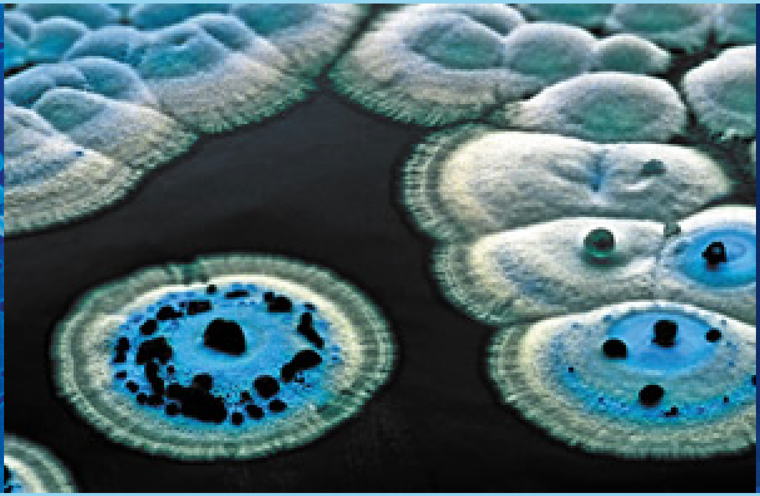
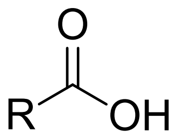




WP15 - Natural Product Chemistry

Prof. Dr. Jeroen S. Dickschat

1. Fatty acids and derivatives

1.1 Nomenclature

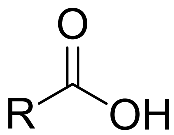


saturated, unbranched

R=H	formic acid (lat. formica = ant)
$R=CH_3$	acetic acid (lat. acetum = vinegar)
$R=C_2H_5$	propionic acid (gr. πρωτοσ = first, πιον = fat, first acid behaving like a fat)
$R=C_3H_7$	butyric acid (lat. butyrum = butter)
$R=C_4H_9$	valeric acid (*Valeriana officinalis* = valerian)
$R=C_5H_{11}$	caproic acid (lat. capra = goat)
$R=C_6H_{13}$	enanthic acid (gr. οινοσ = wine)
$R=C_7H_{15}$	caprylic acid (lat. capra = goat)

1. Fatty acids and derivatives

1.1 Nomenclature

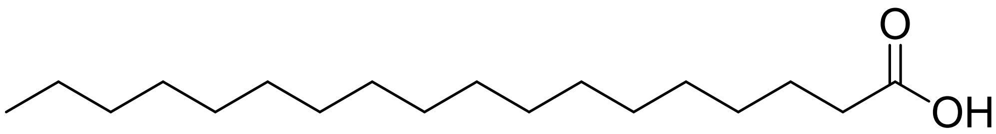


saturated, unbranched

$R=C_8H_{17}$	pelargonic acid (*Pelargonium* = geranium)
$R=C_9H_{19}$	capric acid (lat. capra = goat)
$R=C_{11}H_{23}$	lauric acid (*Laurus nobilis* = laurel)
$R=C_{13}H_{27}$	myristic acid (*Myristica fragrans* = nutmeg)
$R=C_{15}H_{31}$	palmitic acid (*Elaeis guineensis* = oil palm)
$R=C_{16}H_{33}$	margaric acid (first isolated as mixture of palmitic and stearic acid)
$R=C_{17}H_{35}$	stearic acid (gr. στεαρ = tallow)
$R=C_{19}H_{39}$	arachidic acid (*Arachis hypogaea* = peanut)

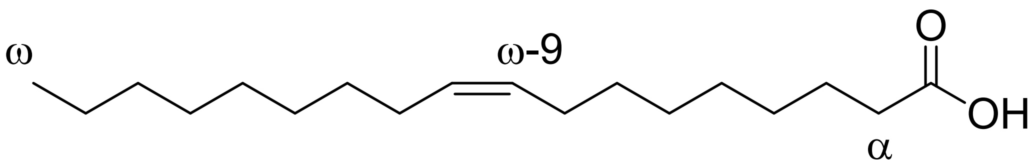
1. Fatty acids and derivatives

1.1 Nomenclature



18:0

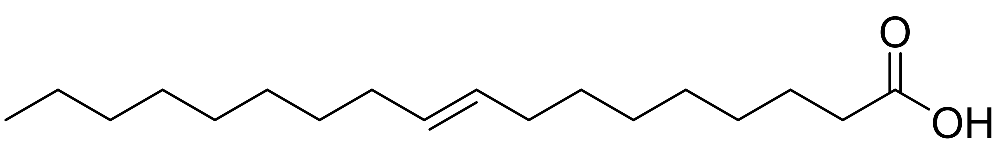
stearic acid



18:1 (ω-9)

oleic acid (lat. oleum = oil), 9*Z*

double bonds usually *Z* configured

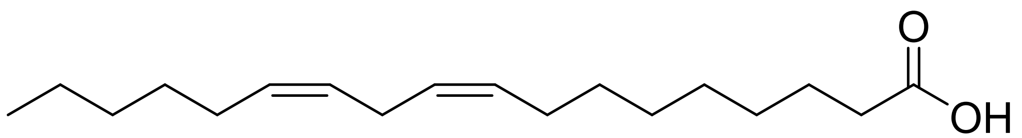


elaidic acid (gr. ελαιον = oil), 9*E*

1. Fatty acids and derivatives

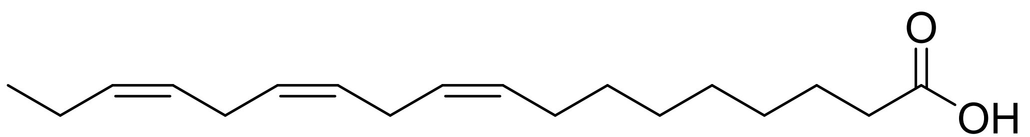
1.1 Nomenclature

double bonds in polyunsaturated compounds usually separated by one methylene group



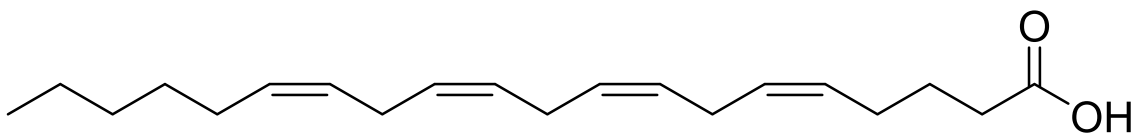
18:2 (ω-6)

linoleic acid (gr. λινον = flax)



18:3 (ω-3)

α-linolenic acid



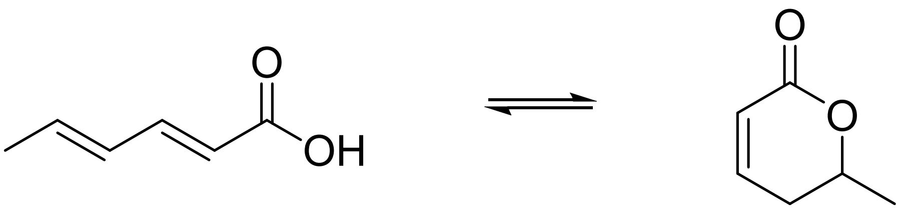
20:4 (ω-6)

arachidonic acid (despite the name this compound does not occur in peanut)

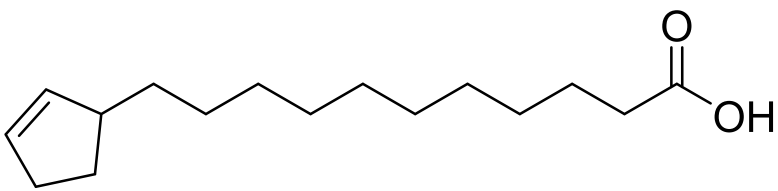
1. Fatty acids and derivatives

1.1 Nomenclature

unusual compounds



sorbic acid (*Sorbus aucuparia* = rowan tree), antibacterial



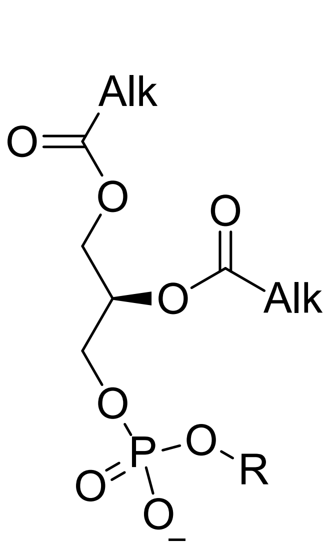
hydnocarpic acid (*Hydnocarpus weightiana*), active against mycobacteria

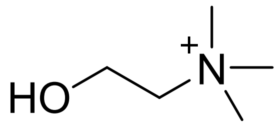
1. Fatty acids and derivatives

1.2 Physiological role

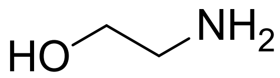
constituents of cell membranes

a) phospholipids of glycerol

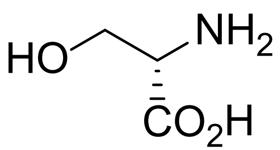




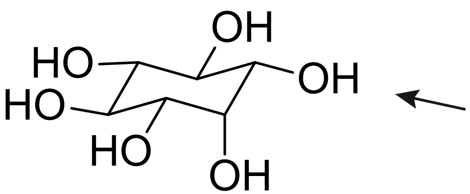
with choline
(lecithins)



with ethanolamine
(cephalins)



with serine
(phosphatidylserine)



esterification

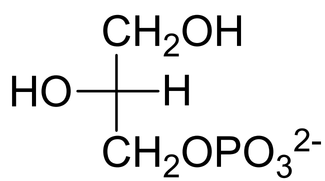
with inositol
(phosphatidylinositol)

1.2 Physiological role

constituents of cell membranes

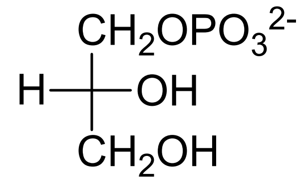
a) phospholipids of glycerol

stereochemistry



L-glycerol 3-phosphate

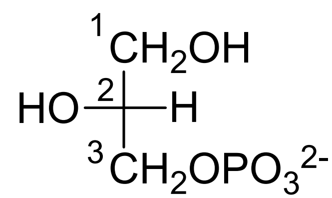
=



D-glycerol 1-phosphate

stereospecific numbering (sn nomenclature)

