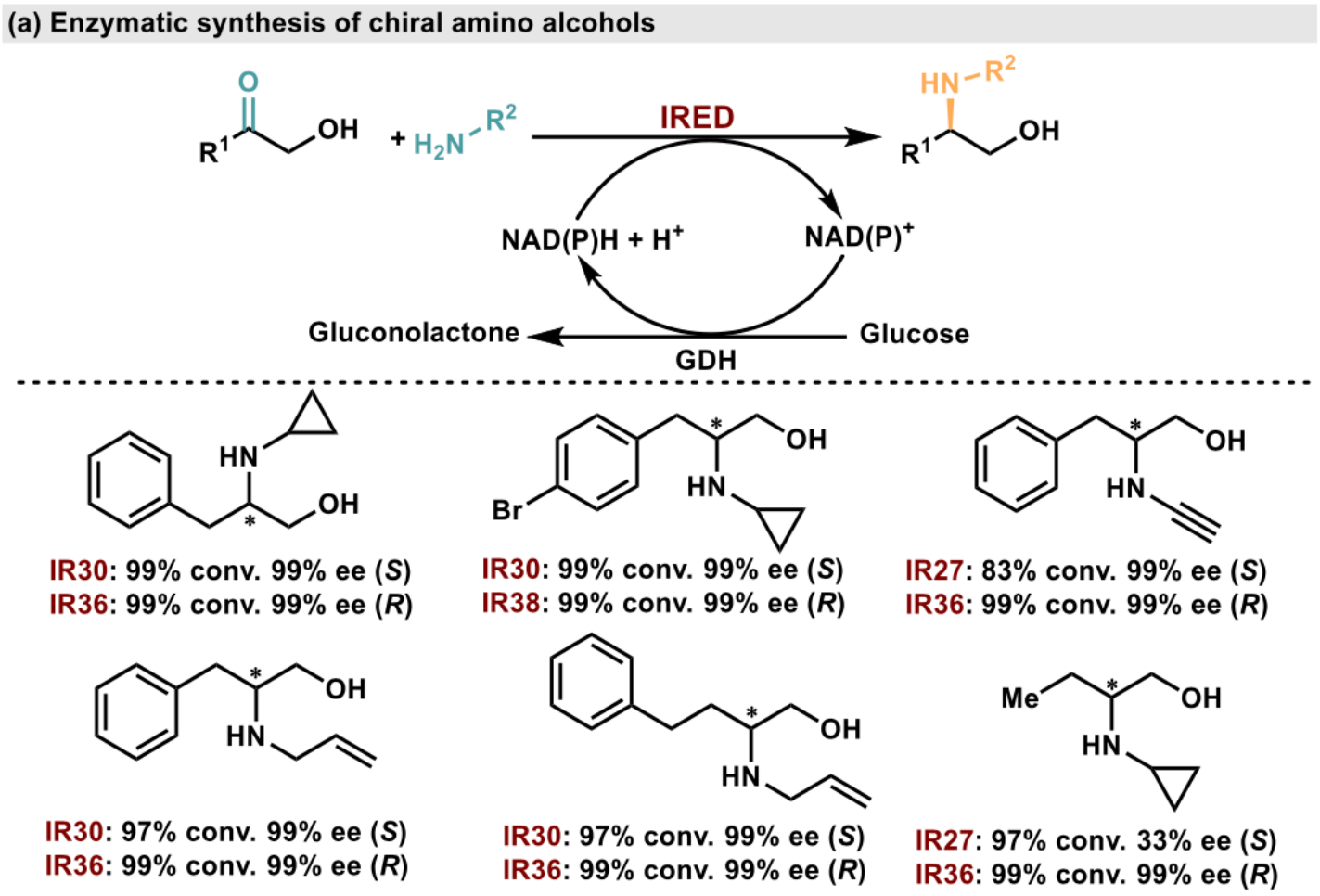
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

Synthesis of 1,4-Diazapanes with IREDs



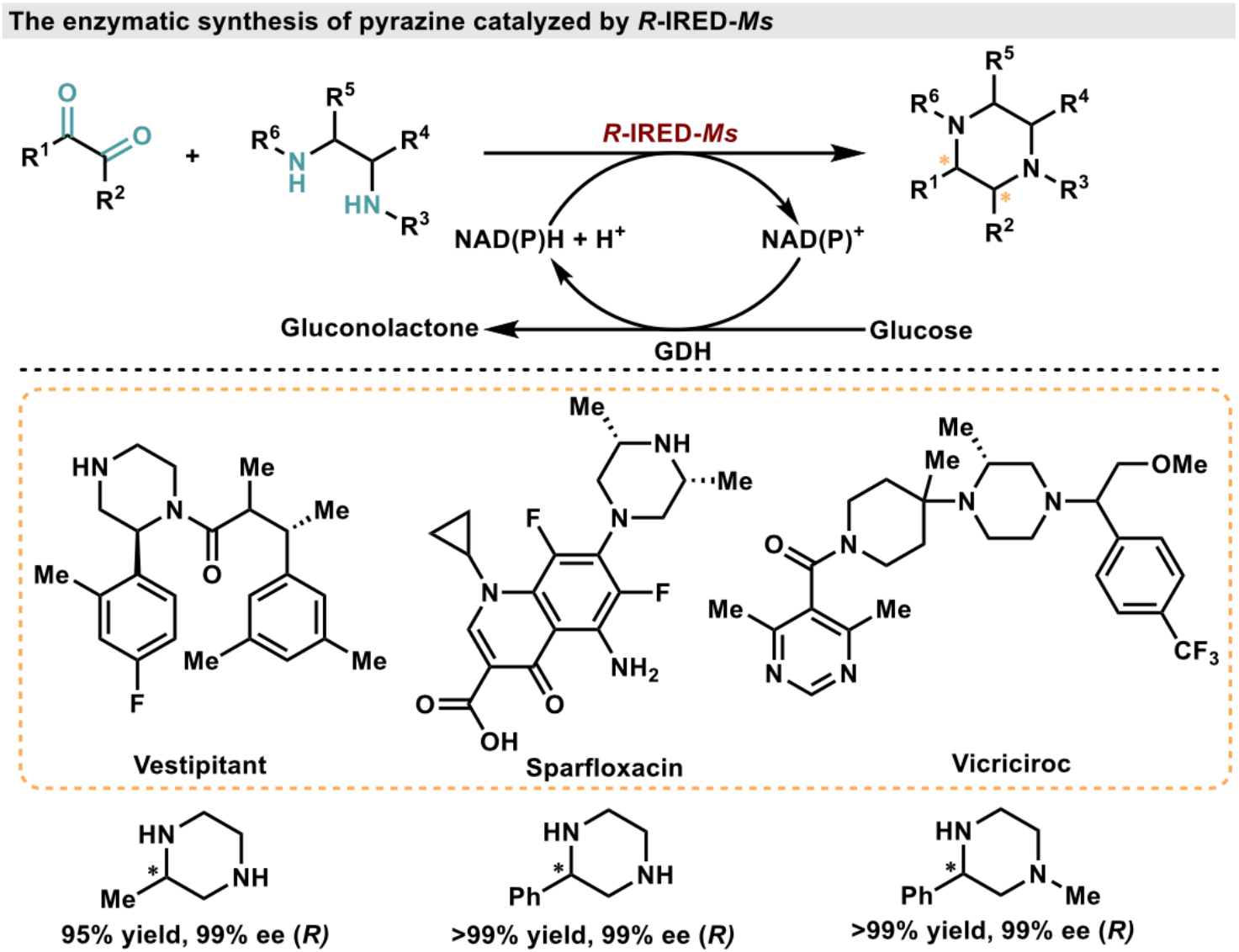
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

Synthesis of 1,2-Amino Alcohols IREDs



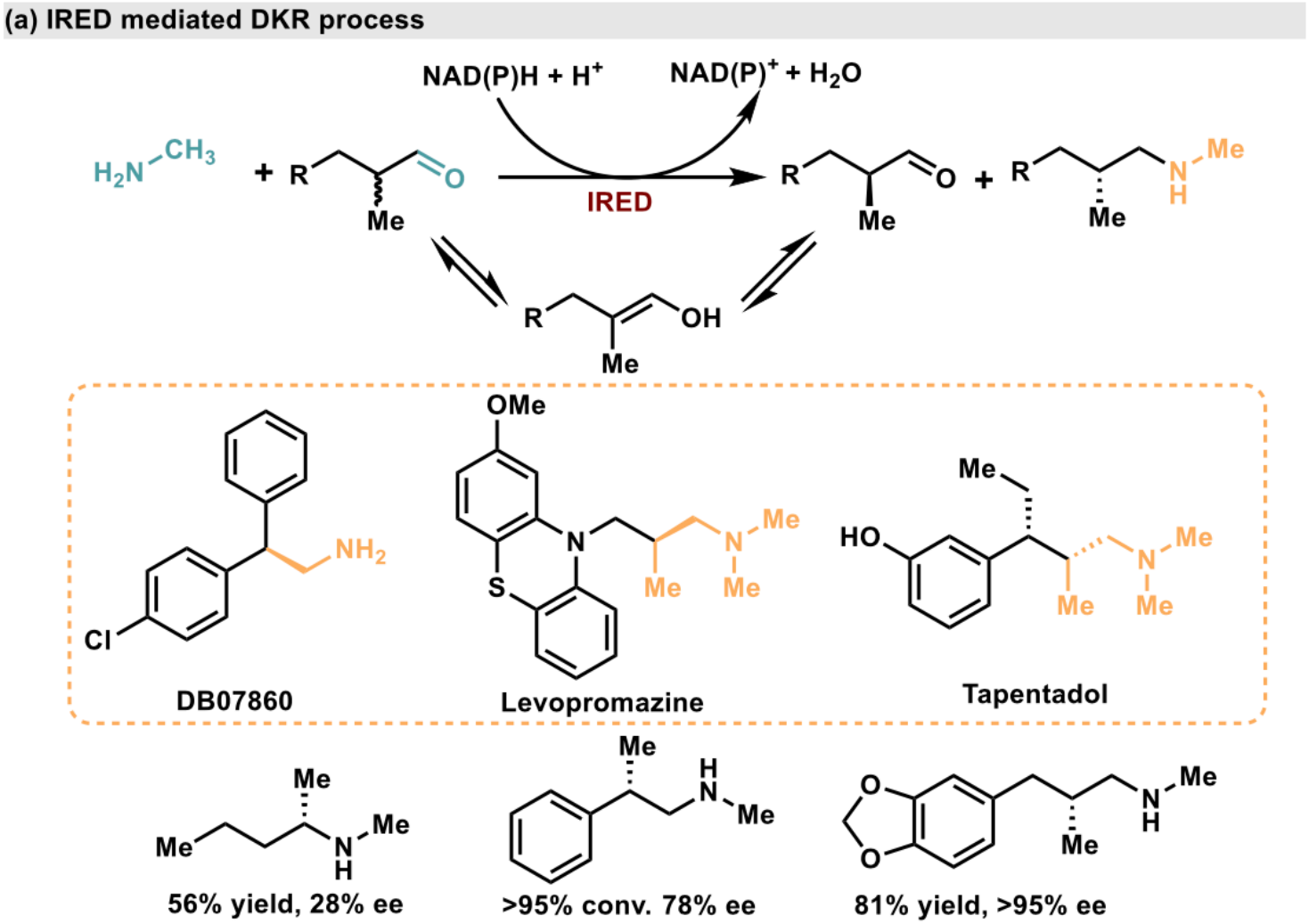
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

