Biosynthesis

Key Aspects

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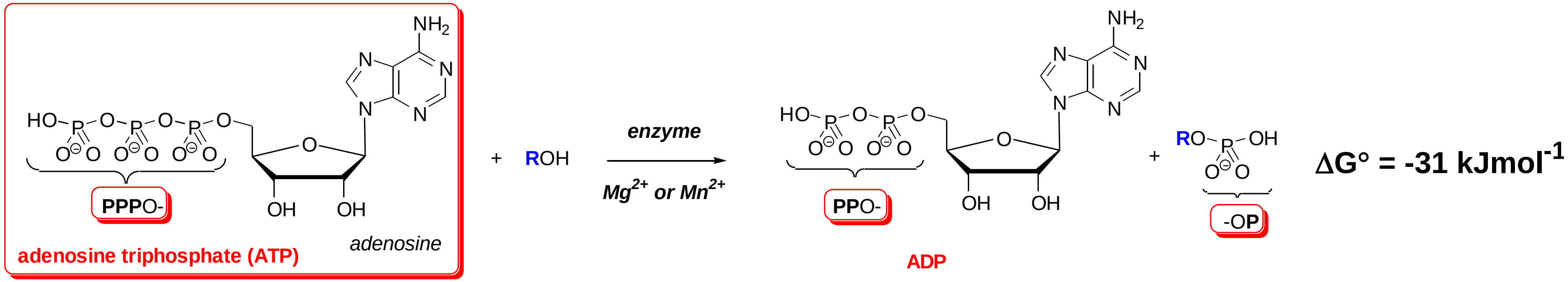
Format & Scope of Lecture

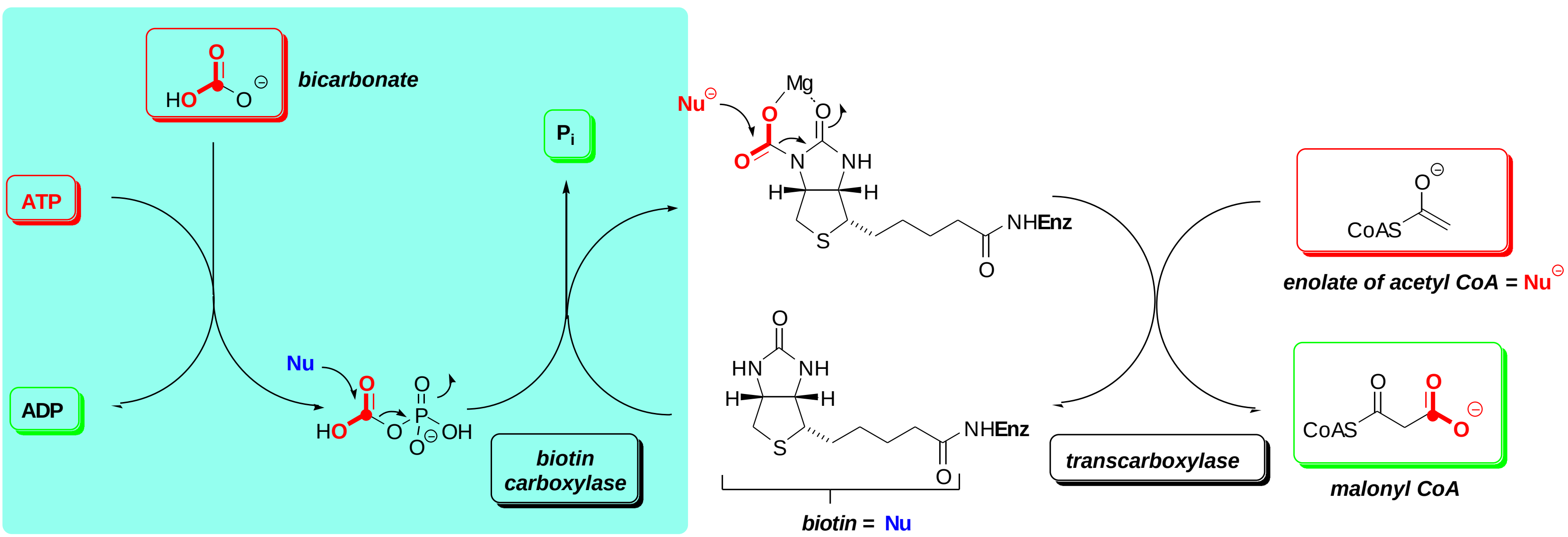
- ***Enzyme cofactor chemistry:***
  - ATP, CoASH, SAM, DMAPP, biotin, $NAD(P)^+$, NAD(P)H, FAD, $FADH_2$, peroxy-$FADH_2$, P_{450} & PLP
- ***Shikimate pathway:***
  - PEP + E-4-P → aromatic α-amino acids
- ***Alkaloids***
  - Lys & Orn pathways to pyrrolidine & piperidine alkaloids – PLP chemistry
  - Phenolic coupling
- ***Fatty acids and polyketides:***
  - The fatty acid synthase (FAS) iterative cycle
  - The polyketide synthase (PKS) iterative cycle(s)
- ***Isoprenoids:***
  - The mevalonate pathway: 3× acetyl CoA → IPP & DMAPP
  - Pathways to linear C10, C15 & C20 isoprenoids: geranyl PP, farnesyl PP, geranylgeranyl PP
  - C30 isoprenoids *via* squalene synthase C15 head-to-head dimerisation
  - Basic carbocation chemistry: alkene cyclisation, elimination, trapping water, 1,2-alkyl & hydride shifts and modes of enzyme control thereof

ATP - Free Energy Releasing Couple

– **Key process:**

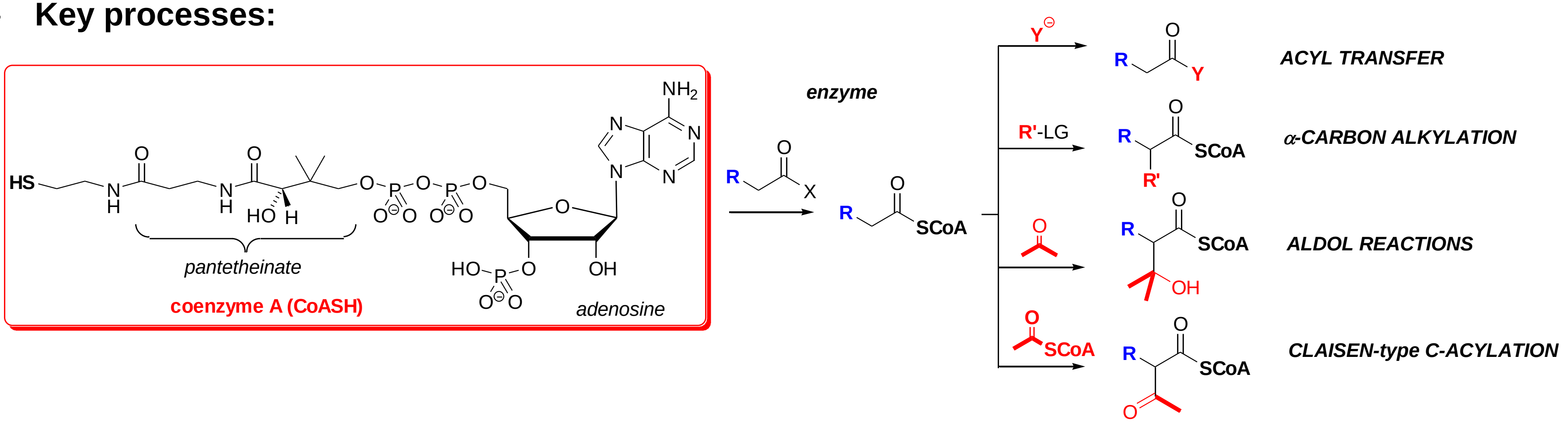


– ***e.g.*** **activation of bicarbonate in malonyl CoA biosynthesis (FA + PK lectures)**

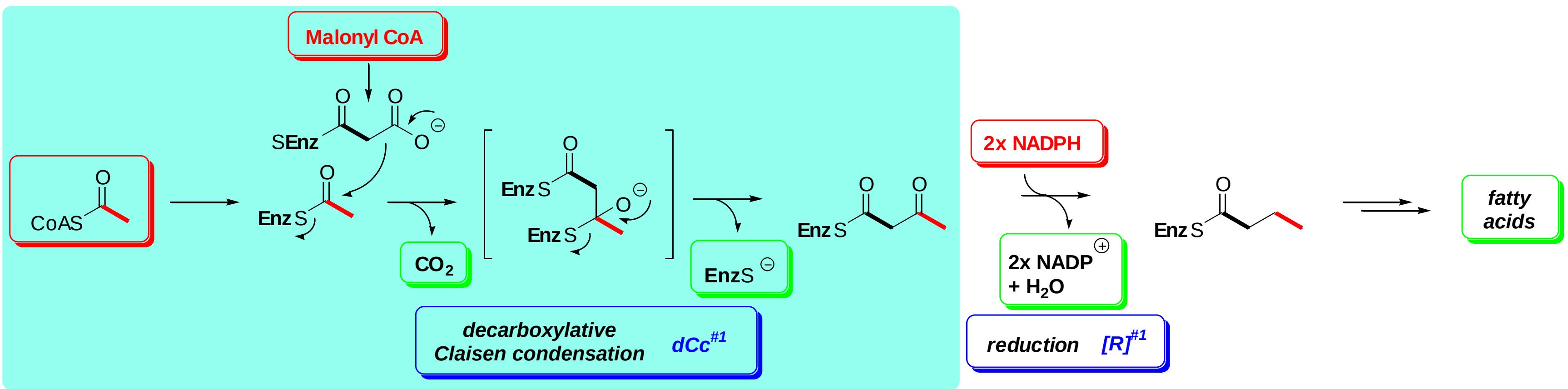


CoASH - C-C Bond Formation

– **Key processes:**

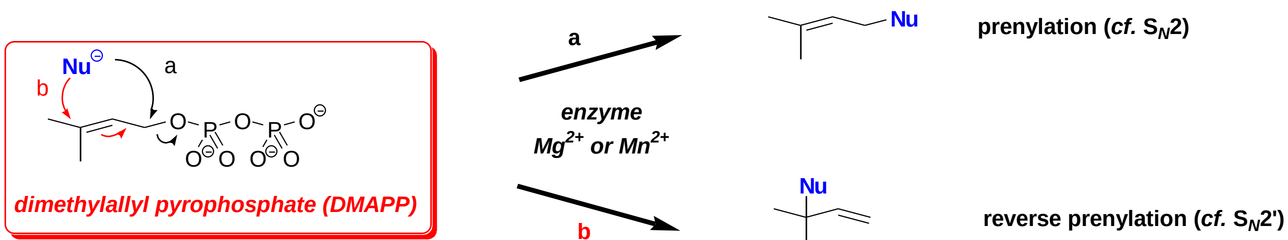


– ***e.g.*** **iterative decarboxylative Claisen condensations in fatty acid biosynthesis (FA + PK lectures)**

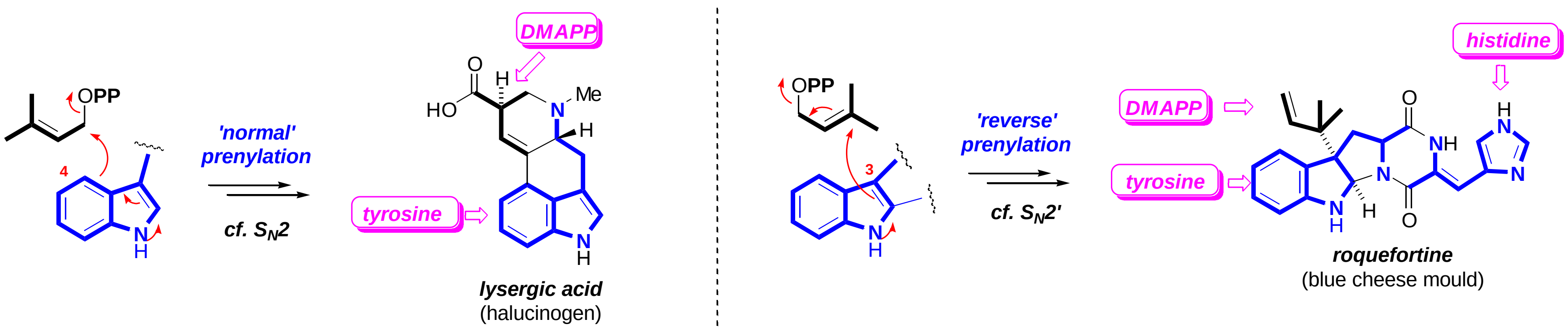


DMAPP - Dimethylallylation

- **Key process:**
  - *NB. via* allylic carbocation with trapping by nucleophile at either most or least substituted end...