convention:
secondary OH stands on left side
top C atom is C-1



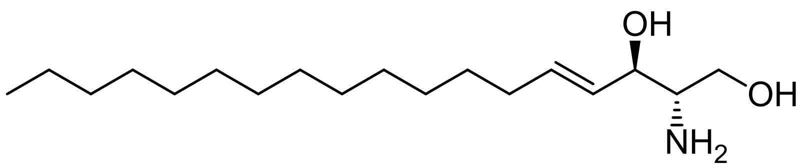
sn-glycerol 3-phosphate

1. Fatty acids and derivatives

1.2 Physiological role

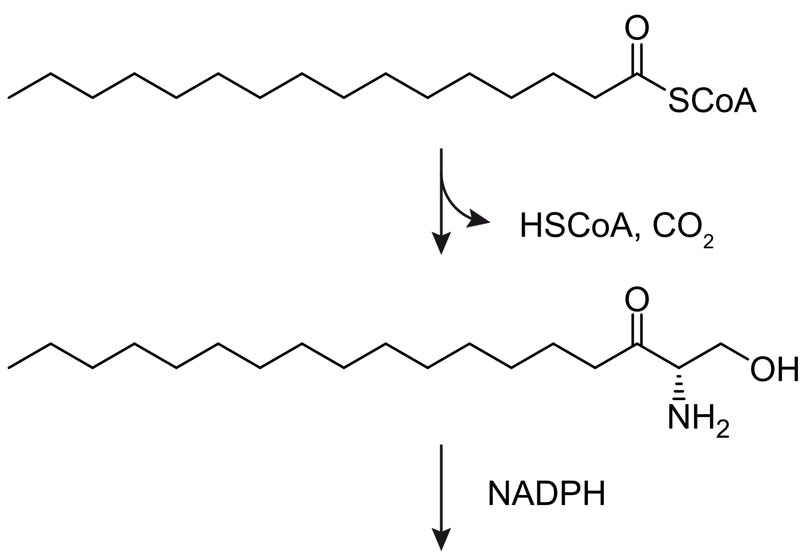
constituents of cell membranes

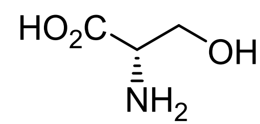
b) phospholipids of sphingosine



sphingosine

biosynthesis





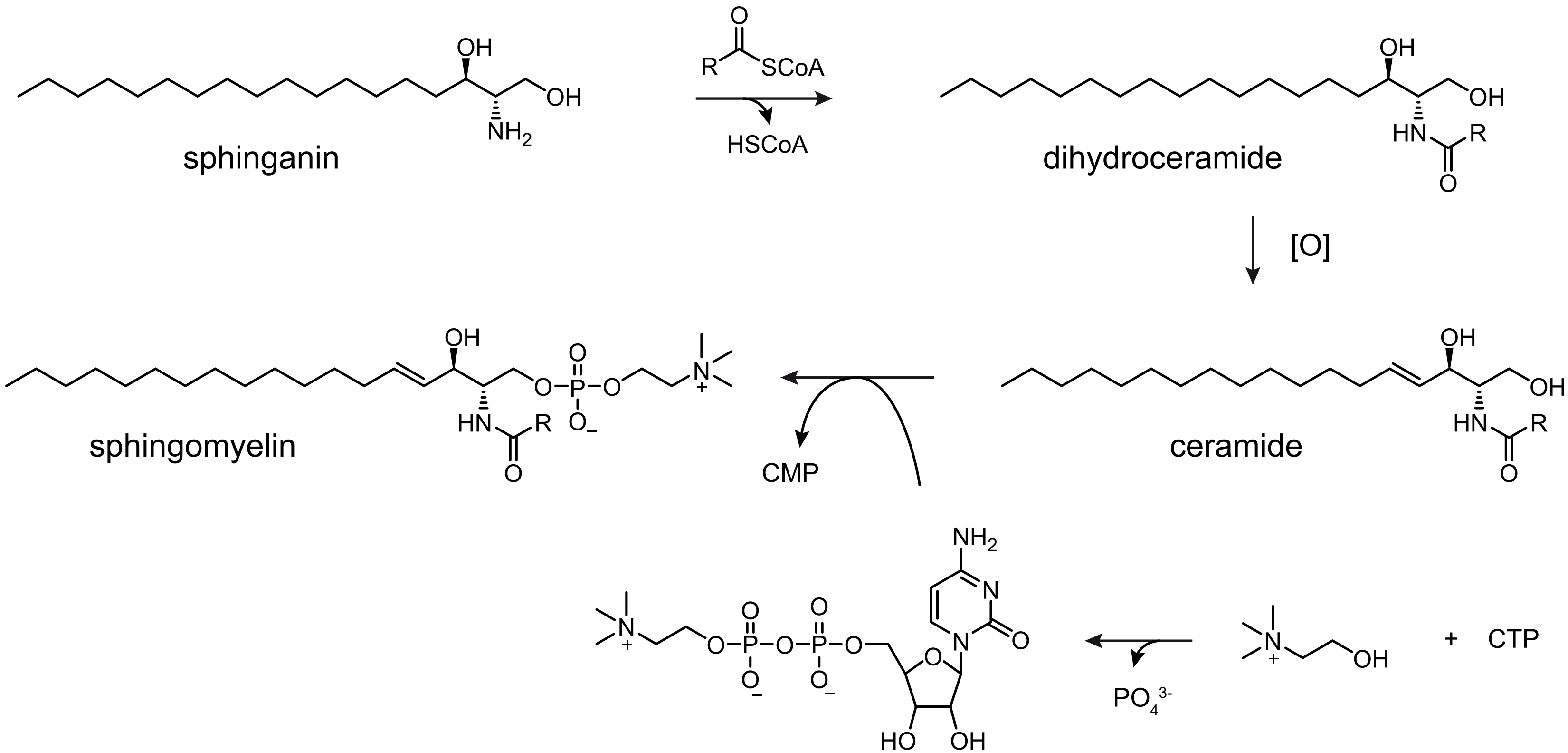
dehydrosphinganin

1. Fatty acids and derivatives

1.2 Physiological role

constituents of cell membranes

b) phospholipids of sphingosine



1. Fatty acids and derivatives

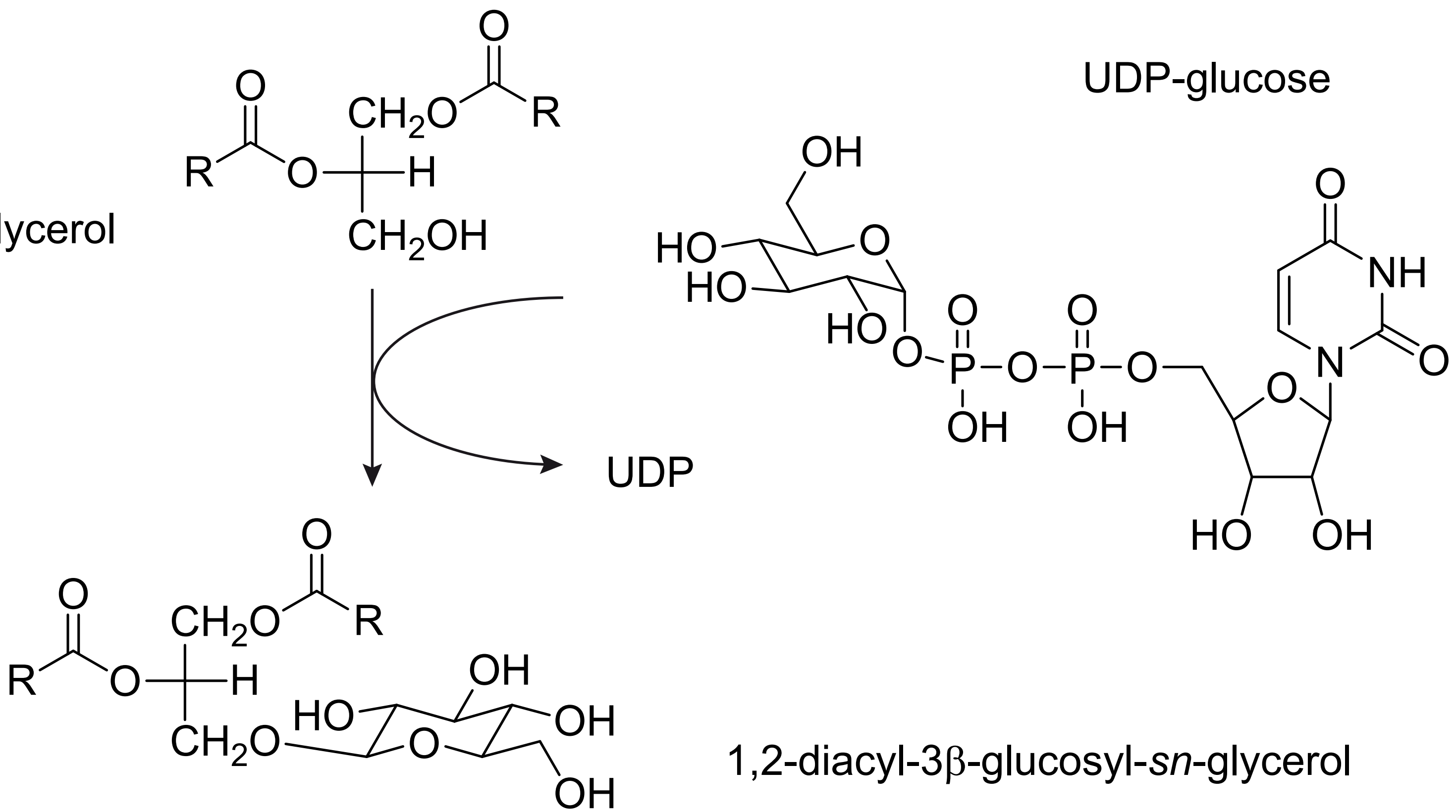
1.2 Physiological role

constituents of cell membranes

c) glycolipids of glycerol

1,2-diacyl-*sn*-glycerol

UDP-glucose

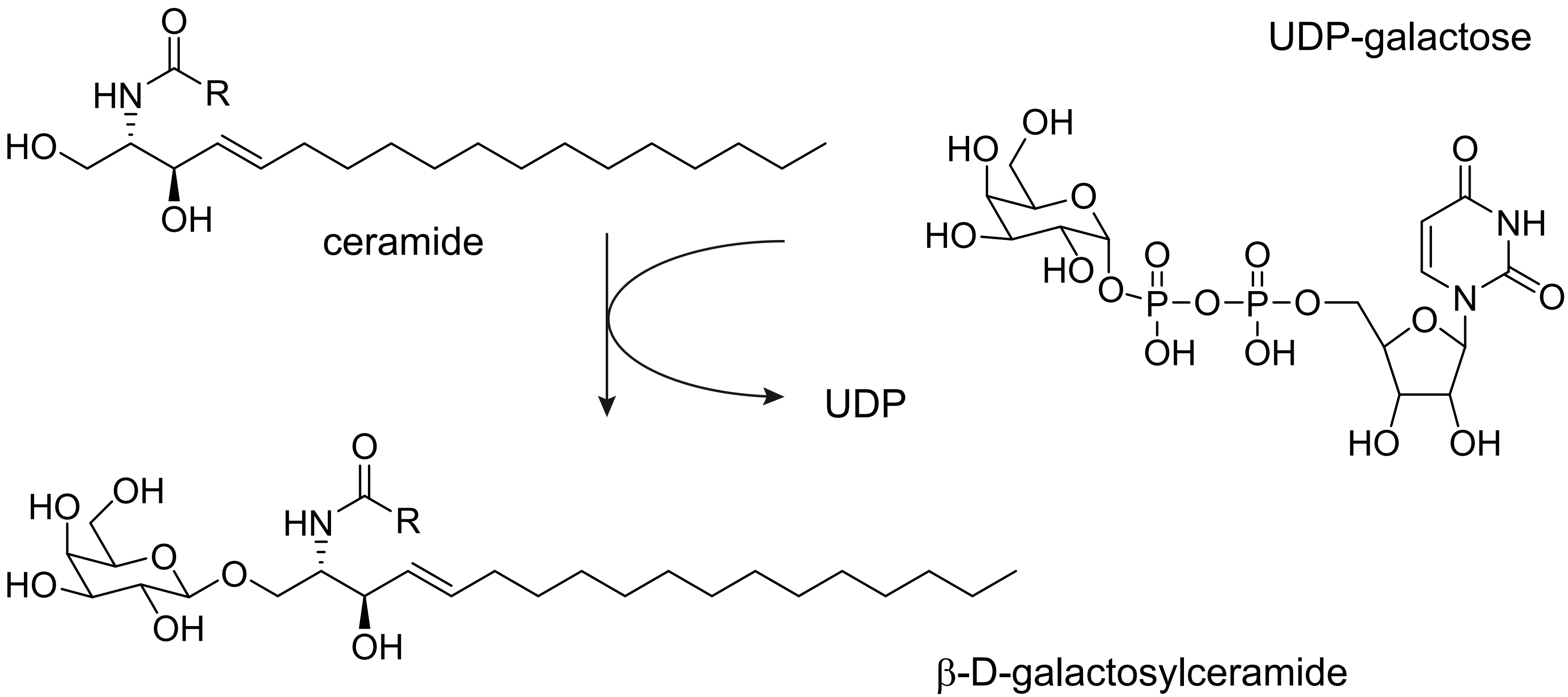


1,2-diacyl-3β-glucosyl-*sn*-glycerol

1.2 Physiological role

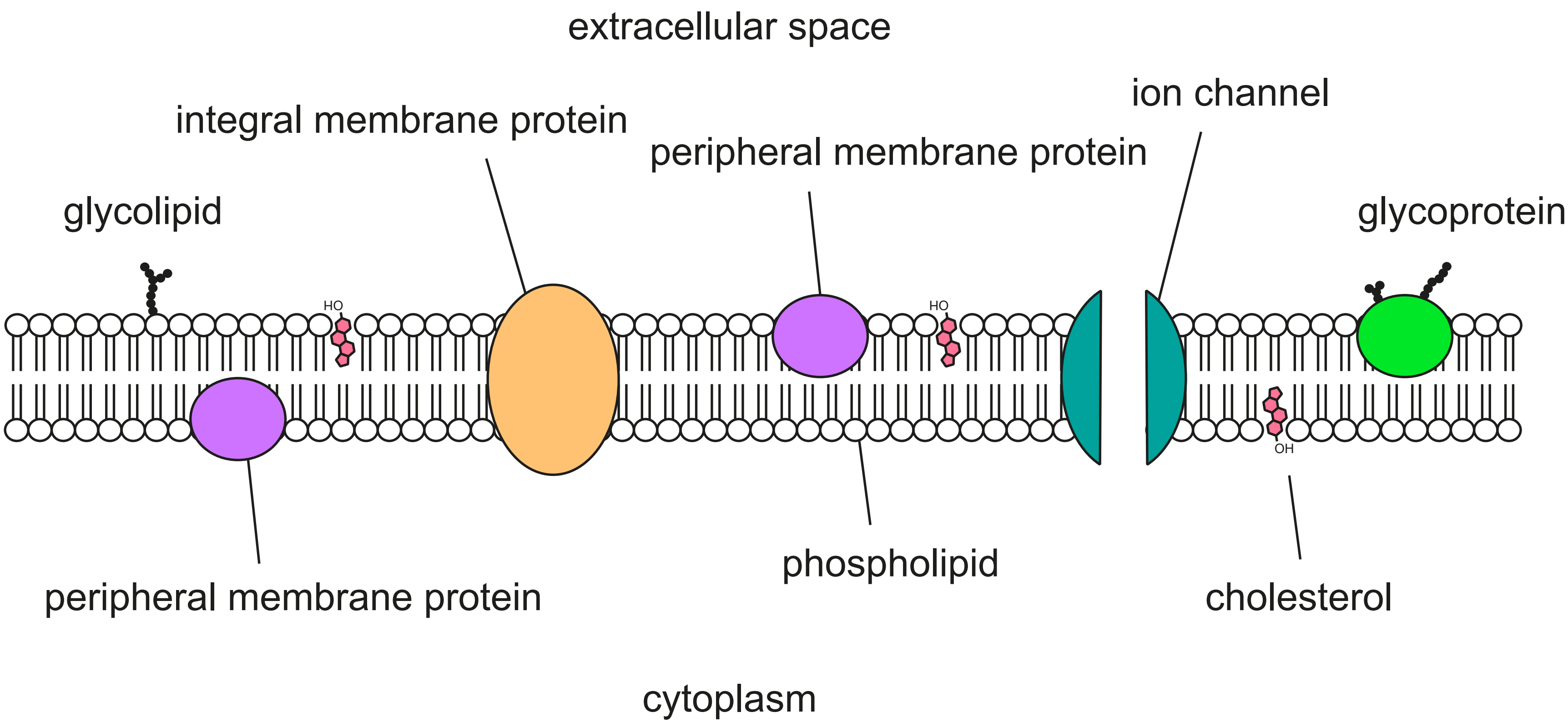
constituents of cell membranes

d) glycolipids of sphingosine



1.2 Physiological role

constituents of cell membranes



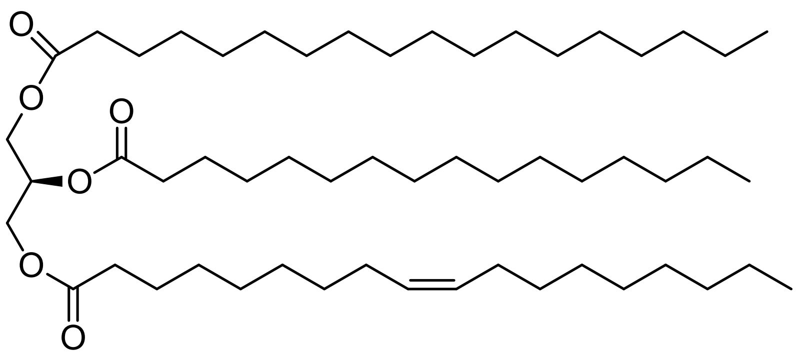
1. Fatty acids and derivatives

1.2 Physiological role

energy storage

fats: triacylglycerols

chiral, if three different acids are used



stearic acid

palmitic acid

oleic acid

Z configured unsaturated fatty acids lower the melting point (plant oils, e. g. olive oil)

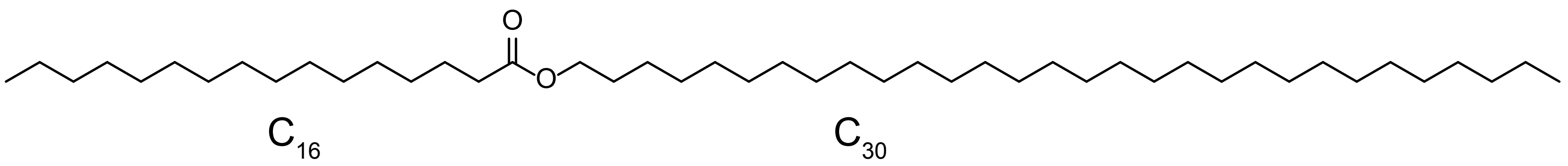
fat hardening by catalytic hydrogenation (margarine)

1. Fatty acids and derivatives

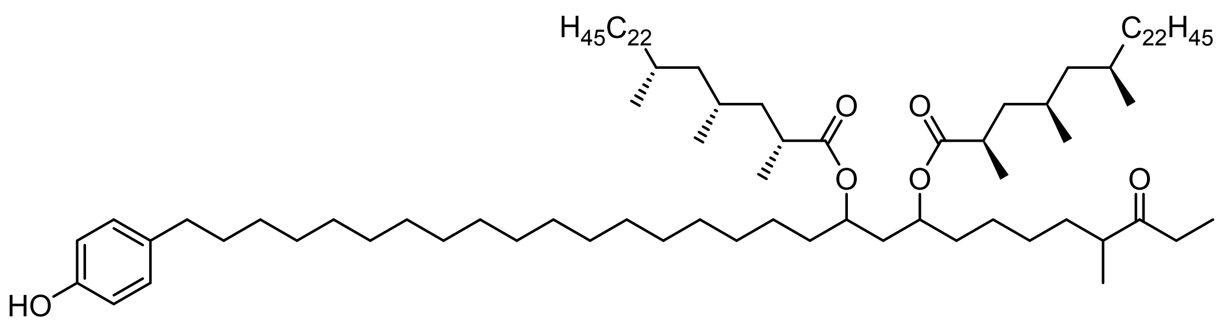
1.2 Physiological role

waxes: esters of fatty acids and fatty alcohols

from bees wax:



phthiocerols - waxes from mycobacteria



2x mycoseric acid

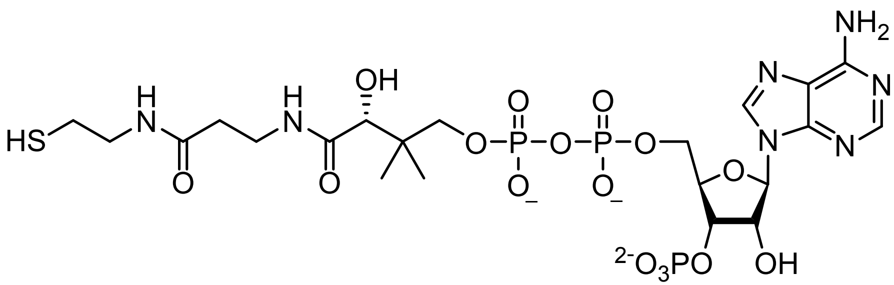
phenolphthiodiolone

cell wall components, suppress immune response of infected organism
about 1/3 of world population latently infected with mycobacteria