Pyrazine Synthesis with IREDs



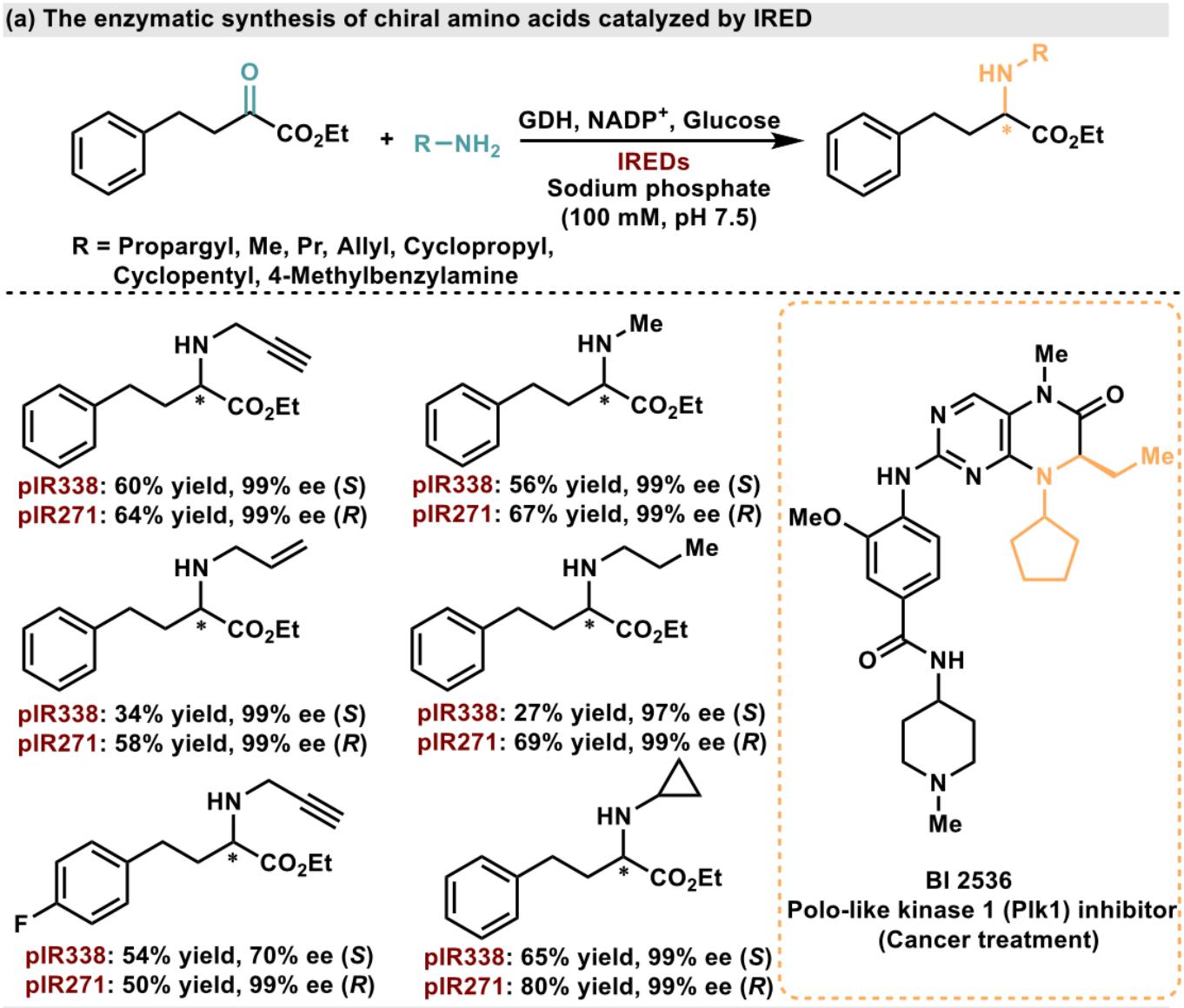
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

DKR of Aldehydes with IREDs



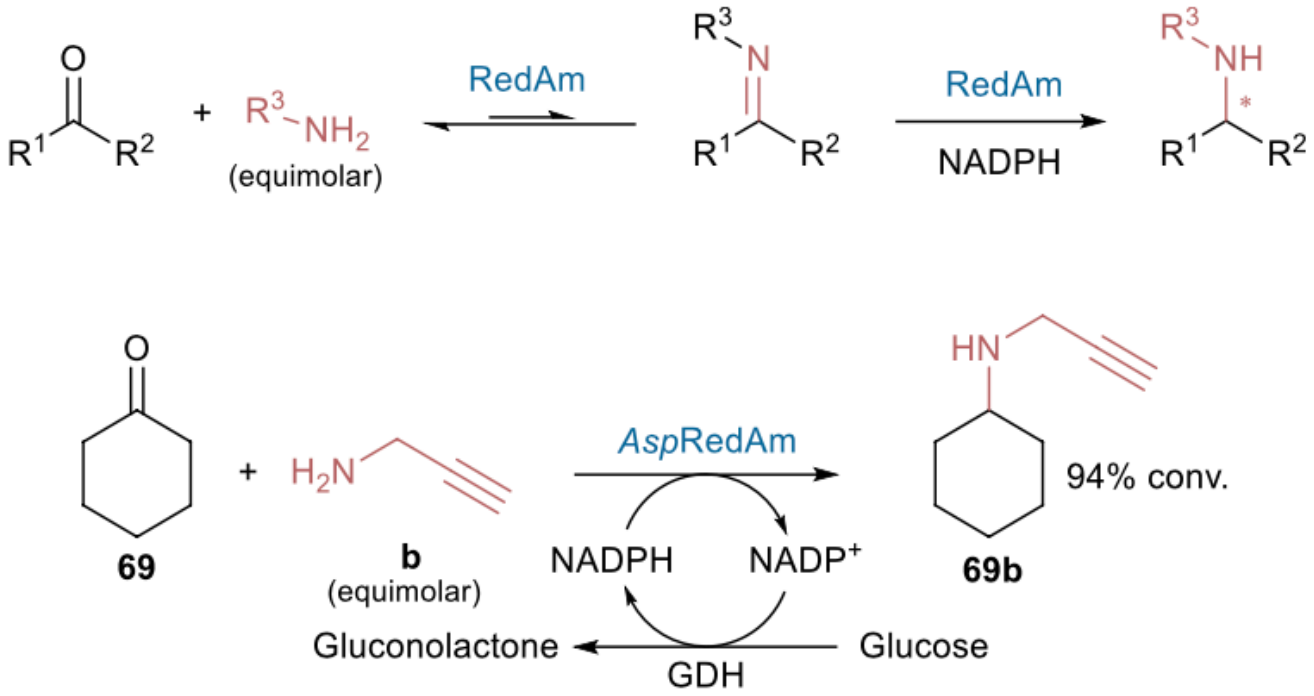
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

Noncanonical Amino Acid Synthesis with IREDs



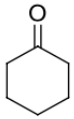
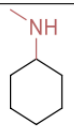
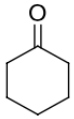
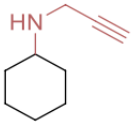
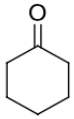
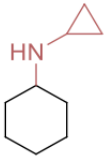
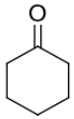
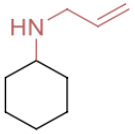
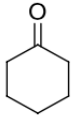
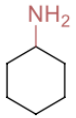
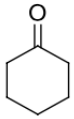
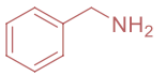
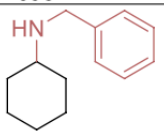
Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

RedAm Catalyzed Equimolar Reductive Amination



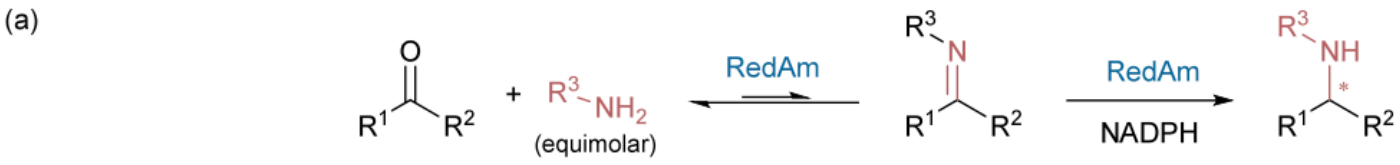
ACS Catal. ***2024***, *14*, 14308-14329.

RedAm Catalyzed Equimolar Reductive Amination

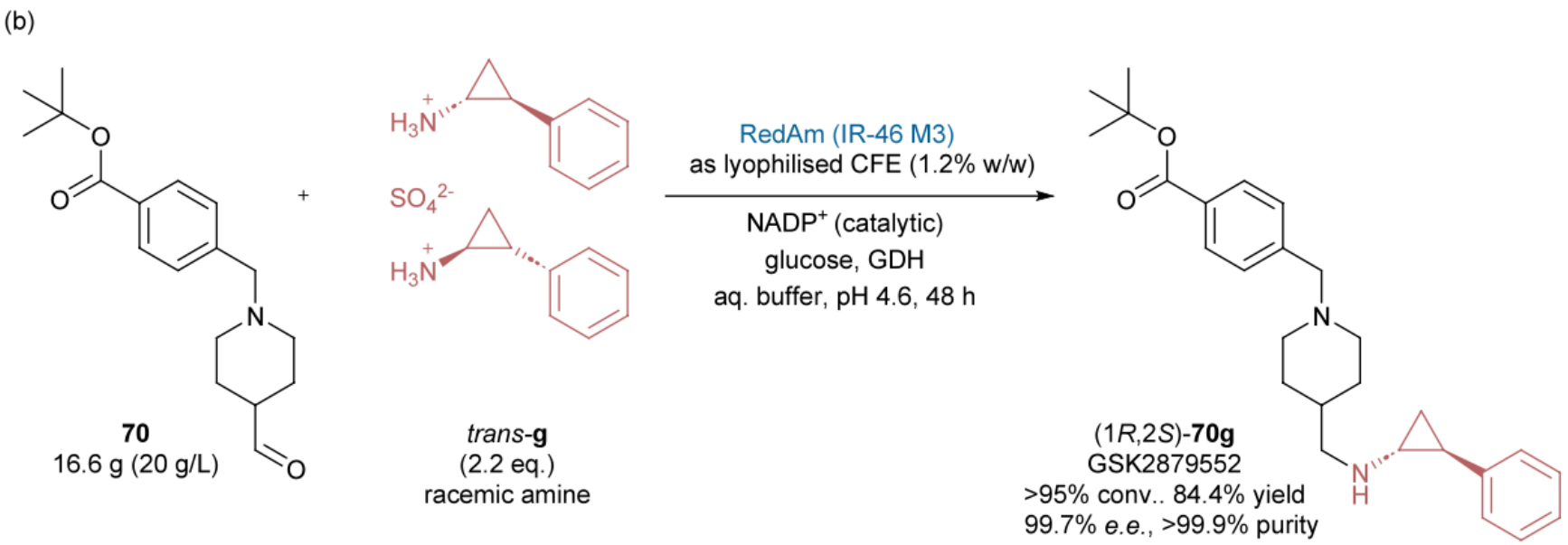
Carbonyl acceptor	Amine	Carbonyl: Amine ratio	Product	Conversion (%)
 **69**	 **a**	1:2	 **69a**	95
 **69**	 **b**	1:1	 **69b**	94
 **69**	 **c**	1:1	 **69c**	90
 **69**	 **d**	1:1	 **69d**	73
 **69**	 **e**	1:4	 **69e**	47
 **69**	 **f**	1:1	 **69f**	84

ACS Catal. ***2024***, *14*, 14308-14329.