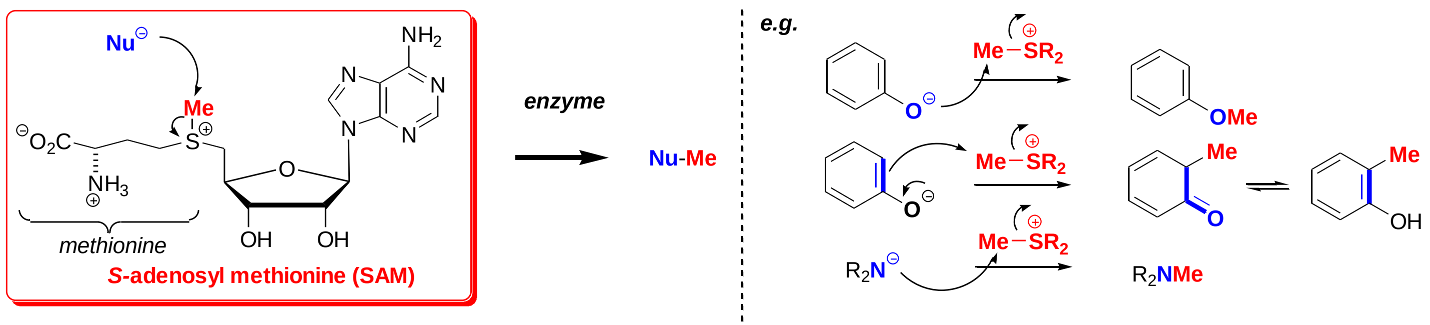


- ***e.g.*** **prenylation & reverse prenylation of alkaloids (alkaloid lectures)**

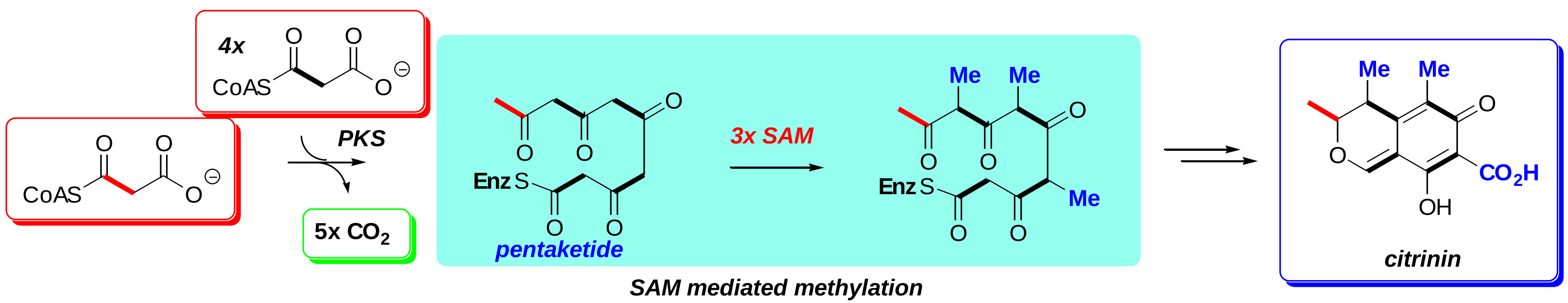


SAM - Methylation

– **Key processes:**

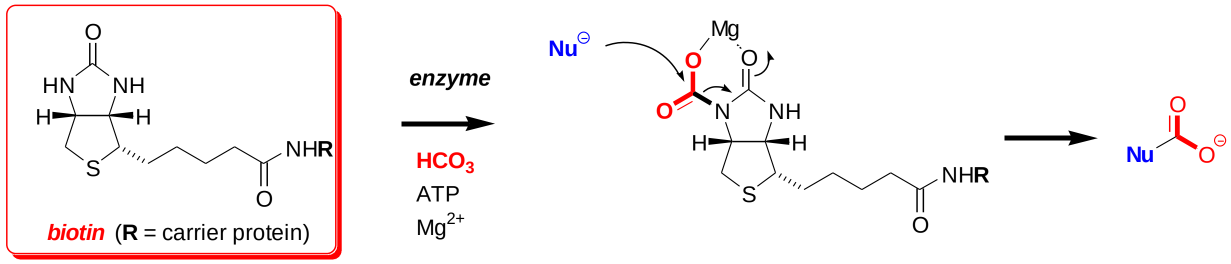


– ***e.g.*** **ketone *C*-methylation in citrinin biosynthesis (FA + PK lectures)**

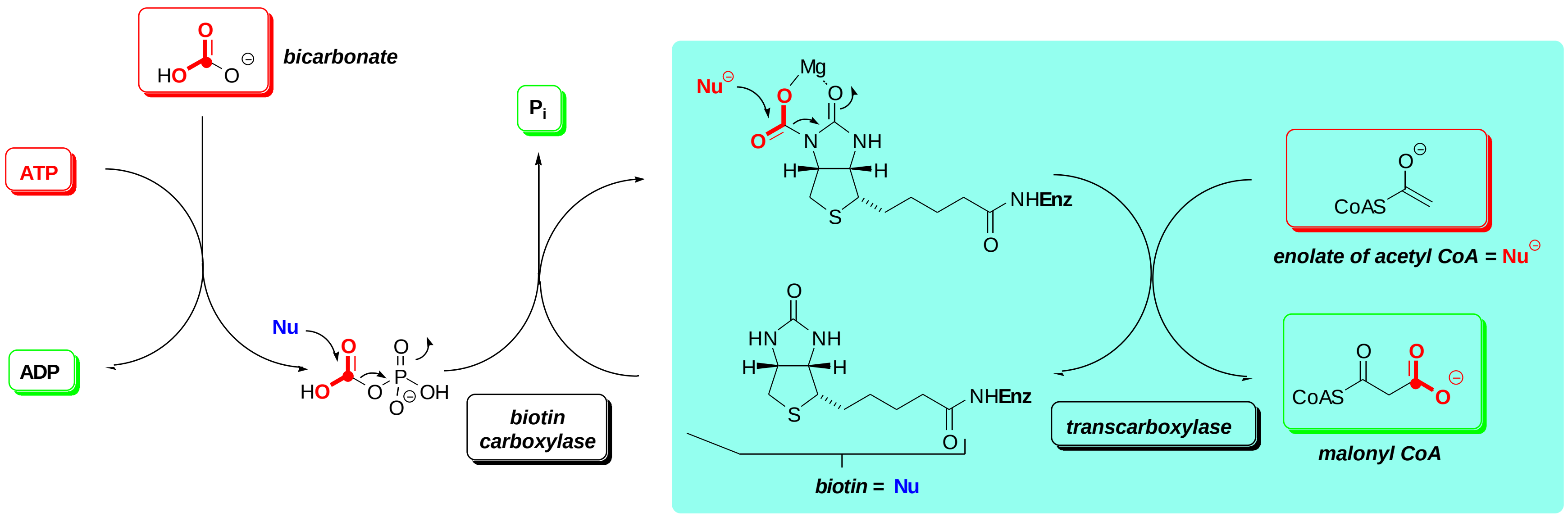


Biotin - Carboxylation

– **Key process:**

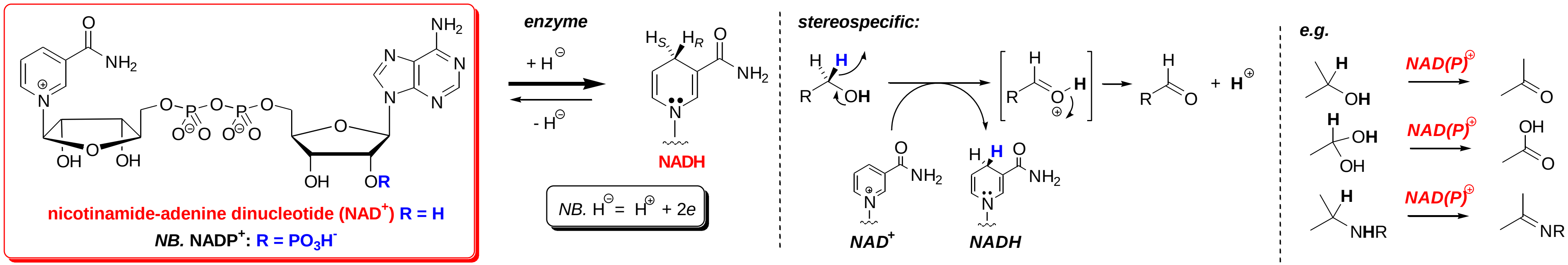


– ***e.g.* carboxylation of acetyl CoA to give malonyl CoA (FA + PK lectures)**

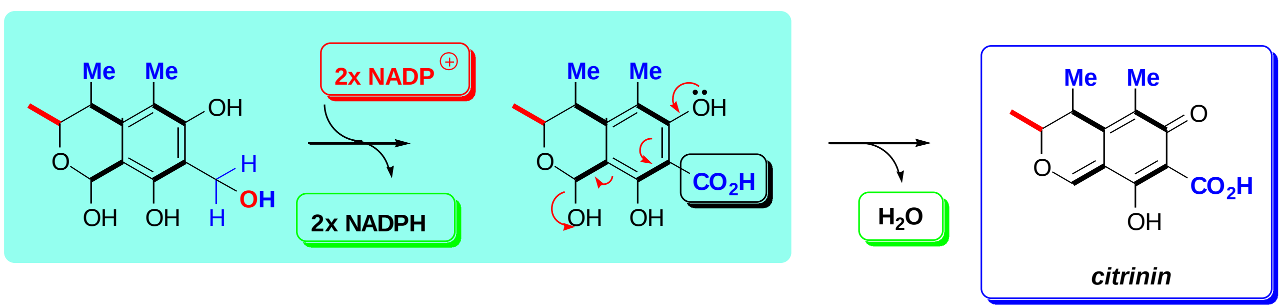


NAD(P)$^+$ - Oxidation

– **Key process:**

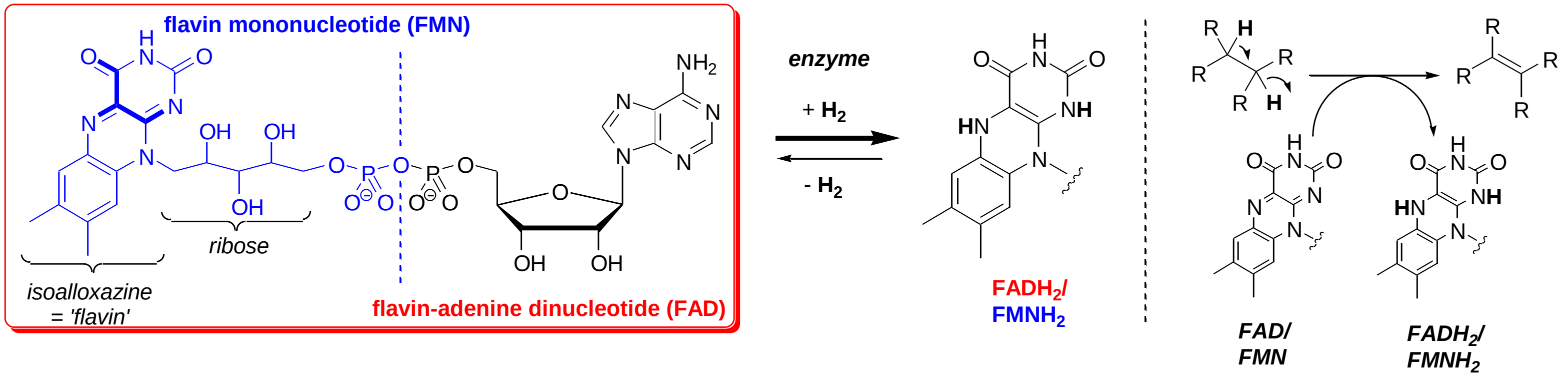


– ***e.g.*** **1° alcohol to carboxylic acid oxidation in citrinin biosynthesis (FA + PK lectures)**