1. Fatty acids and derivatives

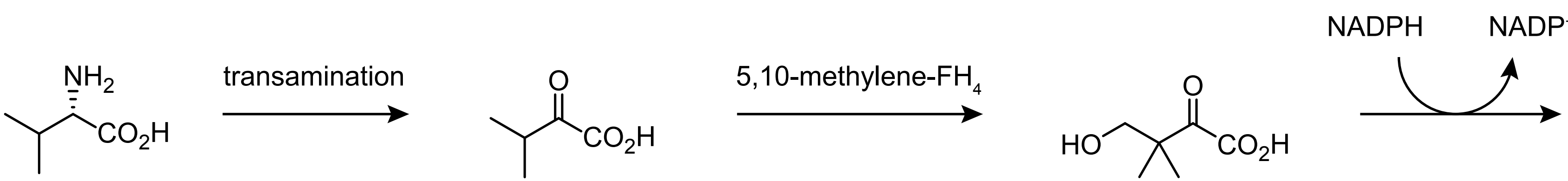
1.3 Fatty acid biosynthesis

1.3.1 Coenzymes



coenzyme A

Biosynthesis

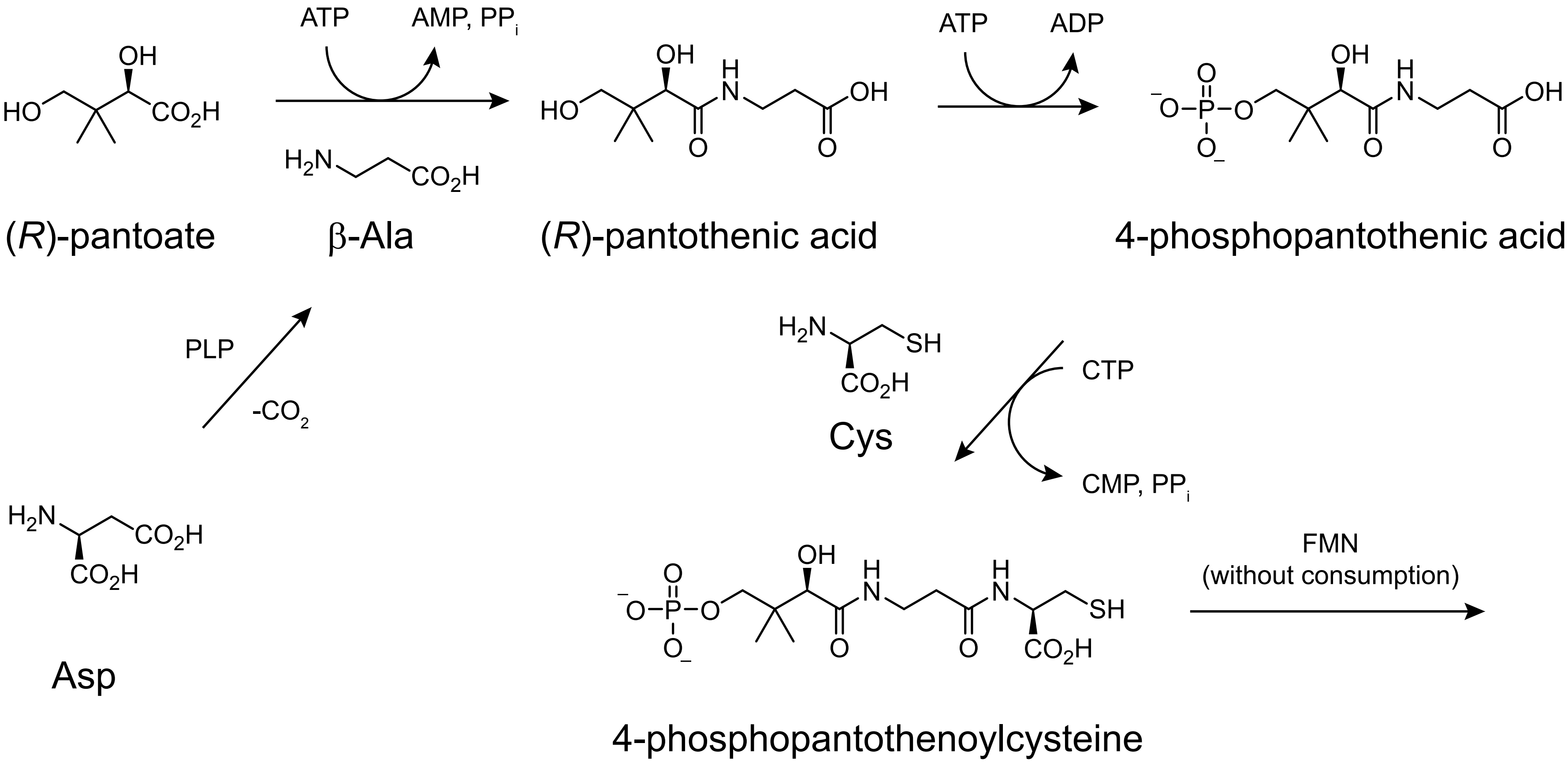


Val

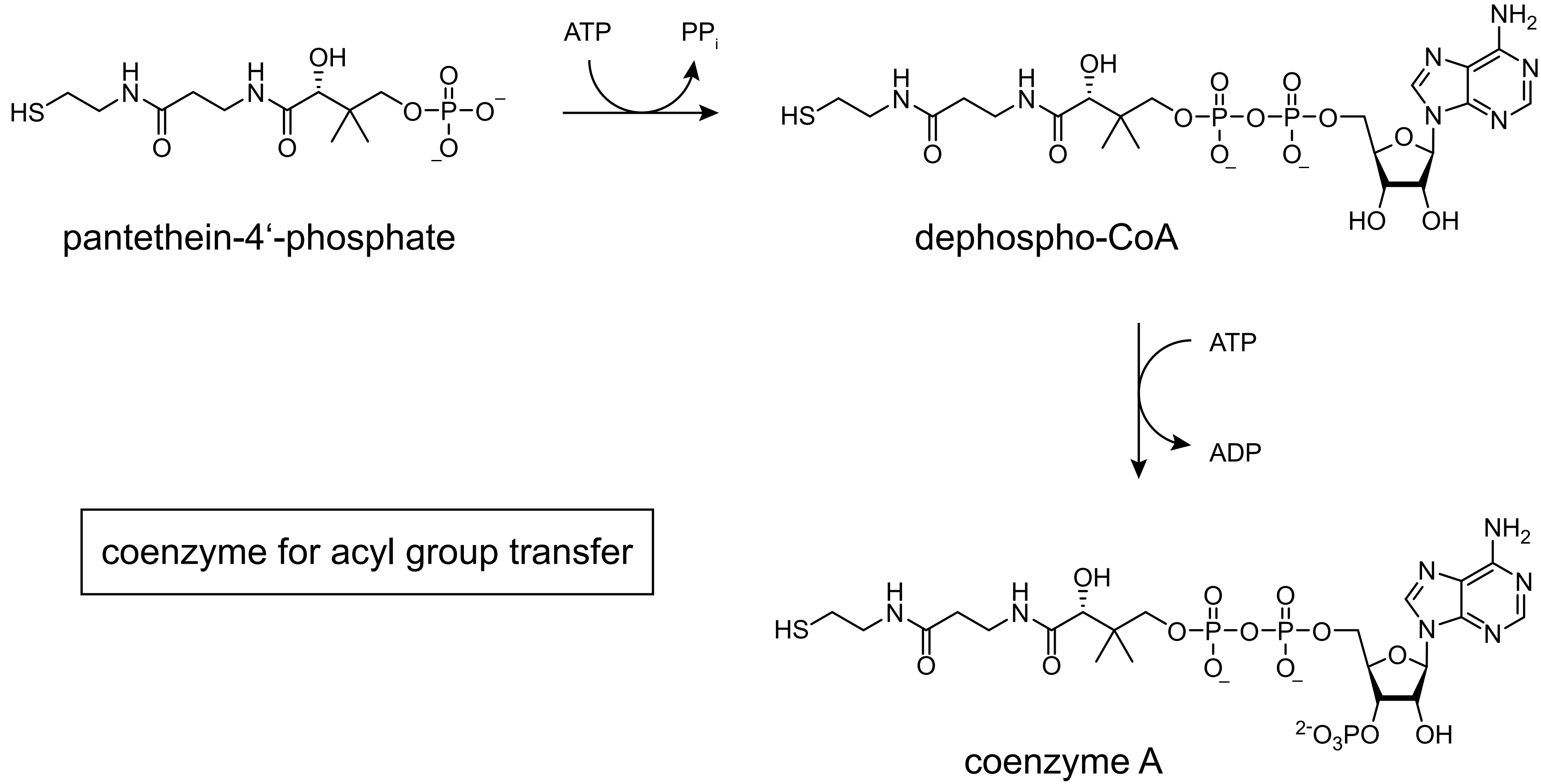
α-ketoisovalerate

dehydropantoate

1.3 Fatty acid biosynthesis



1.3 Fatty acid biosynthesis



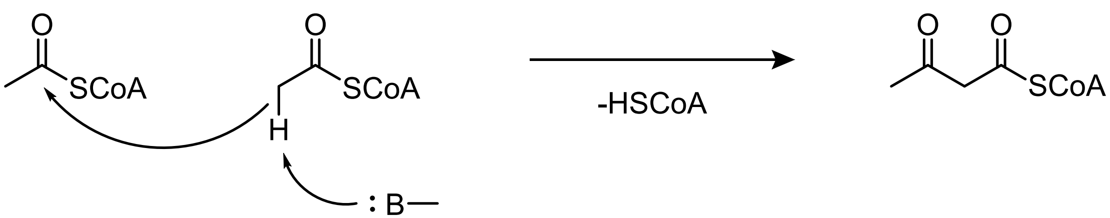
coenzyme for acyl group transfer

1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

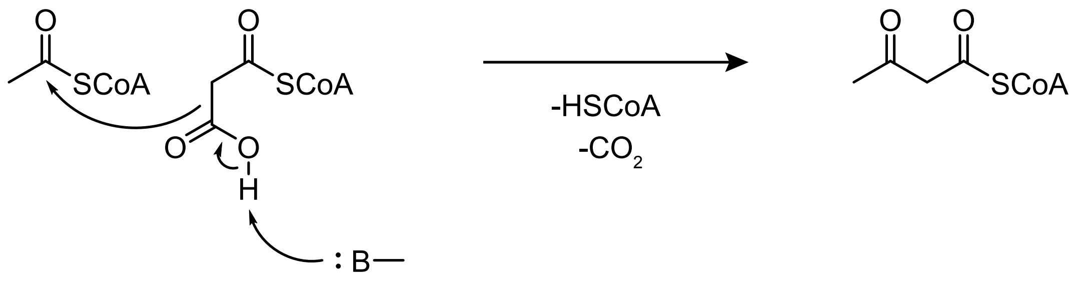
thioesters exhibit

- lower resonance (S >> C, O ≈ C)
- higher C,H acidity
- better leaving group RS^{-}
- higher reactivity in Claisen- and aldol reaction



1.3 Fatty acid biosynthesis

further increase of reactivity:



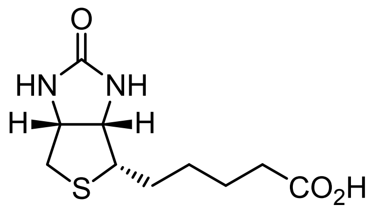
acetyl-CoA   malonyl-CoA   acetoacetyl-CoA

effects of CO_2 formation

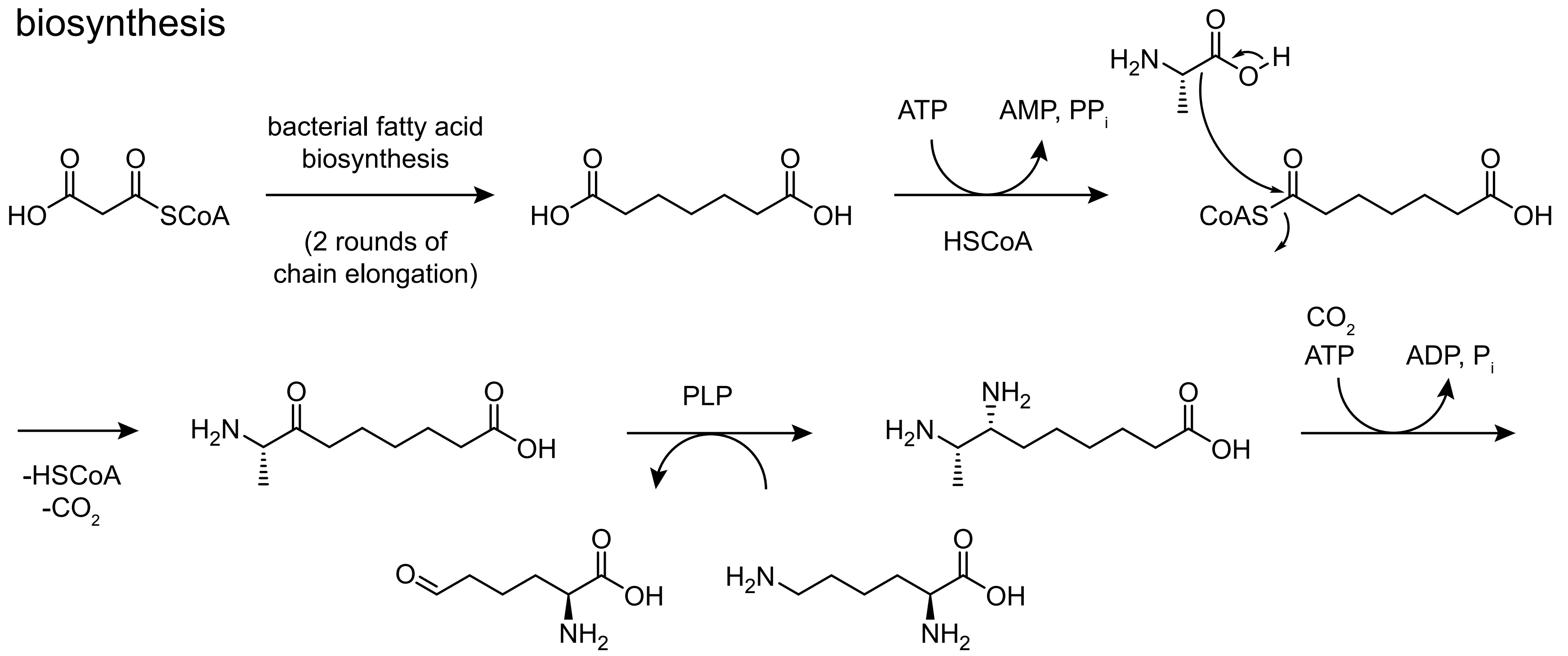
- reaction driven by entropy
- no back reaction possible

1.3 Fatty acid biosynthesis

Biotin: carboxylation of acetyl-CoA

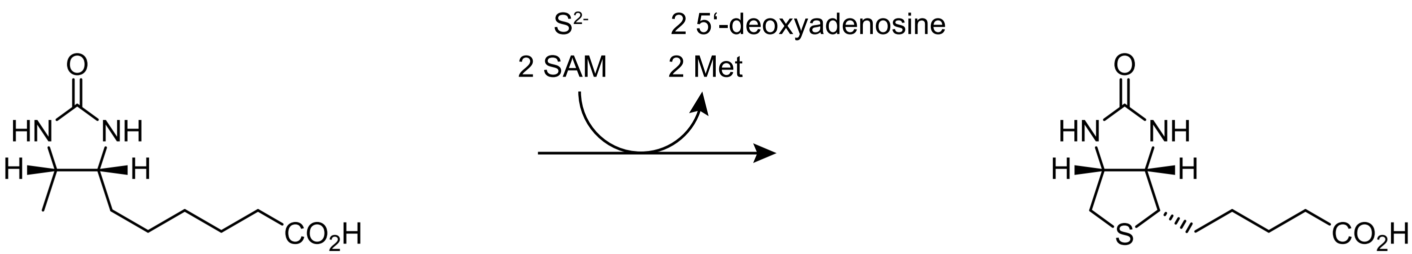


biosynthesis



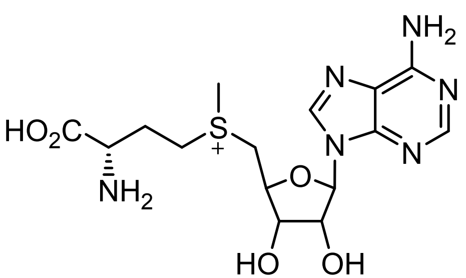
1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

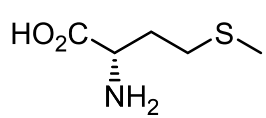


dethiobiotin

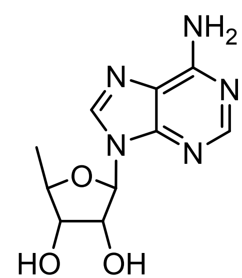
biotin



SAM



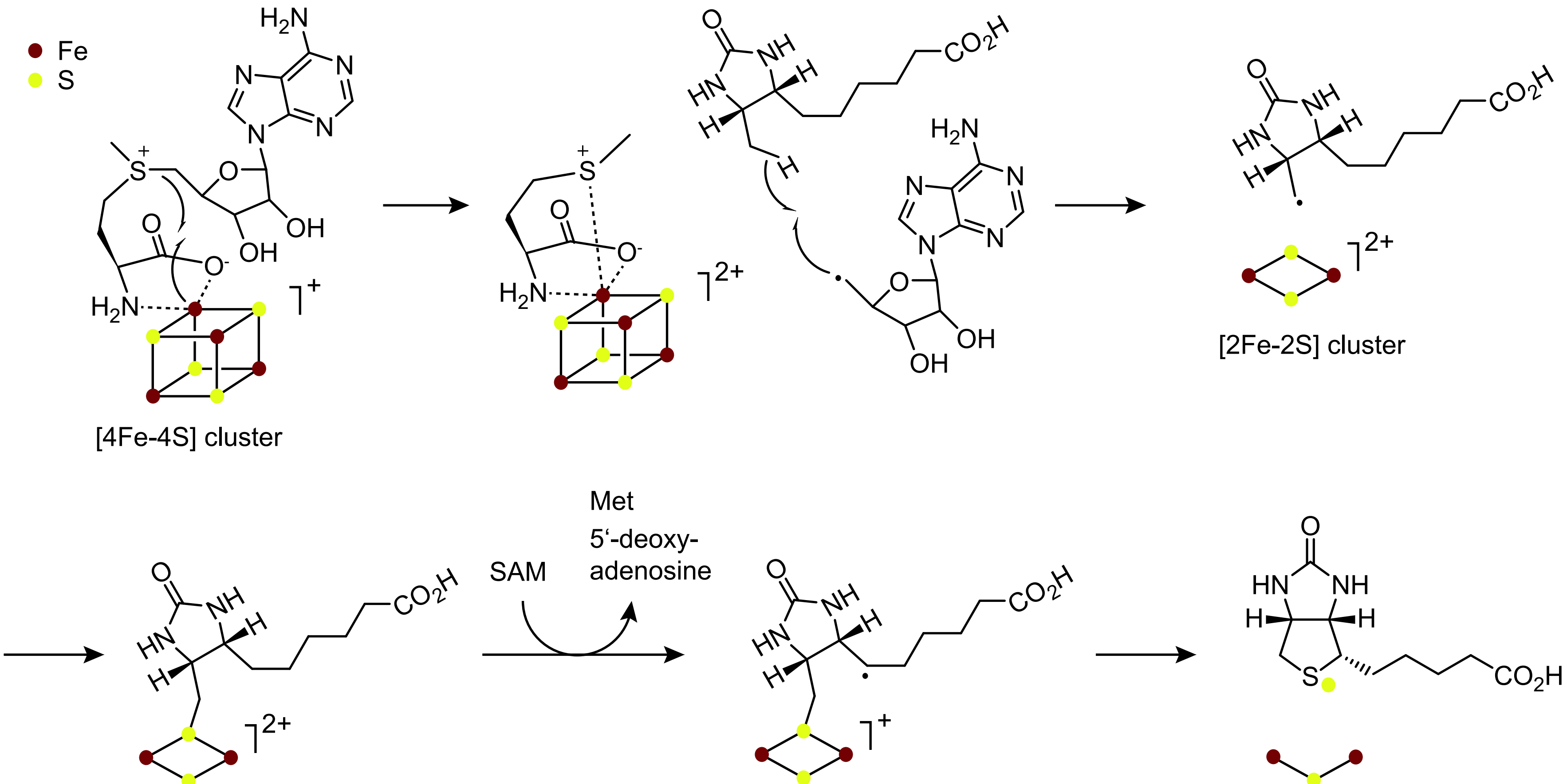
Met



5‘-deoxyadenosine

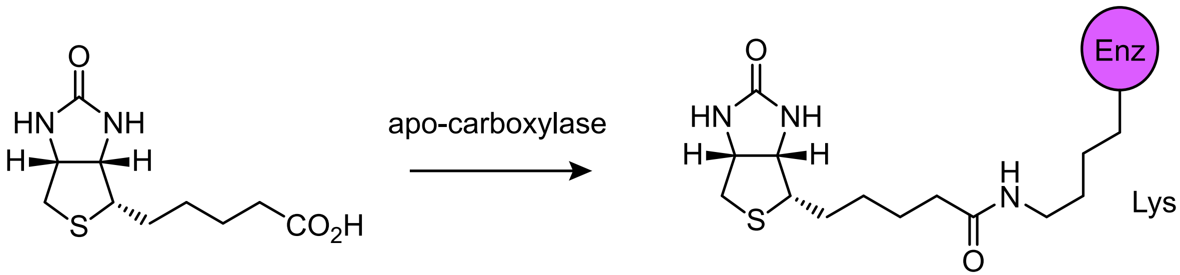
1.3 Fatty acid biosynthesis

radical SAM mechanism

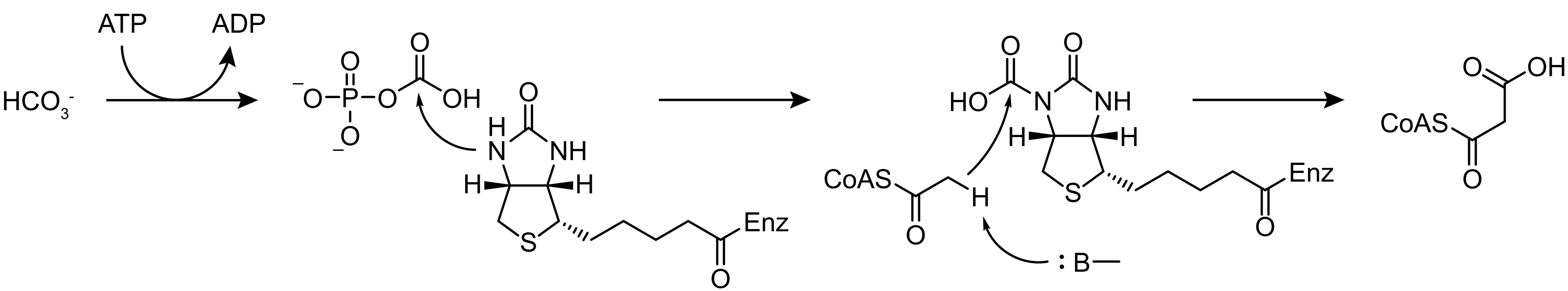


1.3 Fatty acid biosynthesis

carboxylation by biotin



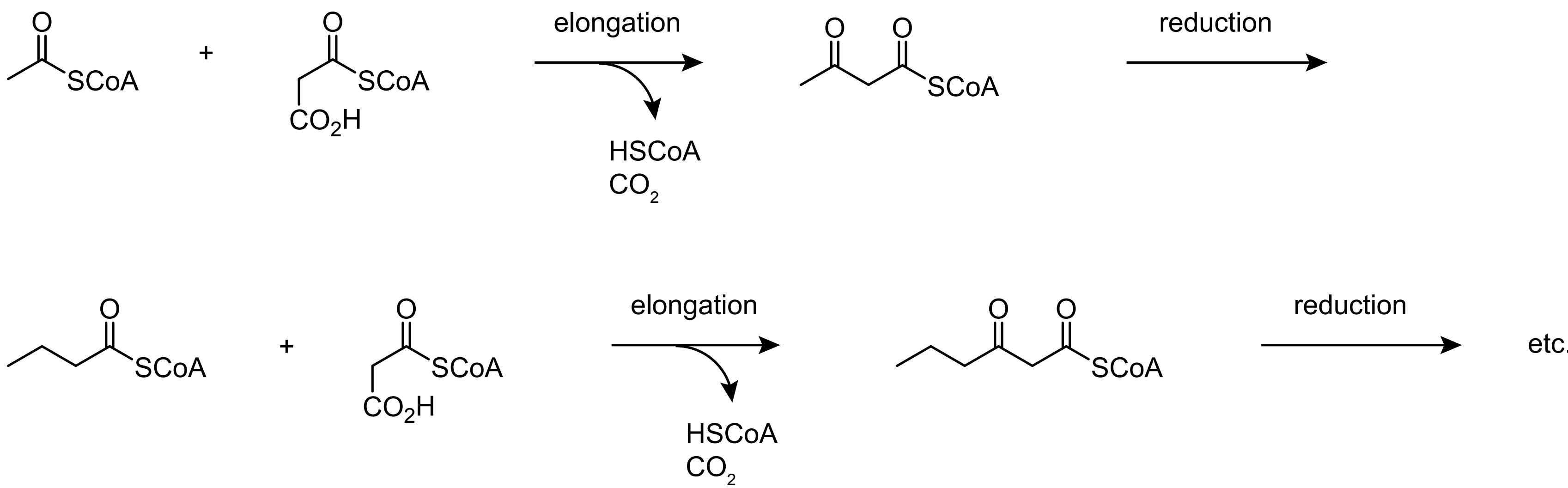
holo-carboxylase



1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

1.3.2 Principle of fatty acid biosynthesis



yields even fatty acids!