Industrial-Scale Applications of RedAms



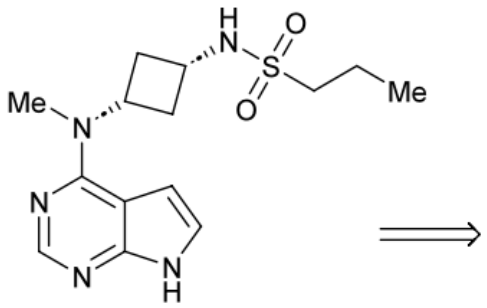
Examples of industry scale application



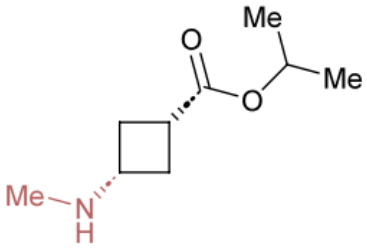
ACS Catal. ***2024***, *14*, 14308-14329.

Industrial-Scale Applications of RedAms

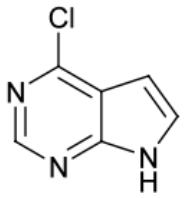
(c)



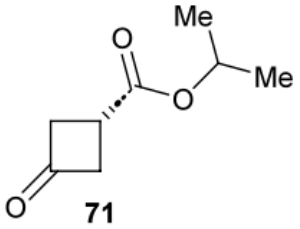
Abrocitinib



cis-cyclobutyl-N-methylamine



pyrrolopyrimidine core



71

230 kg per batch
(100 g/L)

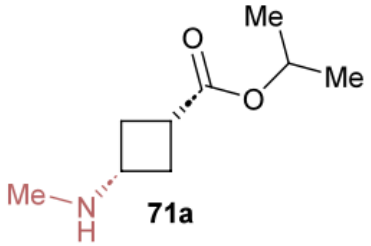
+ $MeNH_2$

a

1.5 eq.

SpRedAm (R3-V6)
as lyophilised CFE (1.5 g/L)

$NADP^+$ (catalytic)
glucose, GDH
aq. buffer, pH 7.0, 48 h

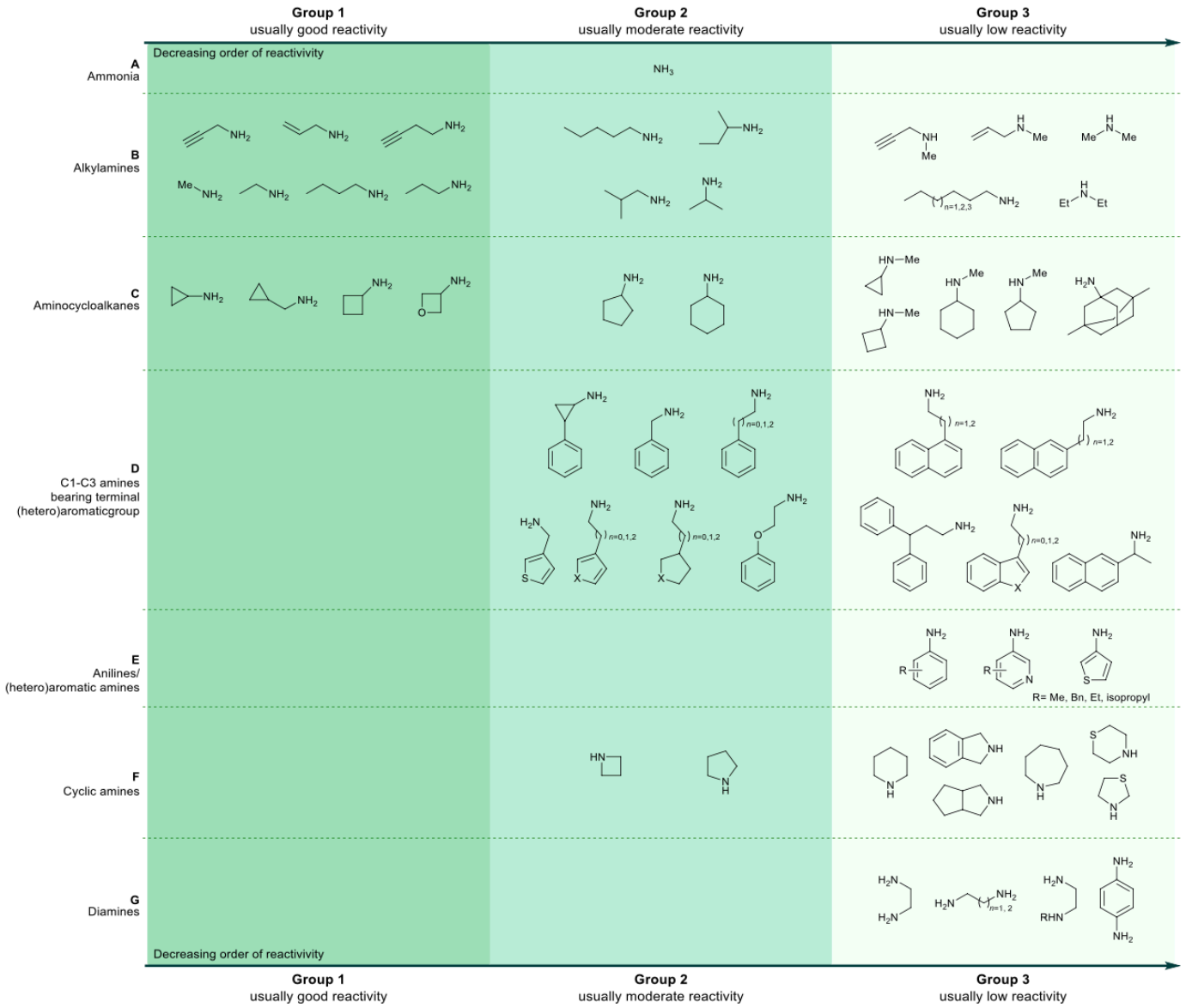


71a

92.5% conv., 73% yield.
>99.5% *d.r.*, >99% purity

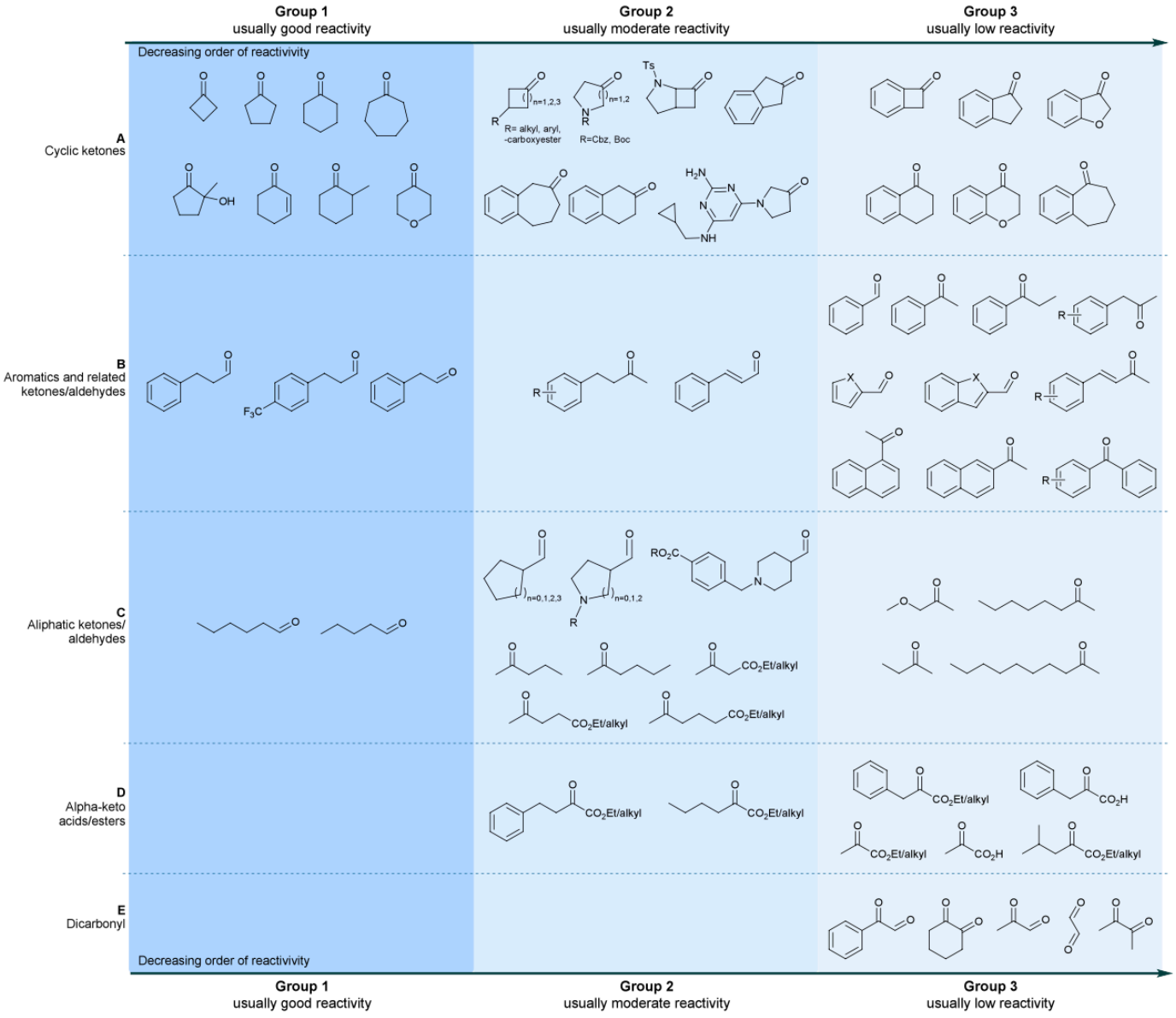
ACS Catal. ***2024***, *14*, 14308-14329.