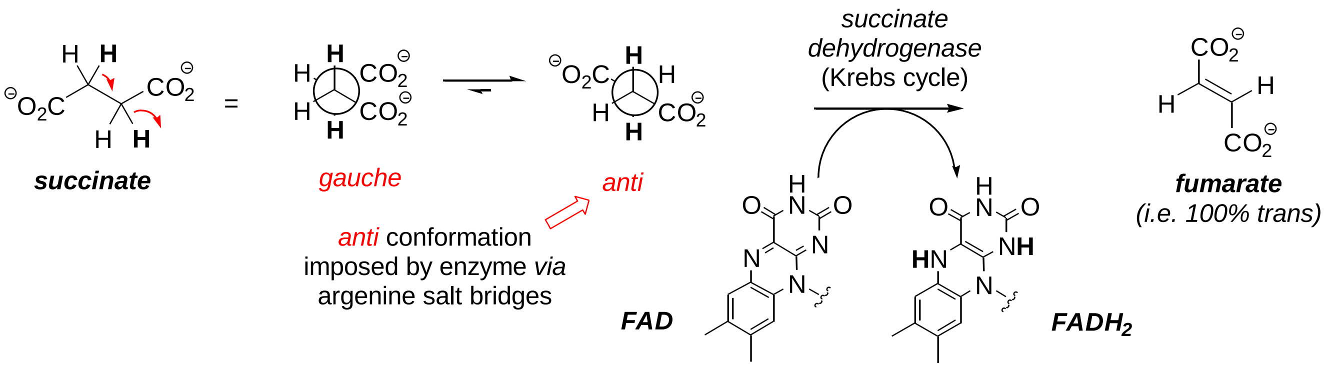


Flavin - Oxidation

– **Key process:**

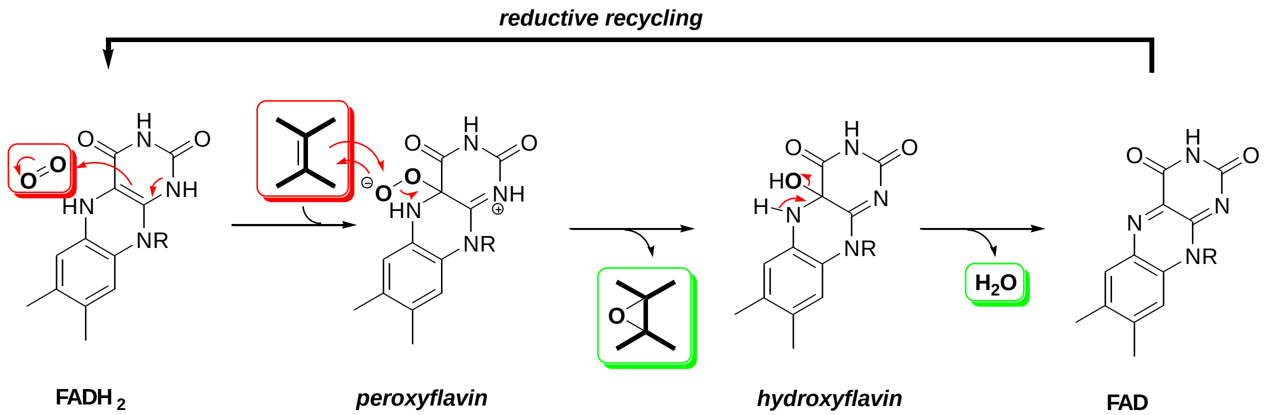


– ***e.g.*** **dehydrogenation of succinate → fumarate (citric acid cycle, alkaloid lectures)**

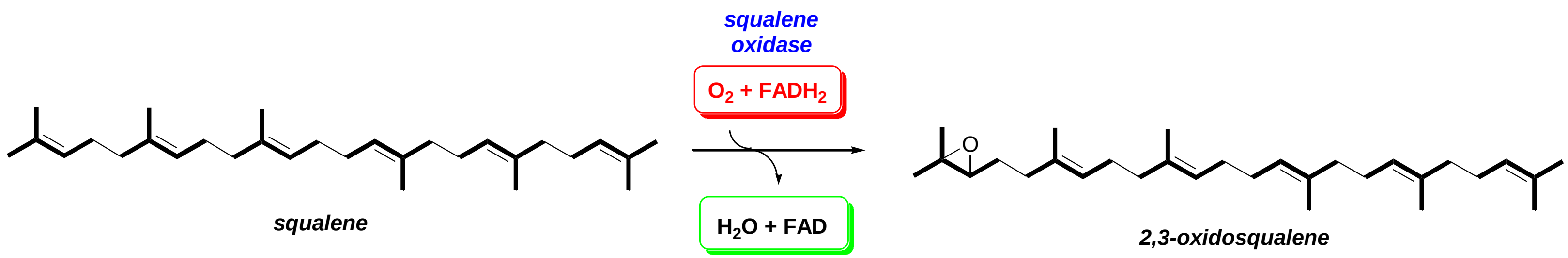


Peroxyflavin - Oxidation

– **Key process:**

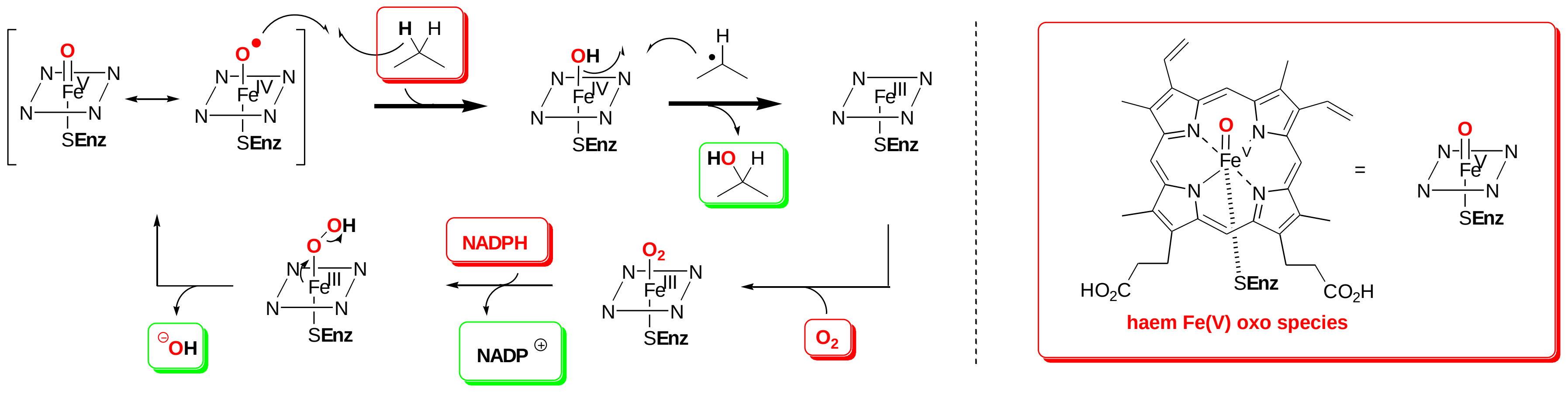


– ***e.g.*** **squalene → 2,3-oxidosqualene (isoprenoid lectures)**

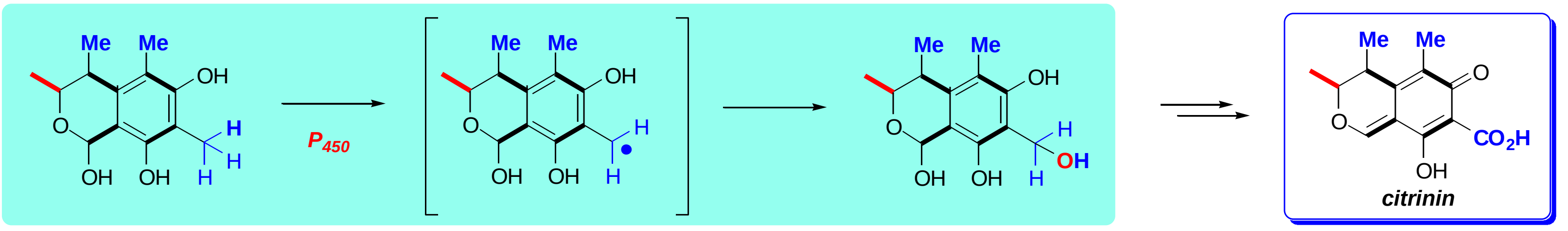


P_{450} *Iron oxo* - Oxidation

– **Key process:**

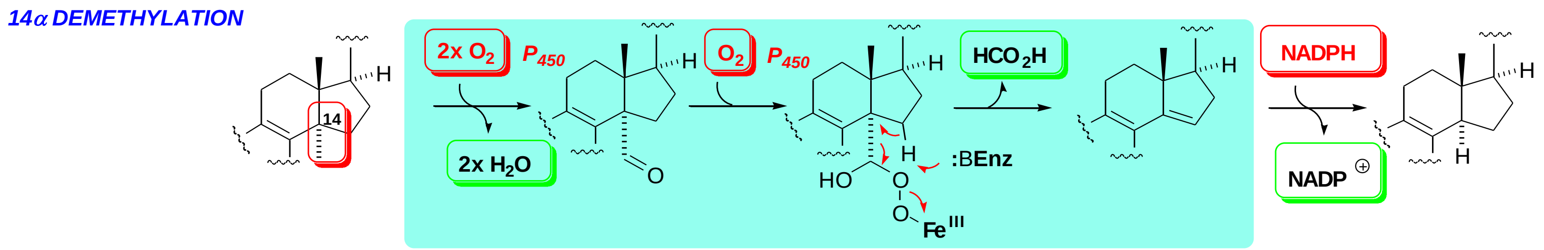


– ***e.g.*** **1)** **<u>benzylic hydroxylation</u>** **in citrinin biosynthesis (FA + PK lectures)**

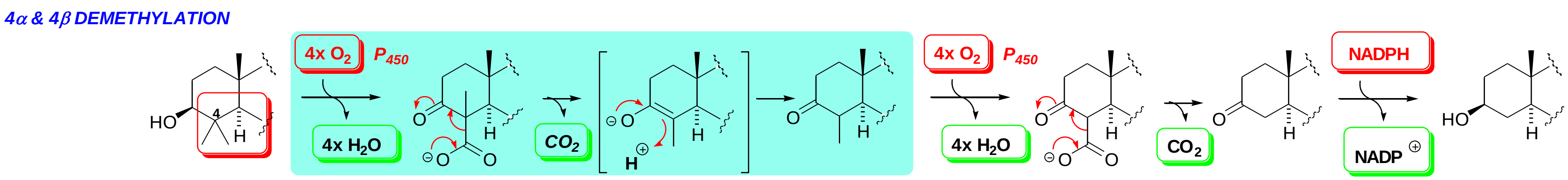


P_{450} *Iron oxo* – Oxidation cont.