1.3 Fatty acid biosynthesis

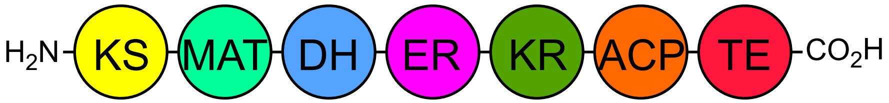
1.3.3 Fatty acid synthase

a) Type I (megasynthases, multienzyme complex)

animals: homodimer

fungi: $\alpha_6\beta_6$ heterododecamer

mammalian fatty acid synthase



KS: ketosynthase

MAT: malonyl/acetyltransferase

DH: dehydratase

ER: enoyl reductase

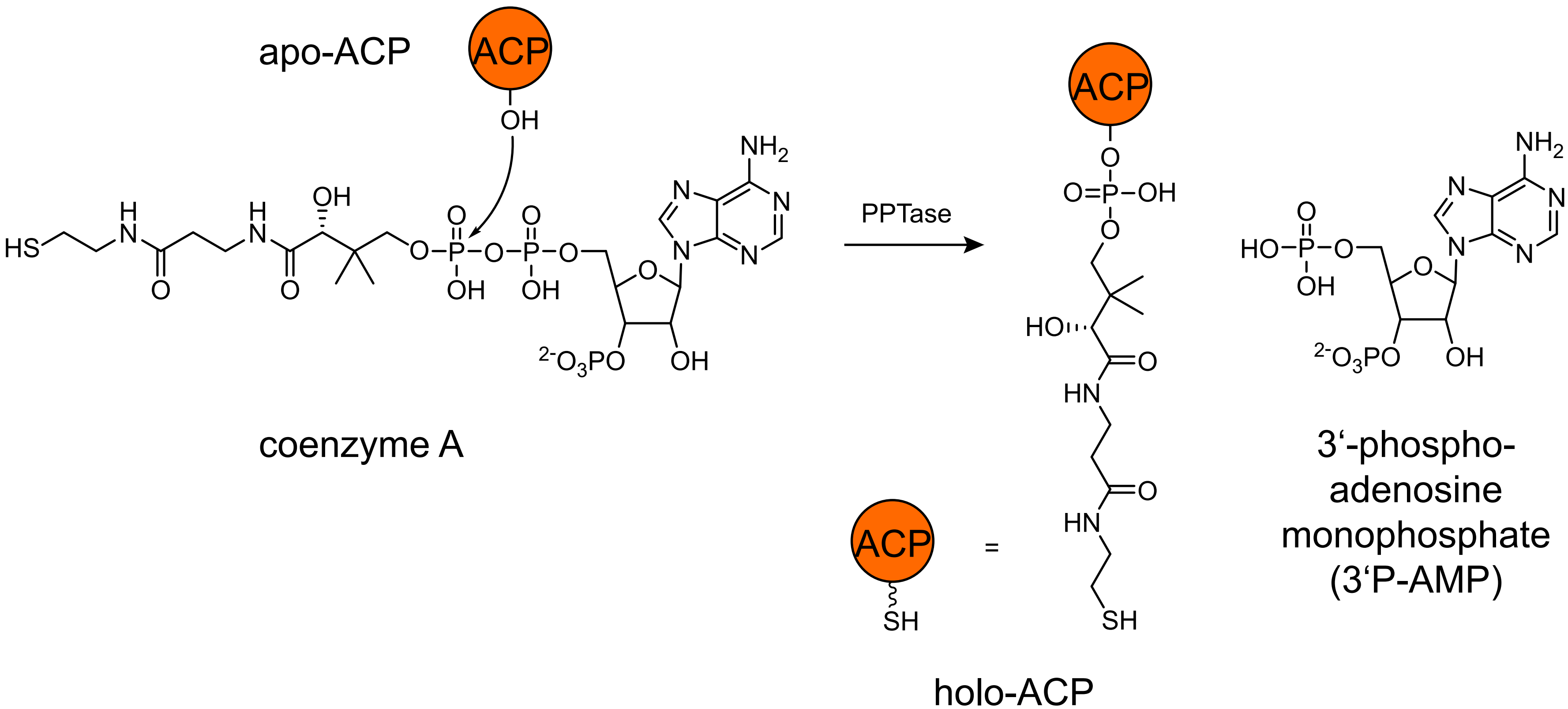
KR: ketoreductase

ACP: acyl carrier protein

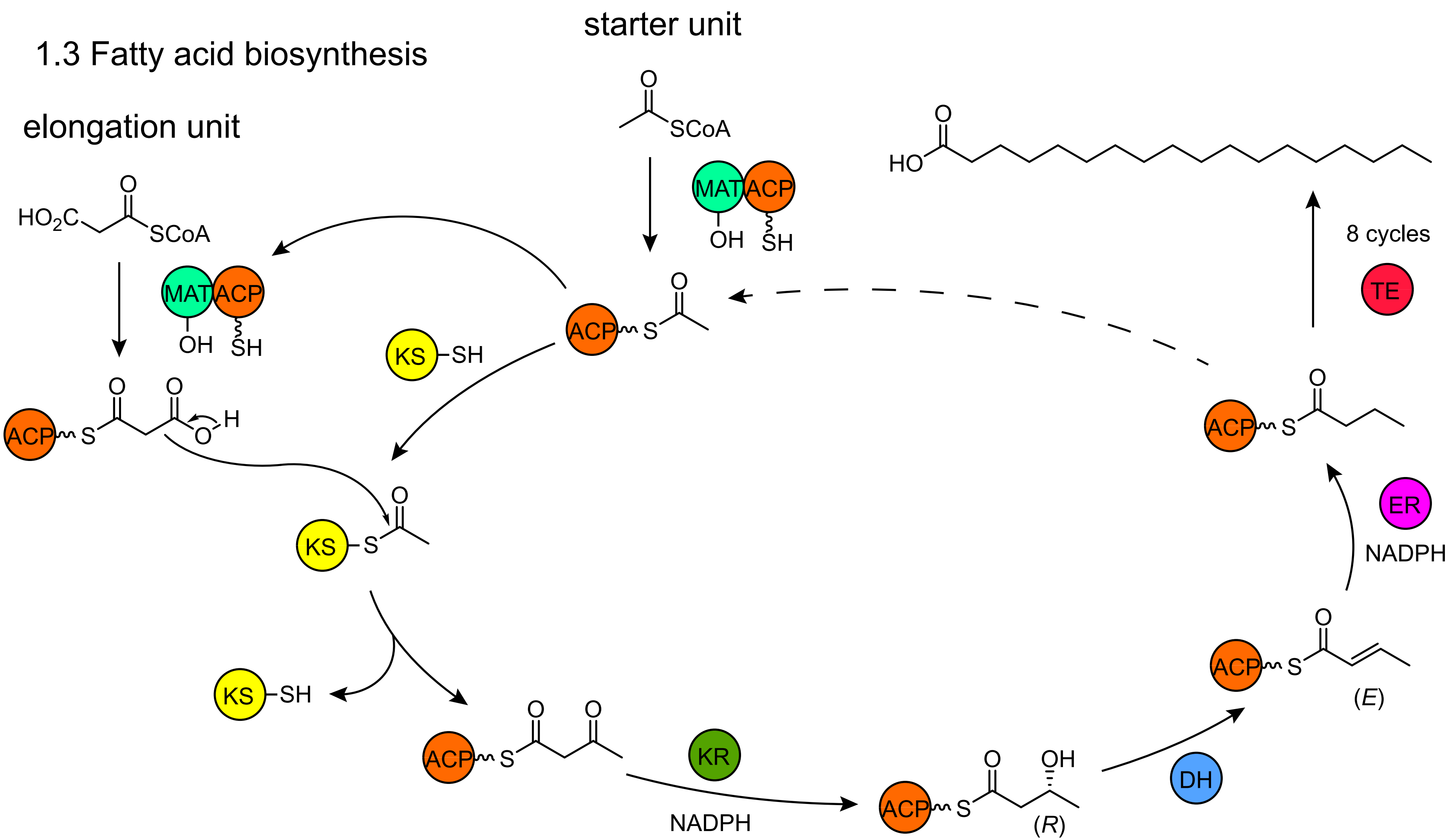
TE: thioesterase

1.3 Fatty acid biosynthesis

activation of ACP by phosphopantetheinyl transferases

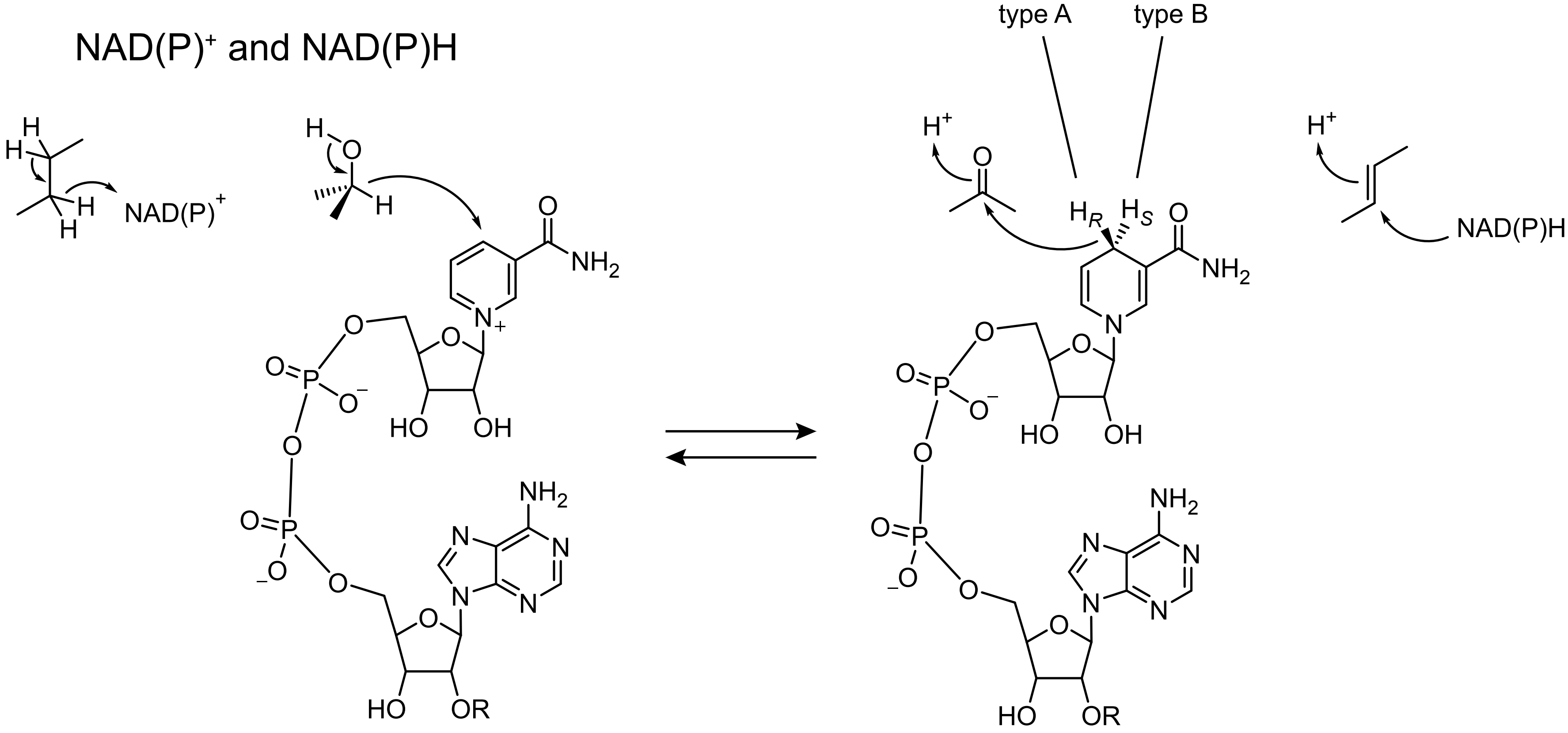


1.3 Fatty acid biosynthesis



1.3 Fatty acid biosynthesis

NAD(P)$^+$ and NAD(P)H



NAD$^+$ (R=H)
NADP$^+$ (R=PO_3^{2-})

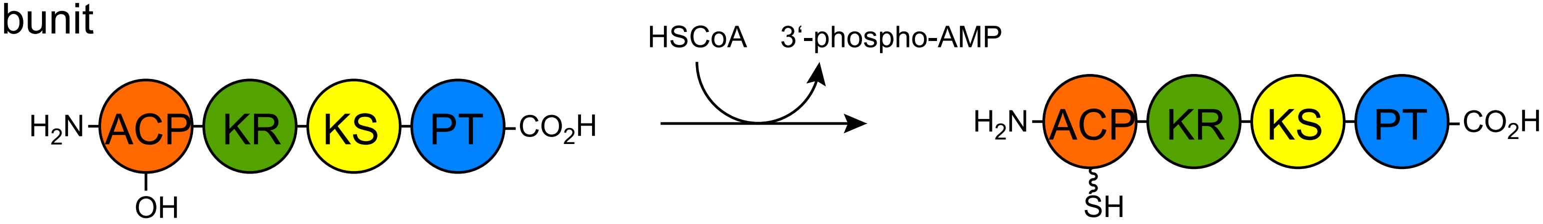
NADH (R=H)
NADPH (R=PO_3^{2-})

1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

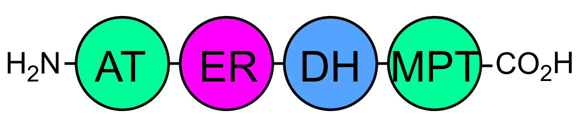
fungal fatty acid synthase

α subunit



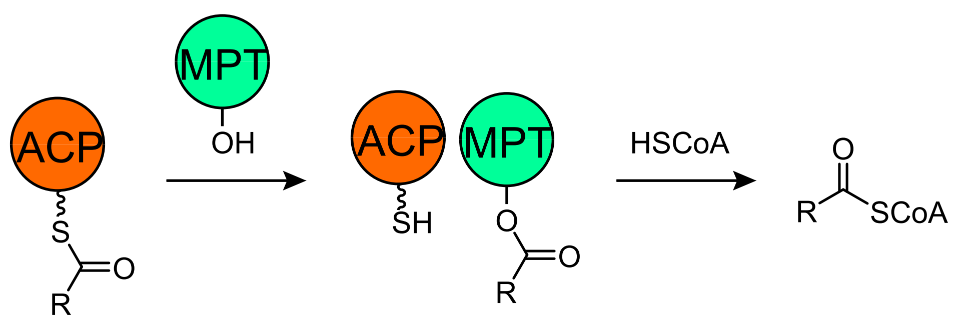
PT: phosphopantetheinyl transferase (self-activation)

β subunit



AT: acetyl transferase (only starter unit)
MPT: malonyl/palmitoyl transferase

product release by reverse loading reaction



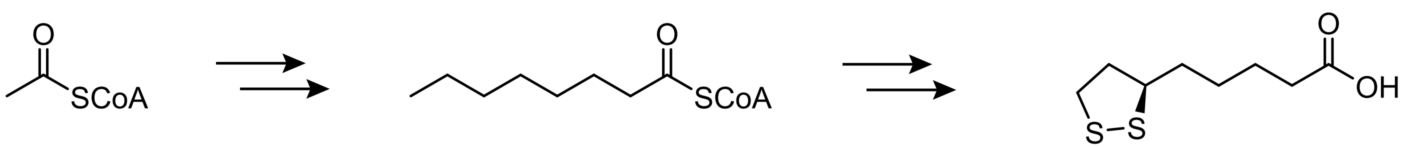
1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

b) Type II (discrete single-domain proteins)

in bacteria: in the cytosol

in eukaryotes: in chloroplasts and mitochondria



lipoic acid

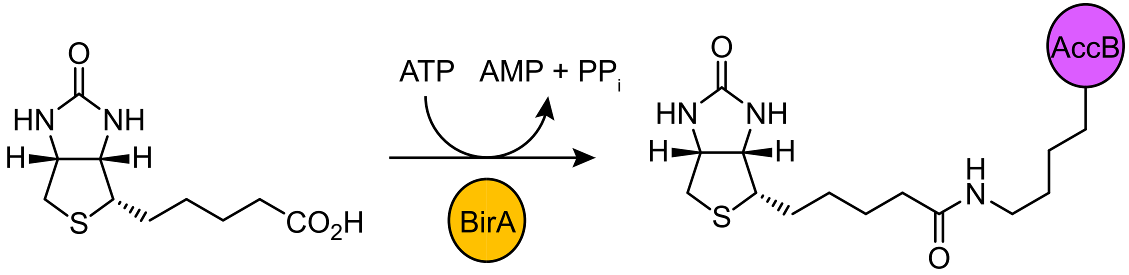
general principle as for type I fatty acid synthases

different enzymology

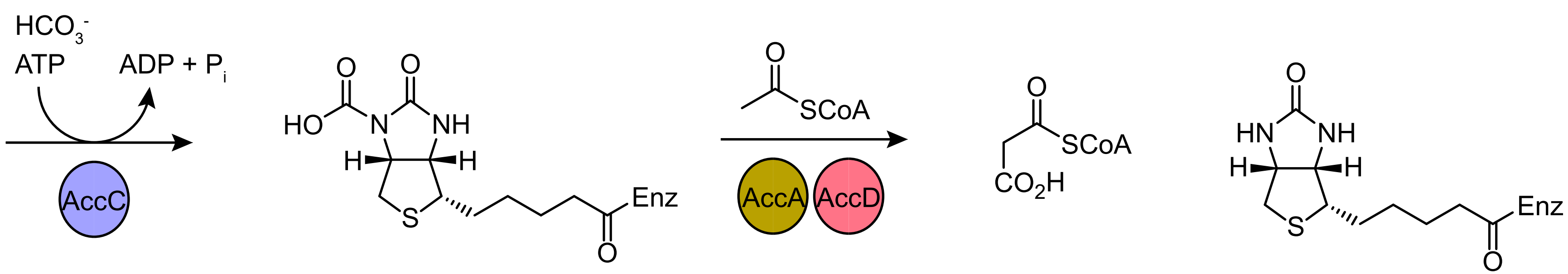
⟶ specific inhibitors (antibiotics)