RedAms: Amine Nucleophile Tolerance



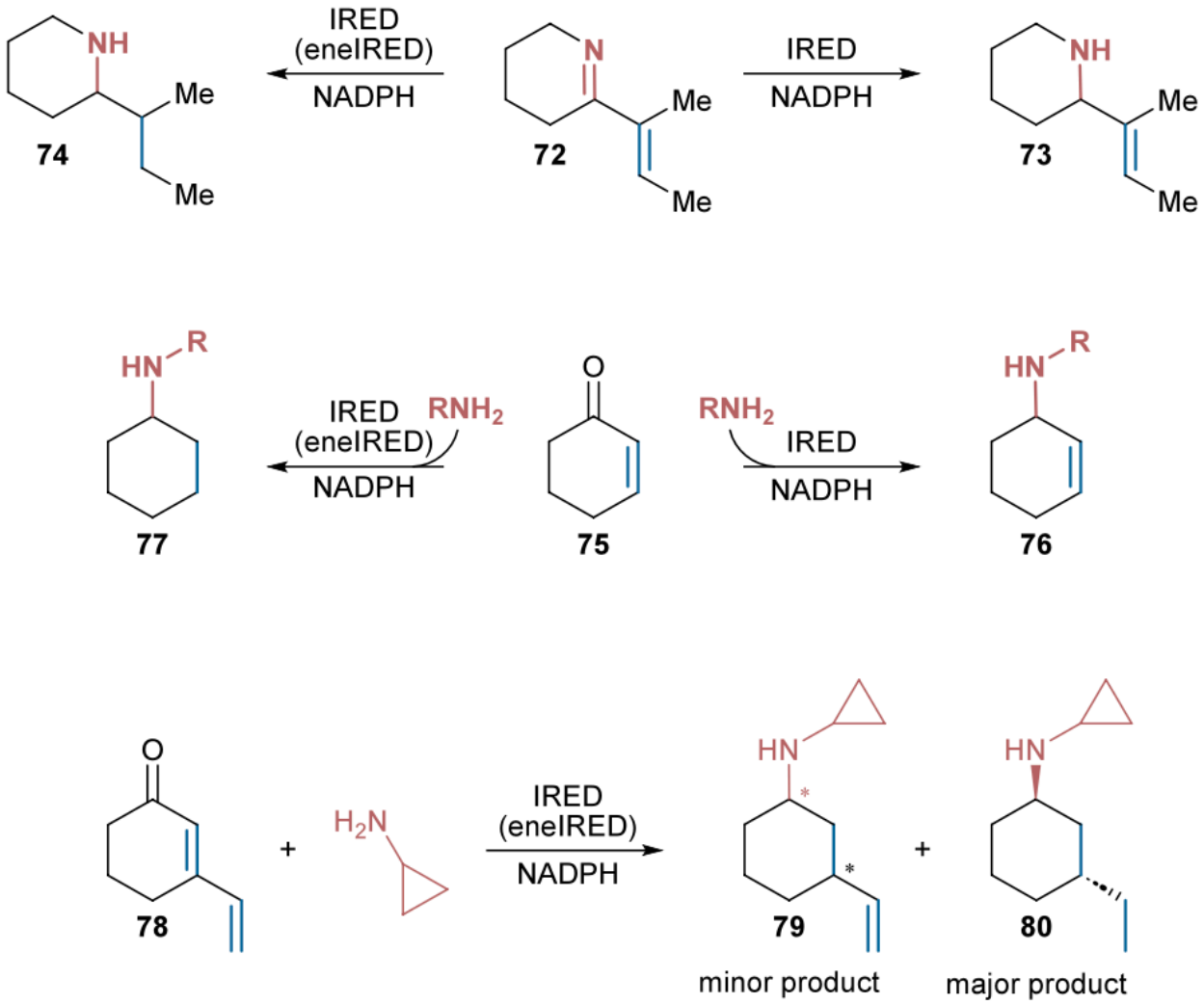
ACS Catal. ***2024***, *14*, 14308-14329.

RedAms: Carbonyl Acceptor Tolerance



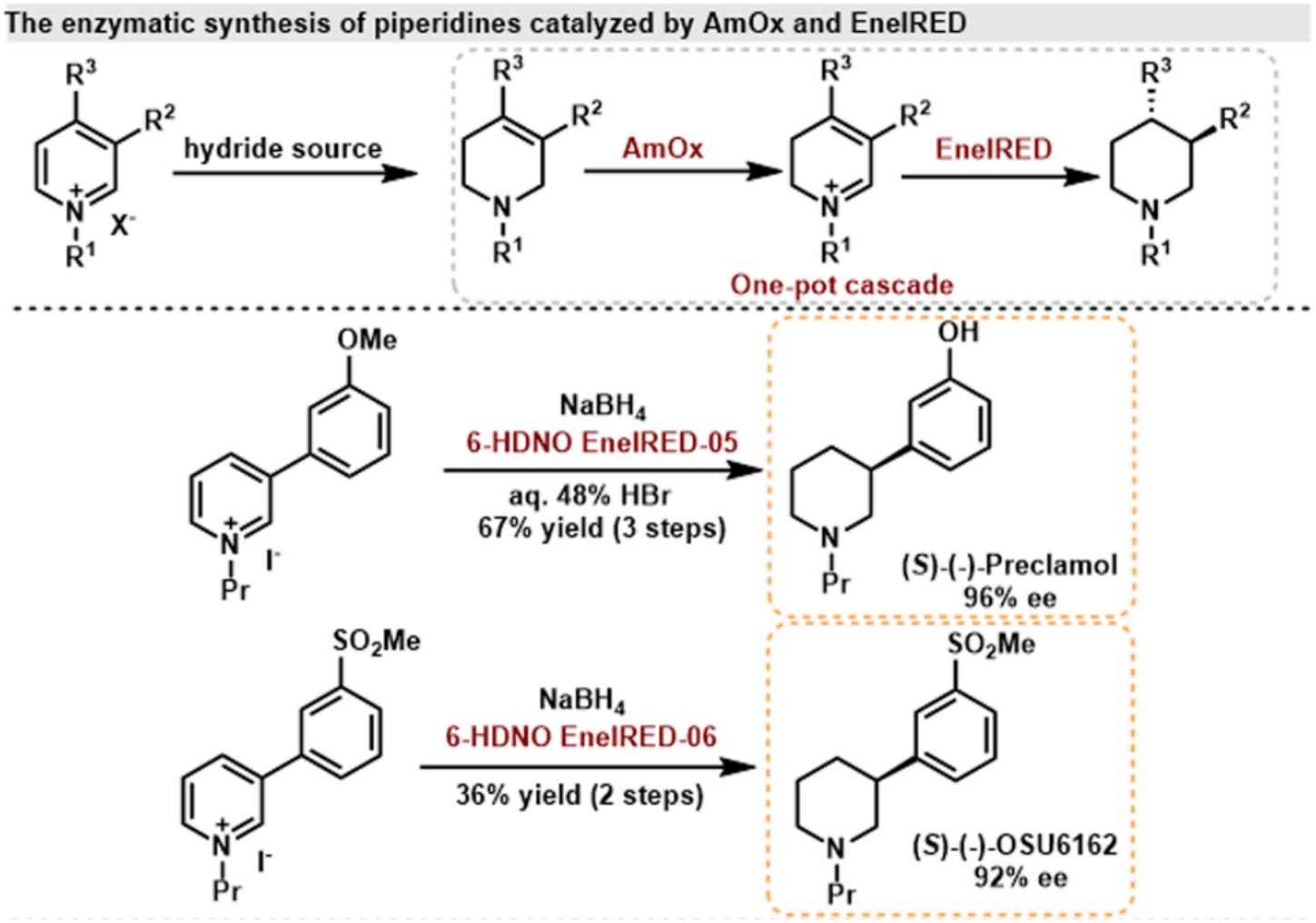
ACS Catal. ***2024***, *14*, 14308-14329.

IRED Catalyzed Four Electron Reduction: eneIREDs



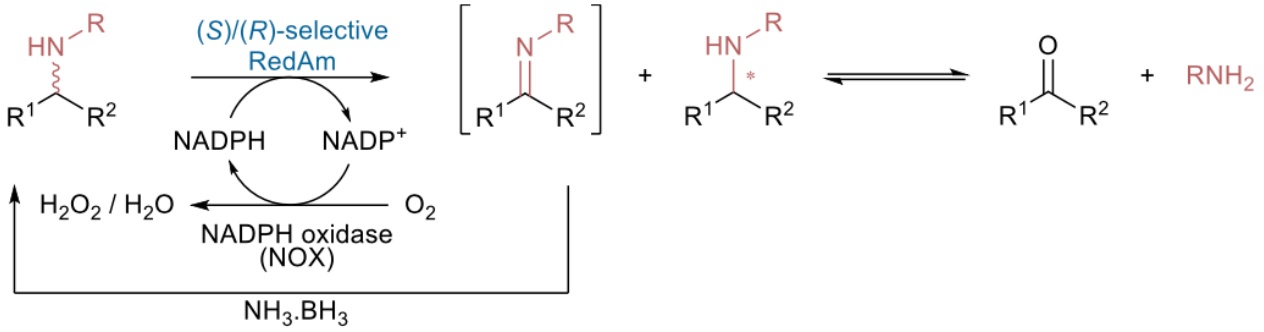
ACS Catal. ***2024***, *14*, 14308-14329.

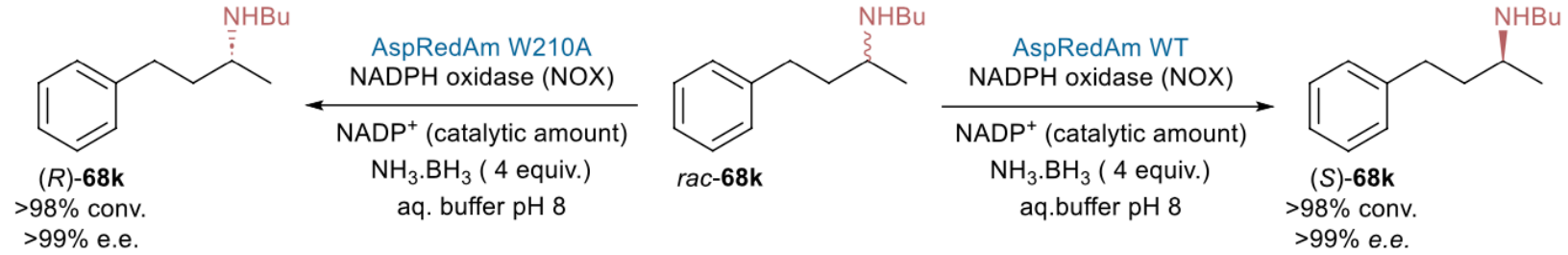
Piperdine Synthesis with eneIREDs



Org. Process Res. Dev. ***2024***, *28*, 3035-3054.

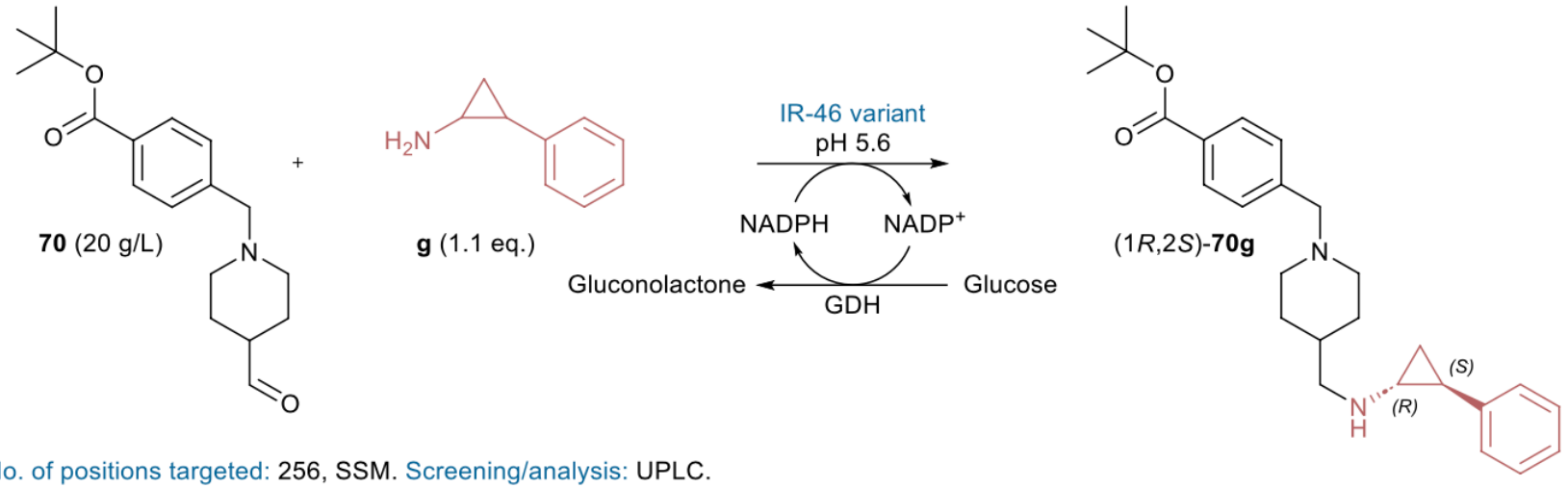
Deracemization with RedAms



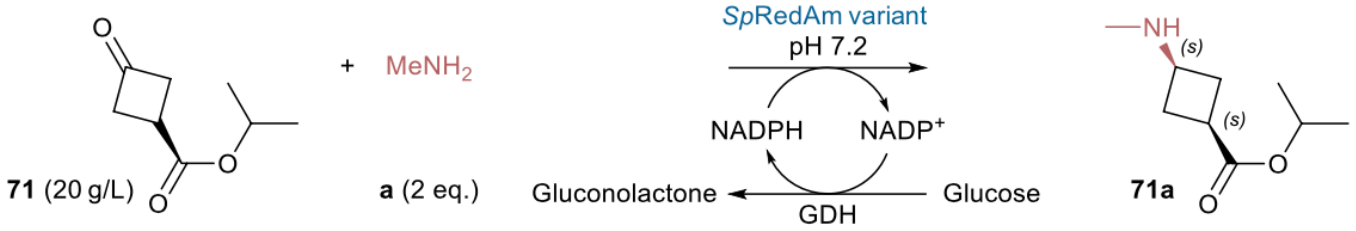


ACS Catal. ***2024***, *14*, 14308-14329.

