Biosynthesis

Primary Metabolism, Enzyme Cofactor Chemistry & Shikimate Metabolites

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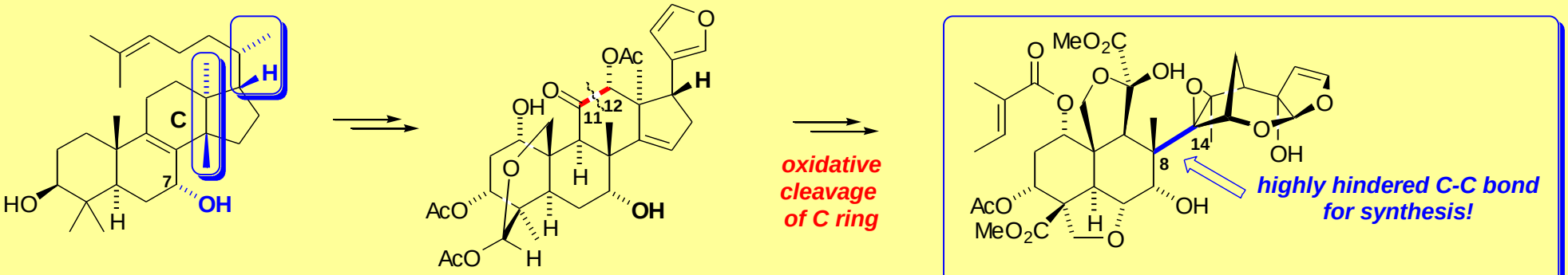
Lessons in synthesis - *Azadirachtin*

- ***Azadirachtin*** is a potent ***insect anti-feedant*** from the Indian ***neem tree:***
  - exact biogenesis unknown but certainly *via* steroid modification:



tirucallol
(*cf.* lanosterol)

azadirachtanin A
(a limanoid =
tetra-*nor*-triterpenoid)

azadirachtin

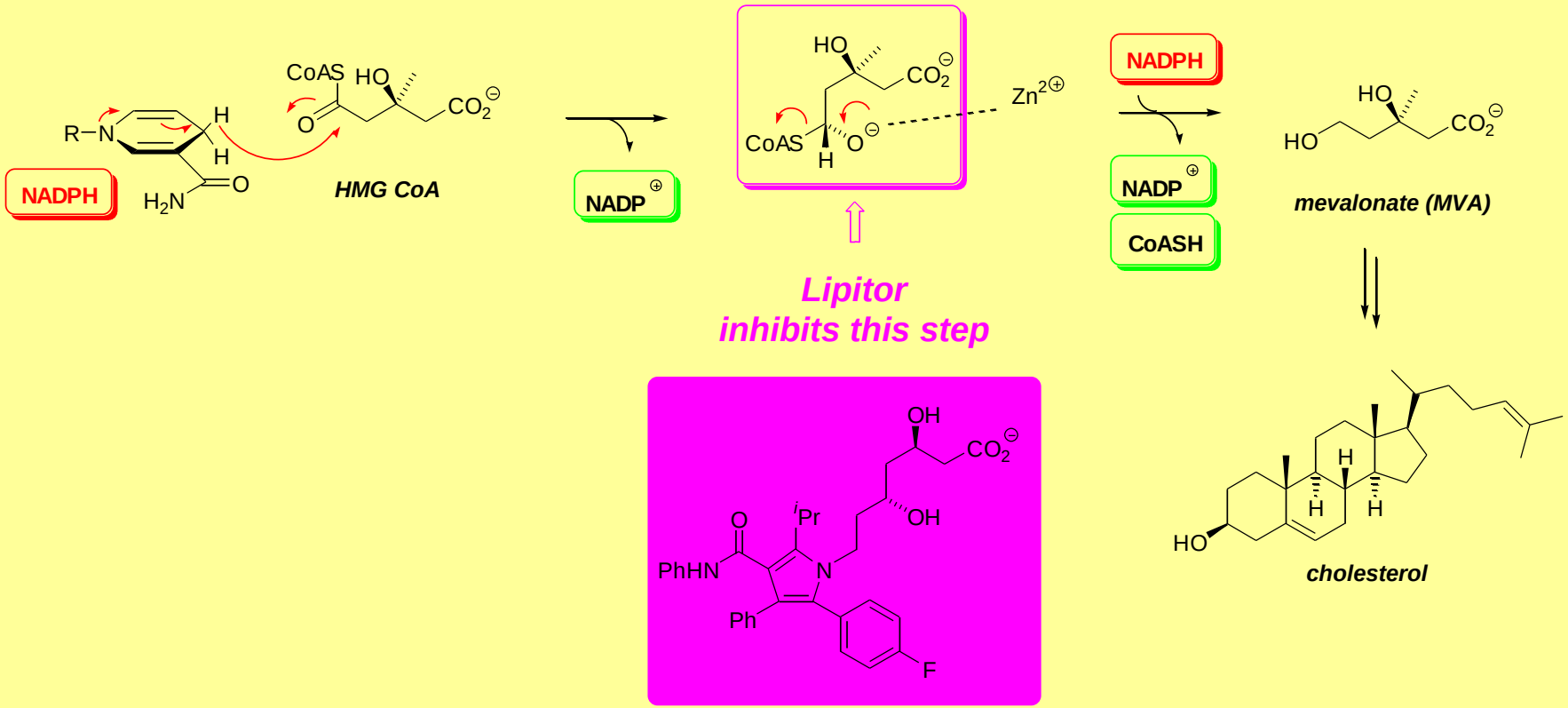
  - Intense synhtetic efforts by the groups of Nicolaou, Watanabe, Ley and others since structural elucidation in 1987.
  - 1st total synthesis achieved in 2007 by Ley following 22 yrs of effort
  - **~40 researchers and over 100 man-years of research! – 64-step synthesis**

  - Veitch Angew. Chem. Int. Ed. **2007**, *46*, 7629 (DOI) & Veitch Angew. Chem. Int. Ed. **2007**, *46*, 7633 (DOI)

Inspiration for med chem - *statins*

- ***HMG CoA*** → ***MVA*** is the ***rate determining step*** in the biosynthetic pathway to ***cholesterol***

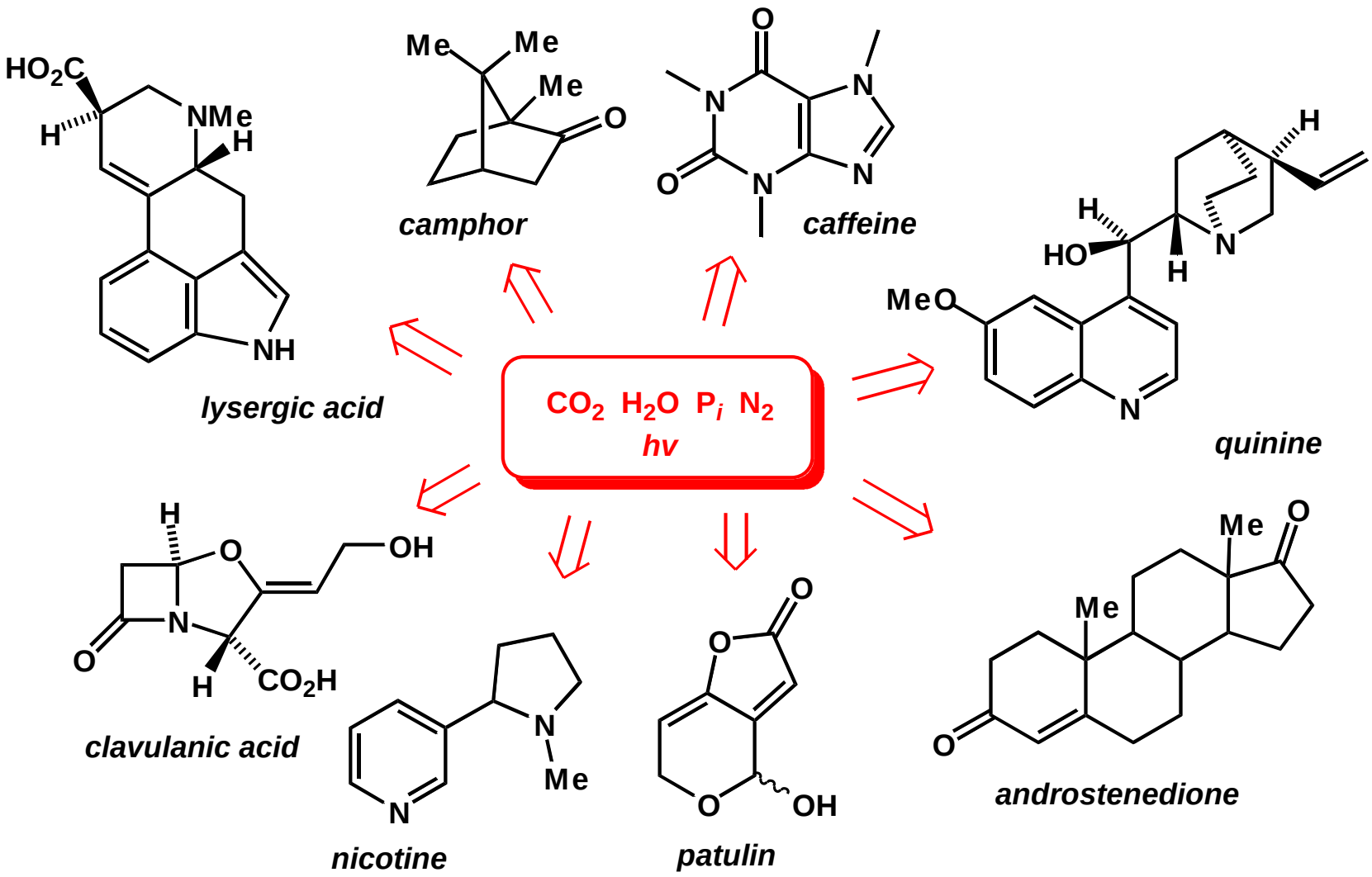
- ***'Statins'*** inhibit HMG CoA reductase and are used clinically to treat ***hypercholesteraemia*** - a causative factor in ***heart disease***
  - *e.g.* ***lipitor*** (Atorvastatin calcium, Pfizer) is a competitive inhibitior of HMG-CoA reductase and the worlds biggest selling drug [first drug to reach $10 billion sales (2004: $10.8 bn]



Format & Scope of Lectures

- ***What is biosynthesis?***
  - some definitions – phototrophs, chemotrophs; metabolism (catabolism/anabolism), 1° & 2° metabolites
- ***Overview of primary metabolism → secondary metabolites***
  - photosynthesis & glycolysis → shikimate formation → shikimate metabolites
  - acetylCoA & the citric acid cycle → α-amino acids → penicillins, cephalosporins, alkaloids
  - acetylCoA → malonylCoA → fatty acids, prostaglandins, polyketides, macrolide antibiotics
  - acetylCoA → mevalonate → isoprenoids, terpenoids, steroids, carotenoids
- ***Biological/biosynthetic reactions – enzyme & cofactor chemistry***
  - *free energy source* – ATP
  - *C-C & C-O bond formation* – CoASH, SAM, DMAPP, biotin
  - *oxidation* – NAD^+, FAD/FMN, haem iron oxo monooxygenases
  - *reduction* – NADPH
  - *C-N bond formation* – pyridoxal
- ***The shikimate biosynthetic pathway***
  - the core shikimate pathway - mechanisms of the key enzymes
  - aromatic amino acids: Phe, Tyr & Trp
  - ArC_3 metabolites – coumarins, lignans & lignins
    - mixed shikimate/malonylCoA (polyketide): flavonoids
  - ArC_2, ArC_1 & ArC_0 metabolites
    - mixed shikimate/mevalonate (isoprenoid): ubiquinones, menaquinones & tocopherols

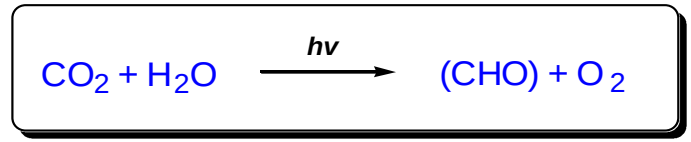
Metabolism & Natural Product Diversity



Phototrophs & Chemotrophs

- ***Living organisms*** are not at equilibrium. They ***require*** a continuous influx of ***free energy*** to perform mechanical work & for cellular growth/repair:

  - ***Phototrophs*** (*e.g.* green plants, algae & photosynthetic bacteria): derive free energy from the sun *via* ***photosynthesis*** ('CO_2 fixation'):
    - 10^{15} kg/year by green plants, which constitute 99% of Earths biomass (*i.e.* 10^{12} tons of dry matter)
    - 1g of carbon processed = >6250 litres of air