– ***e.g.*** **2) unactivated oxidative demethylation: 14α demethylation in steroid biosynthesis (isoprenoid lectures)**

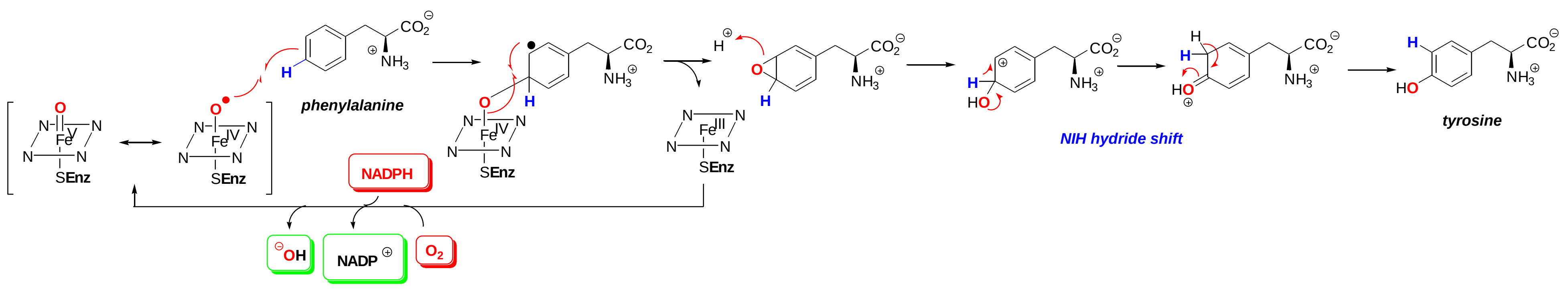


– ***e.g.*** **3) activated oxidative demethylation: 4α/β demethylation in steroid biosynthesis (isoprenoid lectures)**

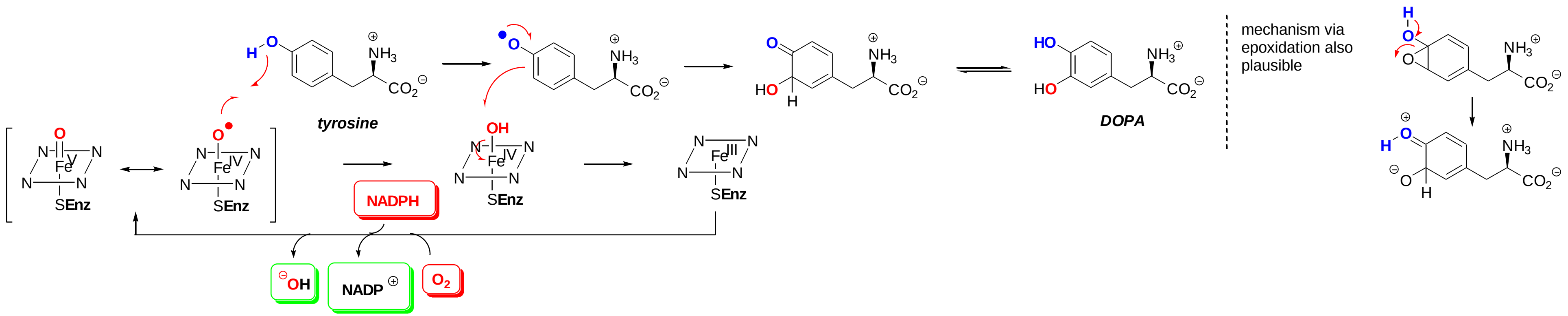


P_{450} *Iron oxo* – Oxidation cont.

– ***e.g.*** **4) unactivated aromatic hydroxylation: phenylalanine → tyrosine in barley (alkaloid lectures)**

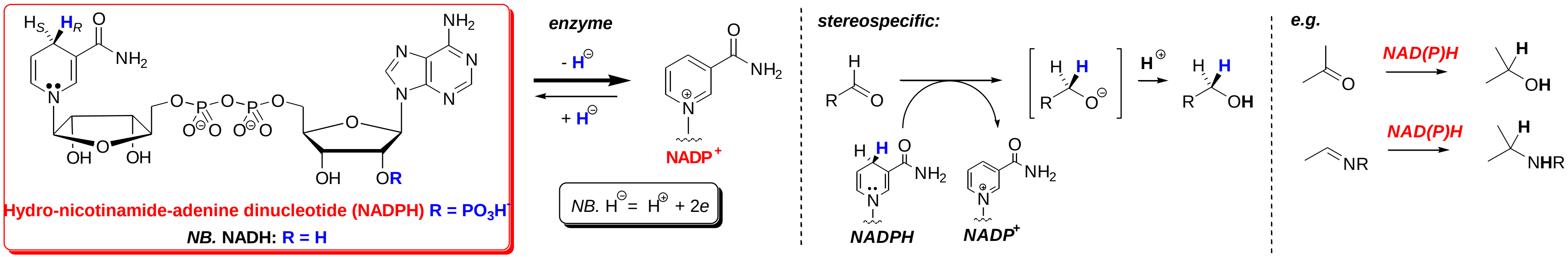


– ***e.g.*** **5) activated aromatic hydroxylation: tyrosine → DOPA in opium (alkaloid lectures)**

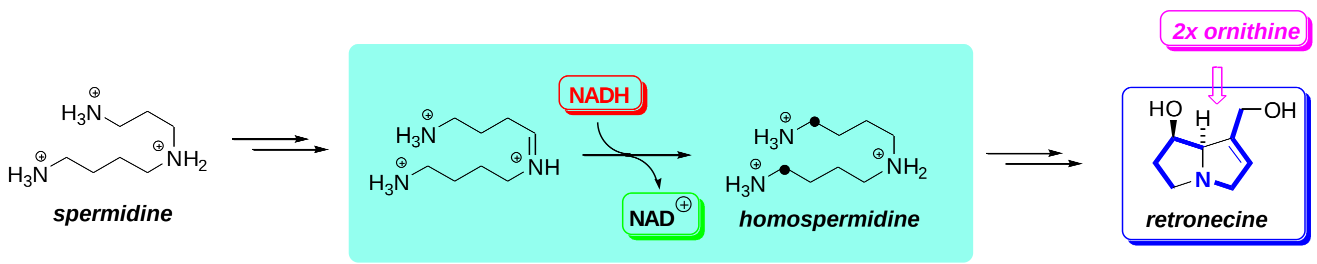


NADPH - Reduction

– **Key process:**

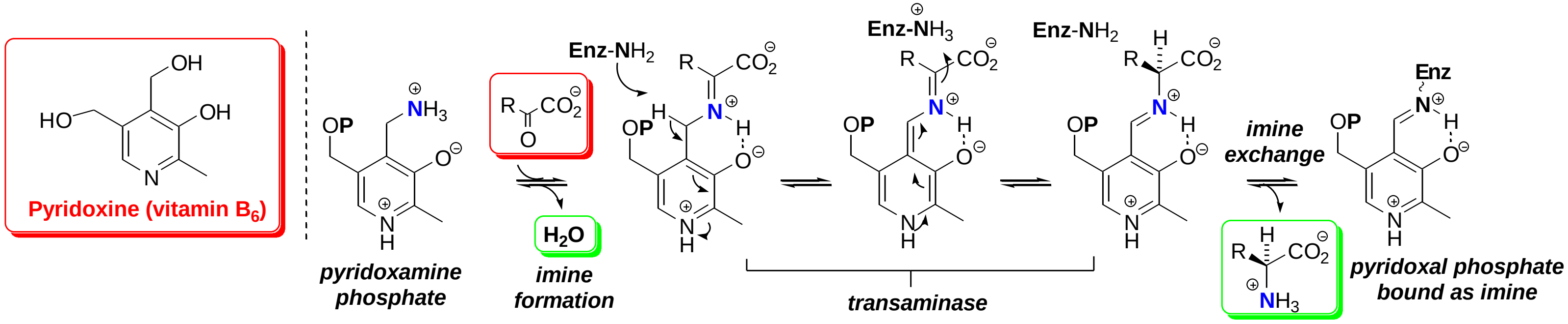


– ***e.g.* iminium ion to amine reduction in pyrrolizidine alkaloid biosynthesis (alkaloid lectures)**

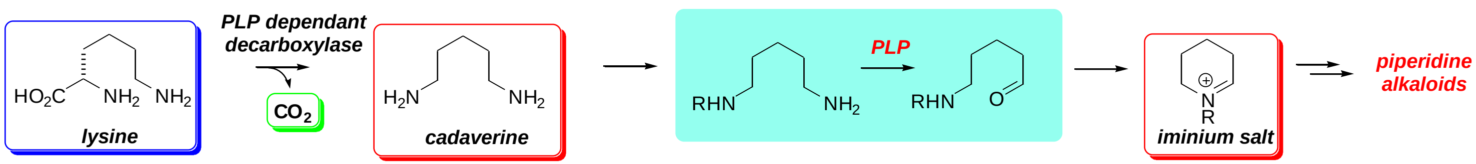


PLP - Transamination

– **Key process:**

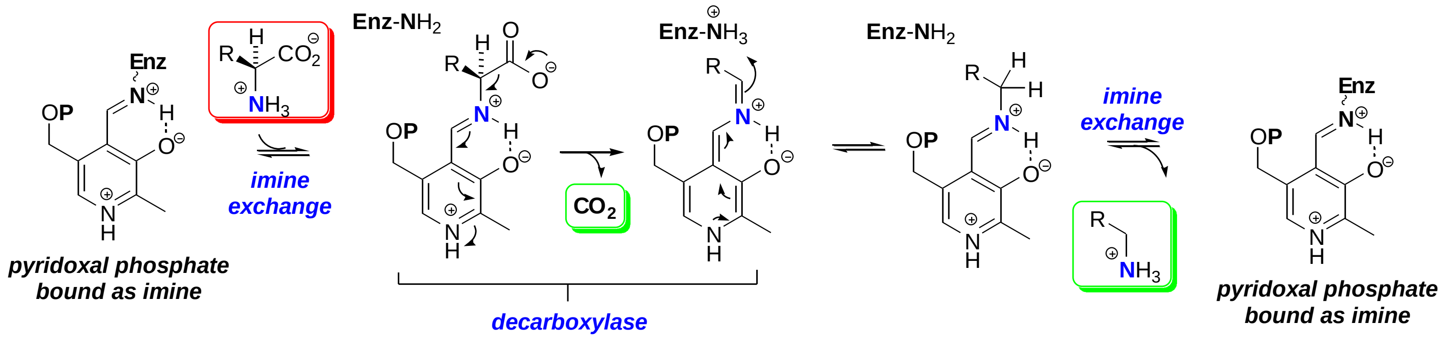


– ***e.g.* oxidative deamination of cadaverine *en route* to piperidine alkaloids (alkaloid lectures)**