Select Engineering Campaigns with IREDs and RedAms



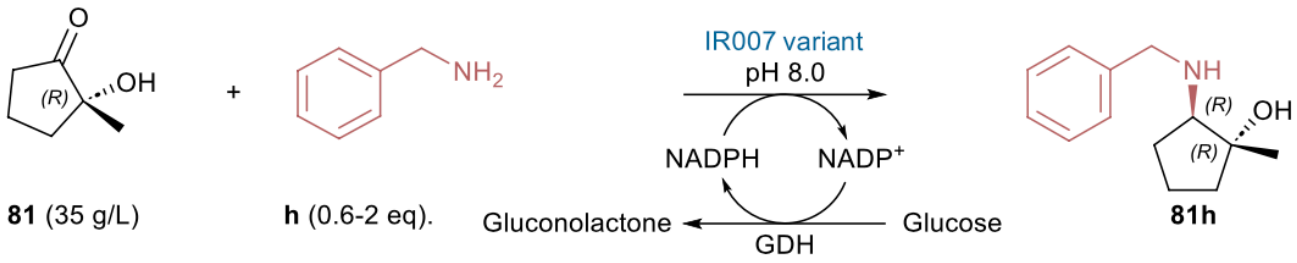
No. of positions targeted: 256, SSM. Screening/analysis: UPLC.
Key hotspots: Y142, D194, L201, G268, L37, Q231, L304, S258, A276, F97, L120.



No. of positions targeted: 93, SSM. Screening/analysis: UPLC.
Key hotspots: F180, A170, F214, Q13, D220, N131, D250, G242, Q237, M176.

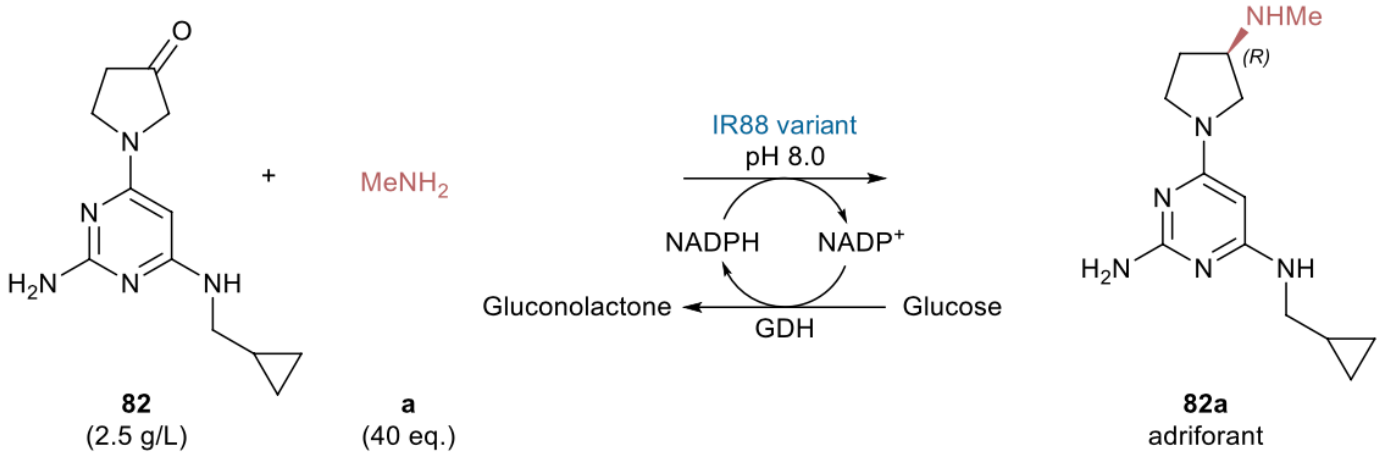
ACS Catal. ***2024***, *14*, 14308-14329.

Select Engineering Campaigns with IREDs and RedAms



81 (35 g/L) **h** (0.6-2 eq). **81h**

No. of positions targeted: 94, SSM. Screening/analysis: UPLC.
Key hotspots: M197, M206, A213, A214, I240, Q217, and S273.

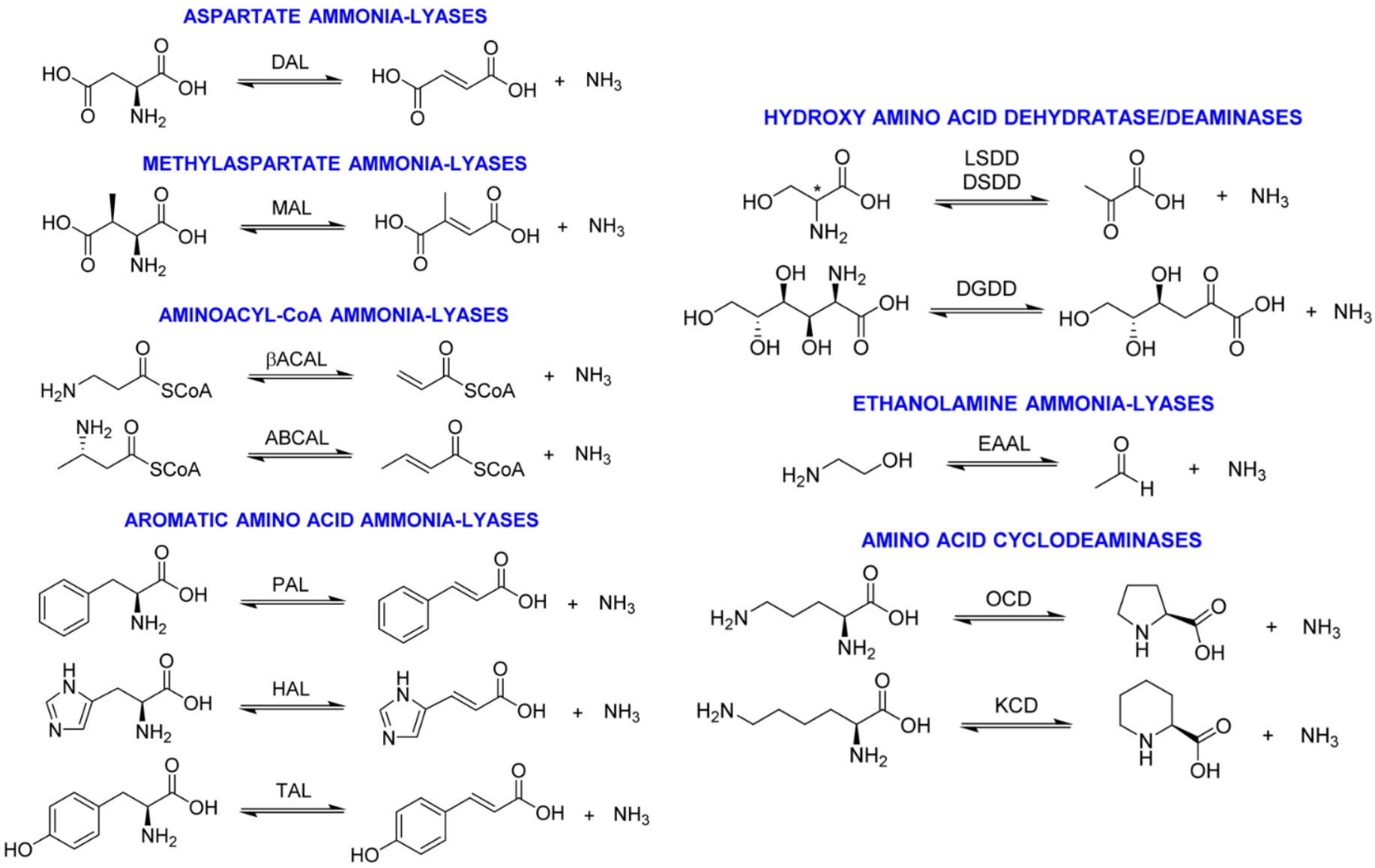


82 (2.5 g/L) **a** (40 eq.) **82a** adriforant

No. of positions targeted: all, DMS. Screening/analysis method: RapidFire MS, UPLC.
Key hotspots: S220, A296, H154, R155, A156, A159, S160, A218, L243, T242, V26, I212, R247, V250, S117, A210, S57, V175, R247, A259, A218.