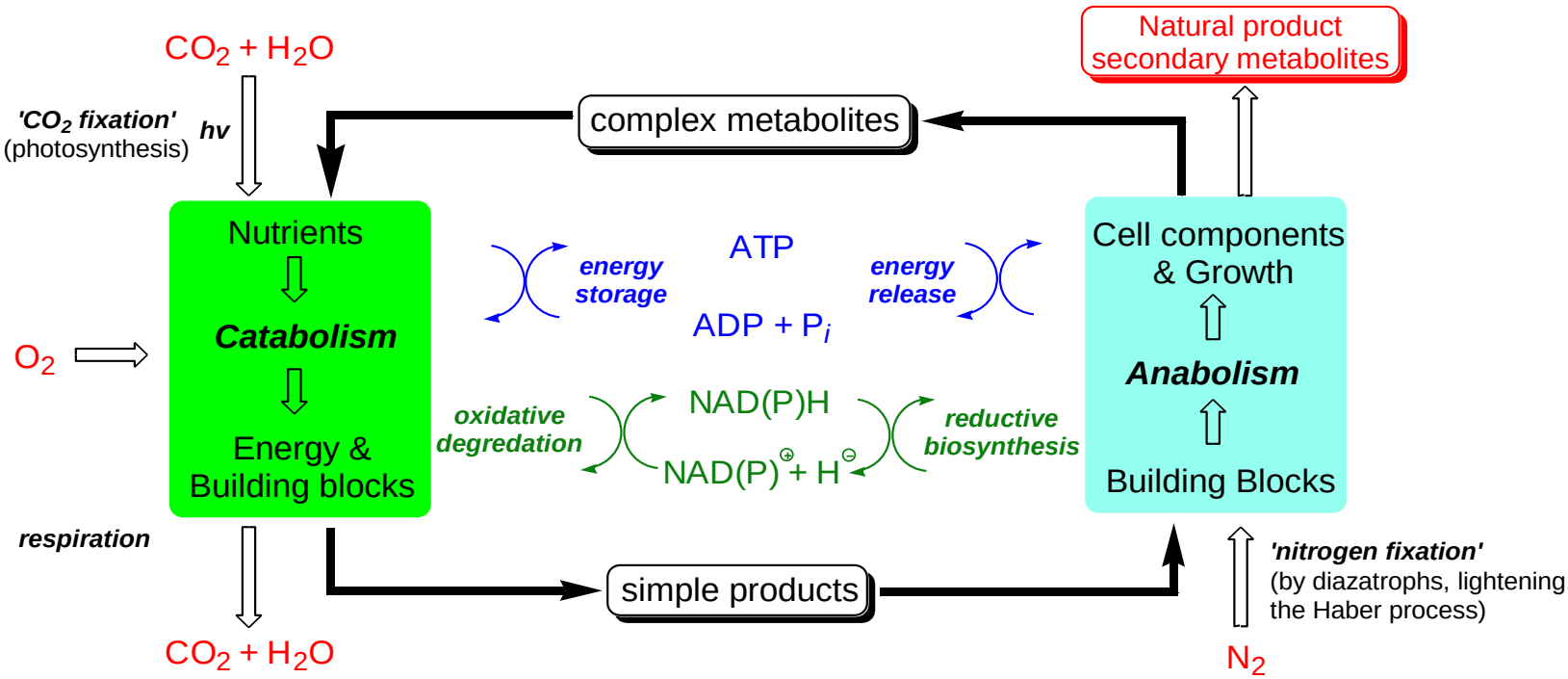
PHOTOSYNTHESIS

  - ***Chemotrophs*** (*e.g.* animals, fungi, most bacteria): derive free energy by ***oxidising nutrients*** (carbohydrates, lipids, proteins) obtained from other organisms, ultimately phototrophs
    - some bacteria & fungi require just D-glucose
    - mammals require sugars, essential amino acids (~half total used) & certain vitamins (enzyme co-factors or precursors)

    - *Degradation of the nutrients is coupled to the stoichiometric production of 'high energy' phosphate compounds, particularly* ***adenosine triphosphate*** *(****ATP****, see later). All metabolic function is underpinned by ATP energetic coupling.*
    - *By analogy with a money-based economy, the metabolic cost of production of a given metabolite from another can be quantified in terms of 'ATP equivalents' defined as the # of moles of ATP consumed/produced per mole of substrate converted in the reaction or sequence*

Metabolism

- ***Metabolism*** is the term used for *in vivo* processes by which compounds are degraded, interconverted and synthesised:
  - ***Catabolic*** or ***degradative***: primarily to release energy and provide building blocks
    - generally ***oxidative*** processes/sequences (glycolysis, Krebs cycle)
  - ***Anabolic*** or ***biosynthetic***: primarily to create new cellular materials (1° & 2° metabolites)
    - generally ***reductive*** processes/sequences
- These two types of process are coupled – one provides the driving force for the other:



Types of Metabolite & Biosynthesis

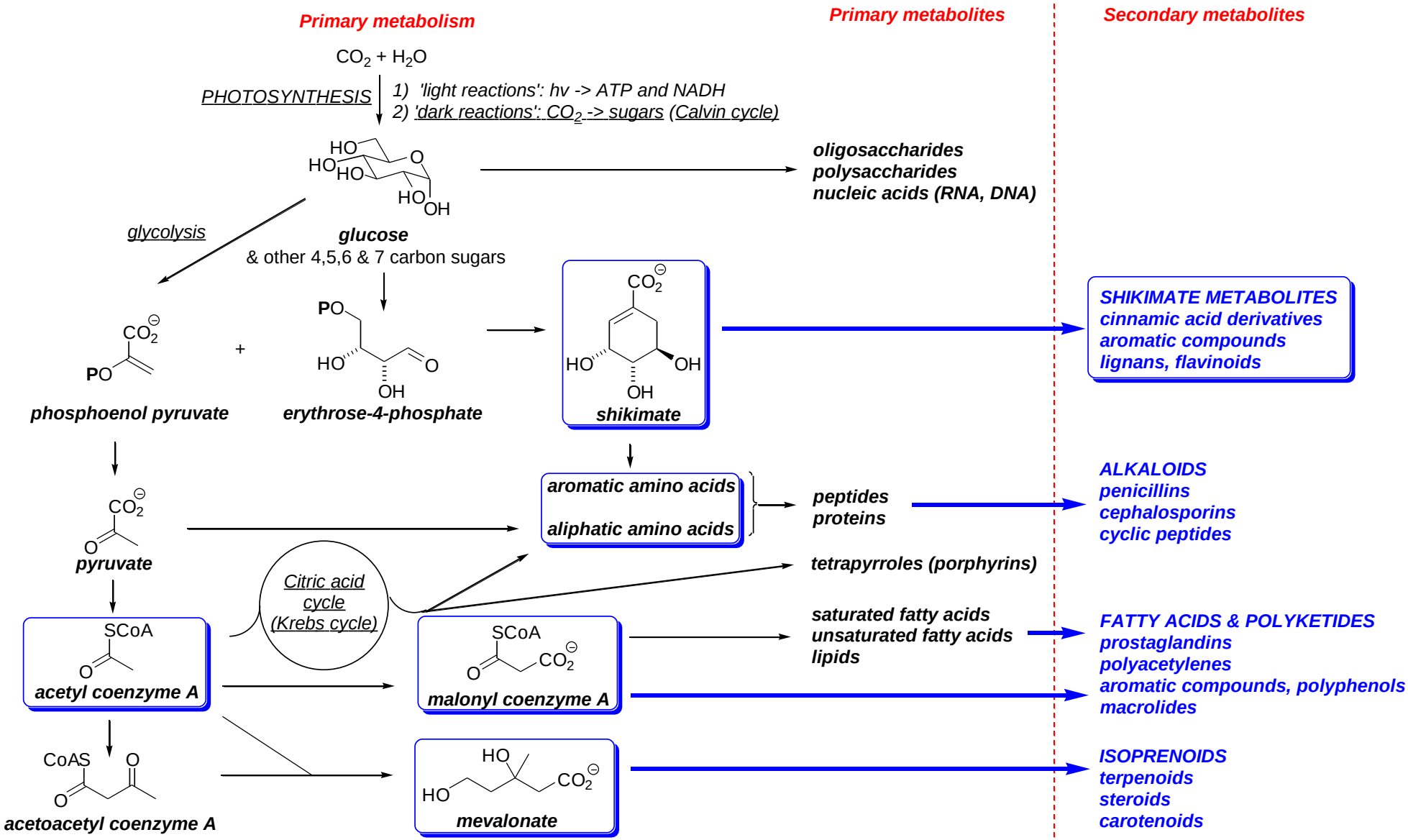
- ***Biosynthesis*** is the term for the *in vivo* synthesis of metabolites/natural products:
  - These are divided into two camps:
    - ***Primary metabolites:*** These are the universal and essential components for the survival of living organisms. *e.g.* sugars, amino acids, nucleotides, 'common' fats and polymers such as proteins, DNA, RNA, lipids and polysaccharides
    - ***Secondary metabolites:*** Compounds produced by organisms which are not required for survival, many of which have no apparent utility to the host organism. Frequently a given metabolite will only be produced in a single organism or in a set of closely related organisms. Provide a rich source of pharmacologically active compounds. *e.g.* ***shikimate derivatives***, ***alkaloids***, ***fatty acids***, ***polyketides***, ***isoprenoids***
  - Although the boundary is imprecise the term ***biosynthesis*** *is most commonly applied, by* ***organic chemists****, to the* ***in vivo synthesis of secondary metabolites***:

"Now ever since Perkin, failing to make quinine, founded the dyestuffs industry, organic chemists have found the study of 'natural products' an inexhaustable source of exercises, which can be performed out of pure curiosity even when paid for in the hope of a more commercial reward. As a result the organic chemist's view of nature is unbalanced, even lunatic but still in some ways more exciting than that of the biochemist. While the enzymologist's garden is a dream of uniformity, a green meadow where the cycles of Calvin and Krebs tick round in disciplined order, the organic chemist walks in an untidy jungle of uncouthly named extractives, rainbow displays of pigments, where in every bush there lurks the mangled shapes of some alkaloid, the exotic perfume of some new terpene, or some shocking and explosive polyacetylene..."

... Since these intriguing derivatives AND e.g. lysine or ATP are ALL in a sense 'natural products' we may prefer the term 'secondary metabolite' for the former

Bu'Lock *Adv. Appl. Microbiol.* **1961**, *3*, 293

Primary Metabolism - *Overview*

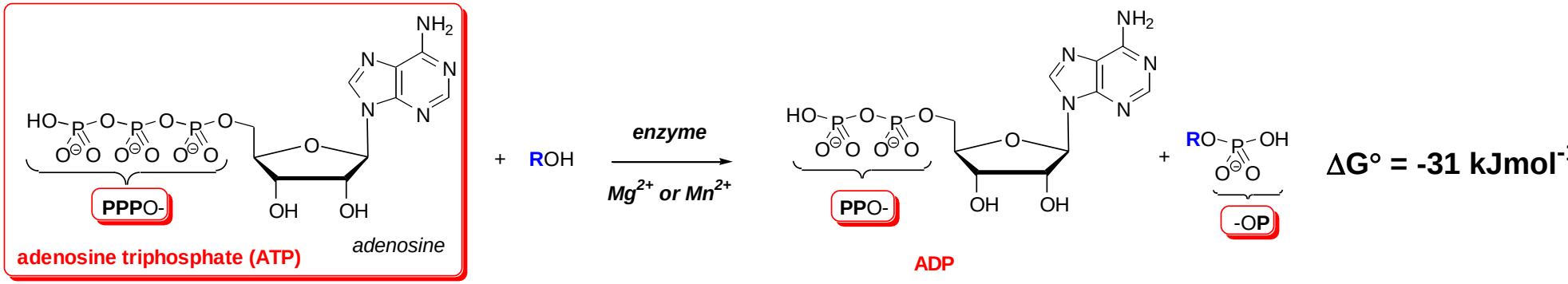


Biological/Biosynthetic Reactions – *Enzyme Catalysis & Cofactors*

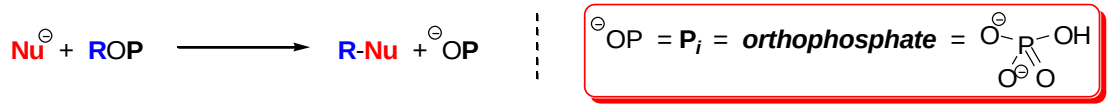
- Most biosynthetic steps are catalysed by specific, individual ***enzymes***. They generally perform familiar processes such as ***oxidation***, ***reduction***, ***alkylation***, ***hydrolysis***, ***acylation***, ***hydroxylation***, ***elimination*** *etc.*
- ***Different enzymes*** carrying out ***related reactions*** often employ ***common co-factors***: small organic functional fragments and/or metal ions. *e.g.*
  - ***FREE ENERGY RELEASING COUPLE:*** ***Adenosine triphosphate (ATP)***
  - ***C-C & C-O BOND FORMATION:*** ***Coenzyme A (CoASH); S-adenosyl methionine (SAM); dimethylallylpyrophosphate (DMAPP); biotin***
  - ***OXIDATION:*** ***NAD(P)$^+$; FAD/FMN; Haem iron oxo species (e.g. P_{450})***
  - ***REDUCTION:*** ***NAD(P)H; ($FADH_2$/$FMNH_2$)***
  - ***C-N BOND FORMATION:*** ***Pyridoxal***

Free Energy Releasing Couple - *ATP*

- ***Adenosine triphosphate (ATP)***
  - phosphorylation of an alcohol by adenosine diphosphate (ADP) is highly ***exothermic*** (*i.e.* liberates energy):



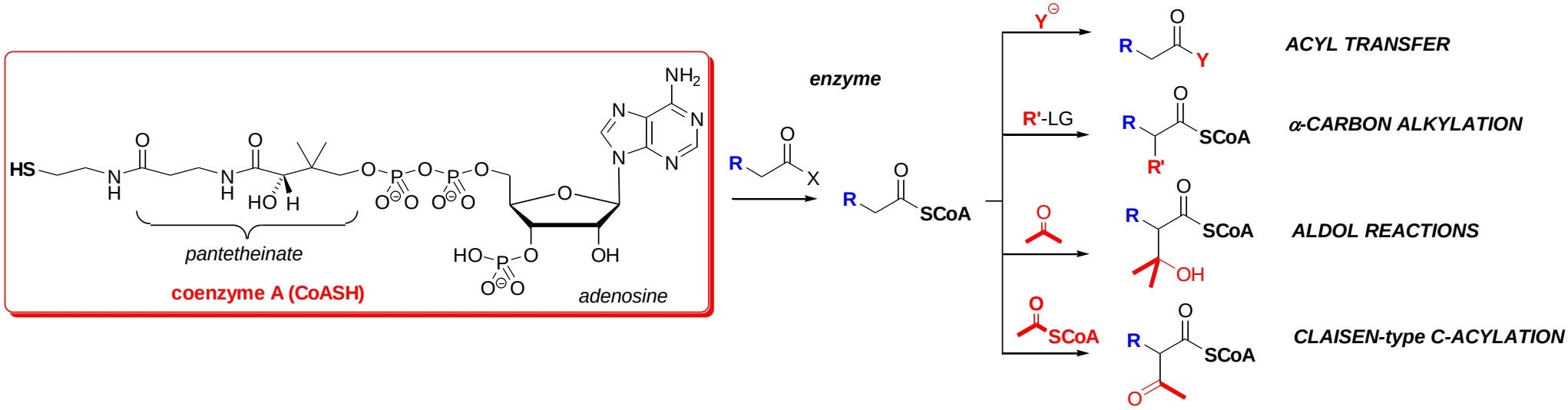
  - The phosphorylated alcohol (**R**O**P**) is then activated towards *e.g.* nucleophilic displacement:



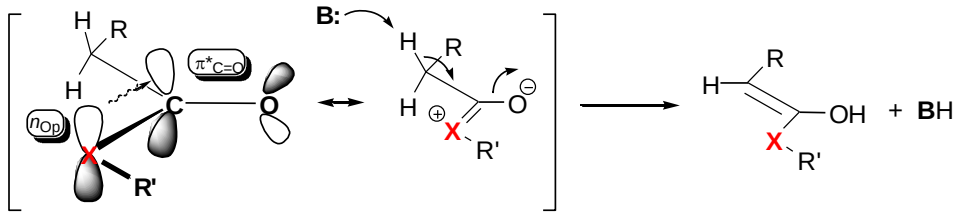
  - So, overall the ***endothermic*** process ROH + Y^- → RY + OH^- has been achieved by 'coupling' the process to the 'hydrolysis of ATP'
  - The situation is analogous to the use of tosylate activation to achieve nucleophilic displacement of an alcohol
  - In general, the exothermicity associated with phosphorylation shifts the equilibria of 'coupled' process by ***a factor of ~10^8***

Acylation & C-C Bond Formation α to C=O – *CoASH*

- ***Coenzyme A (CoASH)***
  - Coenzyme A acts as an acyl transfer/α-carbon activation reagent by forming reactive acyl thioesters:



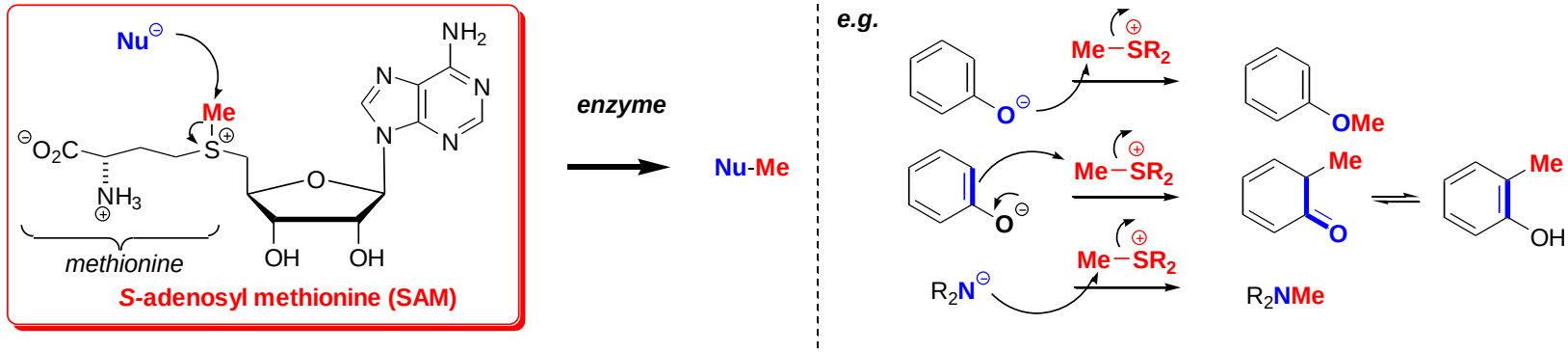
  - Acyl CoA derivatives can act as nucleophiles or electrophiles depending on the circumstances
  - These modes of reactivity are inherent properties of alkyl thioesters:
    - The ***good leaving group ability of RS***$^-$ (*cf.* RO$^-$) reflects: pK_a (RSH) ~10 *cf.* pK_a (ROH) ~16
    - The ***enhanced acidity of protons α to the carbonyl of thioesters*** *cf.* normal esters reflects the poor orbital overlap between the lone pairs on sulfur (n_S) [*cf.* n_O] and the carbonyl anti bonding molecular orbital $\pi^*_{C=O}$



n_X-$\pi^*_{C=O}$ resonance makes carbonyl less susceptible to enolisation
Sulfur is in the 2nd period
so its lone pair has poor size/energy match with the $\pi^*_{C=O}$ orbital
Hence: *pK_a(RCH$_2$COSR') ~20 cf. RCH$_2$COOR' ~25*
i.e. α to a thioester is similar to α to a ketone

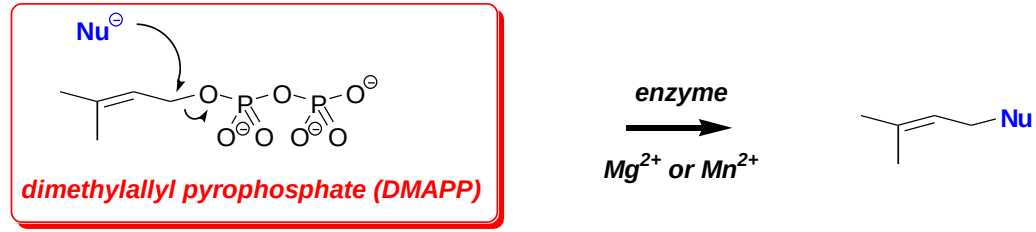
Methylation/Dimethylallylation – *SAM & DMAPP*

- ***S-Adenosyl methionine (SAM)***
  - SAM acts as a versatile *O*-, *C*-, *N*- & *S*- methylating reagent *in vivo*



  - Equivalent to performing an S_N2 methylation using MeI in the laboratory

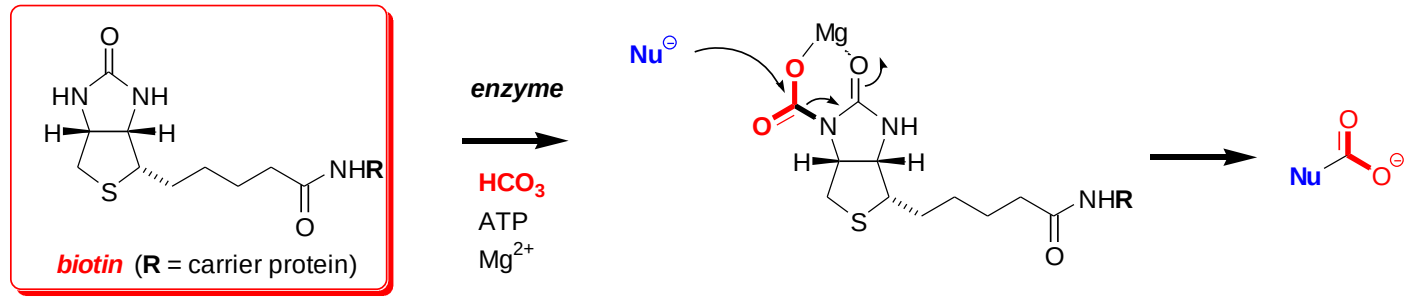
- ***Dimethylallyl pyrophosphate (DMAPP)***
  - DMAPP acts a dimethylallylating reagent – the pyrophosphate (+ Mg^{2+}/Mn^{2+}) is an excellent leaving group



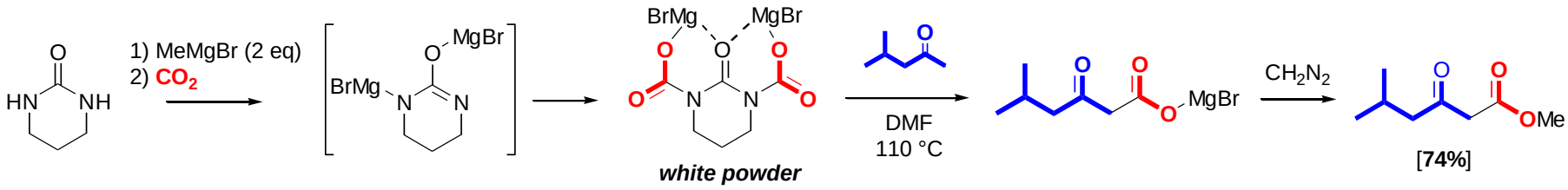
  - Equivalent to performing an S_N2 allylation using allyl bromide in the laboratory

Carboxylation – *Biotin*

- ***Biotin***
  - Biotin in the presence of bicarbonate, ATP and Mg^{2+} enables nucleophile carboxylation *in vivo:*

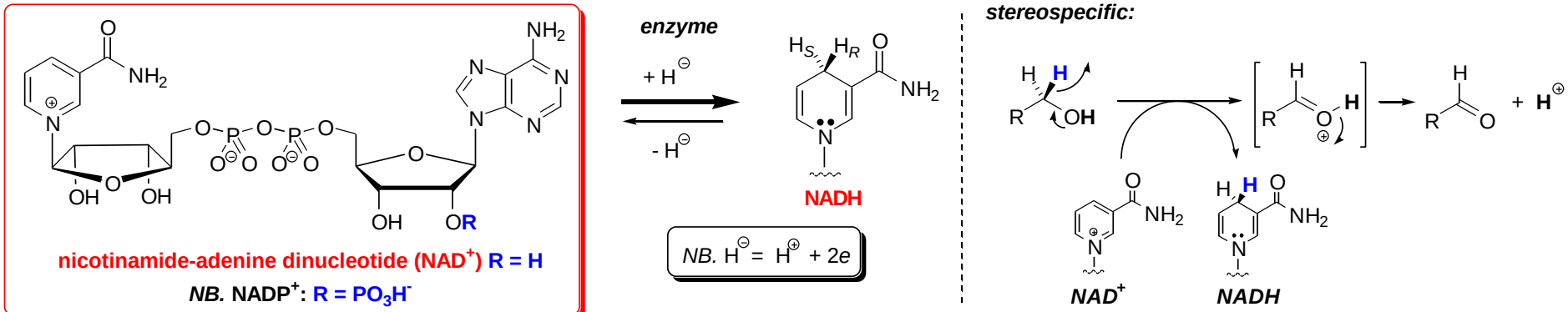


  - a very similar reaction can be carried out in the laboratory
    - Sakurai *et al. Tetrahedron Lett.* **1980**, *21*,1967 (**DOI**)

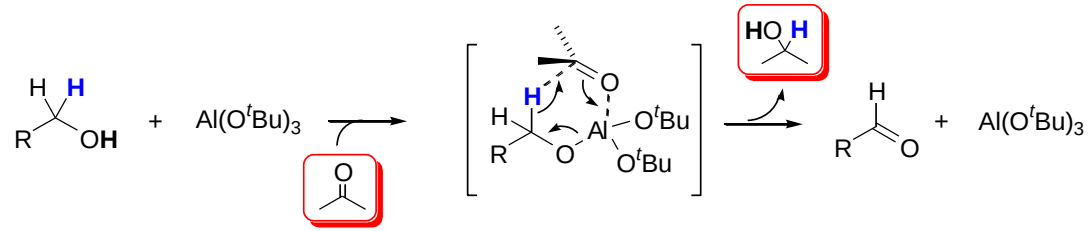


Oxidation – *NAD*$^+$

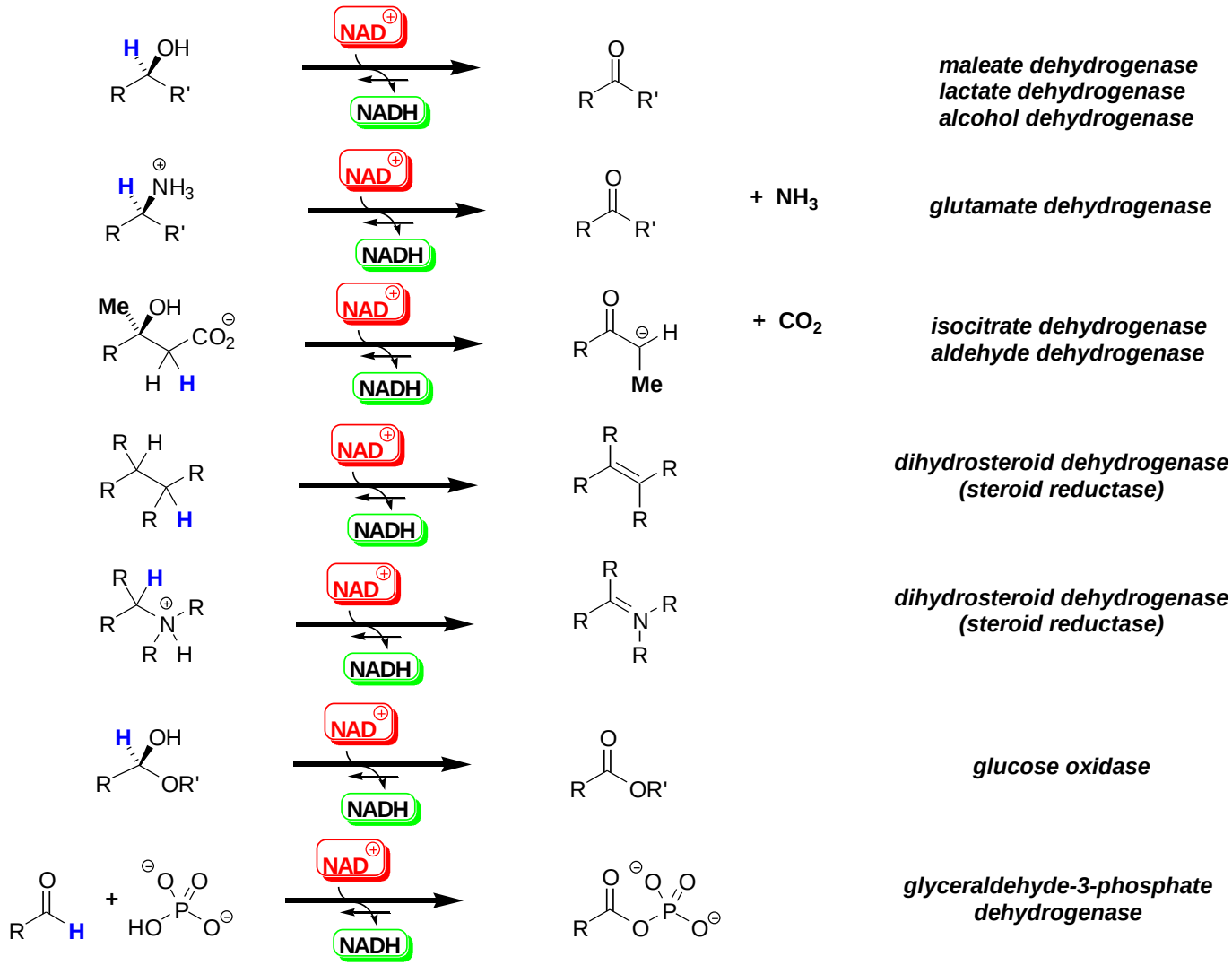
- ***Nicotinamide-adenine dinucleotide (NAD$^+$)*** [and its phosphorylated analogue ***(NADP$^+$)***] are mediators of ***biological oxidation*** (*e.g.* alcohol to ketone oxidation)
  - In general, the couple NAD$^+$/NADH is used by enzymes in ***catabolic oxidation*** (degradation)
  - The reagent is a stereospecific ***hydride acceptor***:



  - Different enzymes show different absolute specificities but are generally specific for the pro-R or pro-S hydrogens both for removal and delivery
  - The Oppenauer oxidation is a similar (*non*-stereoselective) laboratory reaction:
    - for asymmetric variants see: Nishide *et al. Chirality* **2002**, *14*, 759 (**DOI**)



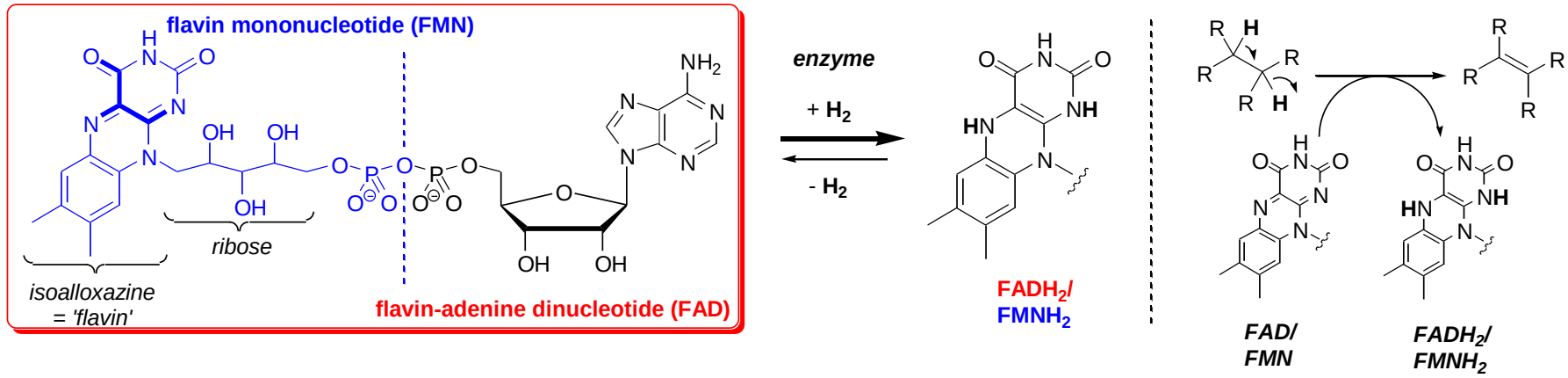
Oxidation Reactions Mediated by *NAD(P)*$^+$



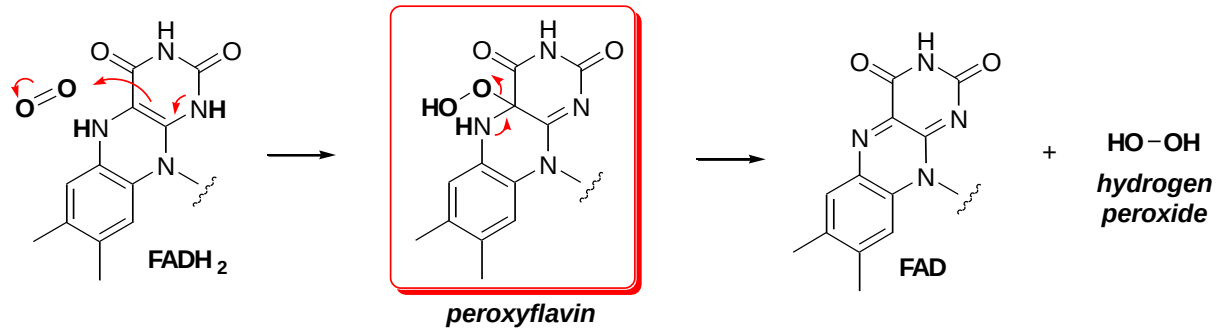
- ***Adapted from C.T. Walshe, 'Enzymatic Reaction Mechanisms', Freeman, 3rd ed.***

Oxidation – *Flavins (FAD & FMN)*

- ***Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)*** are also mediators of ***biological oxidations*** (*e.g.* dehydrogenations – alkane to alkene)
  - Unlike NAD^+, which readily diffuses from enzyme to enzyme, FAD/FMN is usually tightly bound to a given enzyme, sometimes covalently

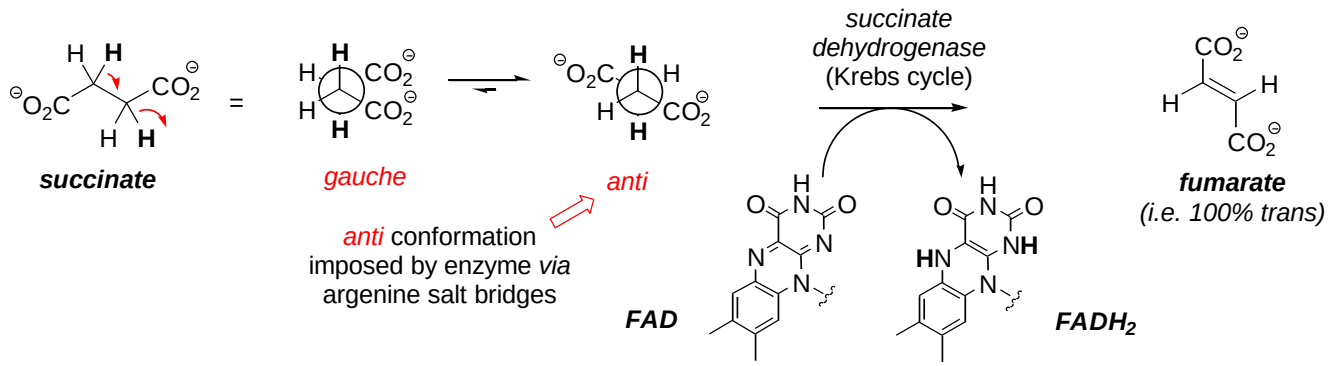


  - Re-oxidation of the $FADH_2$ back to FAD is generally by molecular ***oxygen*** *(although NAD^+ is also sometimes used)*. The intermediate ***peroxyflavin*** can also mediate ***hydroxylation***, ***epoxidation*** & other oxygen transfer reactions (see next slide):

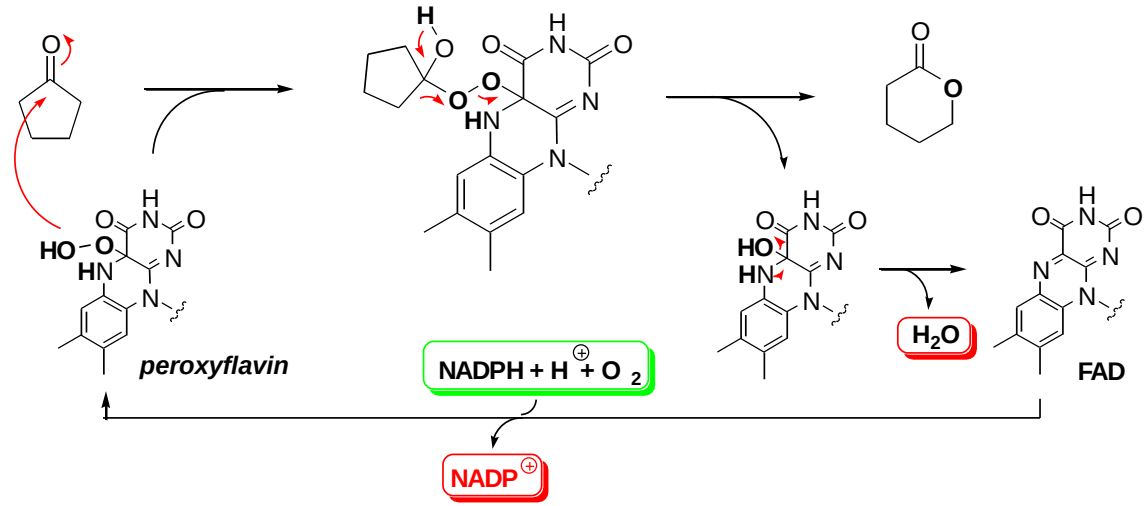


Oxidation Reactions Mediated by Flavins

- ***Dehydrogenation by flavins*** *– e.g. dehydrogenation of succinate → fumarate:*



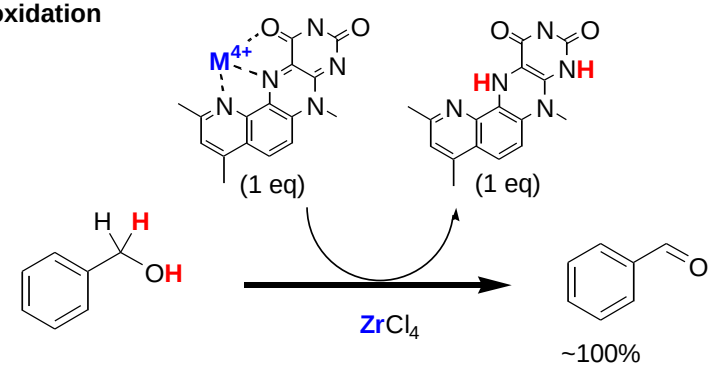
- ***Baeyer-Villiger-type oxidation by peroxyflavins*** *– e.g. ketone monooxygenase:*



Biomimetic Oxidation using FAD Models

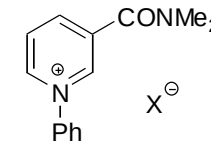
- ***A stoichiometric <u>flavin</u> model oxidising system (alcohol → aldehyde):***
  - Shinkai *Chem. Lett.* **1982**, 812 & *Bull. Soc. Chim. Fr.* **1983**, *56*, 1694

***inter*molecular oxidation**

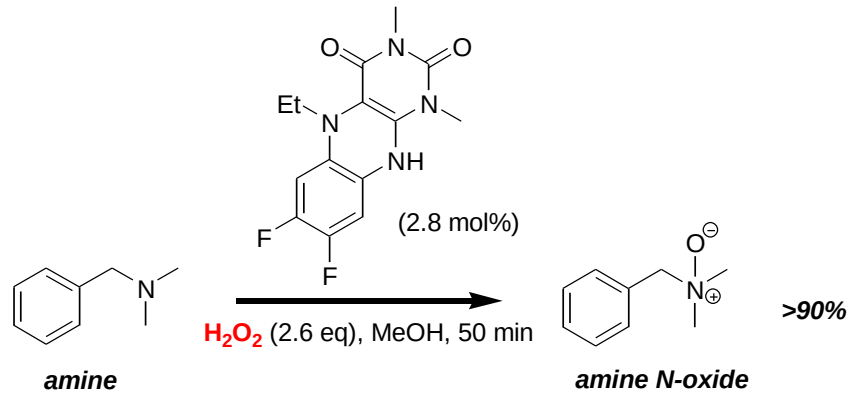


***NB.* simple *N*-alkylated nicotinamide salts (*cf.* NAD^+) perform poorly**

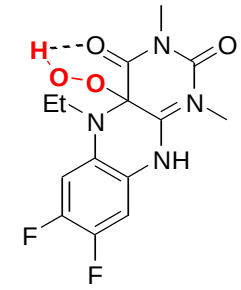
e.g.



- ***A catalytic <u>peroxyflavin</u> model oxidising system (amine → amine N-oxide):***
  - Bäckvall *Chem. Eur. J.* **2001**, *7*, 297 (**<u>DOI</u>**)

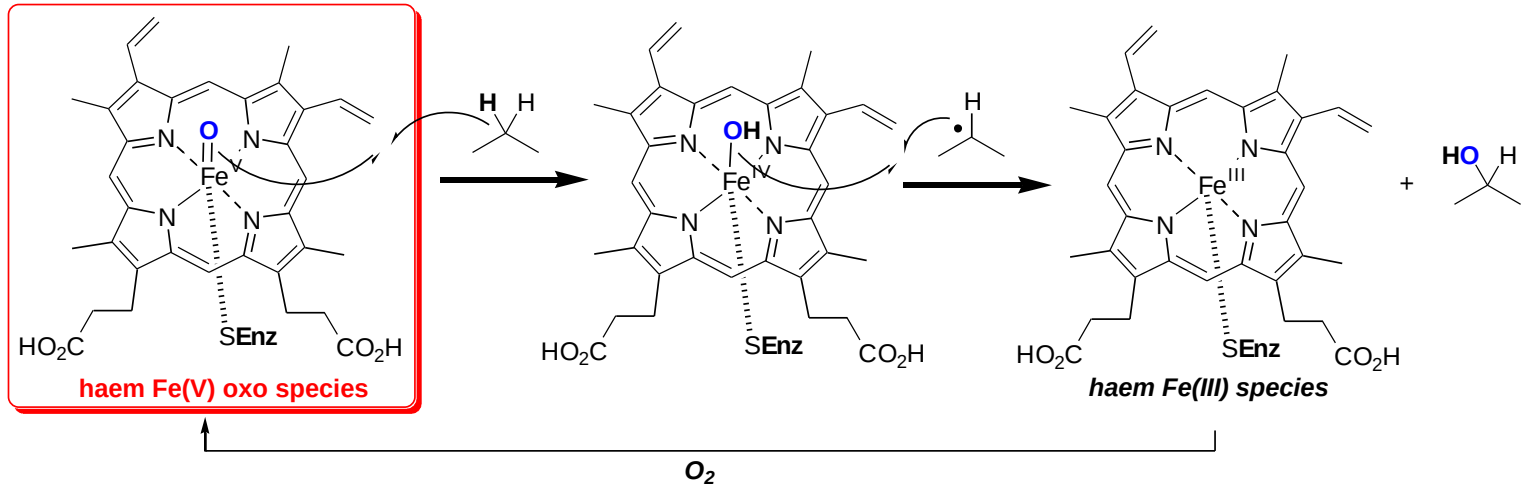


via

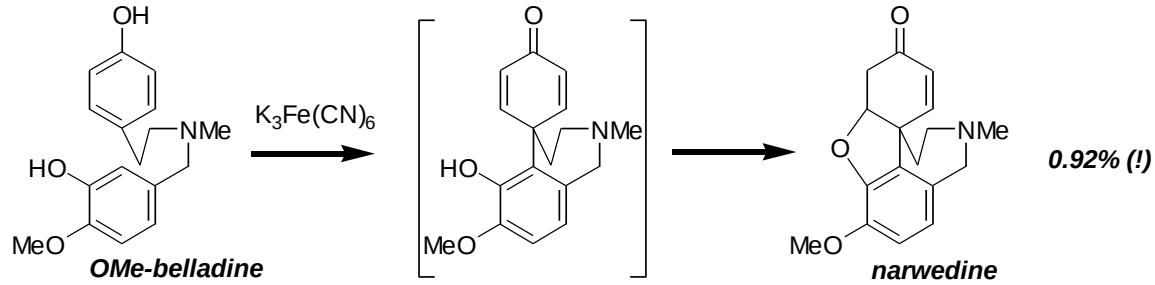


Oxidation – *Haem Iron oxo Species (P_{450})*

- ***Haem iron oxo species*** *e.g.* in ***cytochrome P_{450}*** (a ubiquitous ***heam monooxygenase***) are also mediators of ***biological oxidation*** (*e.g.* phenolic coupling, epoxidation, ***hydroxylation***):