1.3 Fatty acid biosynthesis

Carboxylation of acetyl-CoA



AccA: carboxyltransferase subunit I
AccB: biotin-carboxyl carrier protein (BCCP)
AccC: biotin carboxylase
AccD: carboxyltransferase subunit II
BirA: biotin protein ligase



1. Fatty acids and derivatives

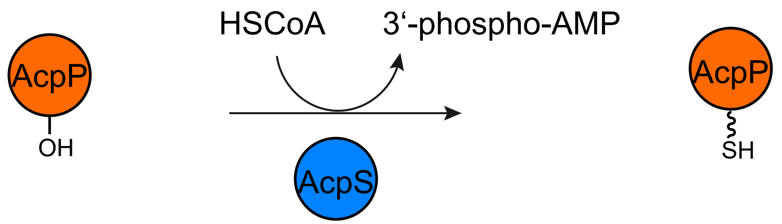
1.3 Fatty acid biosynthesis

Fab enzymes (Fab = fatty acid biosynthesis)

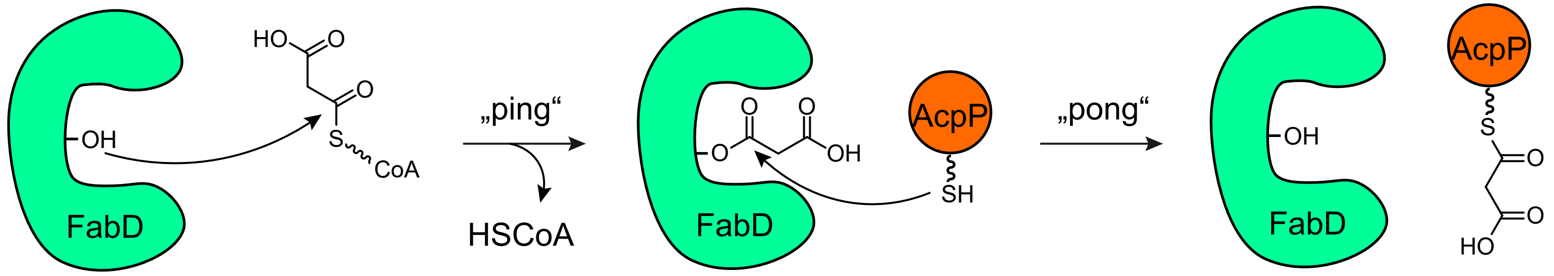
FabH	KS III (chain elongation C_4)		
FabB	KS I (chain elongations $C_6 – C_{14}$)		
FabF	KS II (chain elongations $C_{16} – C_{18}$)		
FabD	malonyl-CoA:ACP transacylase		
FabG	KR		
FabZ	DH	AcpP	apo-ACP
FabI	ER	AcpS	holo-ACP synthase
FabA	3-hydroxydecanoyl-ACP dehydratase/isomerase		

1.3 Fatty acid biosynthesis

- AcpP and AcpS



- FabD: ping-pong mechanism

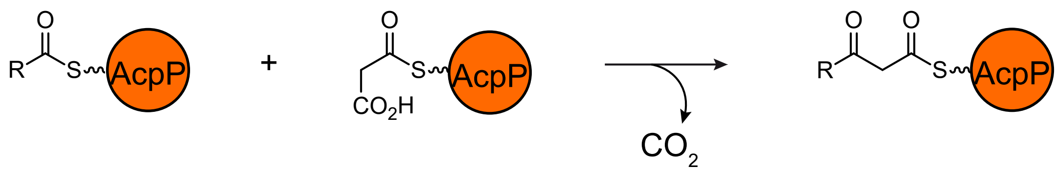


highly conserved GXSXG

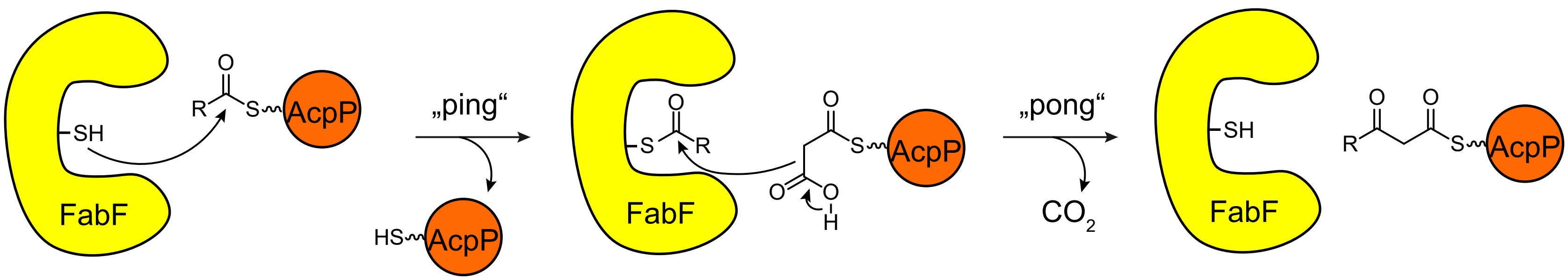
1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

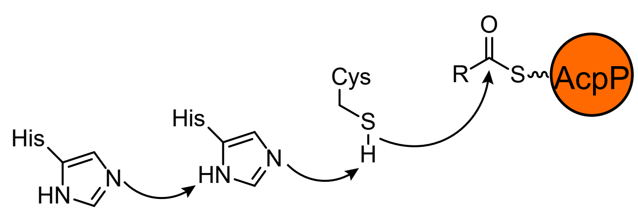
- FabH, FabB, FabF



ping-pong mechanism

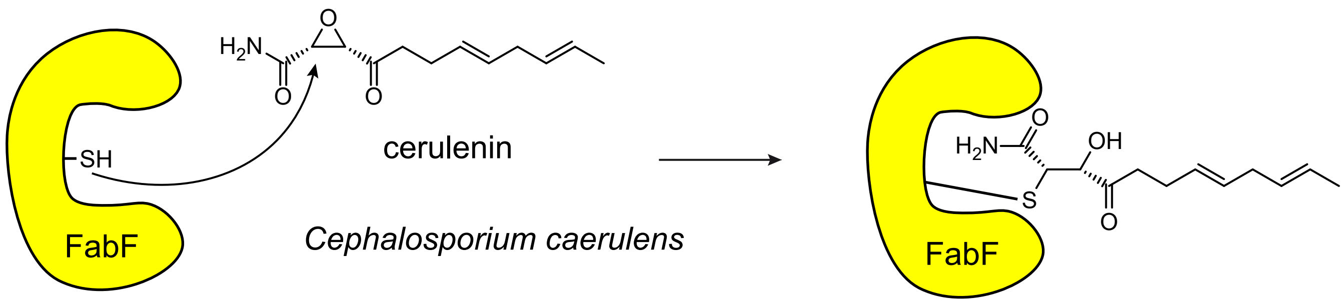


catalytic triad Cys-His-His



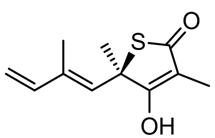
1.3 Fatty acid biosynthesis

inhibitors



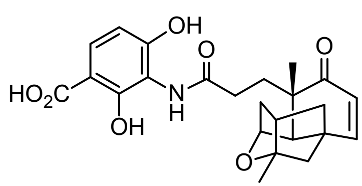
cerulenin

Cephalosporium caerulens



thiolactomycin

Nocardia



platensimycin

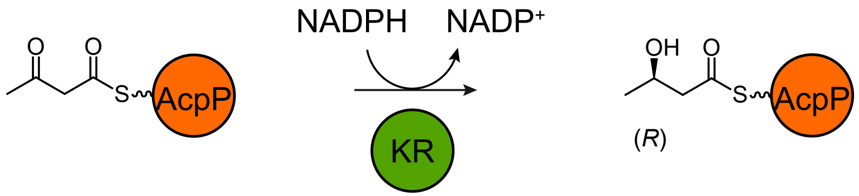
Streptomyces platensis

non-covalent binding
to FabF active site

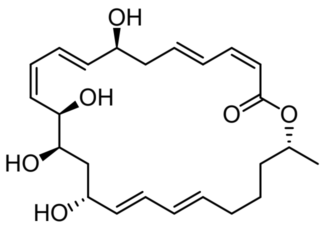
1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

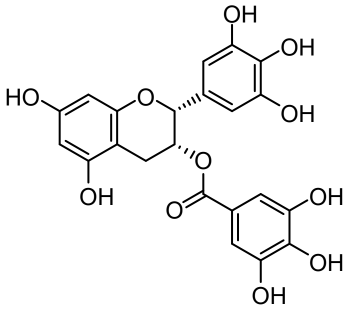
- FabG



inhibitors



macrolactin S (*Bacillus* sp.)

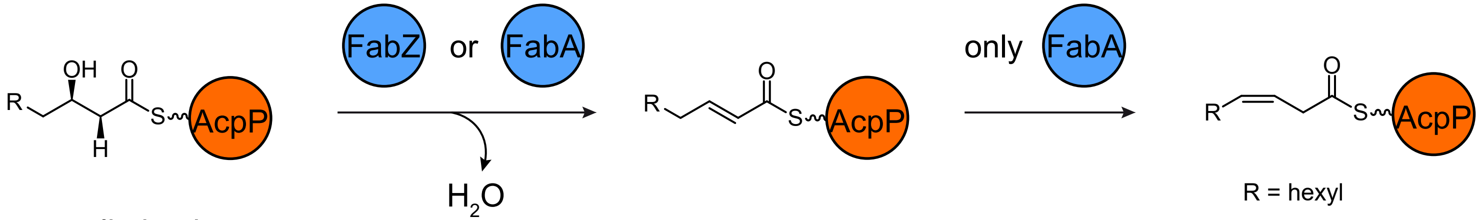


epigallocatechin gallate (*Camellia sinensis*)

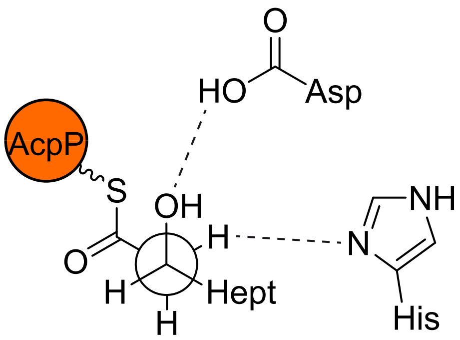
1/3 of dry weight of green tea

1.3 Fatty acid biosynthesis

- FabZ (DH)
  FabA (3-hydroxydecanoyl-ACP dehydratase/isomerase)



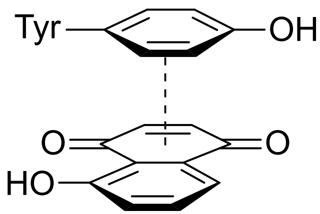
syn elimination



1.3 Fatty acid biosynthesis

- inhibitors

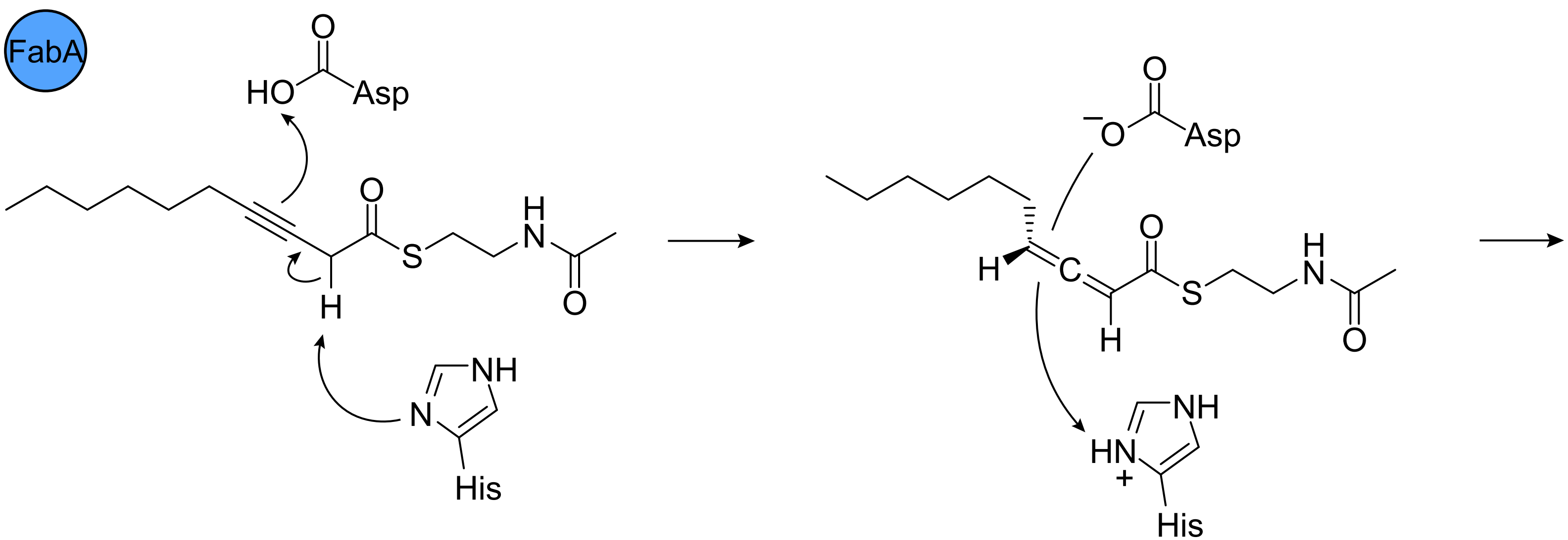
FabZ



π-π interactions

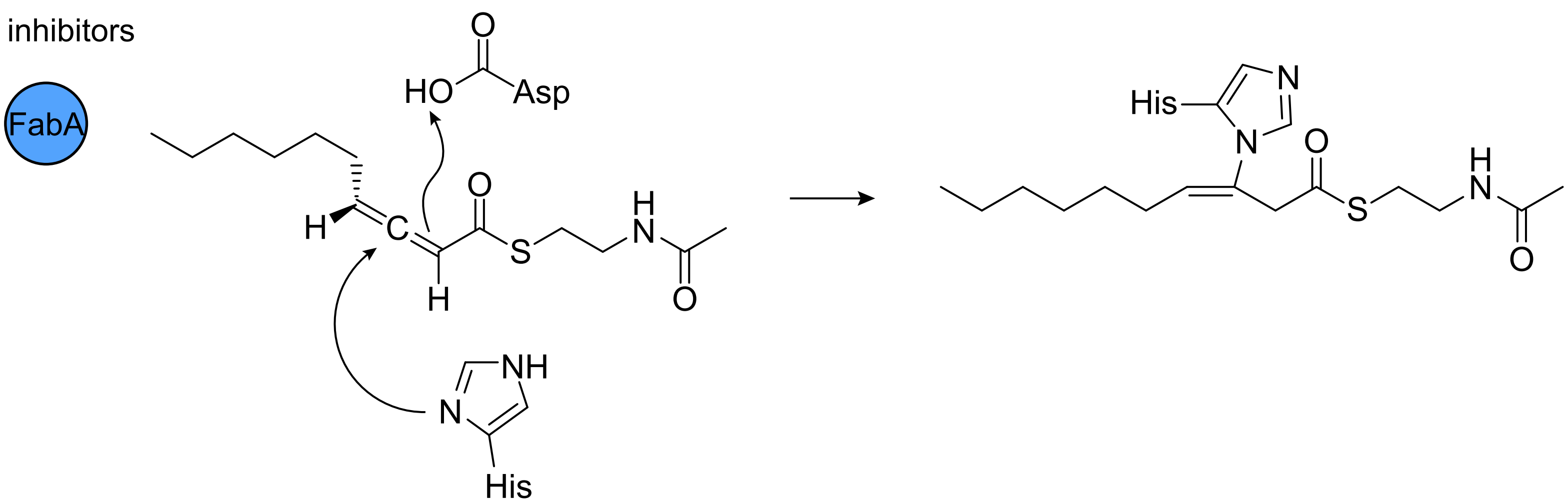
juglone (*Juglans regia*)

FabA

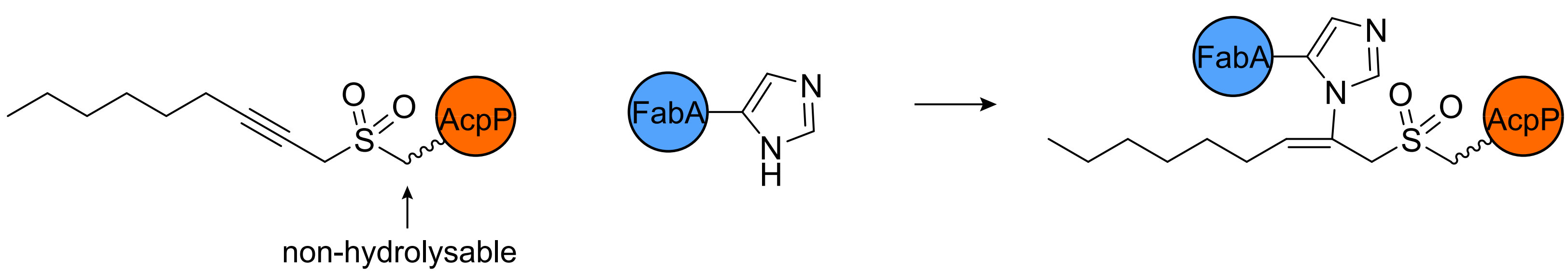


1.3 Fatty acid biosynthesis

- inhibitors



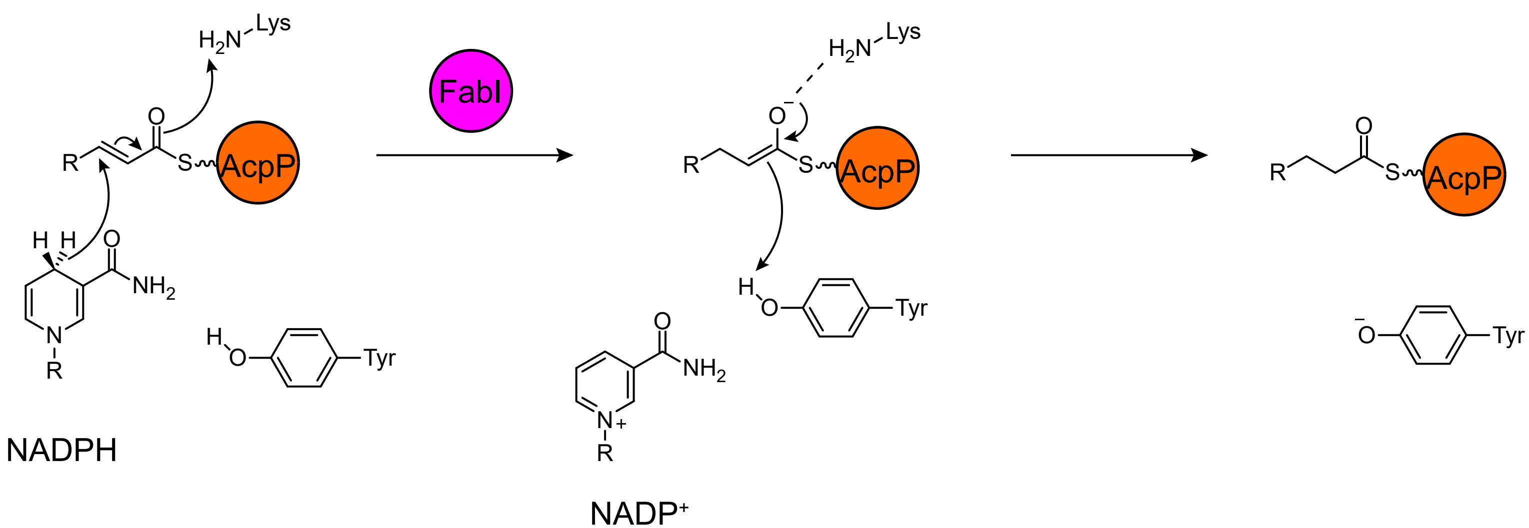
can be used to install covalent bonds between FabA and AcpP for co-crystallisation