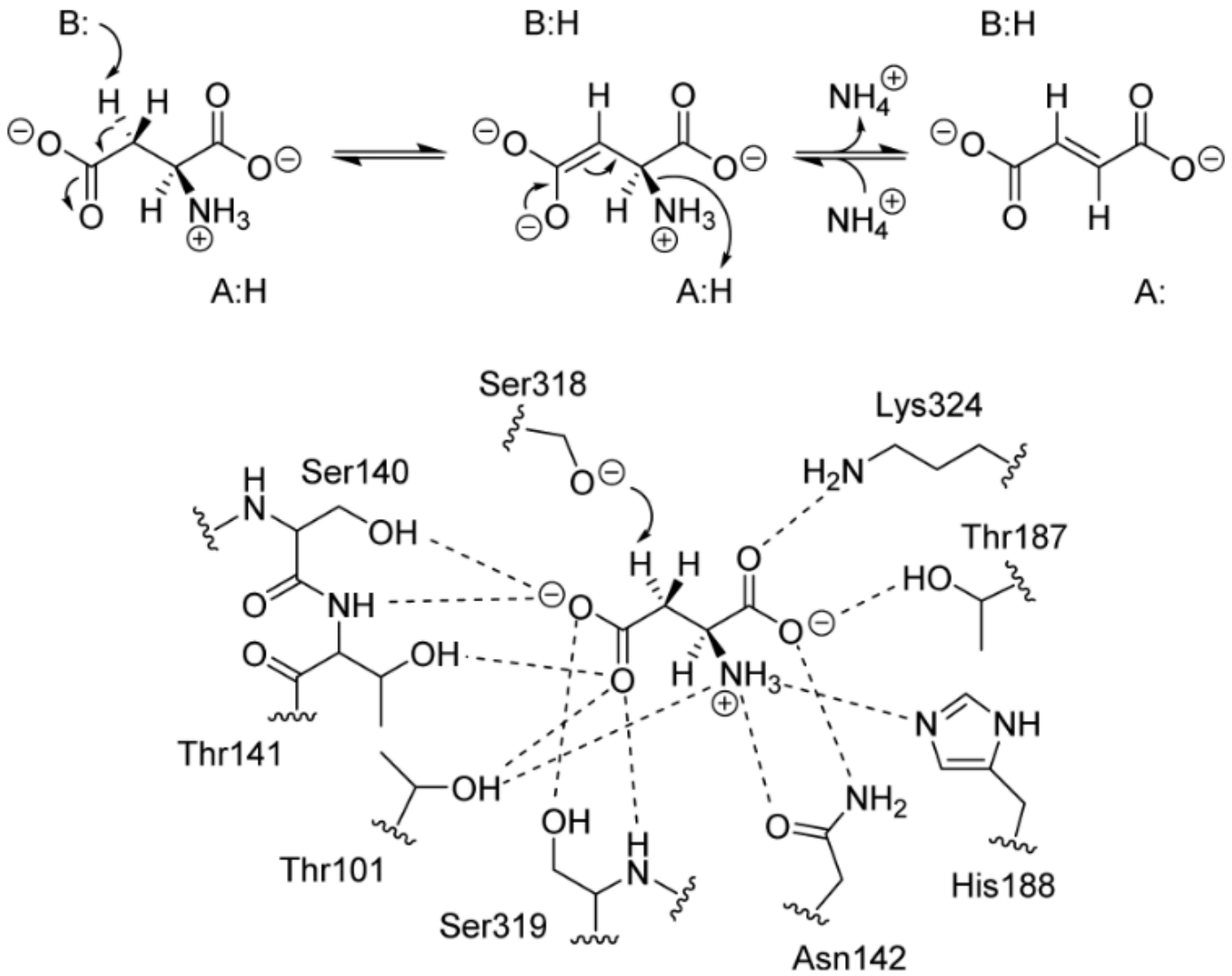
ACS Catal. ***2024***, *14*, 14308-14329.

Reversible C-N Bond Formation with Ammonia Lyases (ALs)



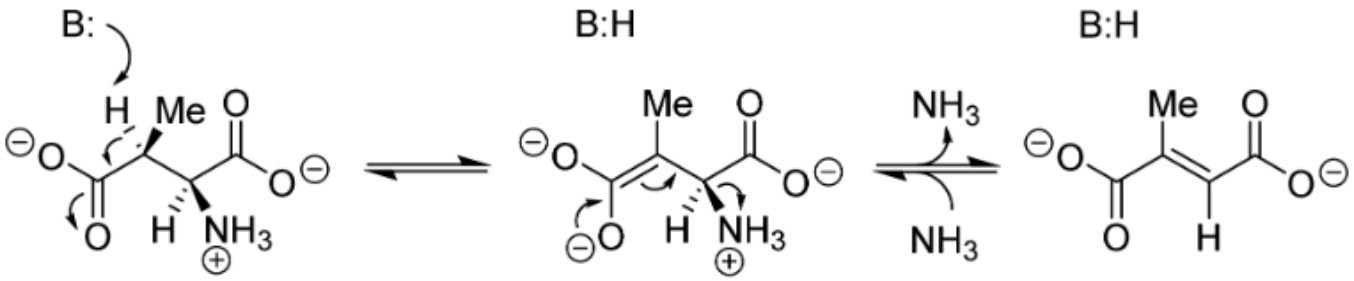
Chem Rev. ***2018***, *118*, 73-118.

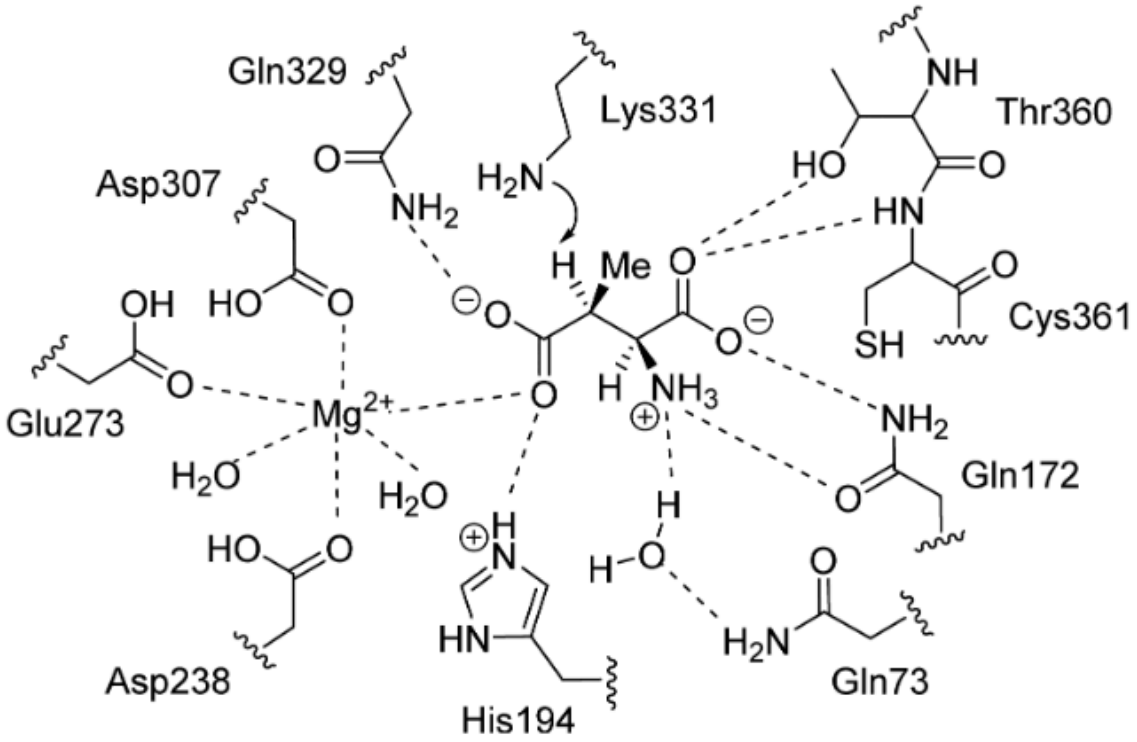
Representative Mechanism for ALs: Aspartate Ammonia Lyase (DAL)



Chem Rev. ***2018***, *118*, 73-118.

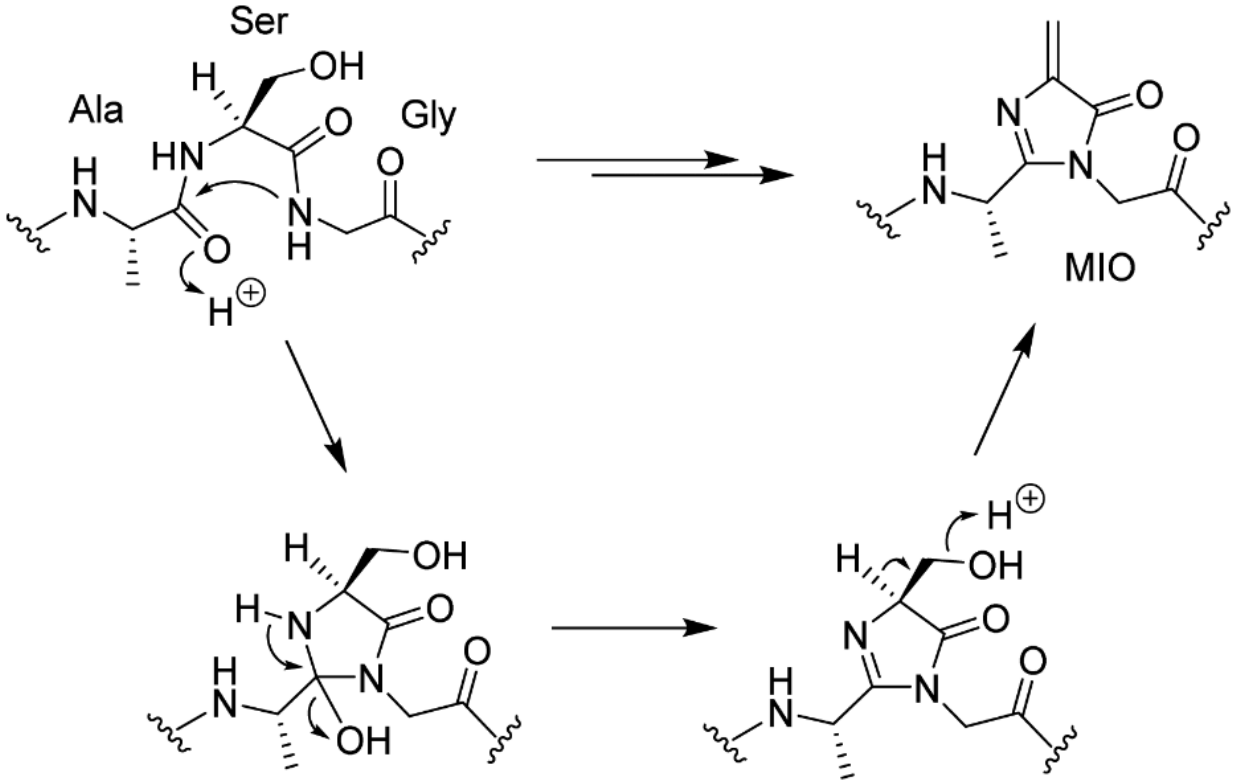
Representative Mechanism for ALs: Methylaspartate Ammonia Lyase (MAL)





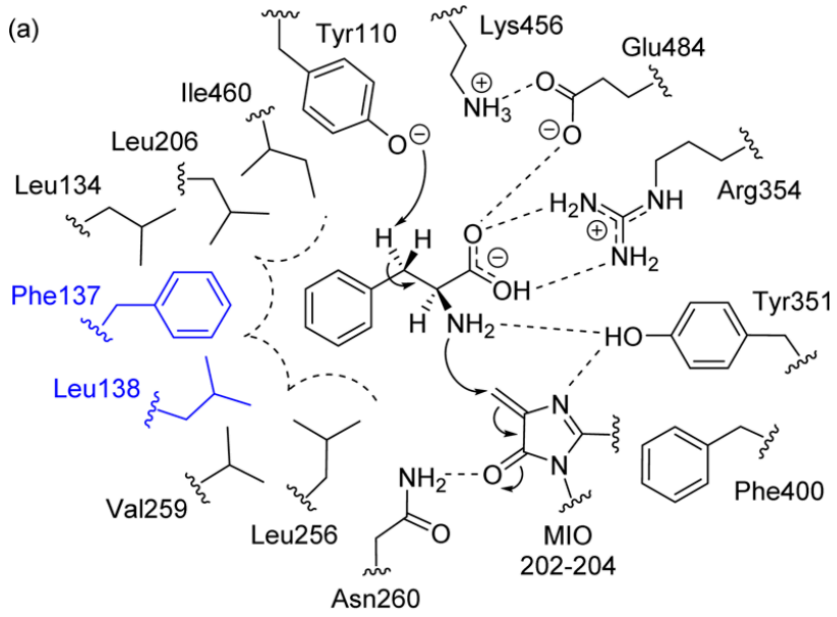
Chem Rev. ***2018***, *118*, 73-118.