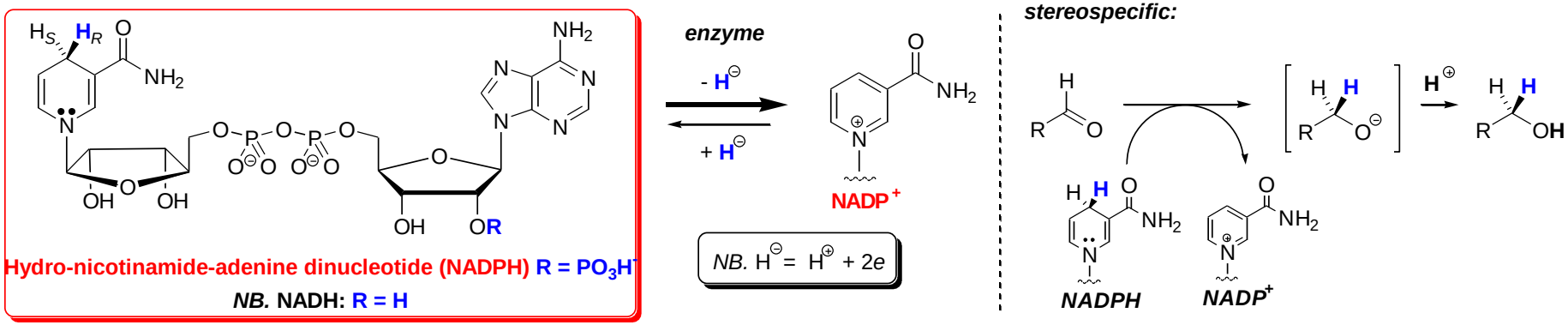


  - The porphyrin ring acts as a tetradentate ligand for the octahedral iron. The two axial positions are occupied by an enzyme amino acid ligand (typically a histidine nitrogen) and hydroxy/hydroperoxy residue respectively
  - Ferricyanide effects similar oxidative processes in the lab (*e.g.* phenolic coupling, see 'alkaloids', later)
    - Barton & Kirby *J. Chem. Soc.* **1962**, 806 (**DOI**)

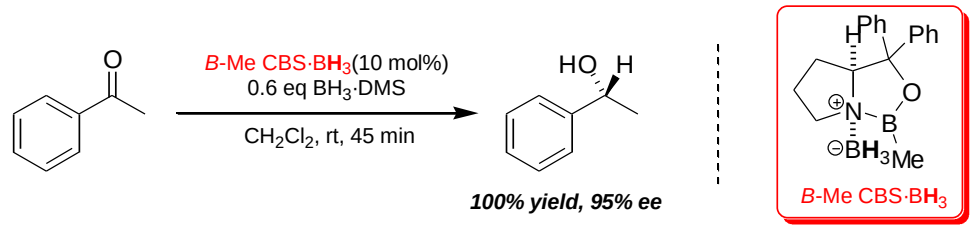


Reduction - *NADPH*

- ***Dihydro-nicotinamide-adenine dinucleotide phosphate (NADPH)*** [and its de-phosphorylated analogue ***(NADH)***] are mediators of ***biological reduction*** (*e.g.* ketone to alcohol reduction)
  - In general, the couple NAPH/$NADP^+$ is used by enzymes in ***anabolic reduction*** (biosynthesis)
  - The reagent is a stereospecific ***hydride donor***:

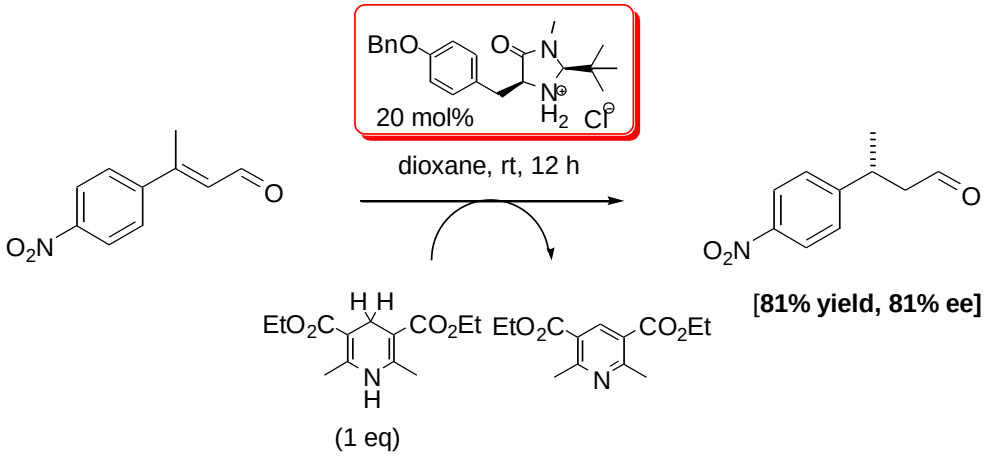


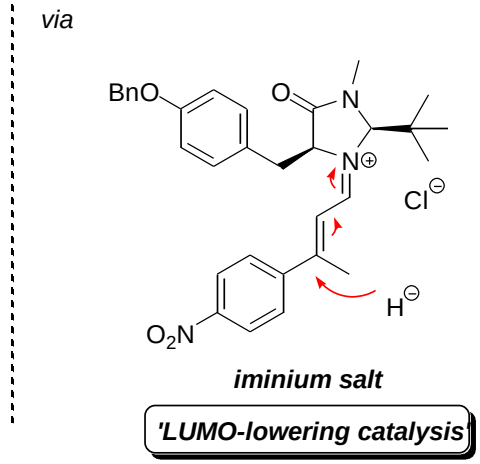
  - As for the reverse process, different enzymes show different absolute specificities but are generally specific for the pro-R or pro-S hydrogens both for removal and delivery
  - NADPH acts like a biochemical equivalent of 'laboratory' metal hydride reductants (*e.g.* $LiAlH_4$, $NaBH_4$) or their chiral equivalents (*e.g.* CBS-borane):



Biomimetic Reduction using NAD(P)H Models

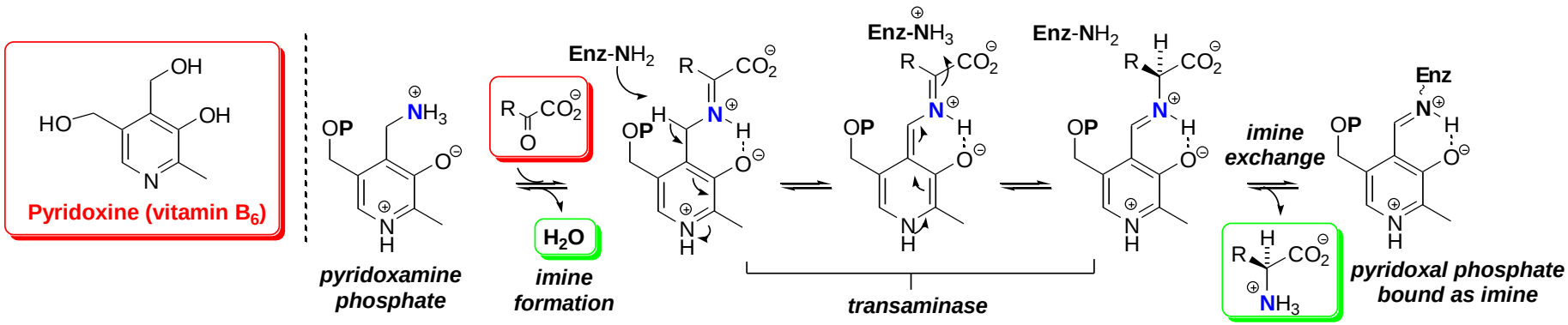
- ***A <u>catalytic</u>, enantioselective <u>NADPH</u> model reducing system (α,β-unsaturated aldehyde → aldehyde):***
  - ***highlight:*** Adolfsson *Angew. Chem. Int. Ed.* **2005**, *44*, 3340 (**<u>DOI</u>**)



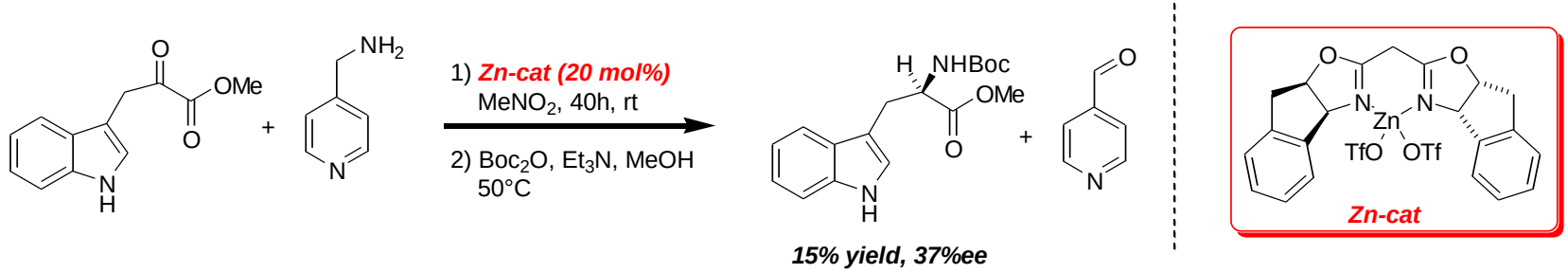


Transamination - *PLP*

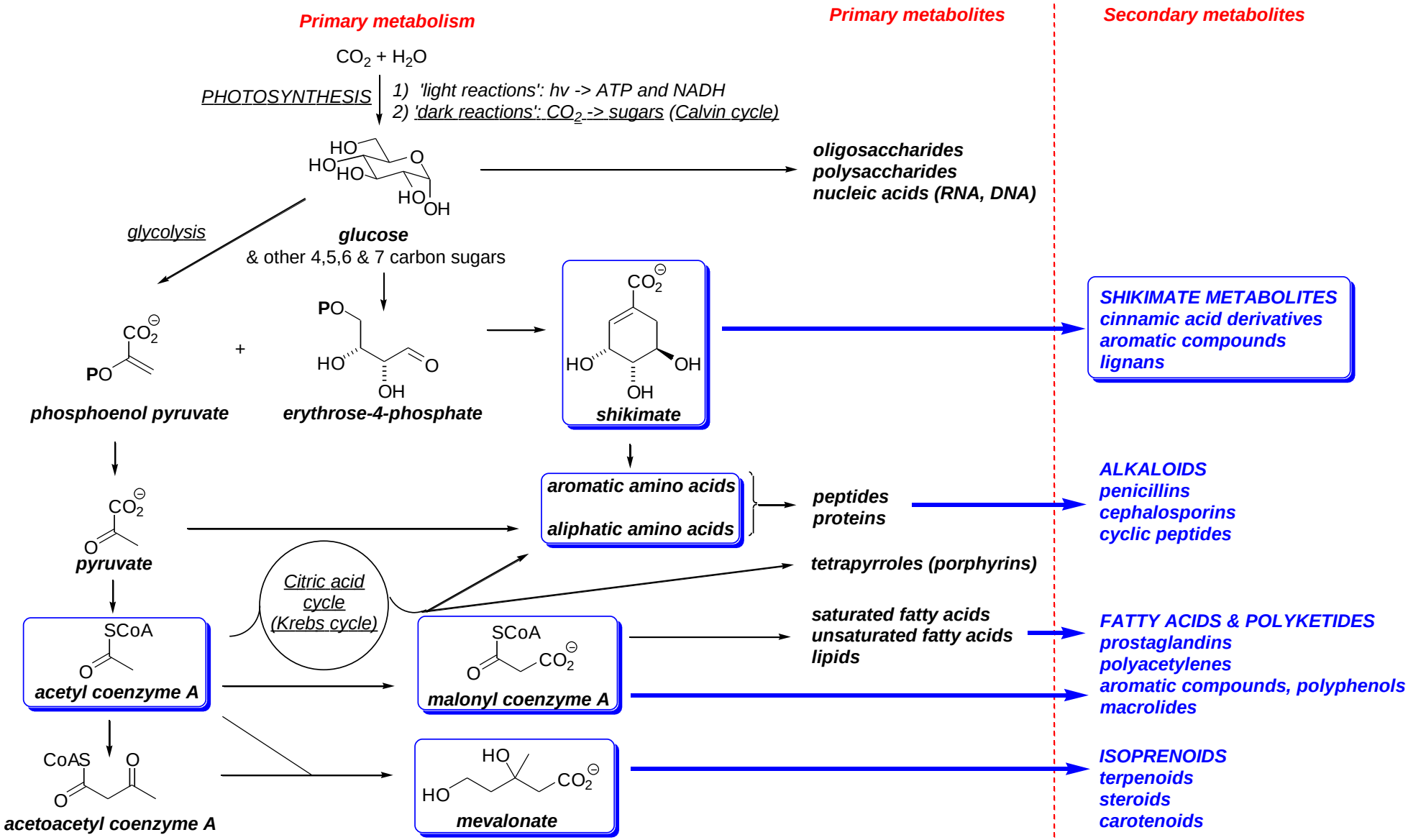
- ***Pyridoxine (vitamin B_6) → pyridoxal-5'-phosphate (PLP)***
  - ***PLP*** forms ***imines*** (Schiffs bases) with ***primary amines***. This forms the basis of *in vivo* ***transamination*** of ***α-ketoacids*** to give ***α-amino acids*** (& also ***racemisation/decarboxylation*** processes, see 'alkaloids')