1.3 Fatty acid biosynthesis

- FabI (ER)

  reactions by KR and DH are reversible, ER is rate determining, irreversible



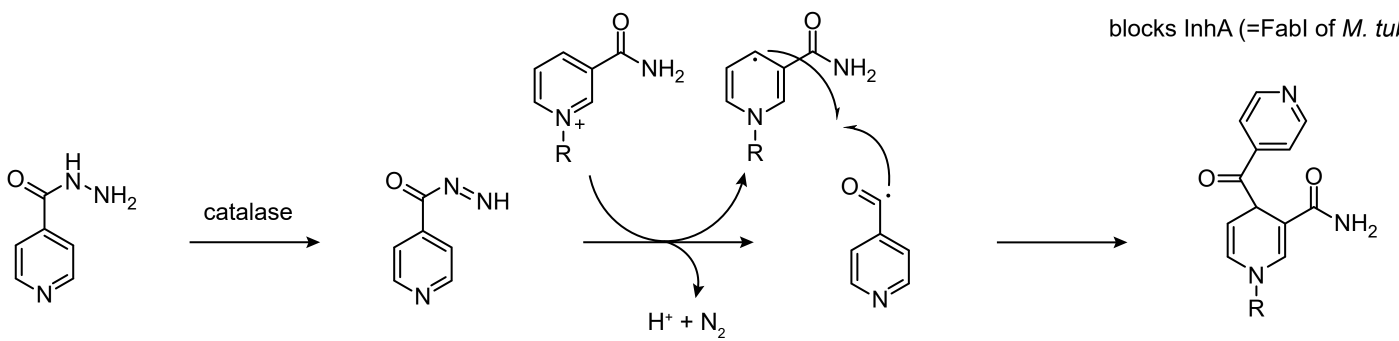
1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

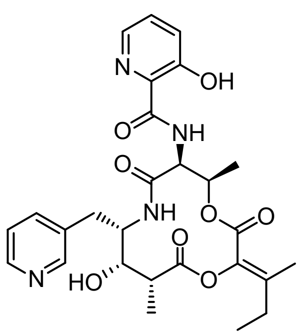
- inhibitors (FabI)

  isoniazid (approved antibiotic against *Mycobacterium tuberculosis*)

blocks InhA (=FabI of *M. tuberculosis*)



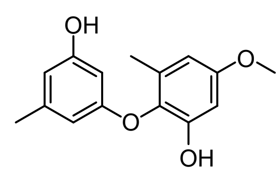
pyridomycin



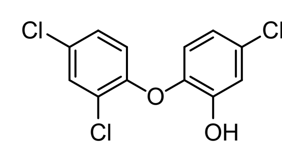
binds to NADH binding site of InhA

from *Dactylosporangium fulvum* (actinobacteria)

cyperin



compare:



FabI inhibitor in plants

from *Ascochyta cypericola* (phytopathogenic fungus)

trichlosan

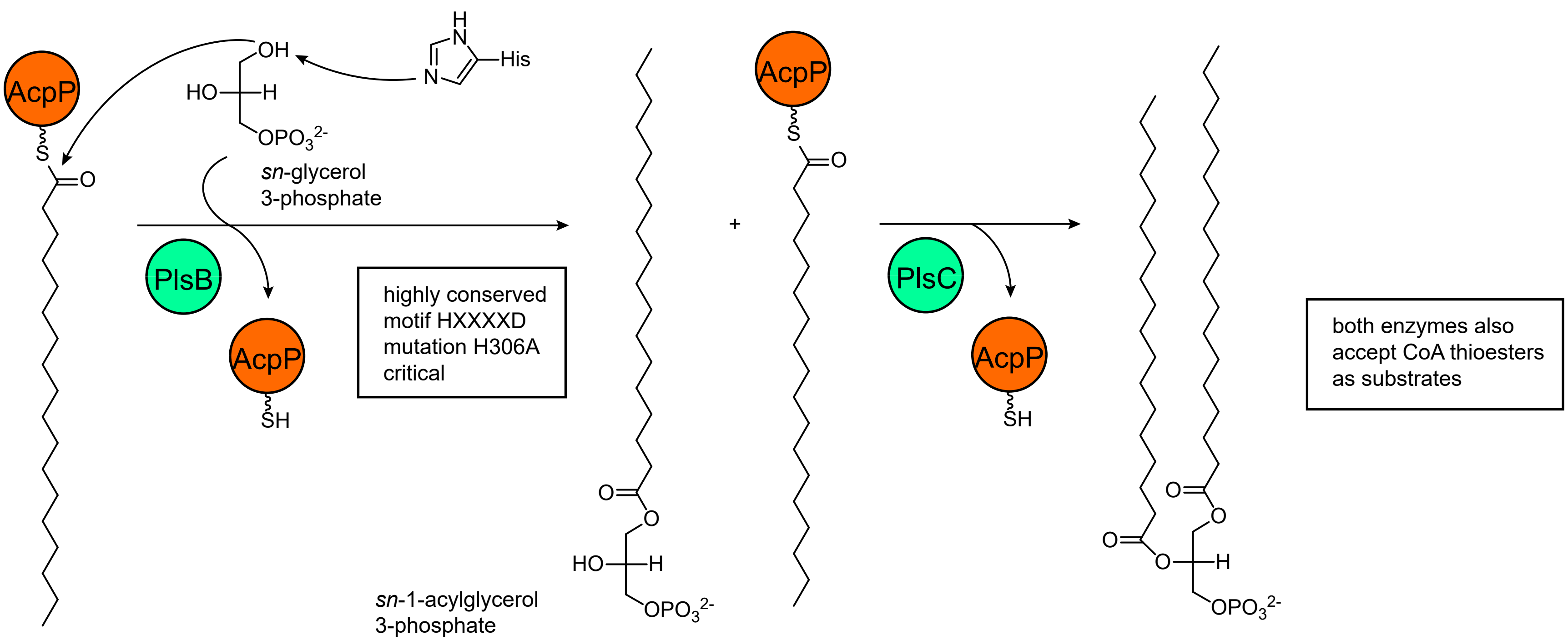
desinfectant
poorly biodegradable

1.3 Fatty acid biosynthesis

- product release

  diverse mechanisms, depending on the organism

  E. coli: PlsB, PlsC (=AT)



1.3 Fatty acid biosynthesis

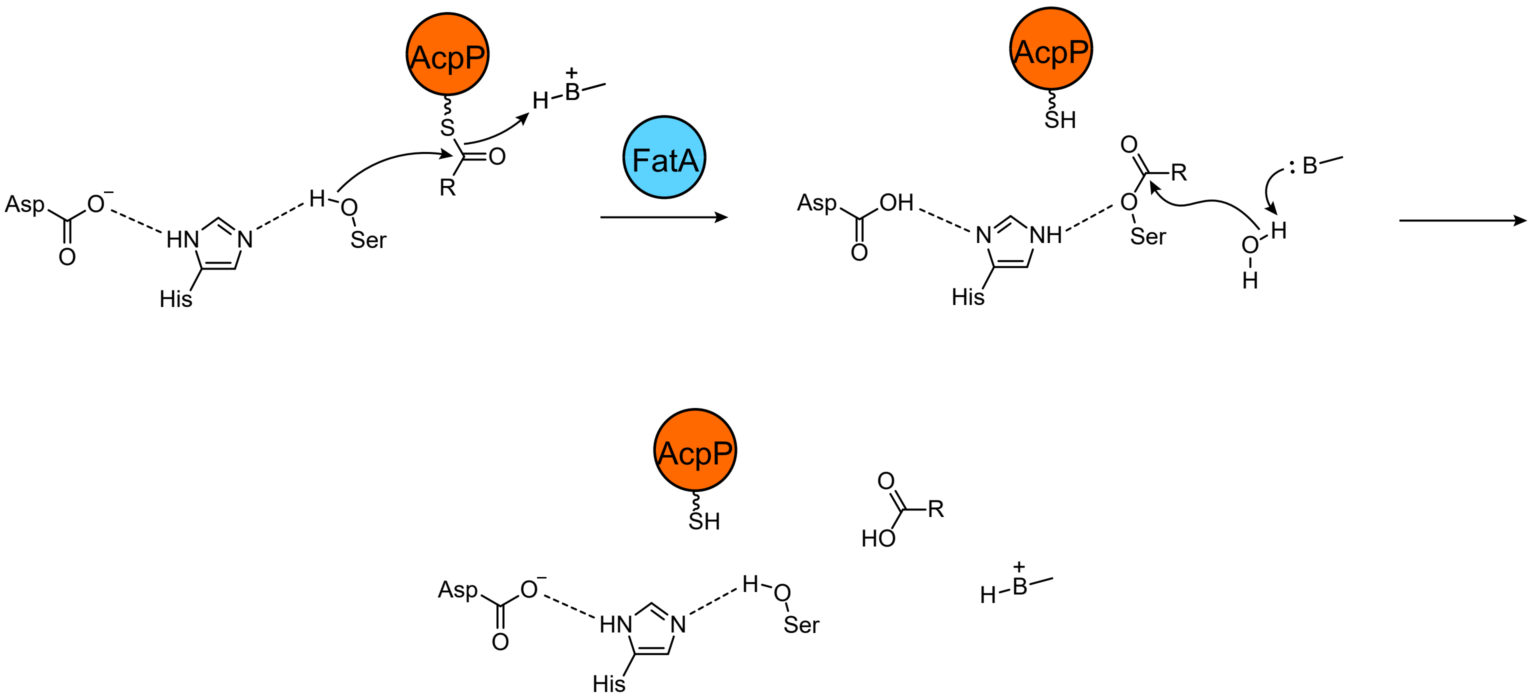
- product release

  in plants: TE (hydrolases)

  catalytical triad Asp-His-Ser or Asp-His-Cys

FatA: prefers 18:1-ACP, 18:0-ACP, 16:0-ACP

FatB: prefers 8:0-ACP – 16:0-ACP

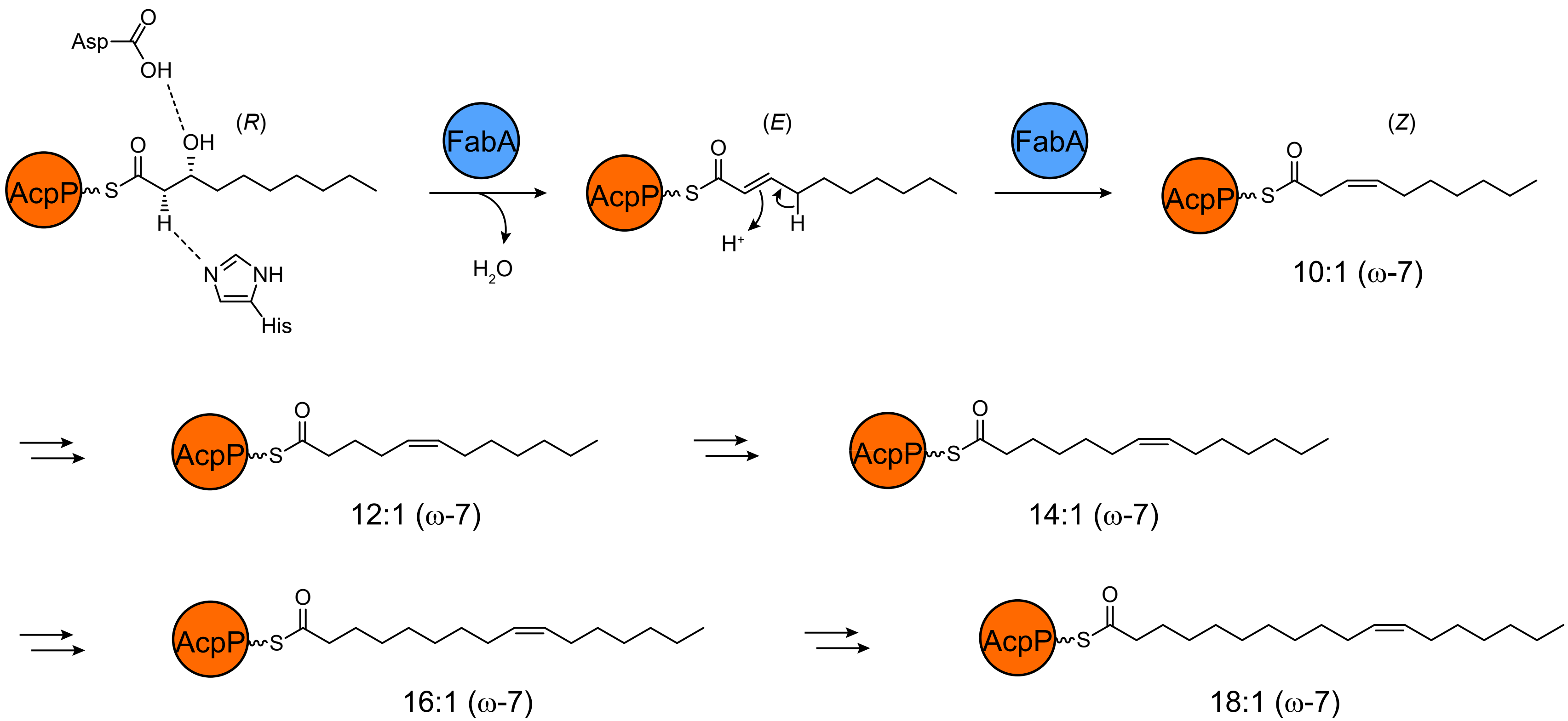


1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

1.3.4 Biosynthesis of unsaturated fatty acids

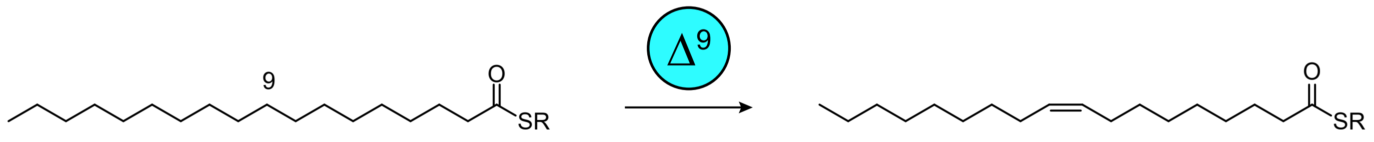
a) anaerobic pathway (bacteria)

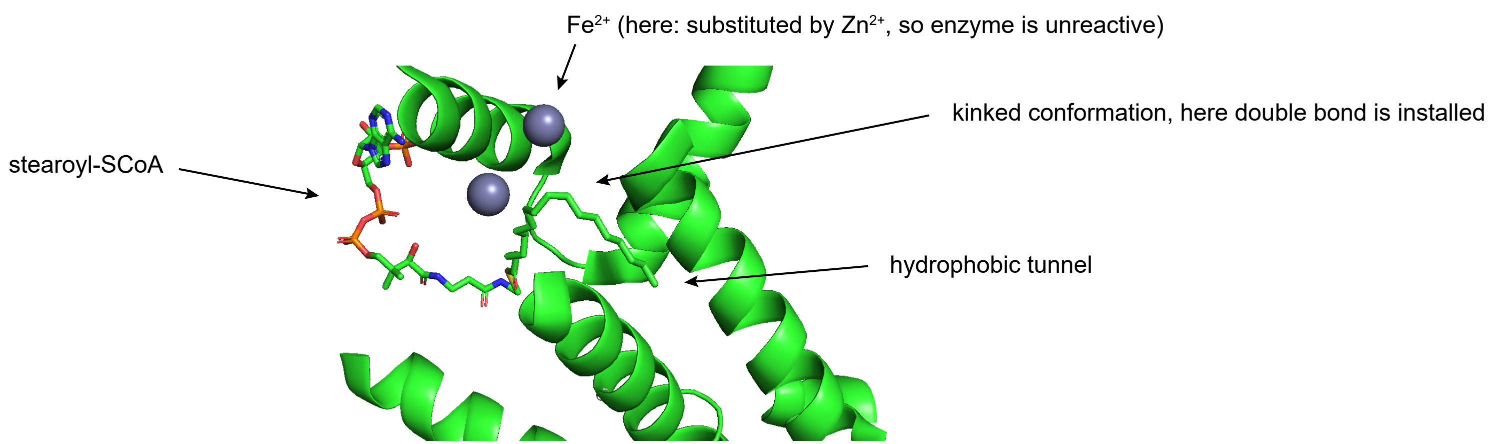


1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- animals, fungi, bacteria: membrane-bound enzymes, substrates = CoA thioesters
- plants: soluble enzymes, substrates = ACP-bound