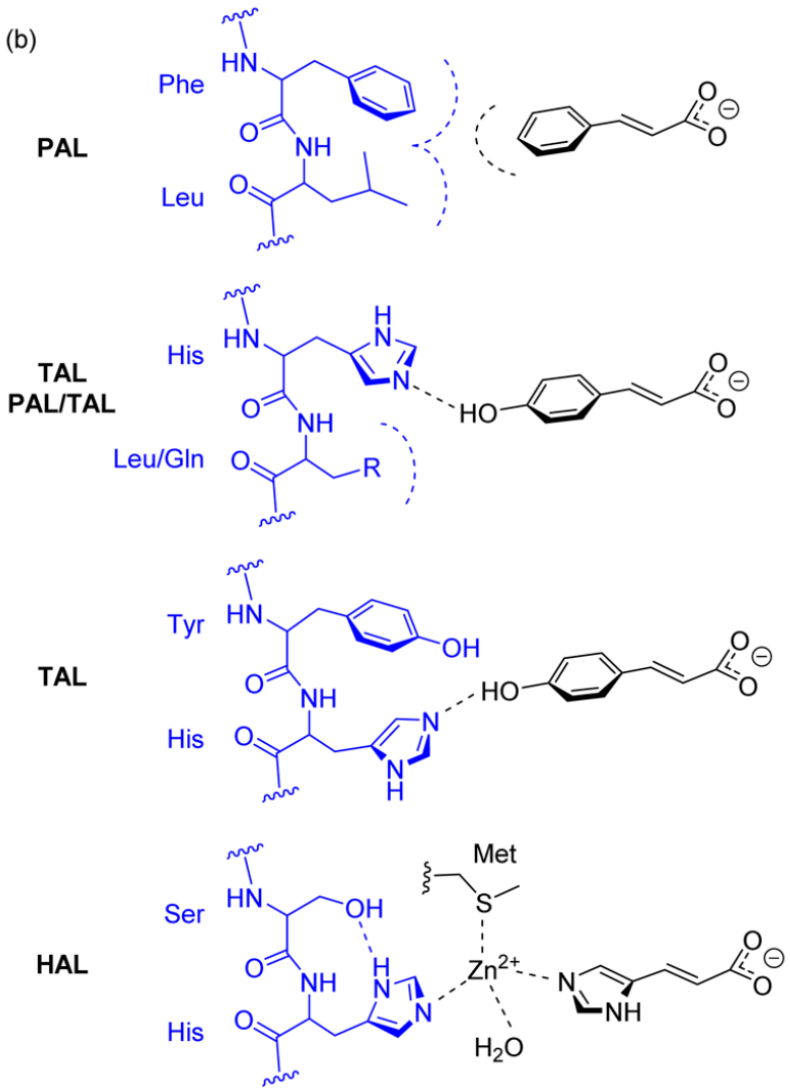
Biosynthesis of Methylideneimidazole-5-one (MIO) Cofactor



Chem Rev. ***2018***, *118*, 73-118.

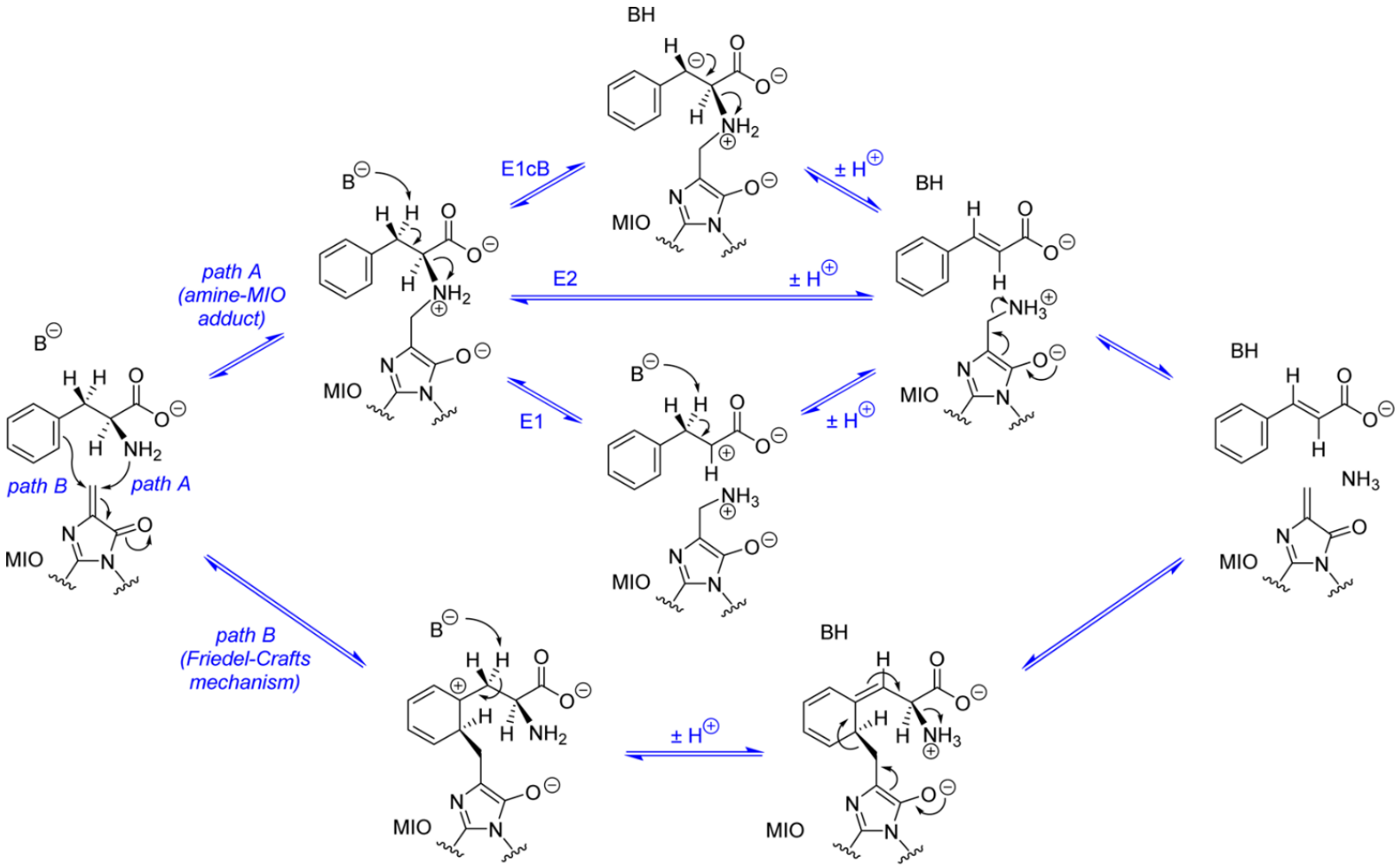
Active Site Contact Points for MIO-Dependent Lyases





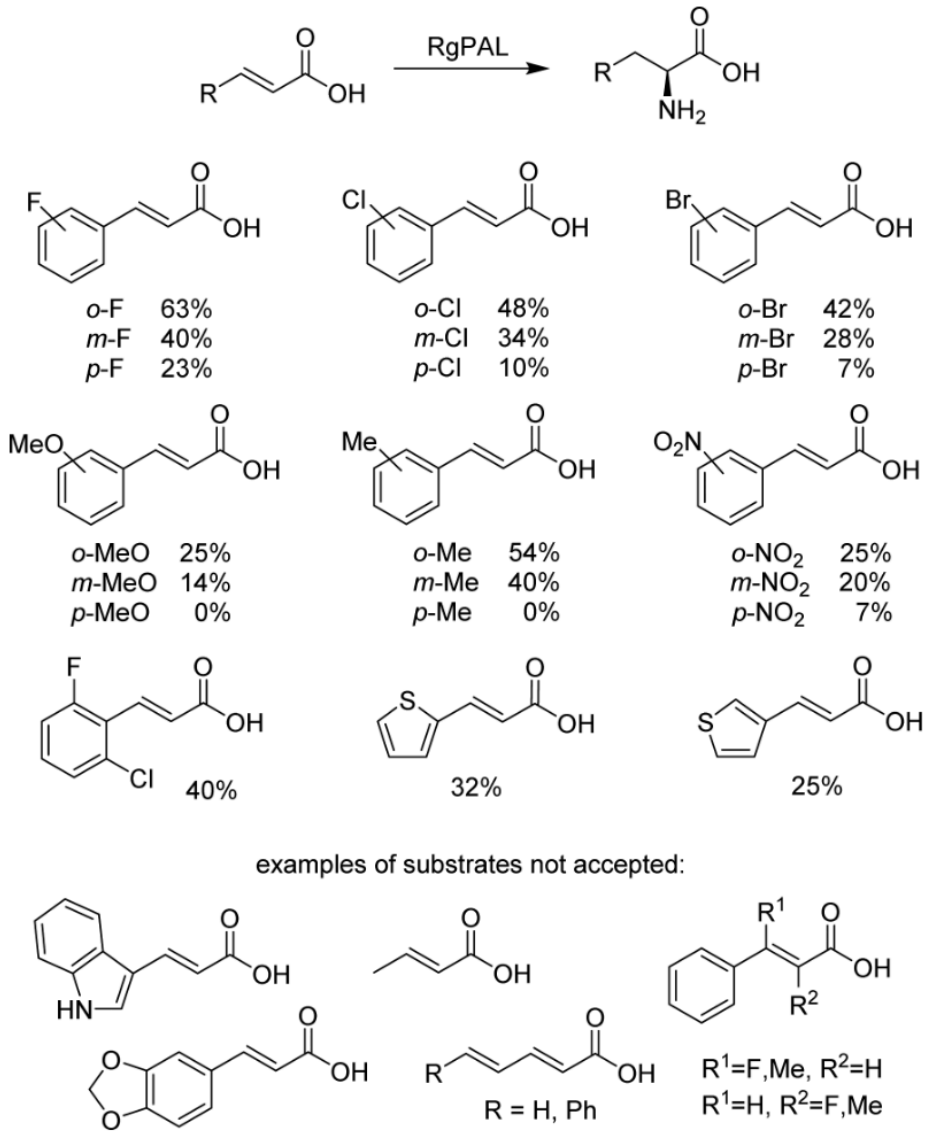
Chem Rev. ***2018***, *118*, 73-118.

Proposed Mechanisms for Deamination by ALs



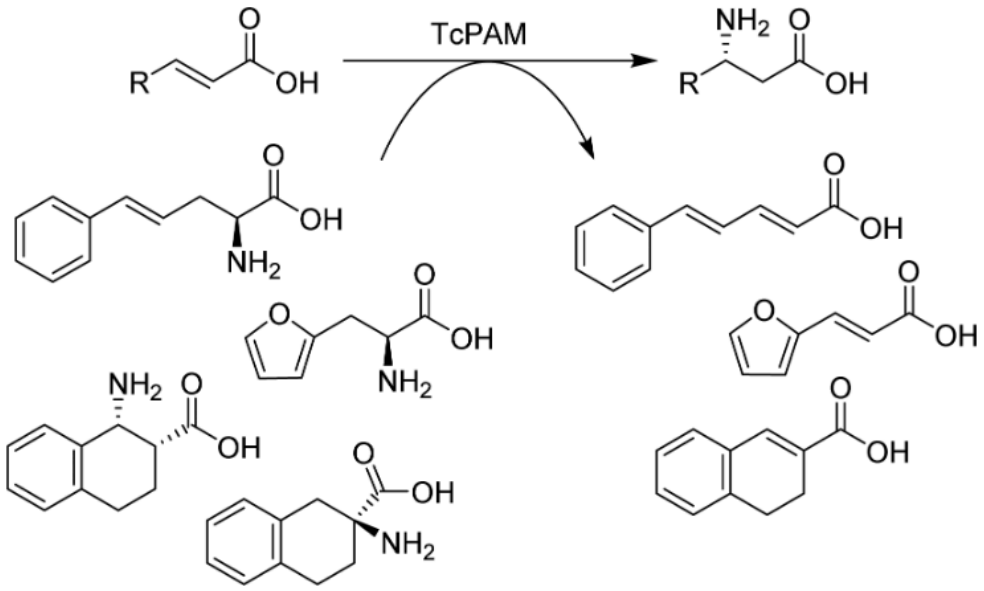
Chem Rev. ***2018***, *118*, 73-118.