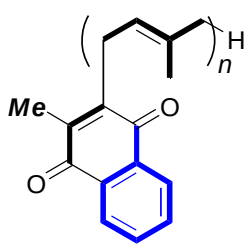
  - The α-carbon protonation is stereospecific and gives the (*S*) configured chiral centre
  - Jørgensen has developed a catalytic, enantioselective lab equivalent of this process:
    - Jørgensen *et al. Chem. Comm.* **2003**, 2602 (**DOI**)



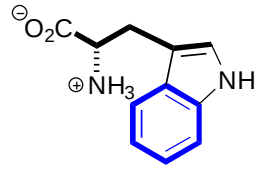
Primary Metabolism - *Overview*



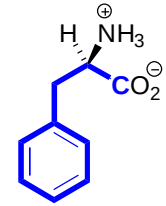
Shikimate Metabolites



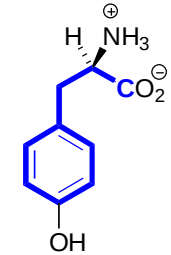
menaquinone (vitamin K_2)
(ArC_1)



(S)-tryptophan
(ArC_0)

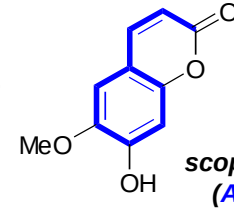


(S)-phenylalanine
(ArC_3)

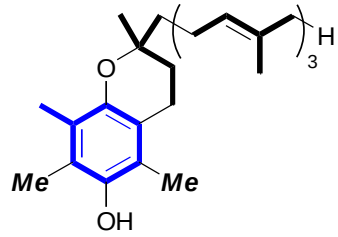


(S)-tyrosine
(ArC_3)

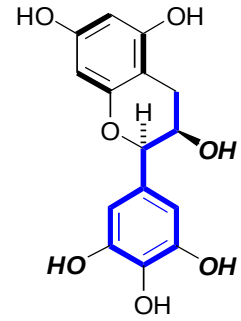
SHIKIMATE METABOLITES



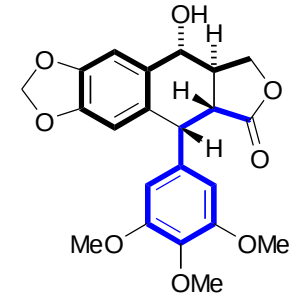
scopoletin
(ArC_3)



α-tocopherol (vitamin E)
(ArC_1)



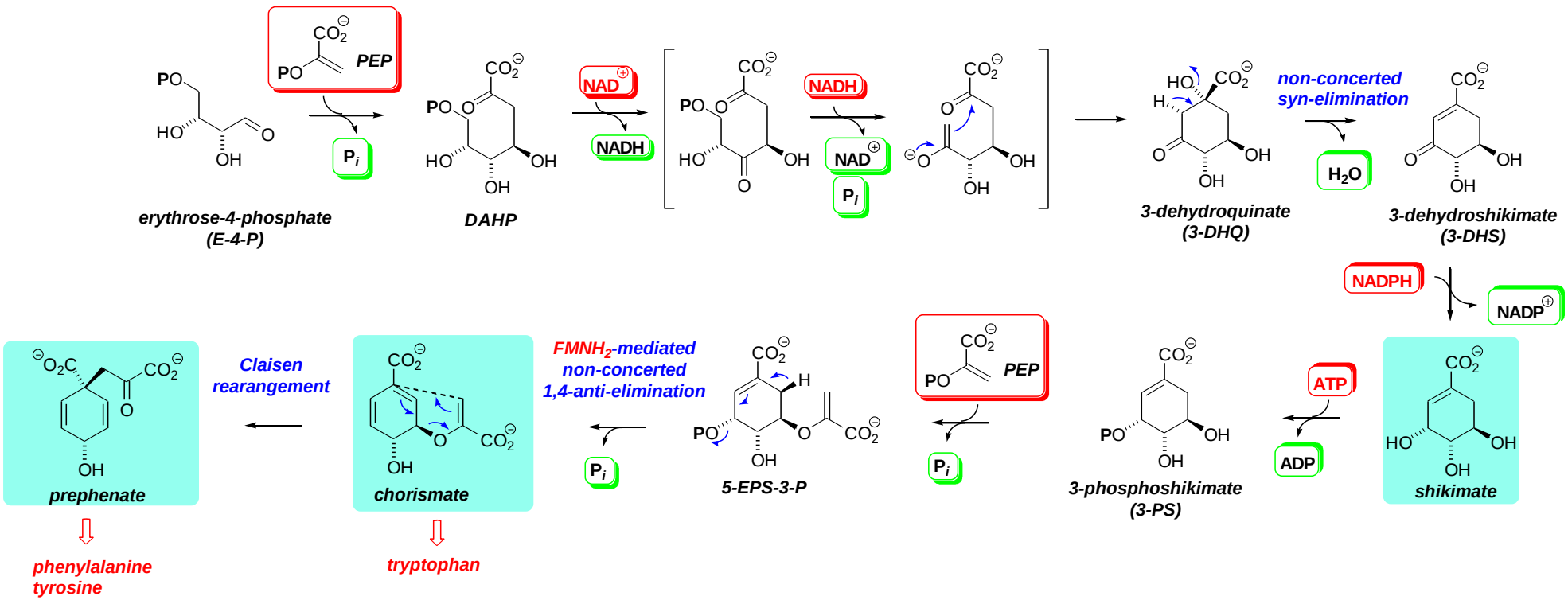
epigallocatechin (EGC)
(ArC_3)



podophyllotoxin
(ArC_3)

The Shikimate Biosynthetic Pathway - *Overview*

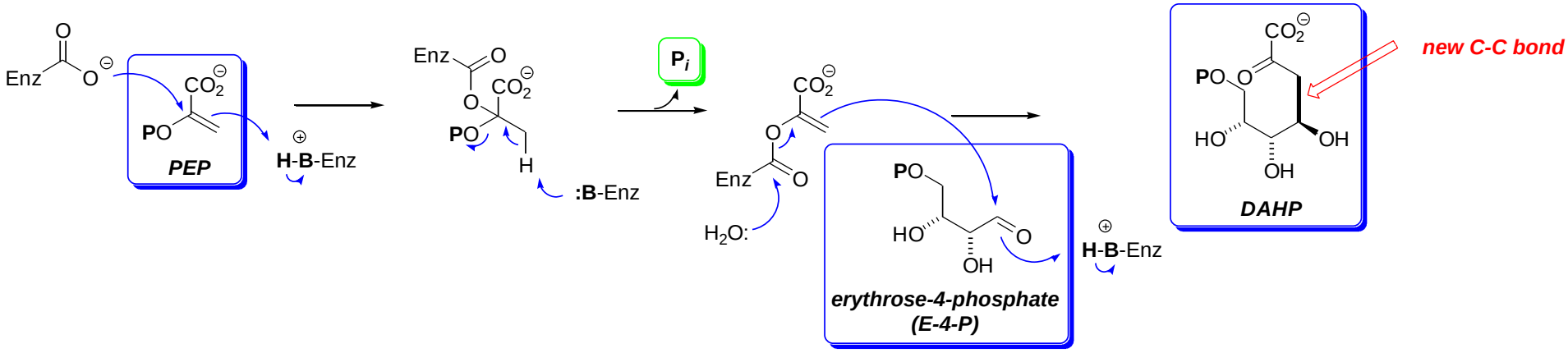
- ***Phosphoenol pyruvate*** *&* ***erythrose-4-phosphate*** → ***shikimate*** → ***chorismate*** → ***prephenate***:



  - The detailed mechanisms of these steps have been studied intensively. Most are chemically complex and interesting. For additional details see:
    - Mann *Chemical Aspects of Biosynthesis* Oxford Chemistry Primer No. 20, **1994** *(key details)*
    - Haslam *Shikimic Acid – Metabolism and Metabolites* Wiley, **1993** *(full details and primary Lit. citations)*
    - http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/misc/shikim.html *(interesting web-site with many biosynethtic pathways)*

PEP + E-4-P → DAHP

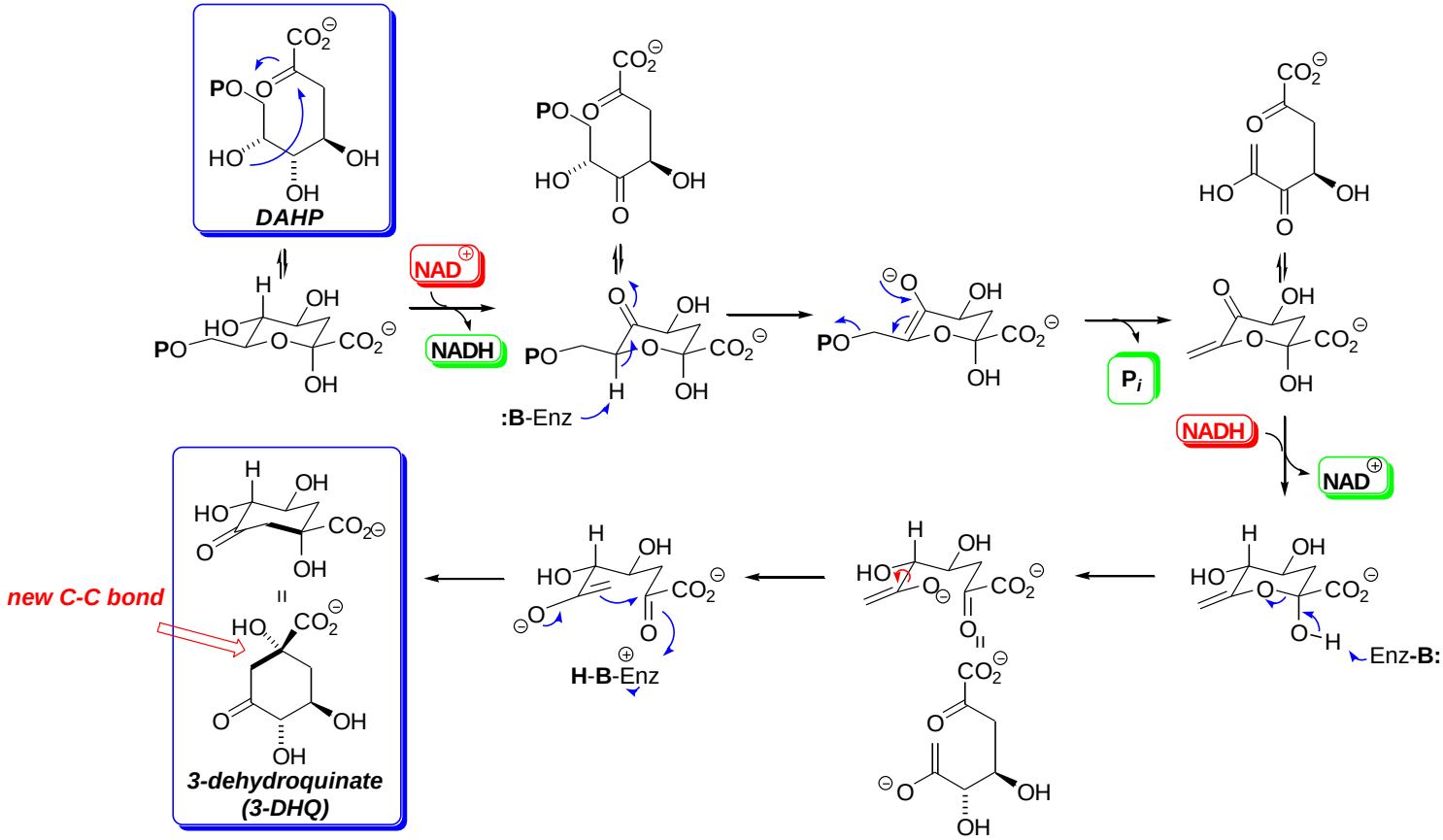
- ***Phosphoenol pyruvate (PEP) + erythrose-4-phosphate (E-4-P) → 3-deoxy-D-arabino-heptulosonate-7-phosphate (DHAP)***
- ***Enzyme: 3-deoxy-7-phosphoheptulosonate synthase* = *DAHP synthase*** [EC 2.5.1.54]
  - *chemistry catalysed:* **an aldol reaction**



  - Floss *et al. J. Biol. Chem.* **1972**, *247*, 736 (**DOI**)

DAHP → 3-DHQ

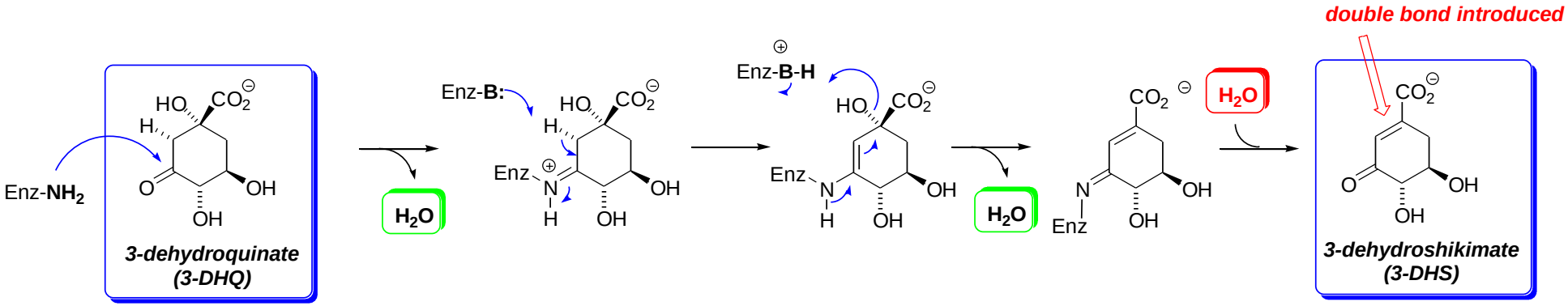
- ***3-Deoxy-D-arabino-heptulosonate-7-phosphate (DHAP) → 3-dehydroquinate (3-DHQ)***
- ***Enzyme*: 3-dehydroquinate synthase** [EC 4.2.3.4]
  - *chemistry catalysed:* **alcohol → ketone → alcohol redox cycle & cyclisation *via* aldol reaction**