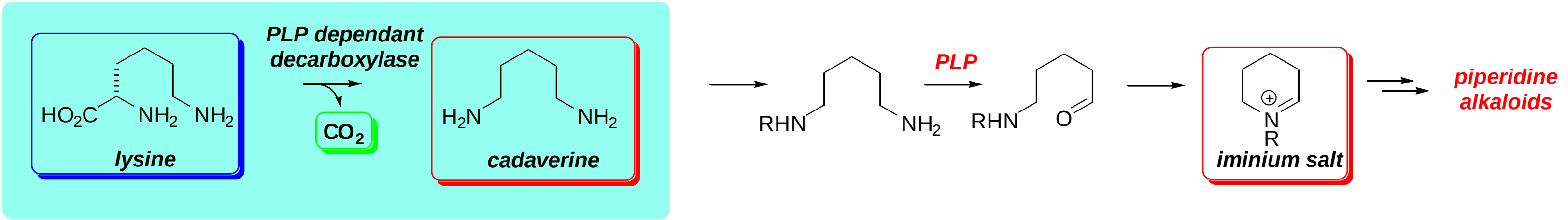


PLP - Decarboxylation

– **Key process:**

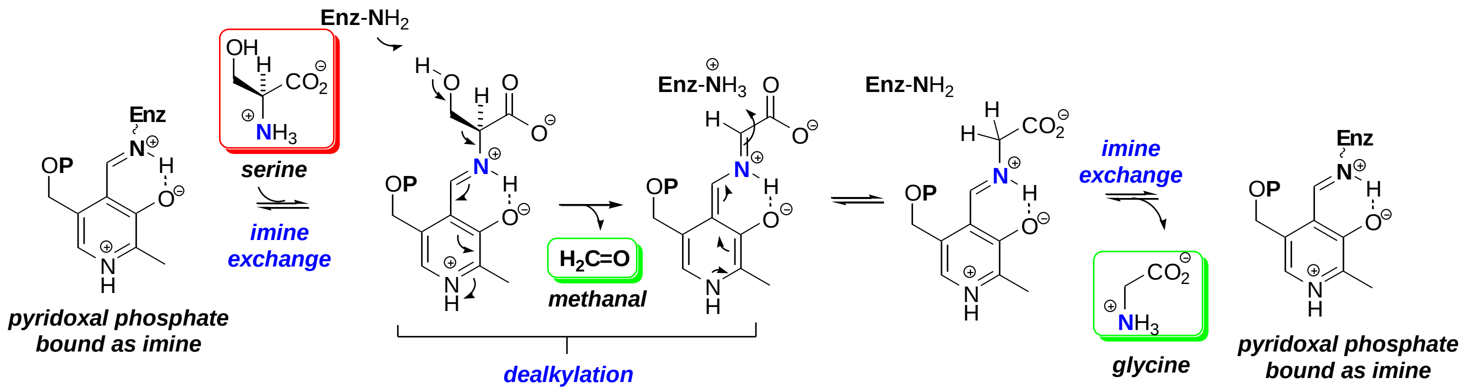


– ***e.g.*** **lysine decarboxylation to cadaverine (alkaloid lectures)**

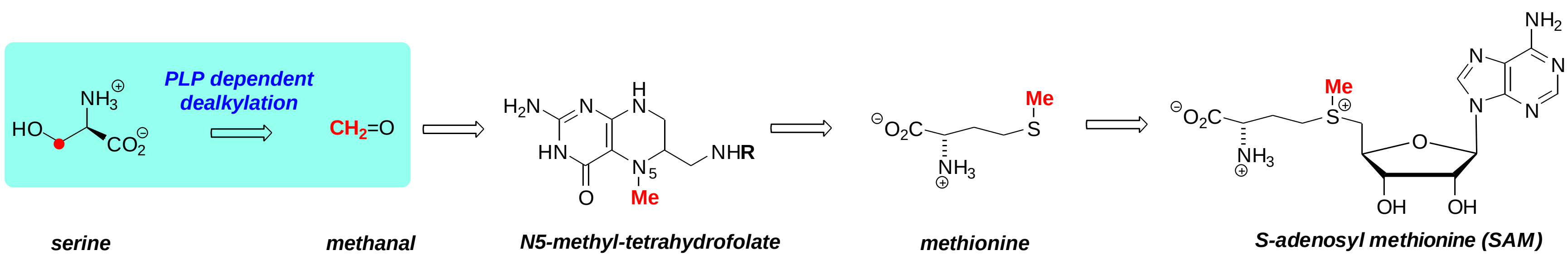


PLP - Dealkylation

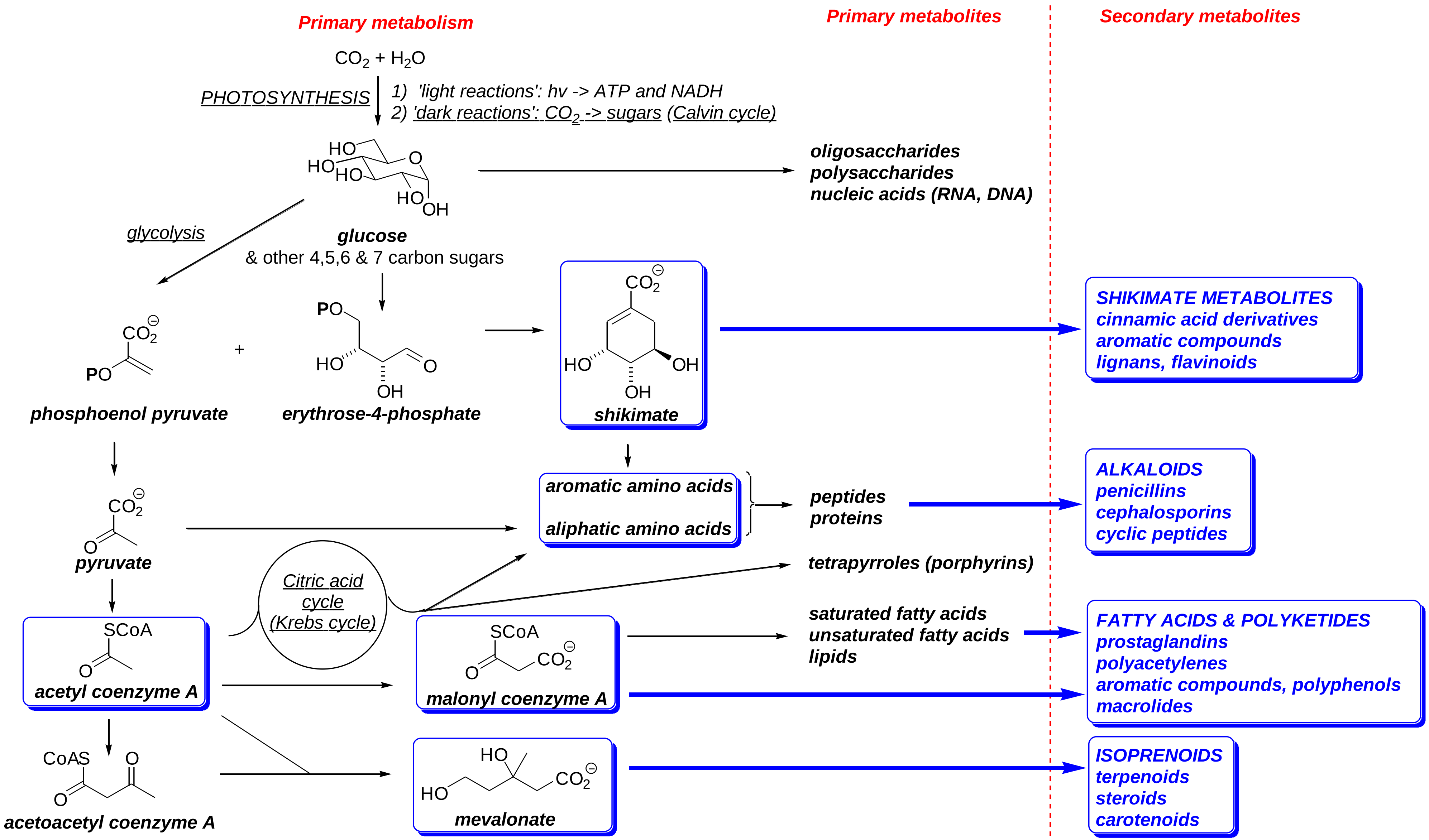
– **Key process:**



– ***e.g.*** **serine side-chain cleavage → methanal (alkaloid lectures)**

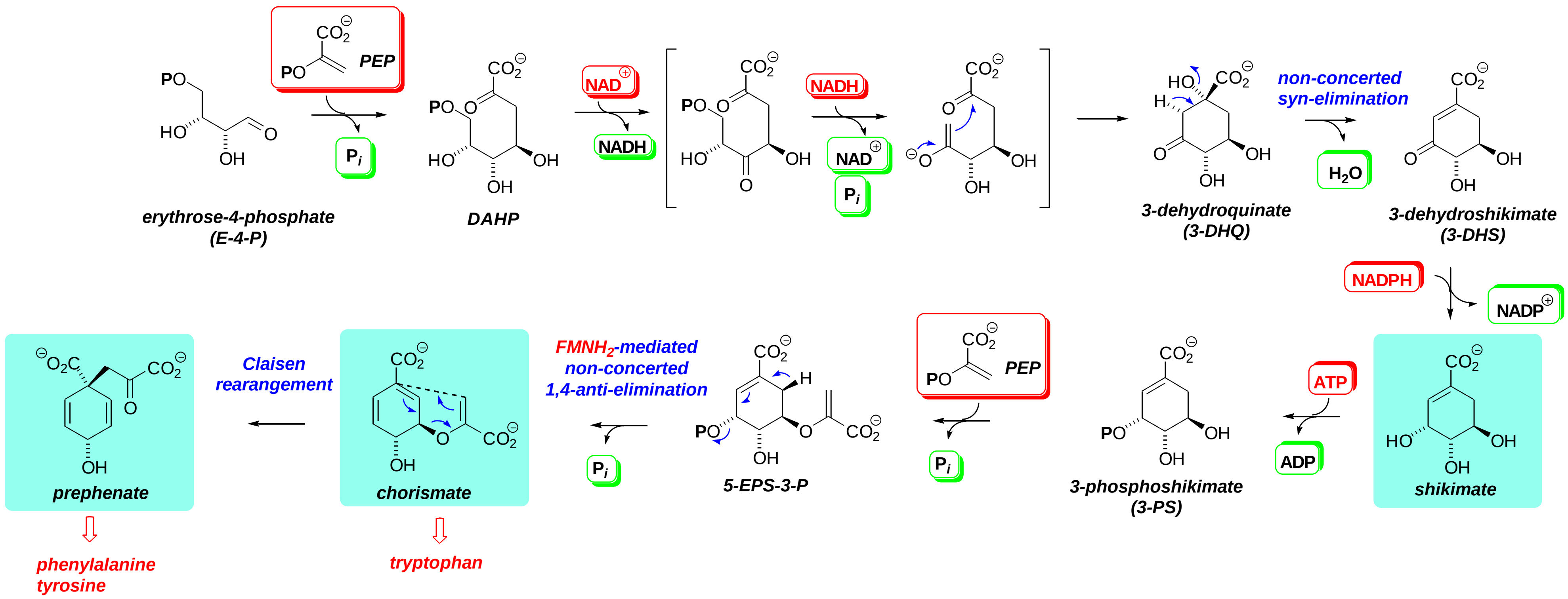


Primary Metabolism - *Overview*



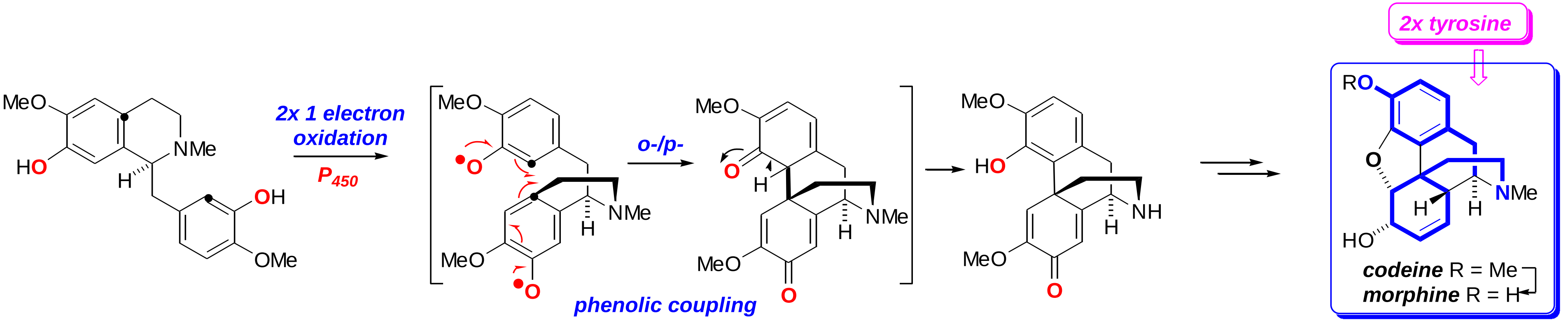
The Shikimate Biosynthetic Pathway

- ***Phosphoenol pyruvate** & **erythrose-4-phosphate** → **shikimate** → **chorismate** → **prephenate***:

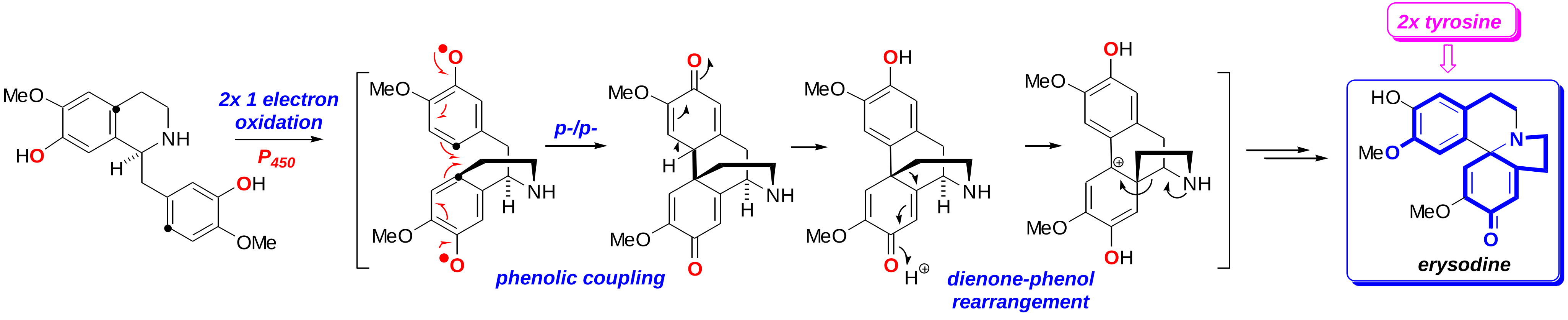


Oxidative Phenolic Coupling

- ***e.g. Morphine biosynthesis:*** *o-/p- oxidative phenolic coupling*:

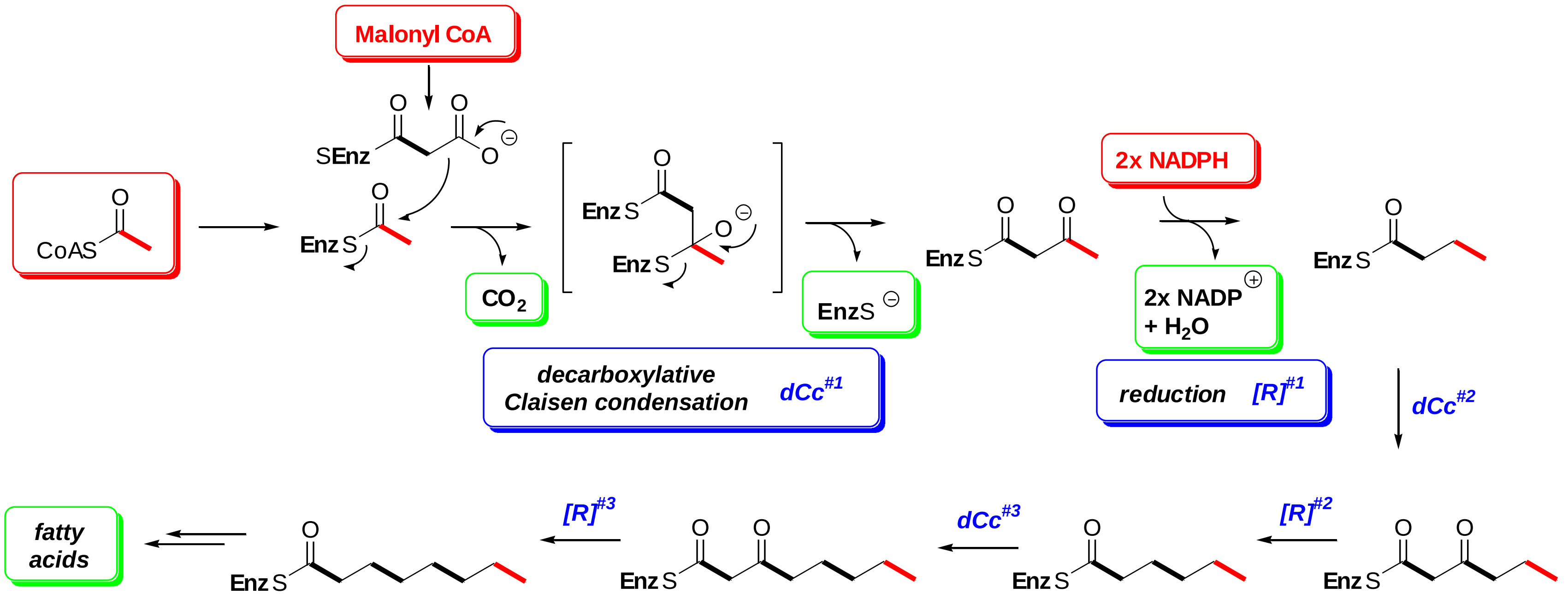


- ***e.g. Erysodine biosynthesis:*** *p-/p- oxidative phenolic coupling & dienone-phenol rearrangement:*



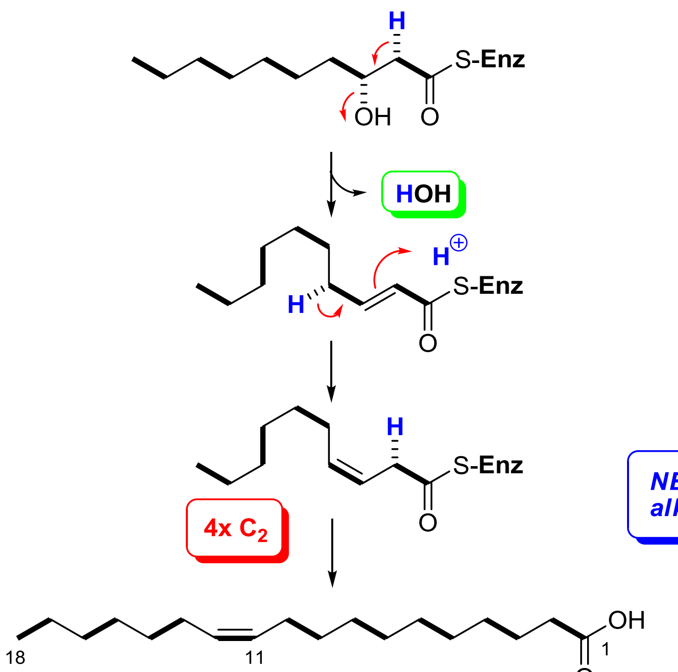
Biosynthesis of Fatty Acids

- **Iterative oligomerisation *via*:**
  - Decarboxylative Claisen condensation
  - 3-step ketone reduction



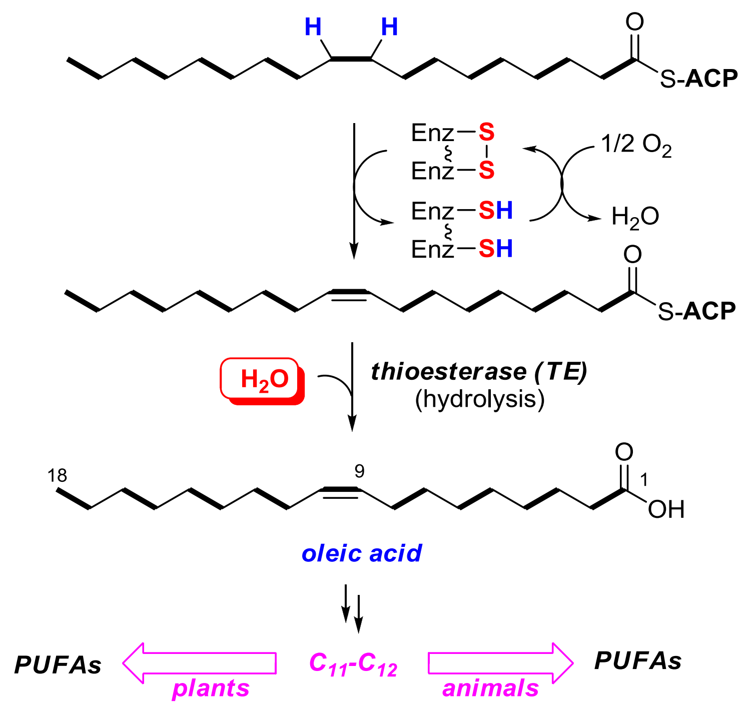
Introduction of Unsaturation

ANAEROBIC ROUTE (bacteria)
(dehydrogenation occurs <u>during</u> chain elongation)
mainly MUFAs but some PUFAs



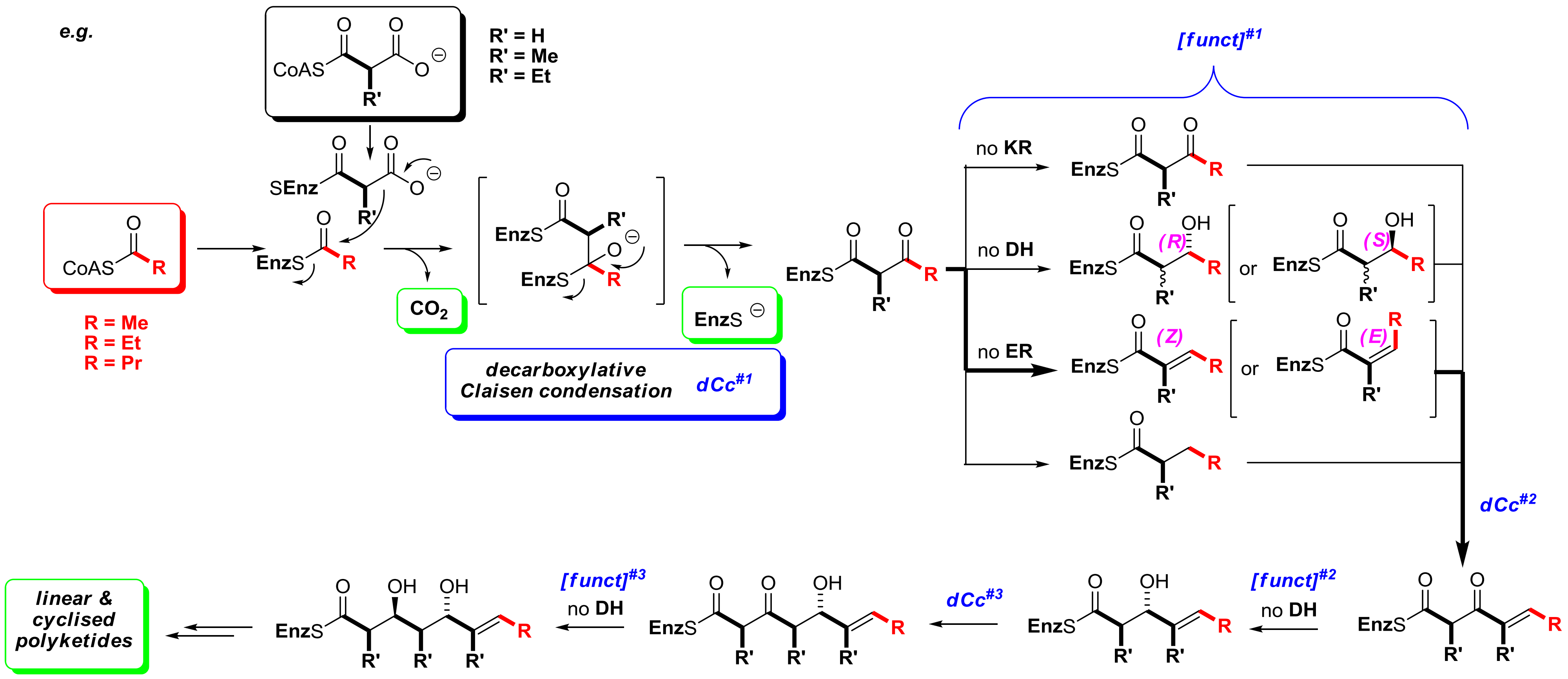
NB. in both cases cis - alkenes are produced