Ammonia Lyase (AL) Catalyzed Synthesis of Phenylalanine Derivatives



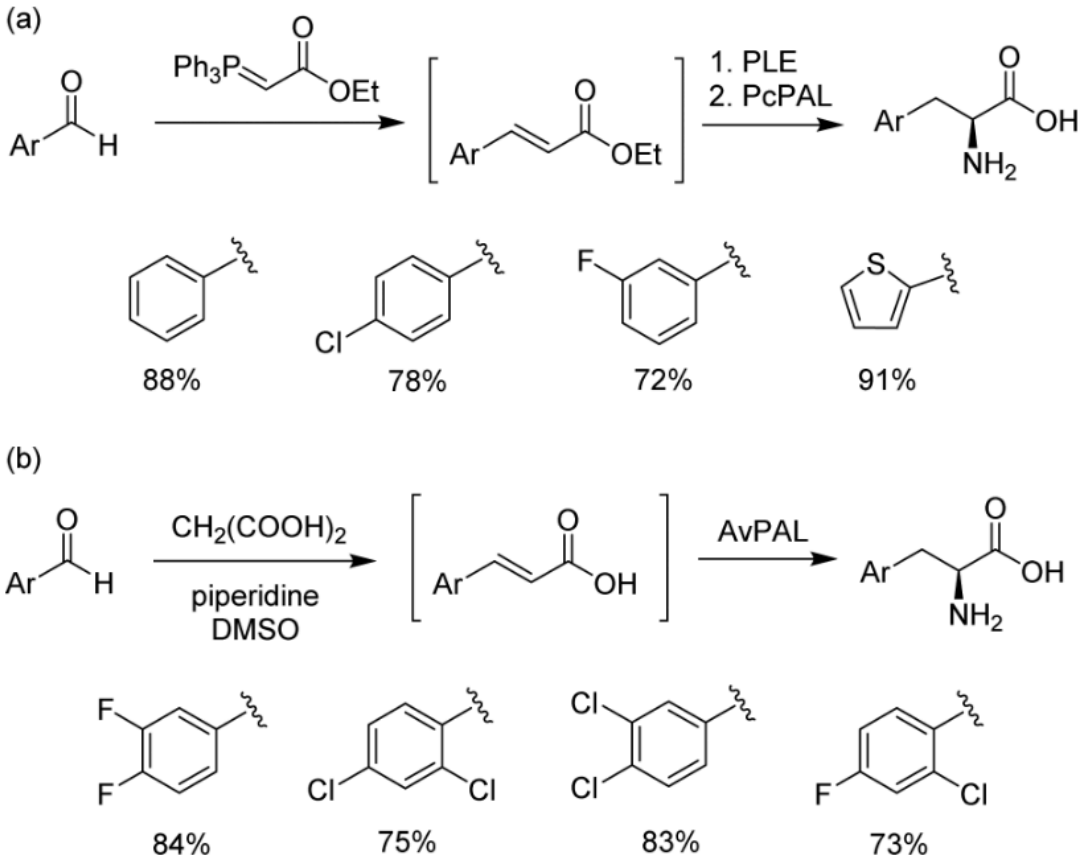
Chem Rev. ***2018***, *118*, 73-118.

Conjugate Addition of Ammonia with ALs



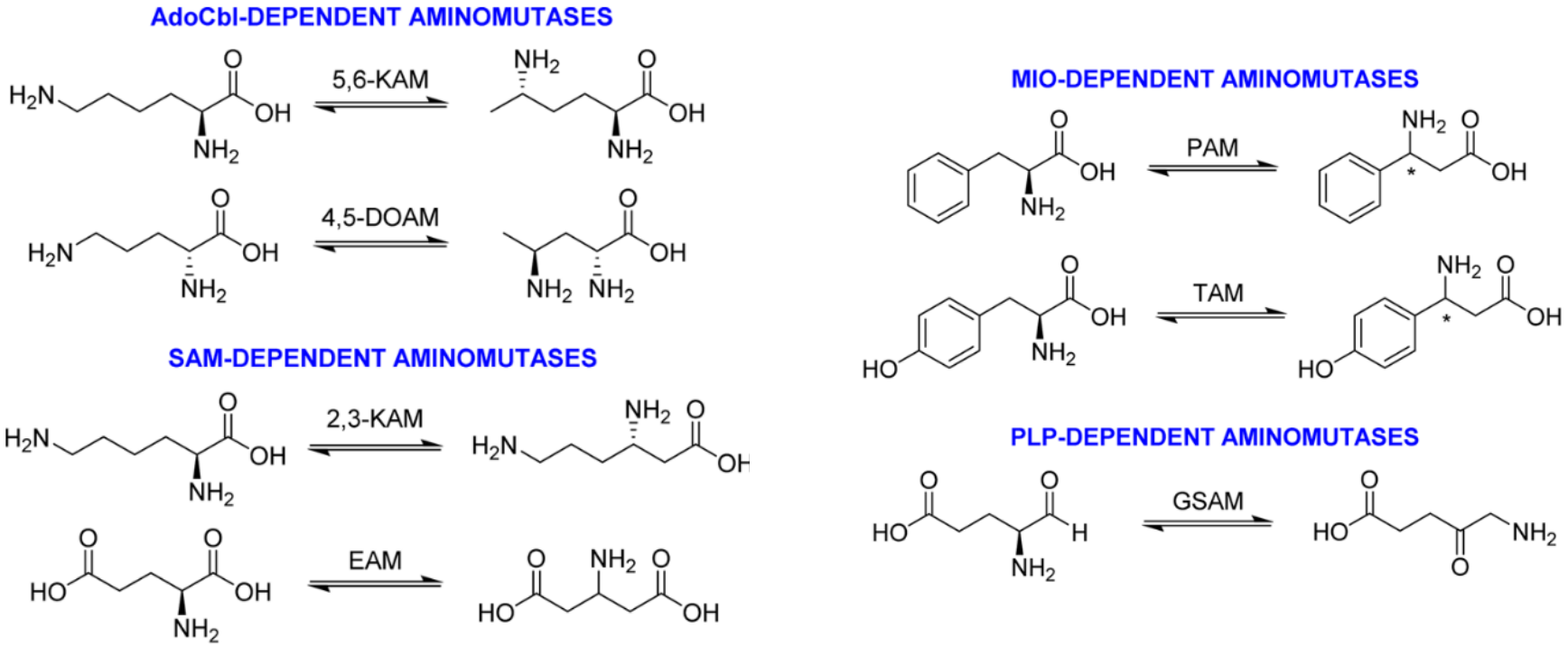
Chem Rev. ***2018***, *118*, 73-118.

One-Pot Olefination/Ammonia Addition



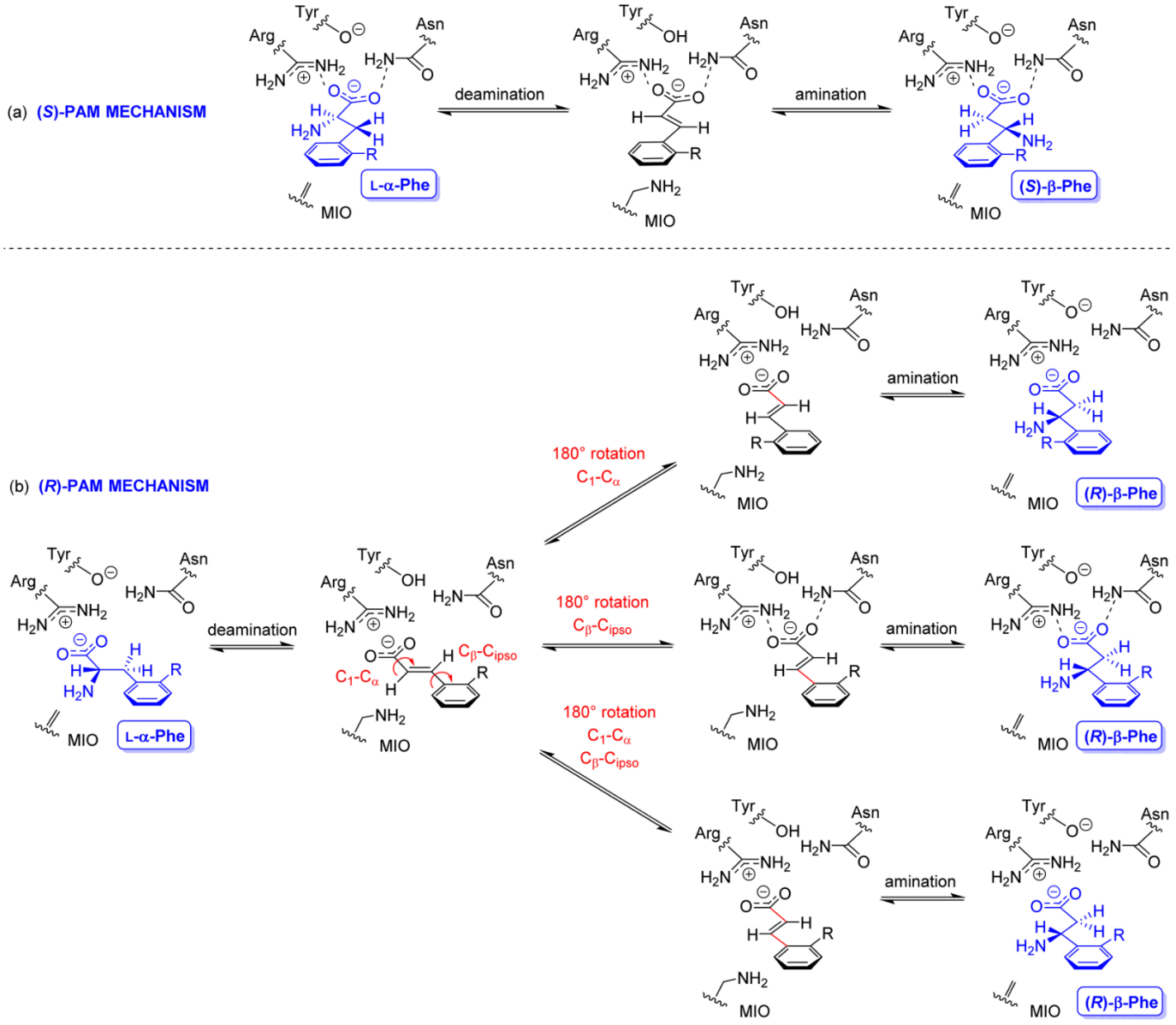
Chem Rev. ***2018***, *118*, 73-118.

Reversible C-N Bond Formation with Aminomutases (AMs)



Chem Rev. ***2018***, *118*, 73-118.

(S) and (R)-Selective Mechanisms for AMs



Chem Rev. ***2018***, *118*, 73-118.