  - Knowles *et al. Biochemistry* **1989**, *28*, 7555 (**DOI**)

3-DHQ → 3-DHS

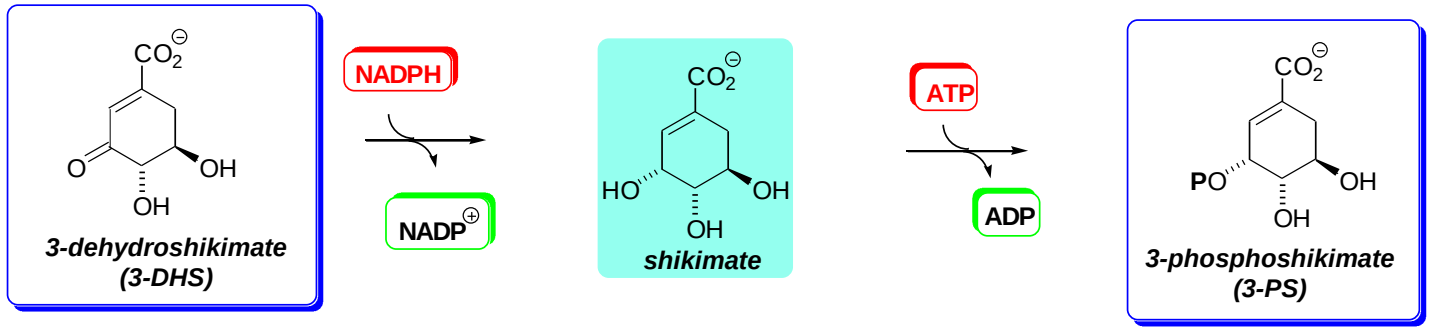
- ***3-Dehydroquinate (3-DHQ) → 3-dehydroshikimate (3-DHS)***
- ***Enzyme: 3-dehydroquinate dehydratase*** [EC 4.2.1.10]
  - *chemistry catalysed:* **stereoselective *syn*-elimination**



- Abell *et al. Biochem. J.* **1996**, *319*, 333 (**DOI**)
- Coggins *et al. J. Biol. Chem.* **1995**, *270*, 25827 (**DOI**)
- Coggins *et al. Nature Struct. Biol.* **1999**, *6*, 521 (**DOI**)

3-DHS → 3-PS

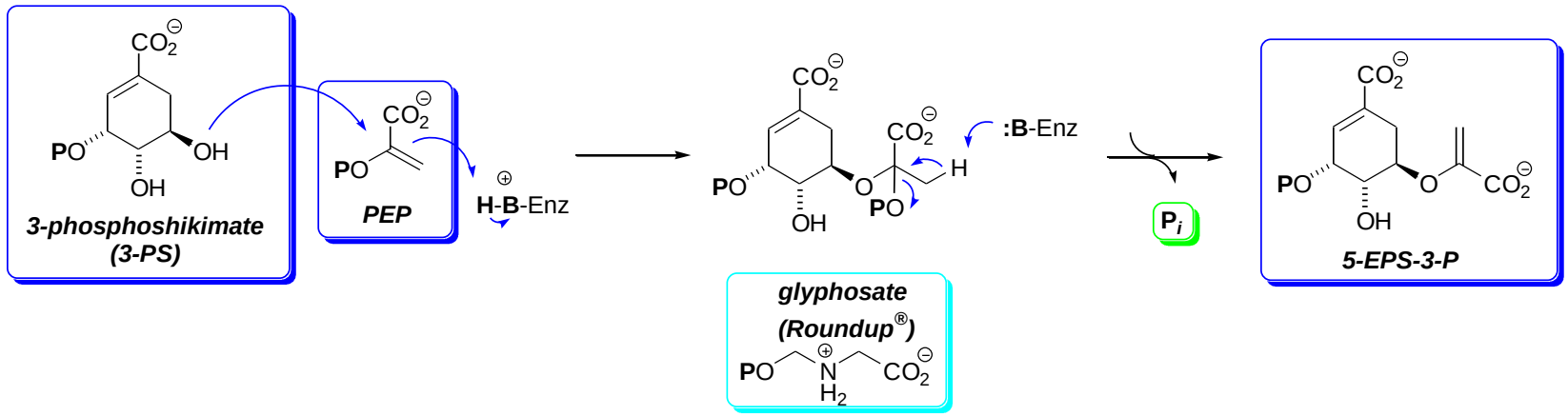
- ***3-Dehydroshikimate (3-DHS)* → *shikimate* → *3-phosphoshikimate (3-PS)***
- ***Enzymes*: *shikimate dehydrogenase*** [EC 1.1.1.25] then ***shikimate kinase*** [EC 2.7.1.71]
  - *chemistry catalysed:* **stereoselective ketone → alcohol reduction then alcohol phosphorylation**



  - Ye *et al. J. Bacteriol.* **2003**, *185*, 4144 (DOI)
  - Morell *et al. J. Biol. Chem.* **1968**, *243*, 676 (DOI)

3-PS → 5-EPS-3-P

- ***3-Phosphoshikimate (3-PS) → 5-enolpyruvylshikimate-3-phosphate (5-EPS-3P)***
- ***Enzyme*: 3-phosphoshikimate 1-carboxyvinyltransferase** [EC 2.5.1.19]
  - *chemistry catalysed:* **vinyl ether formation**

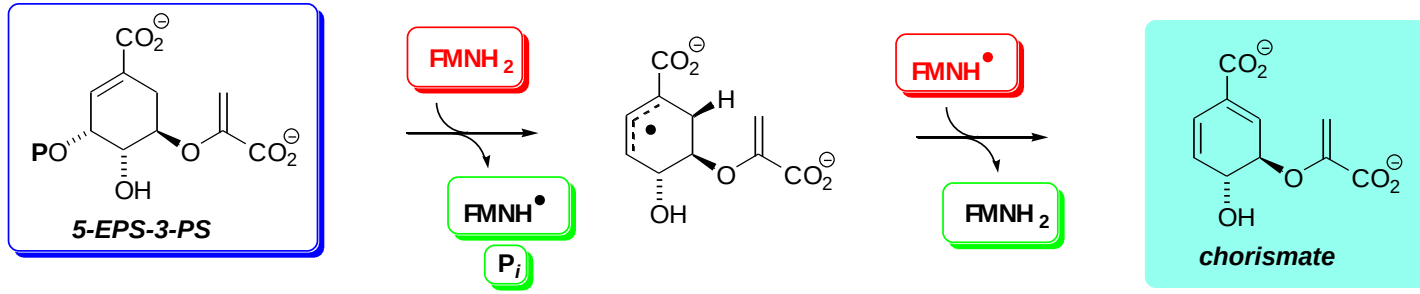


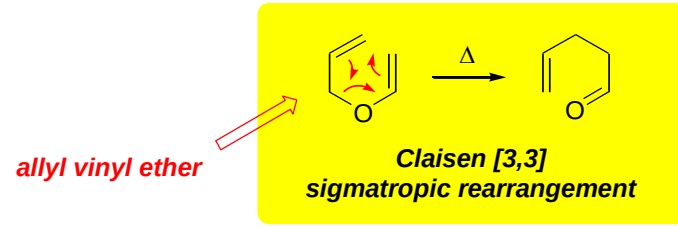
  - ***Glyphosate ('Roundup') – a Monsanto agrochemical is a potent inhibitor of this biosynthetic step***
    - ***a non-selective herbicide***

  - Lewis *et al. Biochemistry* **1999**, *38*, 7372 (DOI)
  - Jakeman *et al. Biochemistry* **1998**, *37*, 12012 (DOI)

5-EPS-3-P → Chorismate

- ***5-Enolpyruvylshikimate-3-phosphate (5-EPS-3P) → chorismate***
- ***Enzyme*: chorismate synthase** [EC 4.2.3.5]
  - *chemistry catalysed:* **non-concerted *anti*-1,4-elimination**

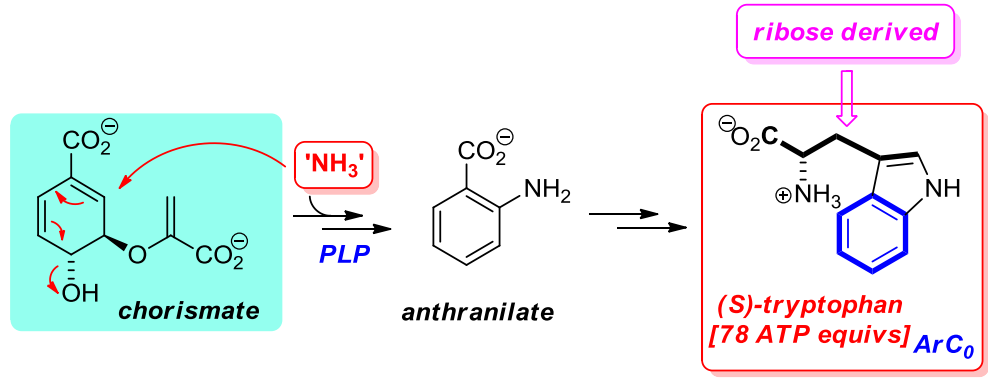




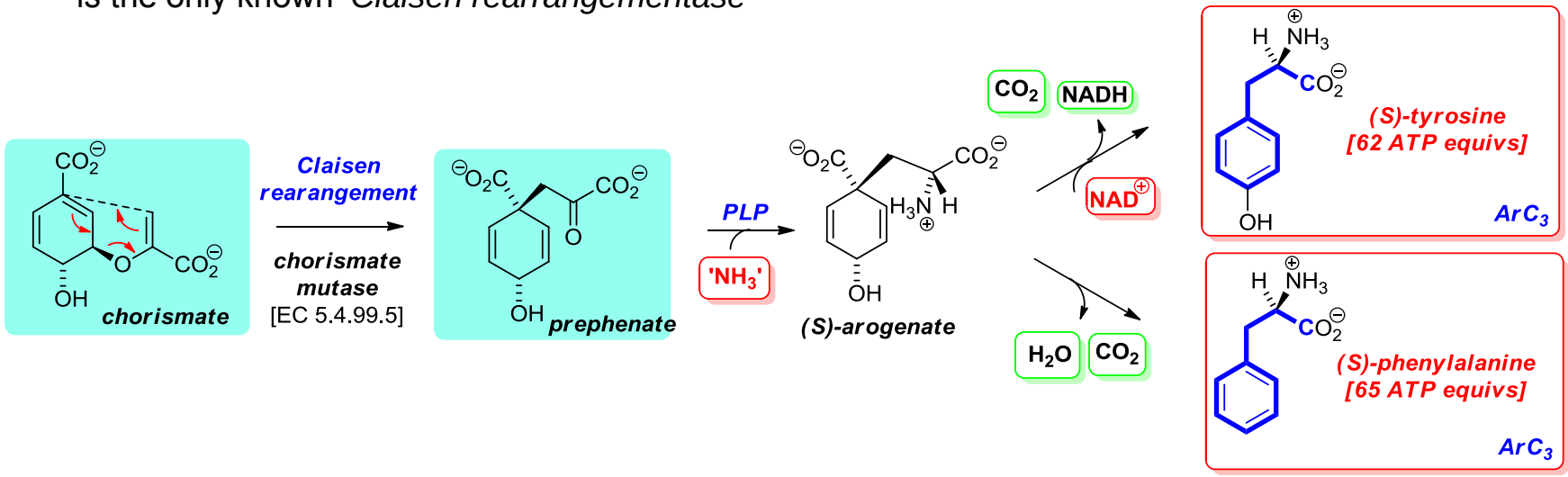
  - Abell *et al. Bioorg. Chem.* **2000**, *282*, 191 (**DOI**)
  - Abell *et al. J. Biol. Chem.* **2000**, *275*, 35825 (**DOI**)
  - Bornemann *et al. Biochemistry* **1996**, *35*, 9907 (**DOI**)

Chorismate → Tryptophan, Tyrosine & Phenylalanine

- ***Chorismate → anthranilate → <u>tryptophan</u>***

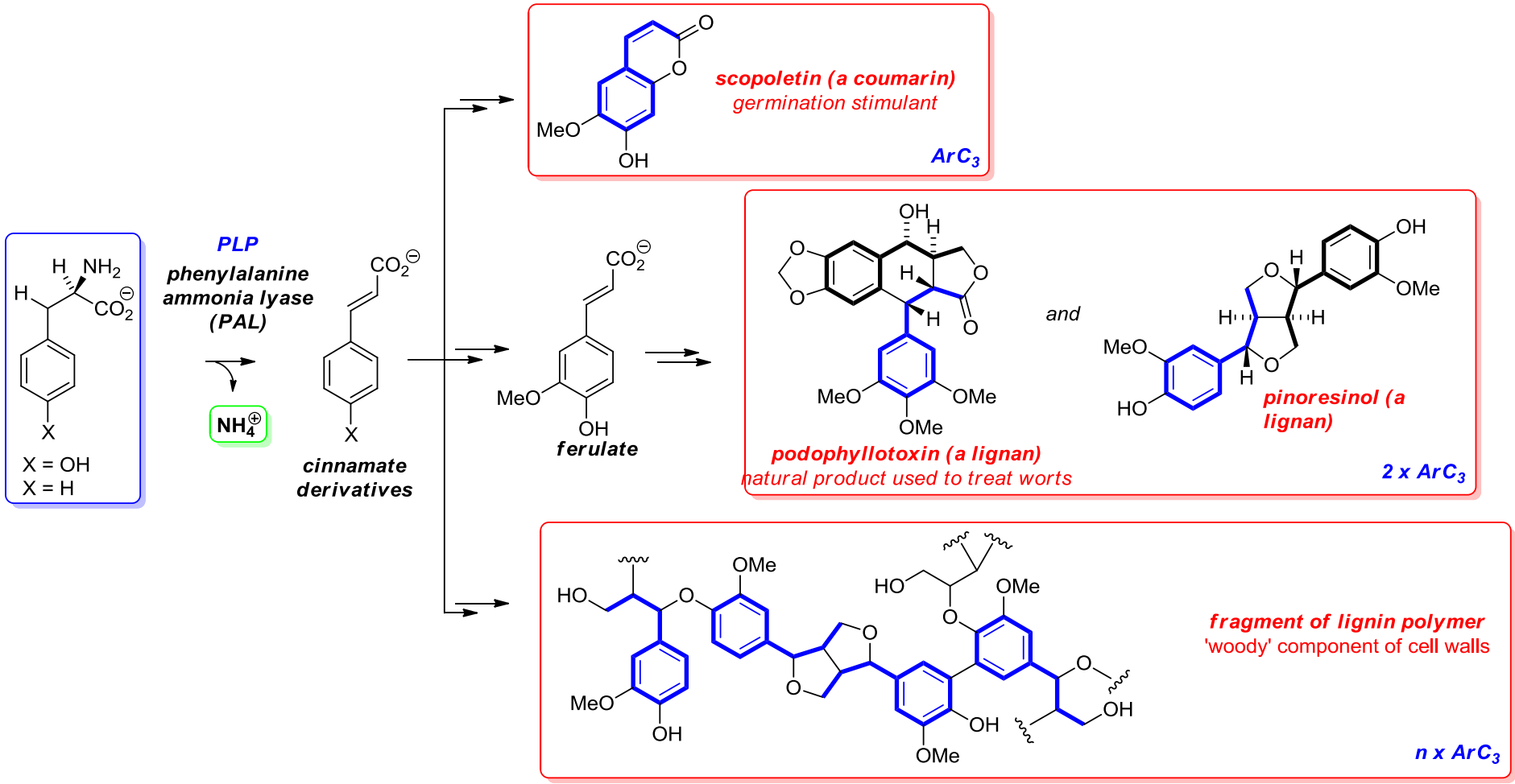


- ***Chorismate → prephenate → <u>tyrosine</u>*** & ***<u>phenylalanine</u>***
  - *NB*. The enzyme *chorismate mutase* [EC 5.4.99.5] which mediates the conversion of chorismate to prephenate is the only known '*Claisen rearrangementase*'