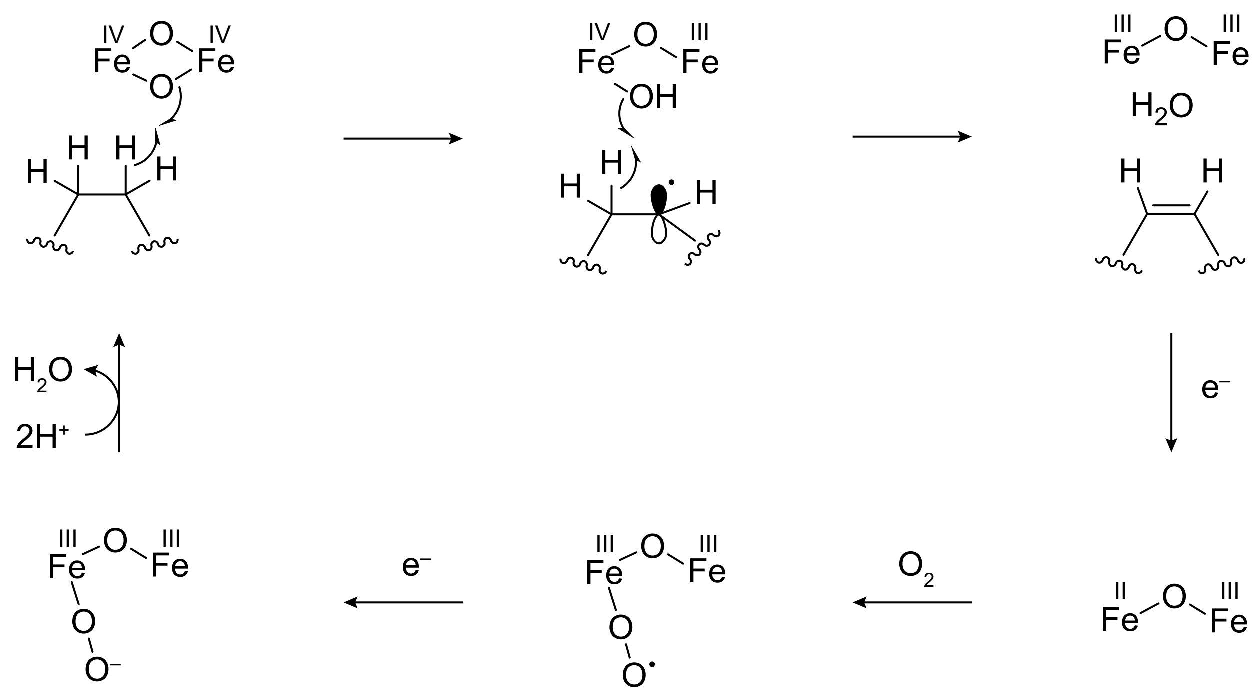


PDB 4YMK

1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- mechanism

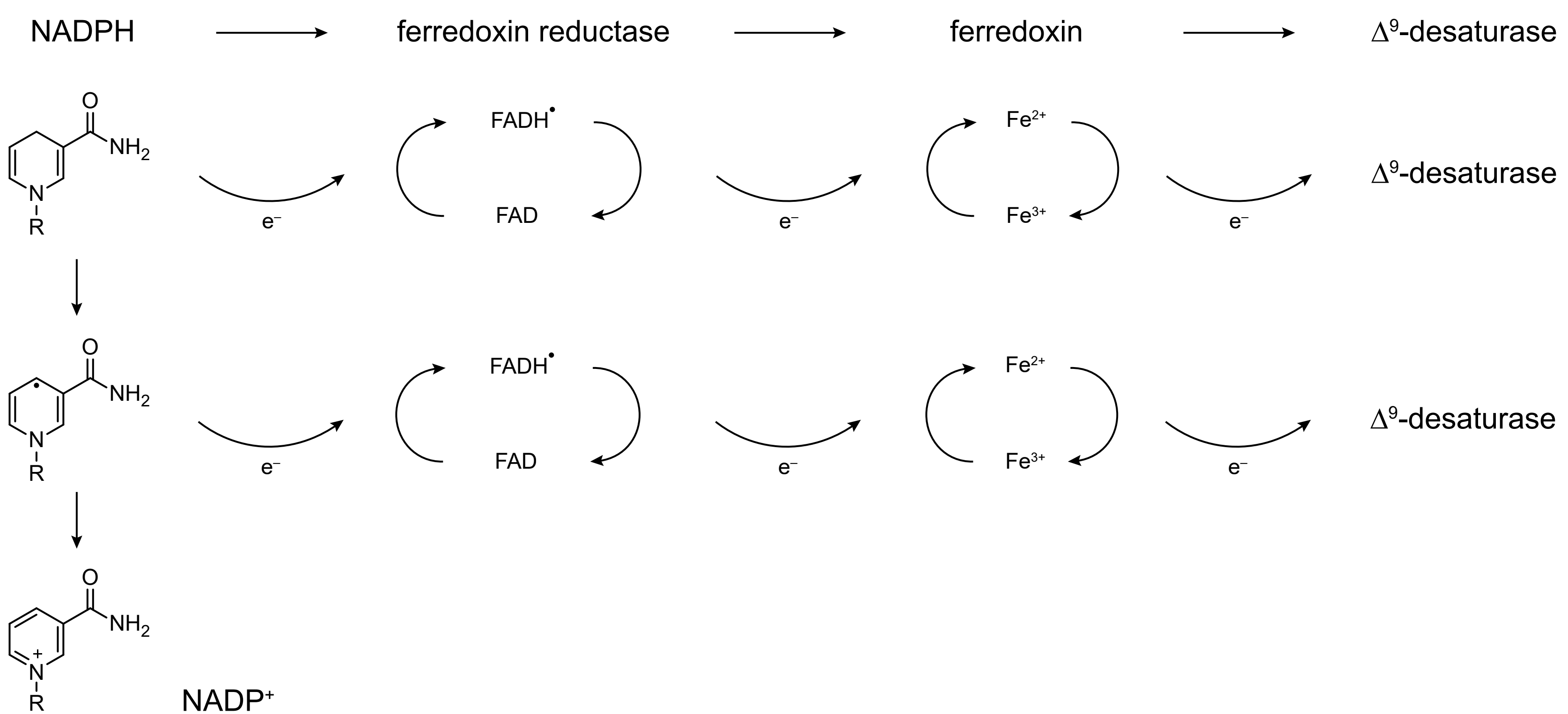


1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- electron transport chain



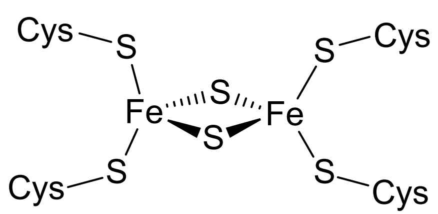
1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

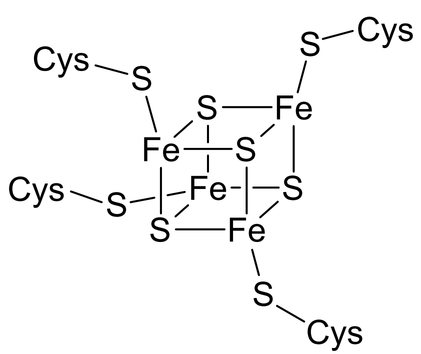
- electron transport chain

ferredoxin

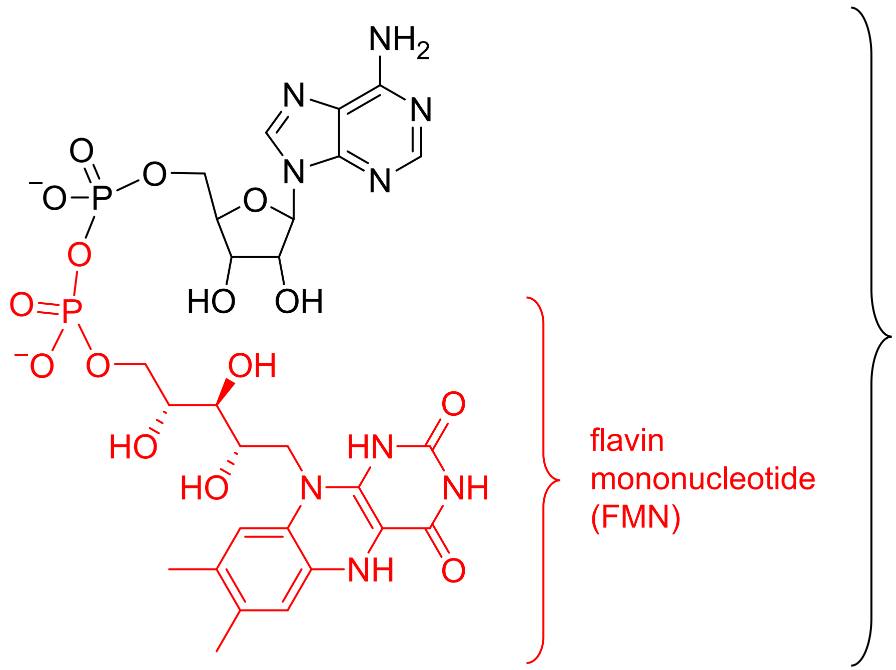
Fe_2S_2



Fe_4S_4



FAD (flavin adenine dinucleotide)



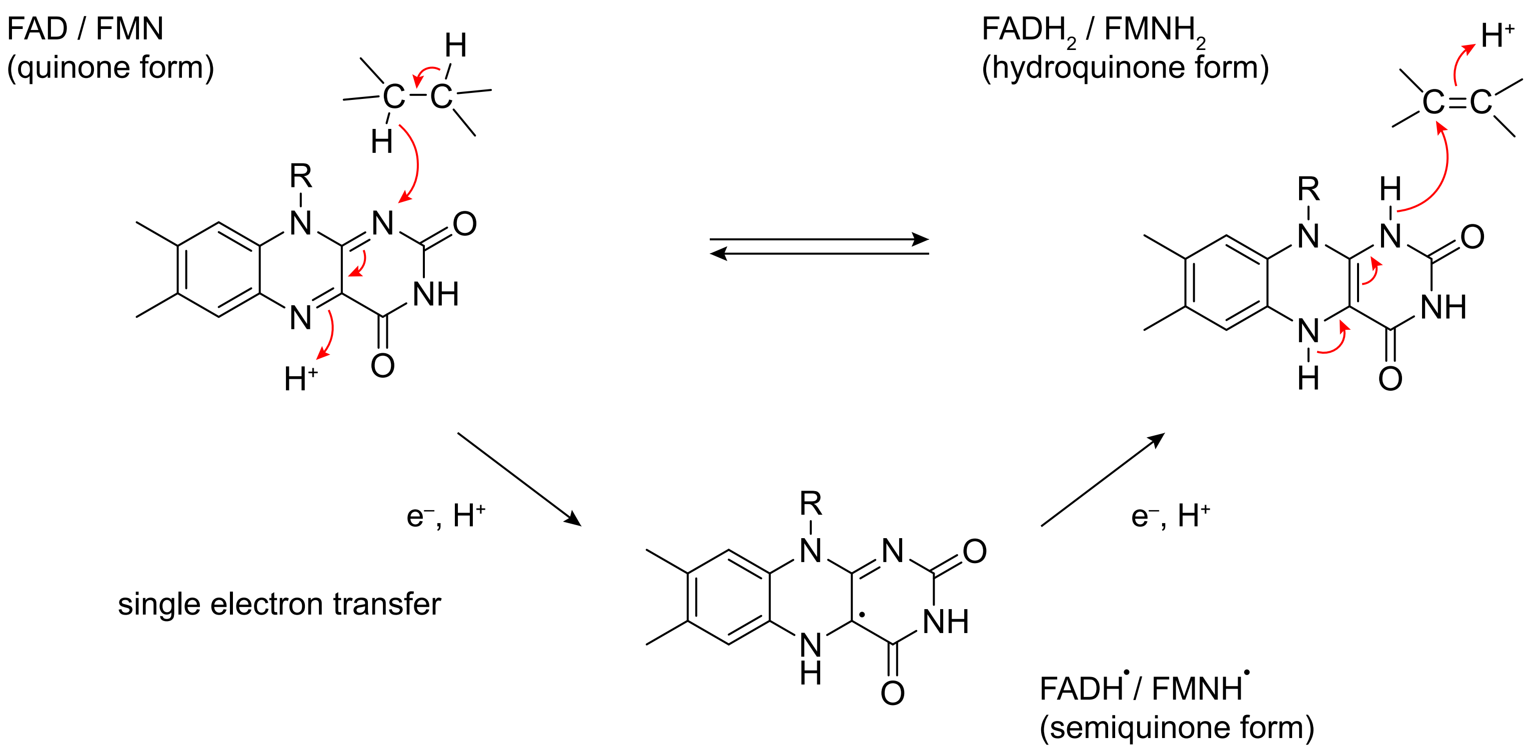
flavin mononucleotide (FMN)

FAD

1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

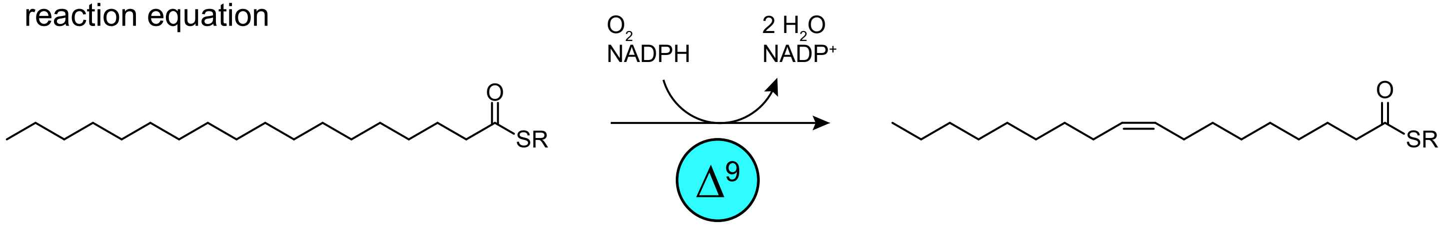
- electron transport chain



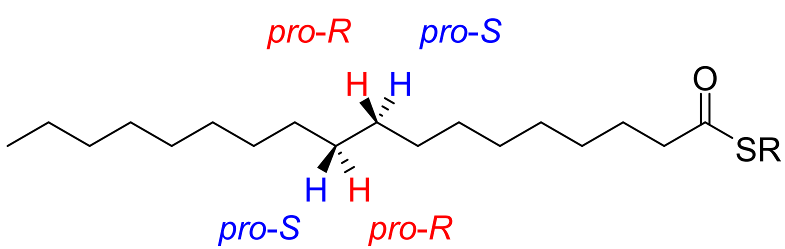
1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- reaction equation



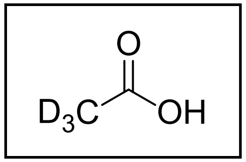
- stereochemistry



investigation possible through stereoselectively deuterated material

accessible by total synthesis, but will require multiple steps

alternative: biosynthesis by feeding of labelled precursors

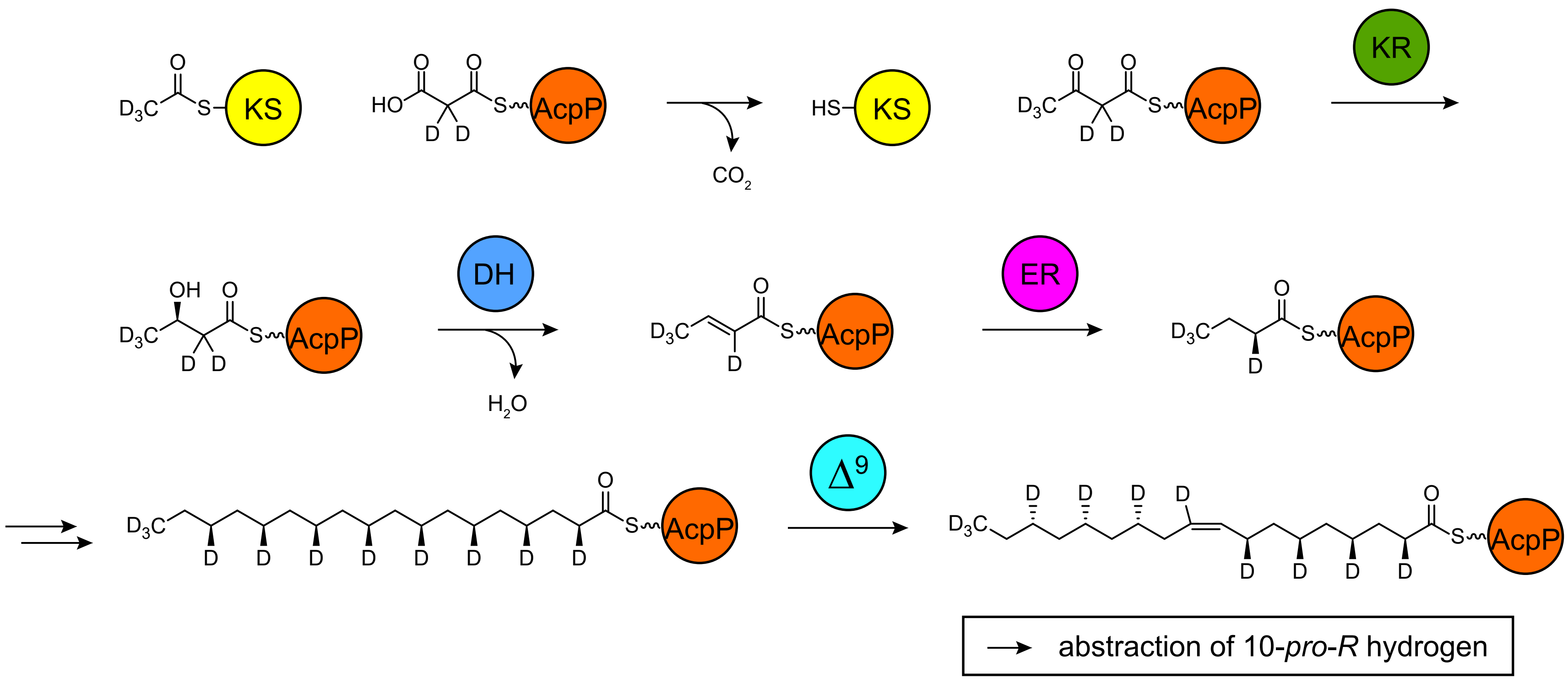


1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- stereochemistry

  feeding of (2,2,2-$^2H_3$)acetic acid to yeast (*Saccharomyces cerevisiae*)



1. Fatty acids and derivatives

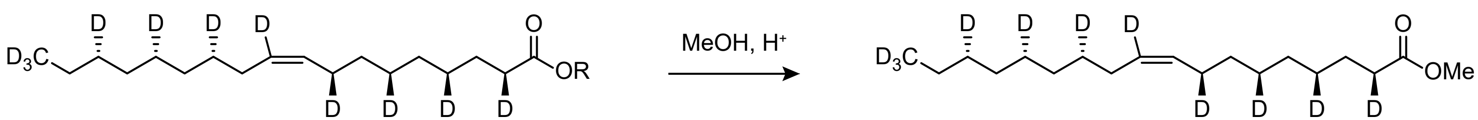
1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- stereochemistry

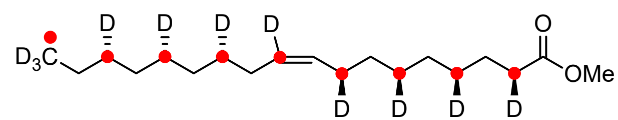
  product analysis

  incorporation into fats



⟶ GC/MS problem: incorporation rates may be low, unclear deuterium content

better approach: feeding of (2-^{13}C,2,2,2-$^{2}H_3$)acetic acid



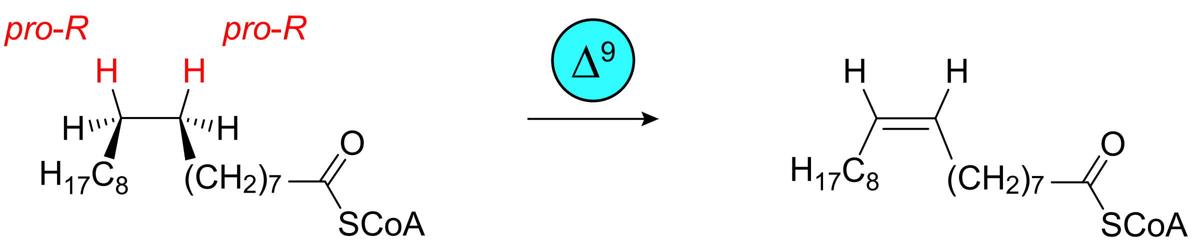
⟶ ^{13}C-NMR (triplet for C10, ^{13}C-^{2}H spin coupling)

similar experiments: $\boxed{\longrightarrow \text{ abstraction of 9-}\textit{pro-R}\text{ hydrogen}}$