AEROBIC ROUTE (mammals, insects & plants)
(dehydrogenation occurs <u>after</u> chain elongation)
MUFAs & PUFAs



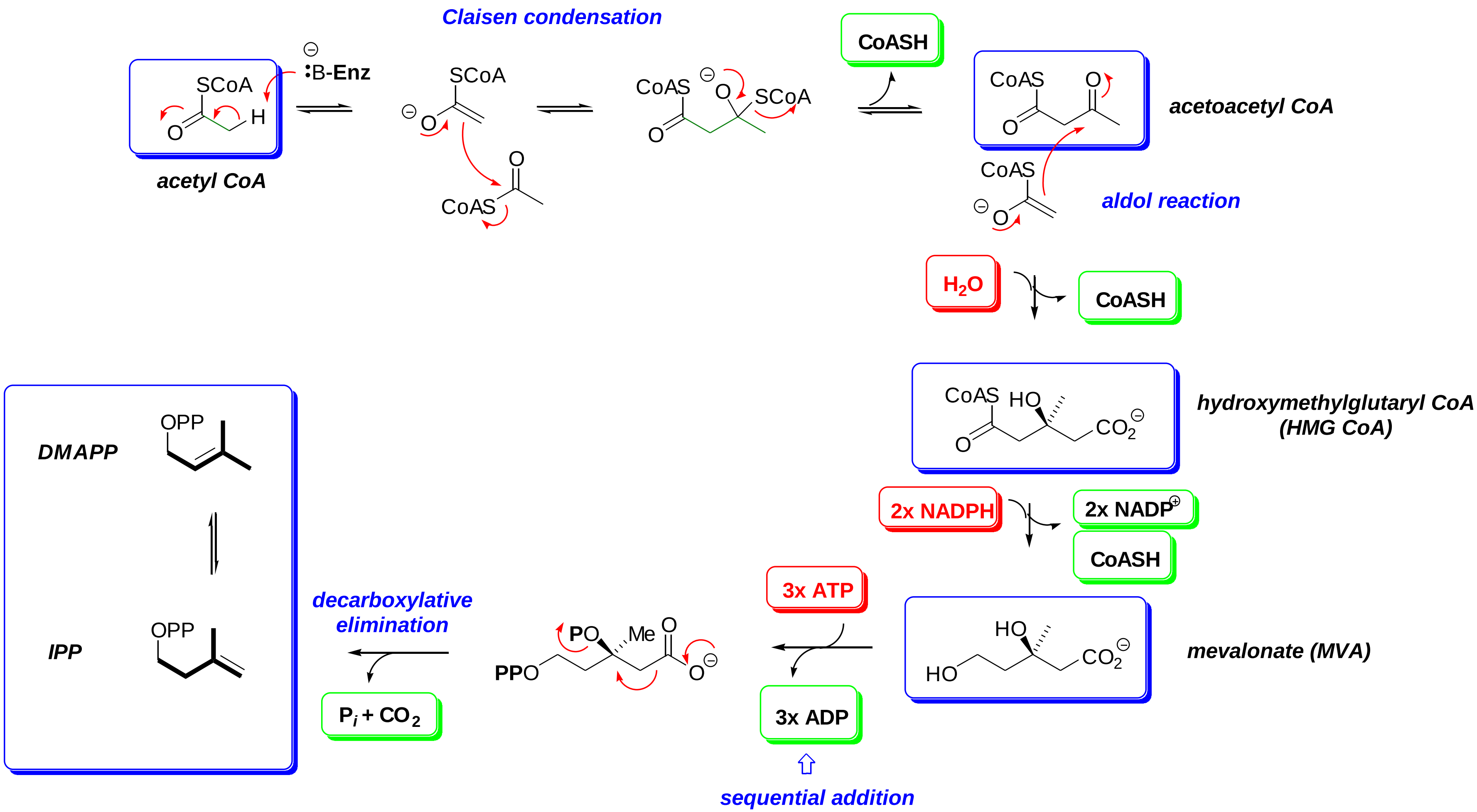
Biosynthesis of Polyketides

- **Iterative oligomerisation *via*:**
  - Decarboxylative Claisen condensation
  - Variable levels of reductive ketone processing in each iteration

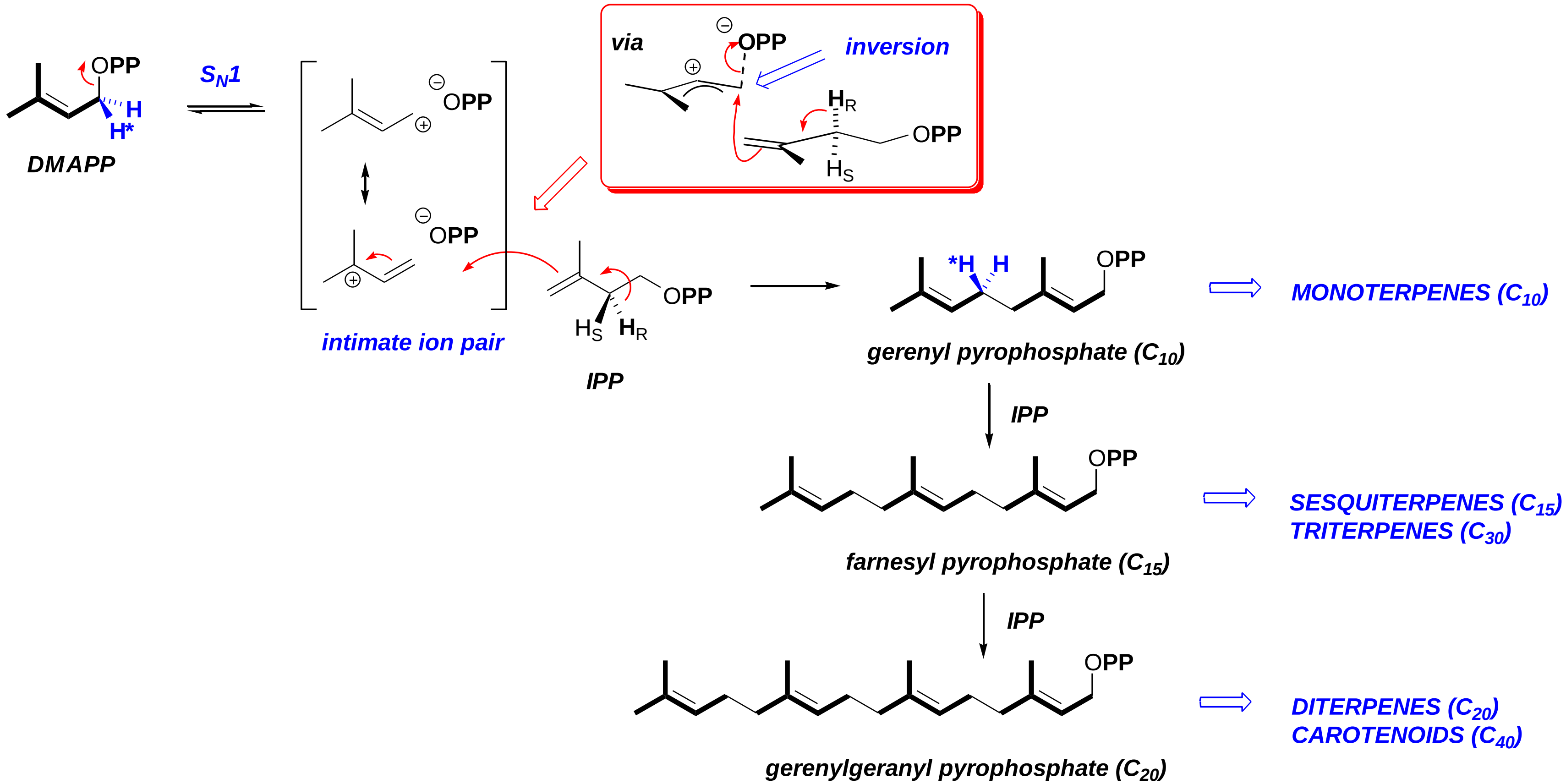


Biosynthesis of IPP & DMAPP

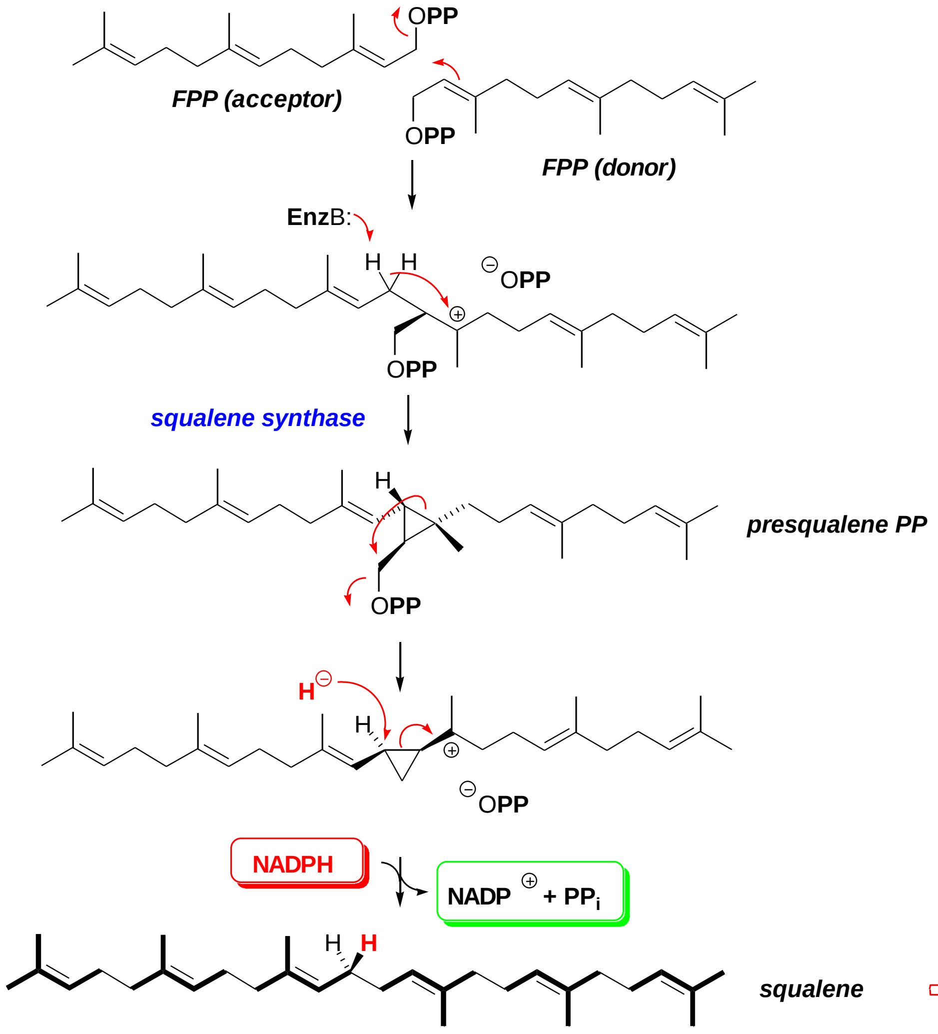
- ***acetyl CoA → acetoacetyl CoA → HMG CoA → mevalonate → IPP → DMAPP:***