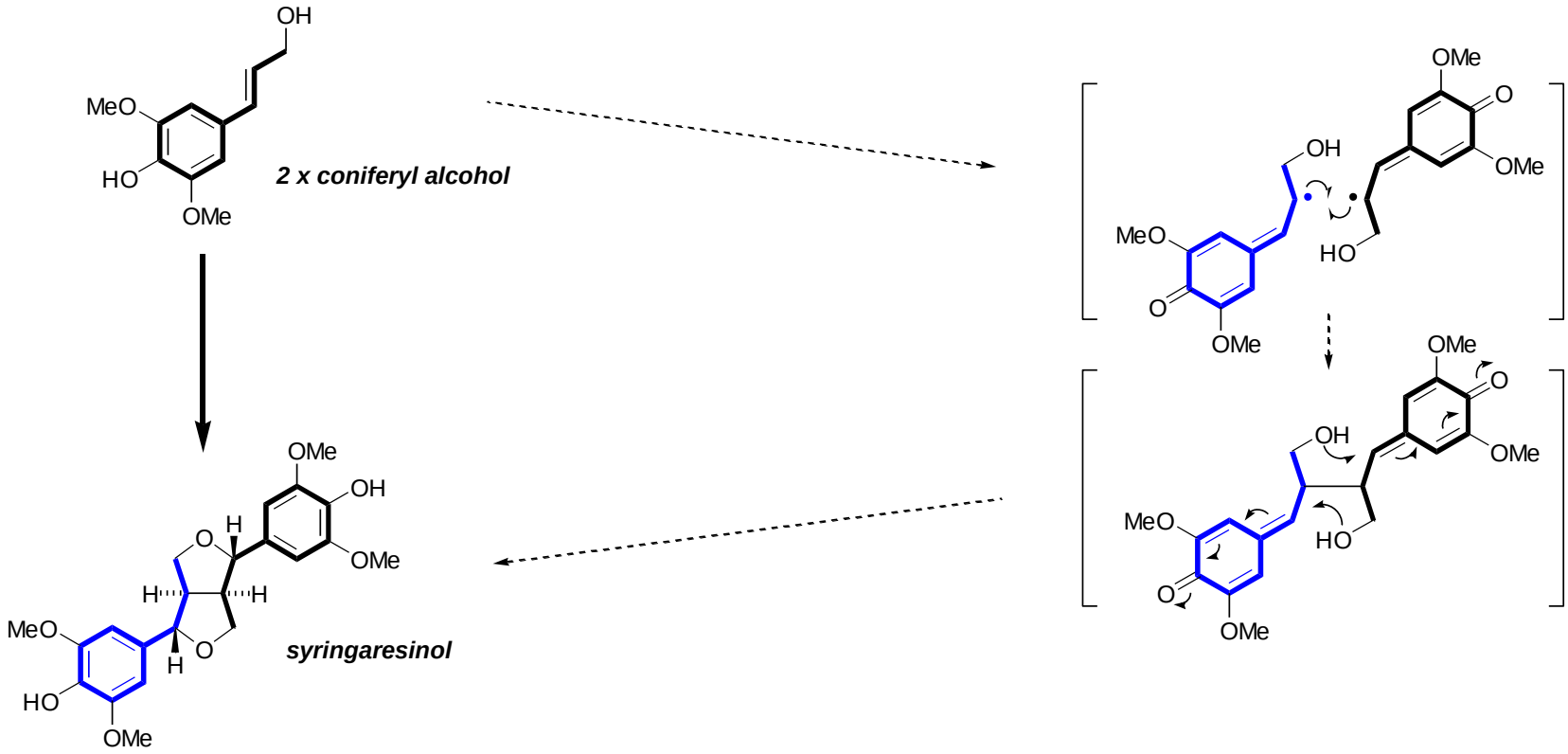
Tyrosine/Phenylalanine → ArC_3 Metabolites

- ***Tyrosine*** & ***phenylalanine*** → ***cinnamate derivatives*** → ***ArC_3 metabolites***
  - ***coumarins***, ***lignans*** (stereoselective enzymatic dimerisation) & ***lignins*** (stereorandom radical polymerisation)



Biomimetic Lignan Synthesis

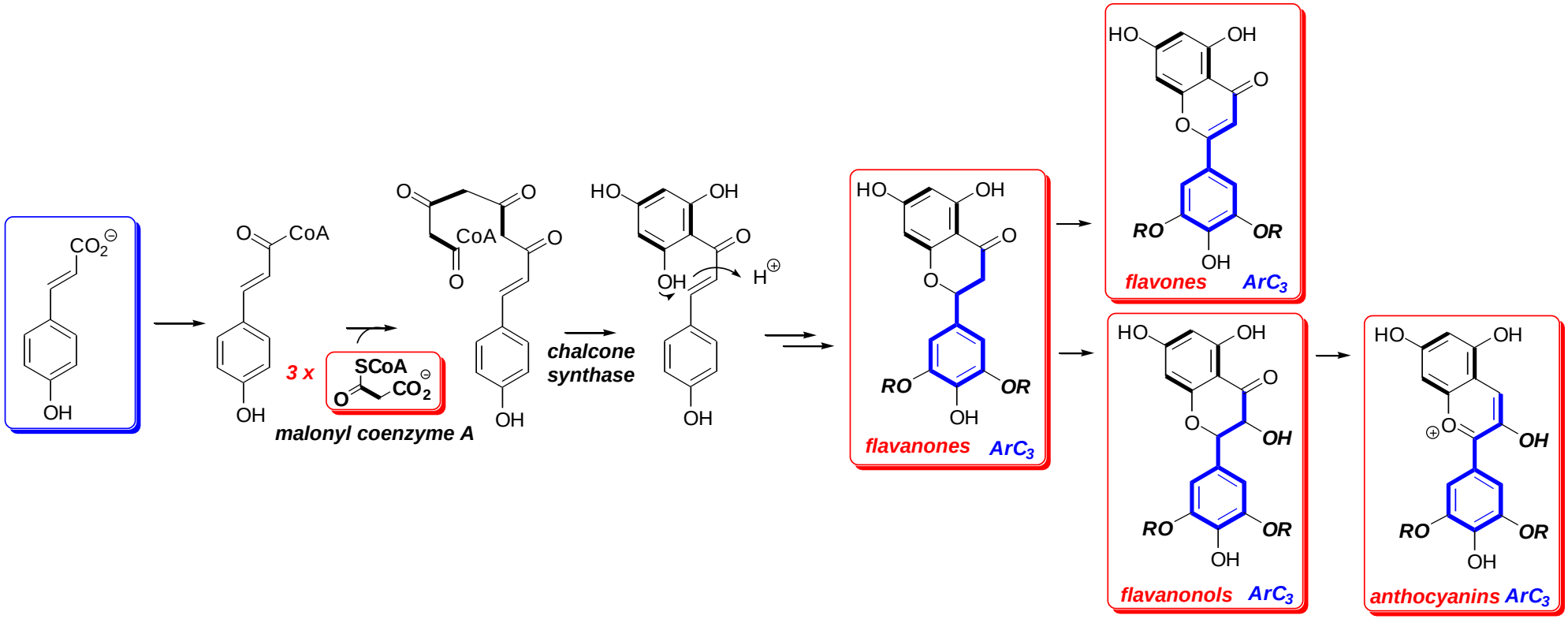
- ***Oxidative dimerisation*** of ***cinnamyl alcohols*** gives ***symmetric furanofuran lignans***
  - ***review:*** Brown & Swain *Synthesis* **2004**, 811 (**DOI**)
  - ***IN VIVO:*** Lewis *et al. Science* **1997**, *275*, 362 (**DOI**) (oxidase → single enantiomer of product)
  - ***IN VITRO:*** Vermes *et al. Phytochem*. **199**1, *30*, 3087 (**DOI**) [$CuSO_4$ (cat.), O_2, acetone-H_2O (**90%**)]



NATURAL = single enantiomer
SYNTHETIC = racemic - BUT a single diastereoisomer

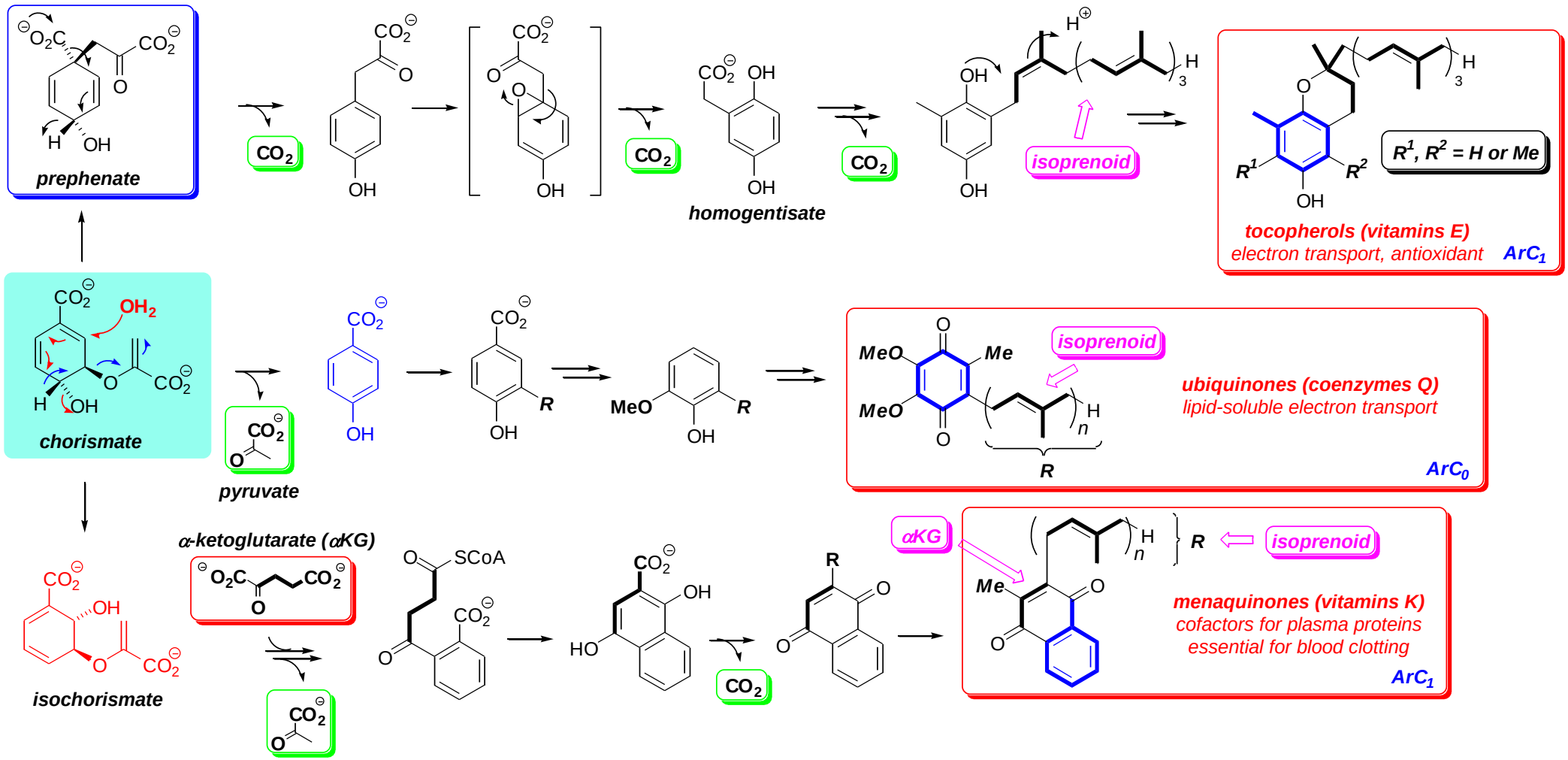
Tyrosine/Phenylalanine → Flavonoids

- ***4-Hydroxycinnamic acid → flavonoids: flavanones, flavanonols, flavones & anthocyanins***
  - Glycosides of these ArC_3 metabolites (*esp.* anthocyanins) constitute coloured pigments in flowers and insects. They also confer bitter and astringent flavours (*e.g.* ***tannins*** & ***catechins*** in tea are polymerised flavonoids)
  - *NB.* 'Mixed' biosynthetic origin: ***shikimate/malonylCoA (polyketide)***



Chorismate → Coenzymes Q & Vitamins E & K

- ***Chorismate → p- & o-hydroxybenzoic acids → coenzymes Q & vitamins E & K***
  - *NB.* 'Mixed' biosynthetic origin: ***shikimate/mevalonate (isoprenoid)***



Primary Metabolism - *Overview*