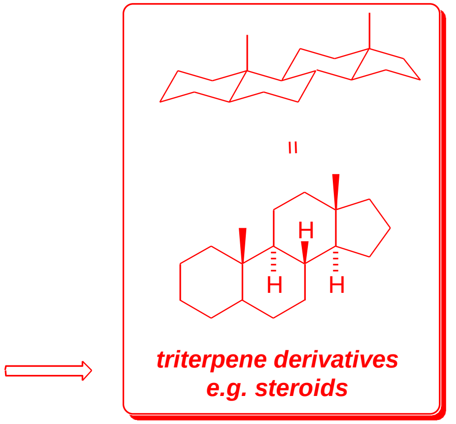


‘Head-to-tail’ Oligomerisation → Isoprenoids



Triterpenes – 2×*FPP 'head-to-head'* → *Squalene*



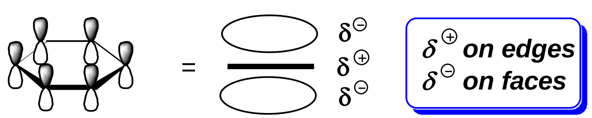


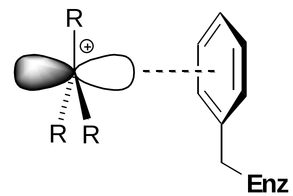
Terpene Cyclases – *Control of Cyclisation*

- ***Functional aspects of terpenoid cyclases:***

  - ***Templating*****:** Active site provides a template for a <u>specific conformation</u> of the flexible linear isoprenoid starting material.
  - ***Triggering*****:** Cyclase <u>initiates carbocation</u> formation.
    - Metal-assisted leaving group departure (*e.g.* pyrophosphate ionization aided by Mg^{2+})
    - C=C bond protonation (*e.g.* squalene-hopene cyclase, see later).
    - Epoxide protonation (*e.g.* oxidosqualene cyclase, see later).
  - ***Chaperoning*****:** Chaperones conformations of carbocationic intermediates through the reaction sequence, ordinarily leading to one specific product.
  - ***Sequestering*****:** Sequesters the carbocation intermediates by burying the substrate in a hydrophobic cavity that is generally solvent-inaccessible. Carbocations are concomitantly stabilized by the presence of aromatic residues in the active site that exert their effects *via* cation-π interactions

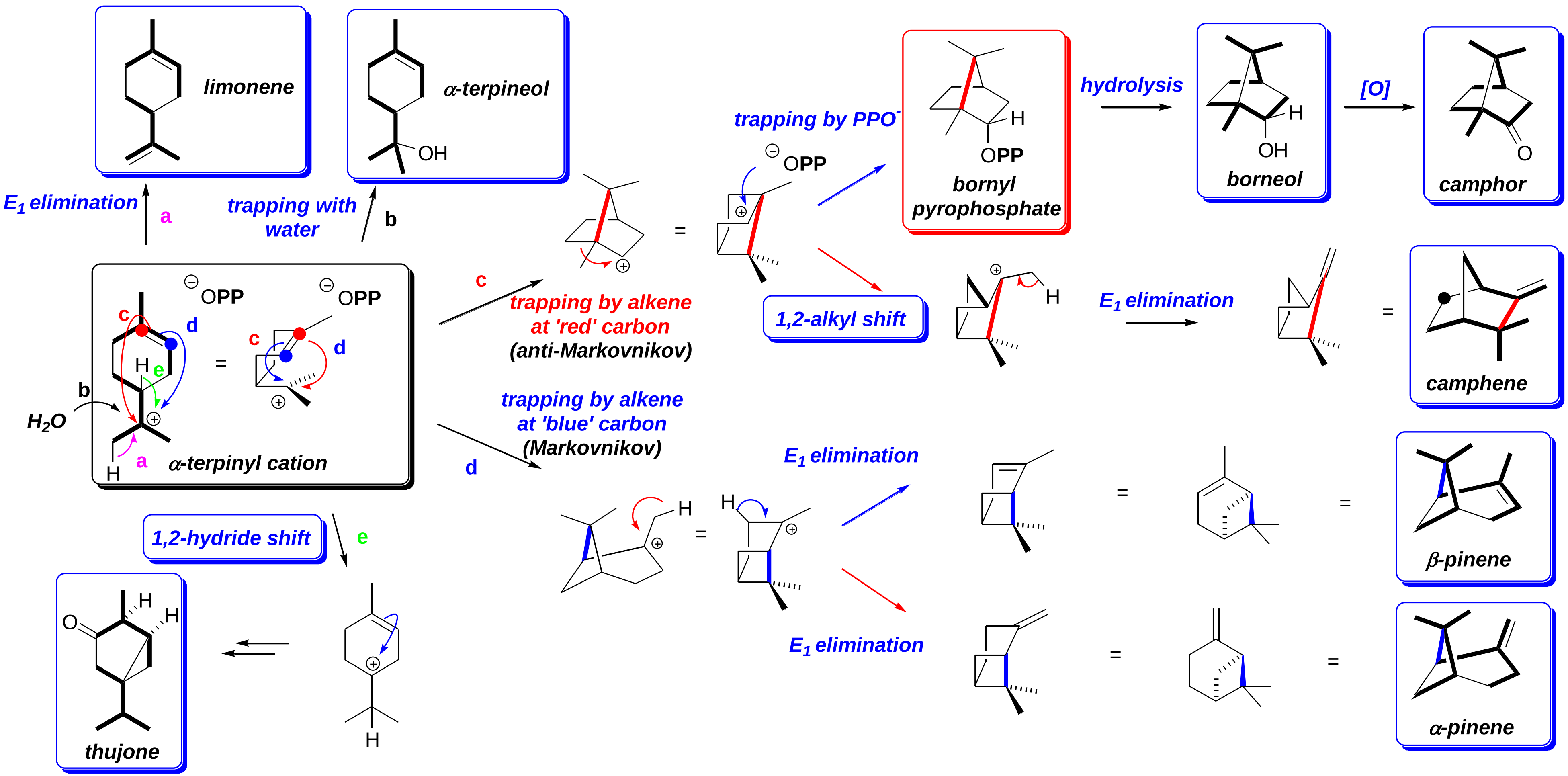
RECALL:



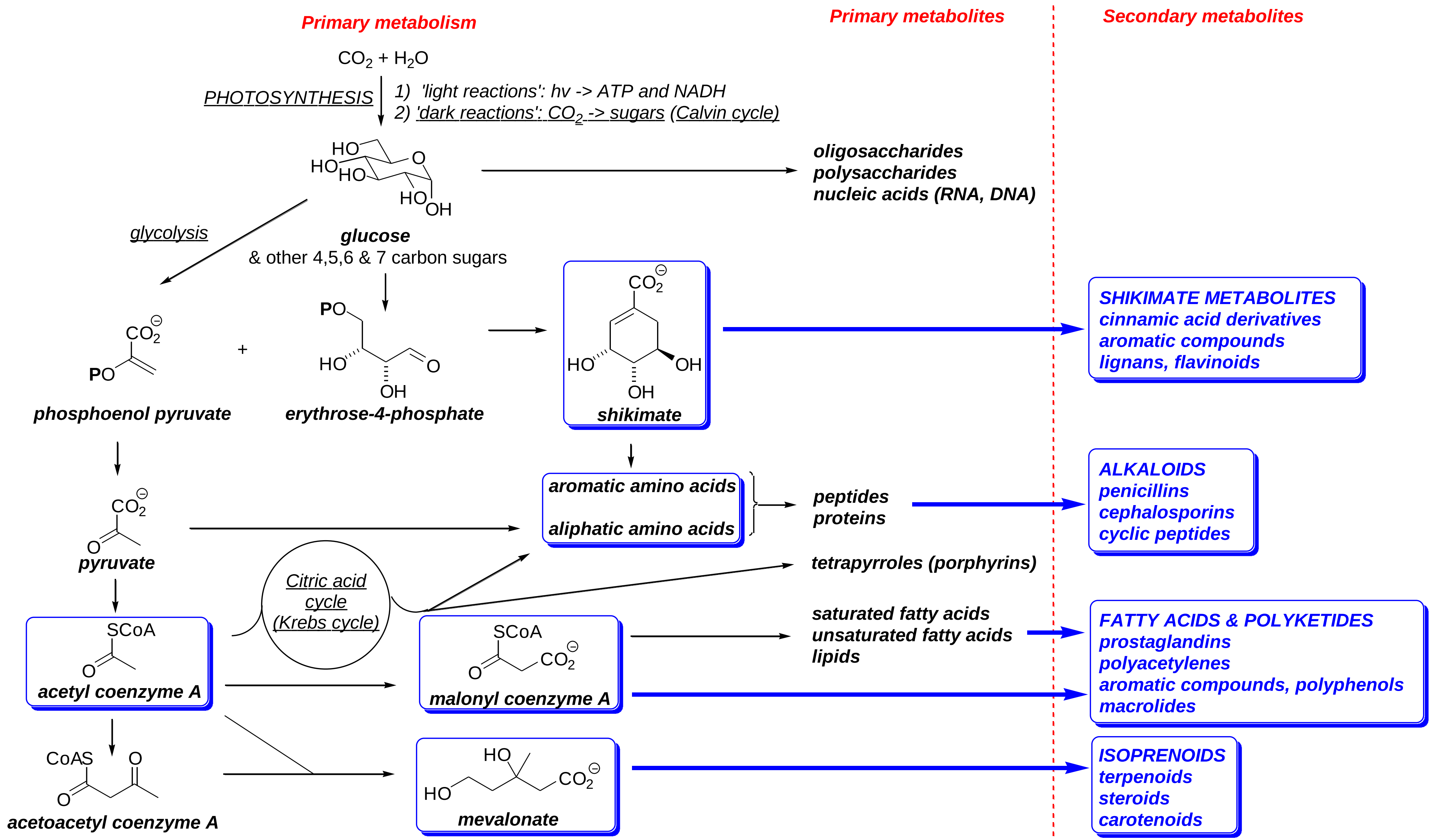


cation - π stabilisation
(~40-80 $kJmol^{-1}$)

Cationic Rearrangements *etc.* → Diversity!



Primary Metabolism - *Overview*



Acknowledgements

- I would like to acknowledge the following for kindly allowing me to consult and use material from their lecture courses on various aspects of biosynthesis:

Dr Finian Leeper, Dept. of Chemistry, University of Cambridge
http://www-leeper.ch.cam.ac.uk

Dr John McKendrick, Dept. of Chemistry, University of Reading
http://www.chem.rdg.ac.uk/dept/staff/org/jem.html

Dr David Widdowson, Dept. of Chemistry, Imperial College London
http://www.ch.ic.ac.uk/widdowson/

- Additionally, I have adapted ideas from several web-sites & in particular I have adapted material from two biological chemistry courses at Harvard University & MIT:
  - ***http://www.courses.fas.harvard.edu/%7echem27/***
  - ***http://ocw.mit.edu/OcwWeb/Chemistry/5-08JSpring2004/CourseHome/index.htm***

- Other reference sources have been the books cited in the 'course overview', particularly:
  - ***J. Mann, 'Chemical Aspects of Biosynthesis', Oxford Chemistry Primer No. 20, 1994***
  - ***J. Mann, 'Secondary Metabolism', Oxford University Press, 2nd ed. 1987***