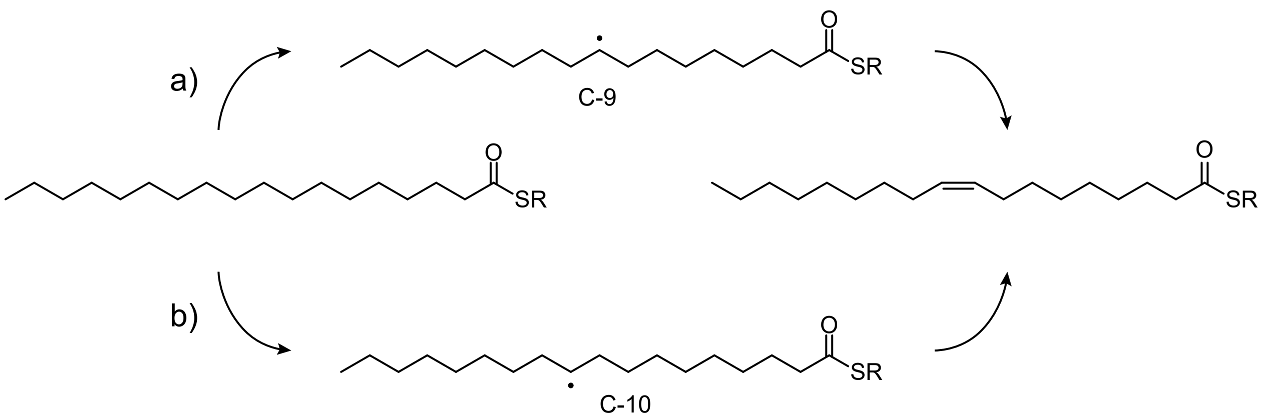
1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- stereochemistry: *syn* elimination



- sequence of H abstractions



model:

E_A for 1st step higher
→ larger KIE

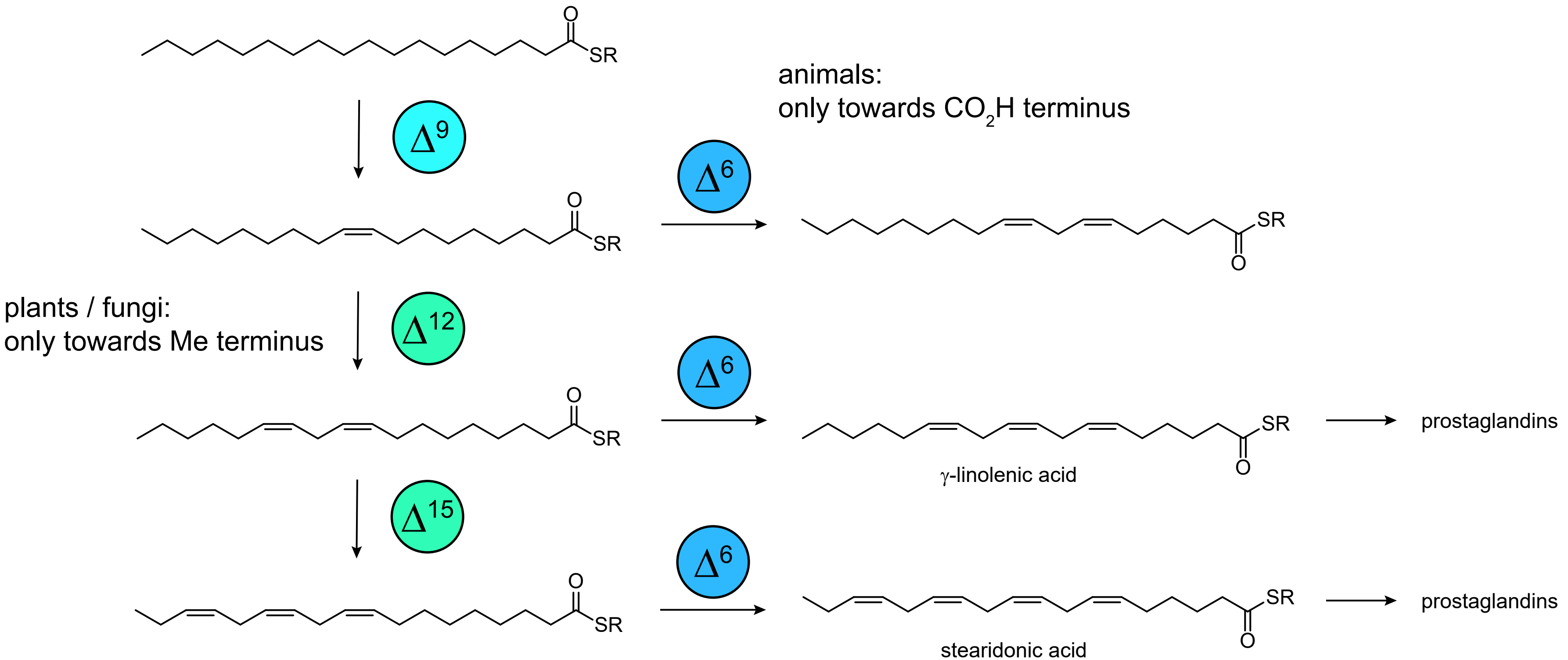
$$\frac{k_{9H}}{k_{9D}} > \frac{k_{10H}}{k_{10D}}$$

→ 1st abstraction at C-9

1.3 Fatty acid biosynthesis

b) aerobic pathway: Δ^9-desaturases

- Δ^9-desaturases ubiquitously present, further desaturases organism specific downstream steps:

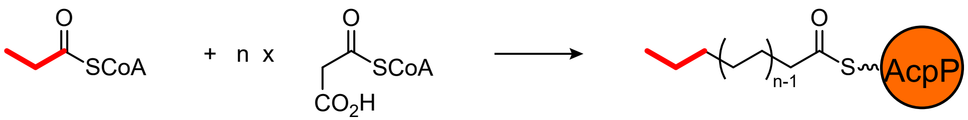


1. Fatty acids and derivatives

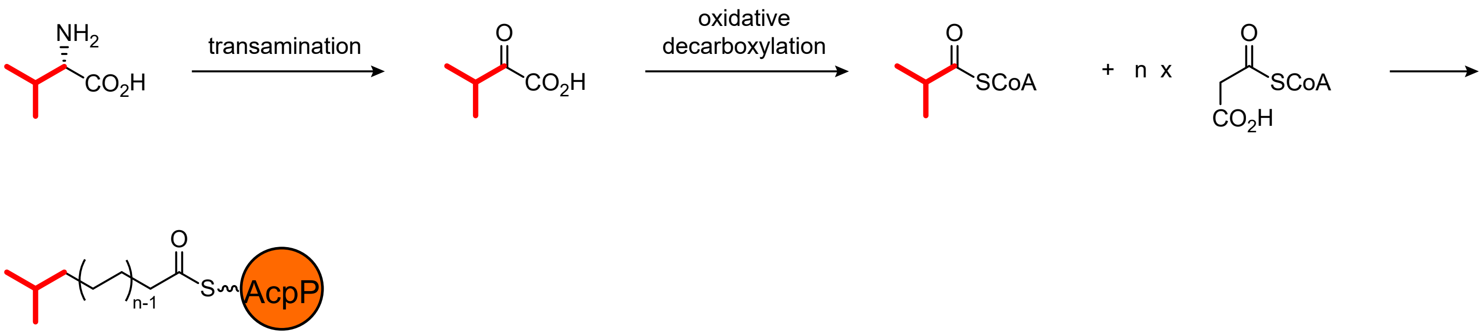
1.3 Fatty acid biosynthesis

1.3.5 Biosynthesis of odd and terminally branched fatty acids

- odd fatty acids: starter unit propionyl-CoA



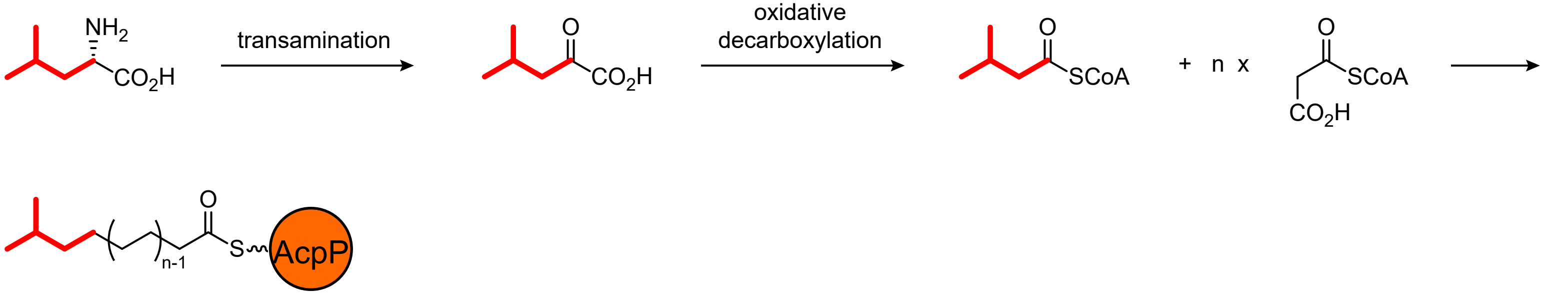
- *iso*-even fatty acids: starter unit from valine



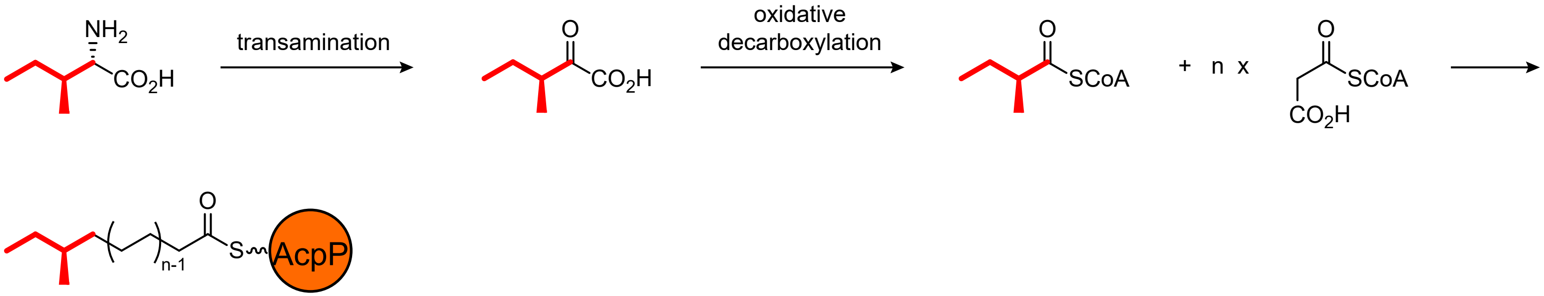
1.3 Fatty acid biosynthesis

1.3.5 Biosynthesis of odd and terminally branched fatty acids

- *iso*-odd fatty acids: starter unit from leucine



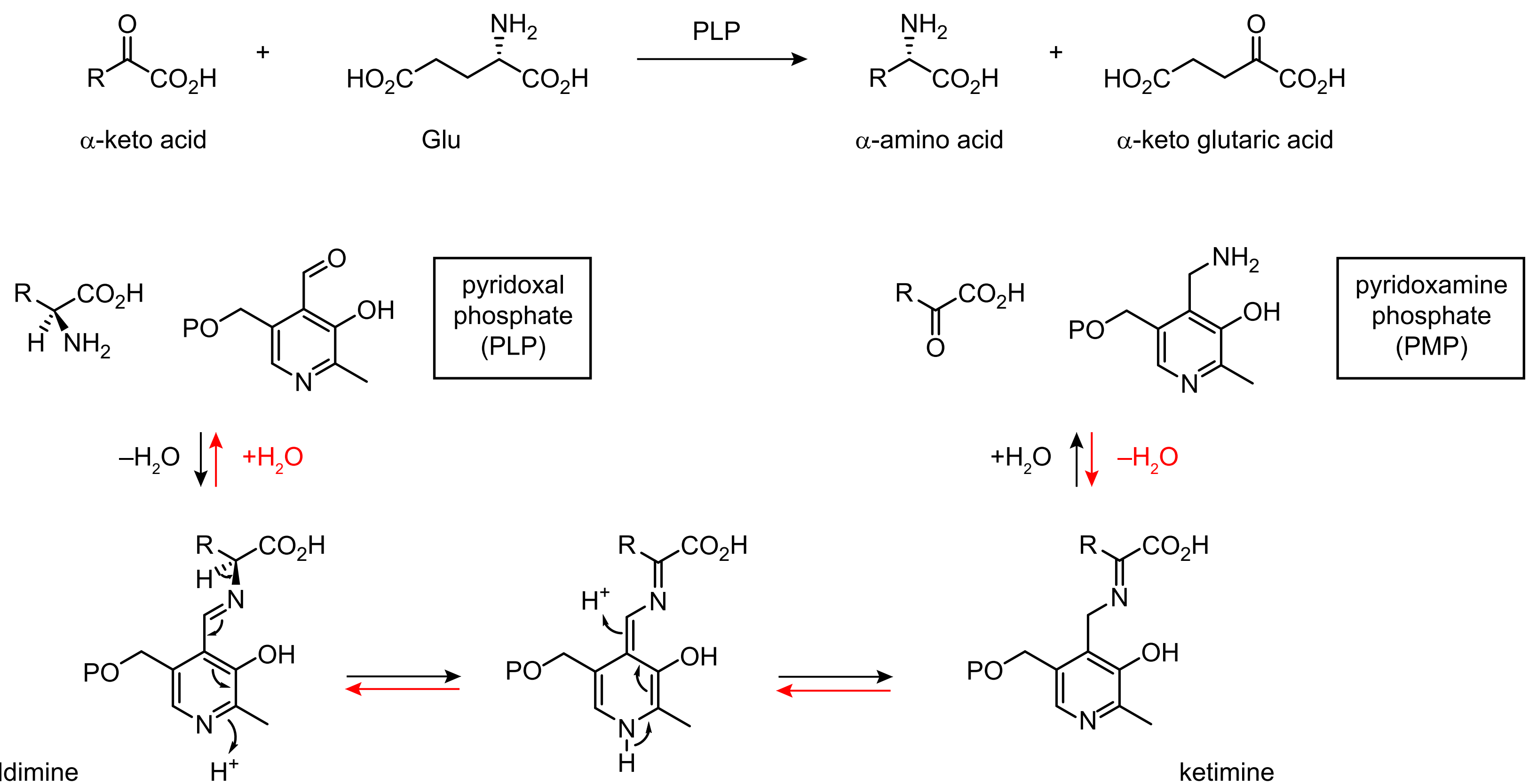
- *anteiso*-odd fatty acids: starter unit from isoleucine



1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

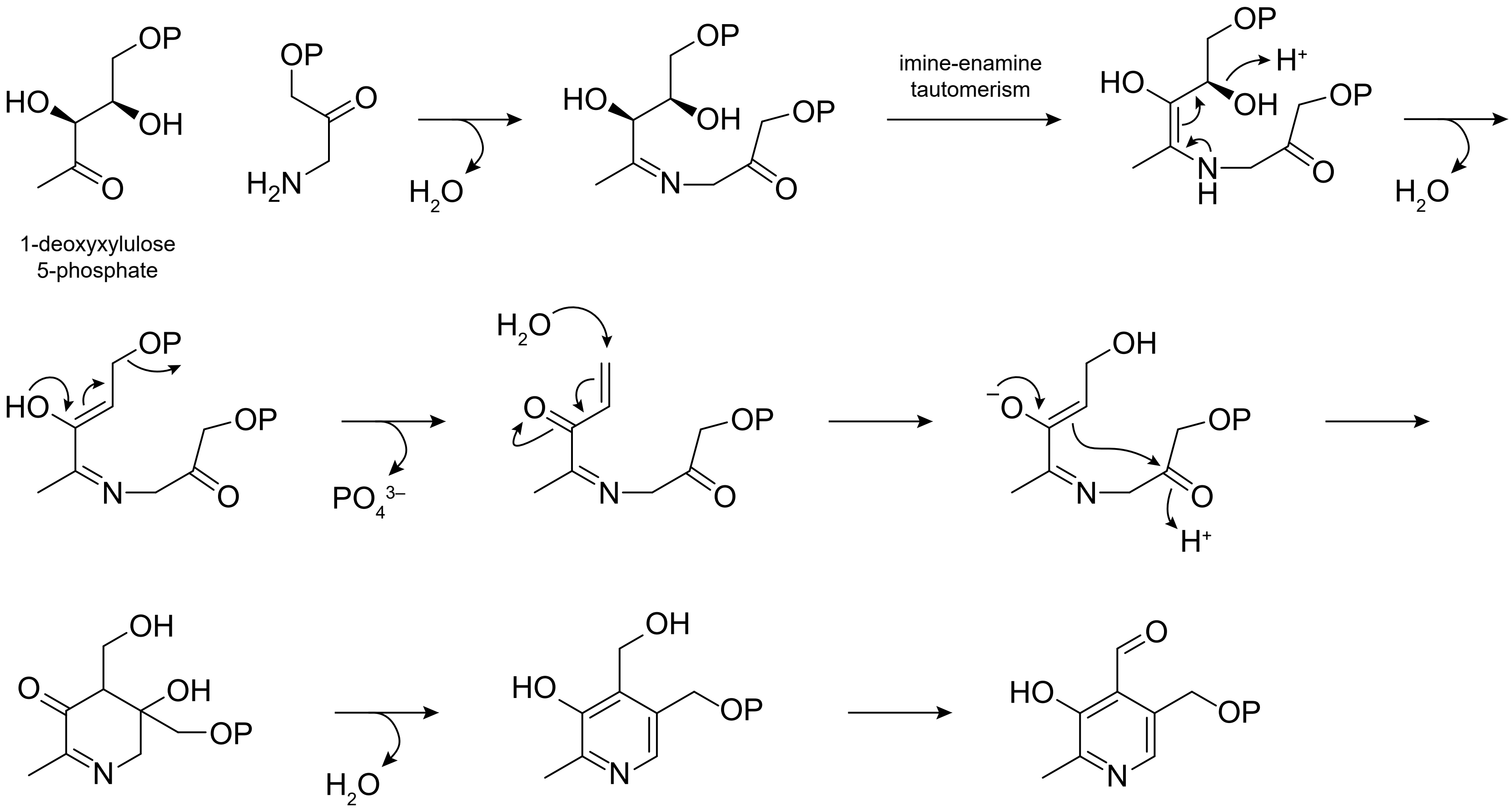
Transamination with pyridoxal phosphate



1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

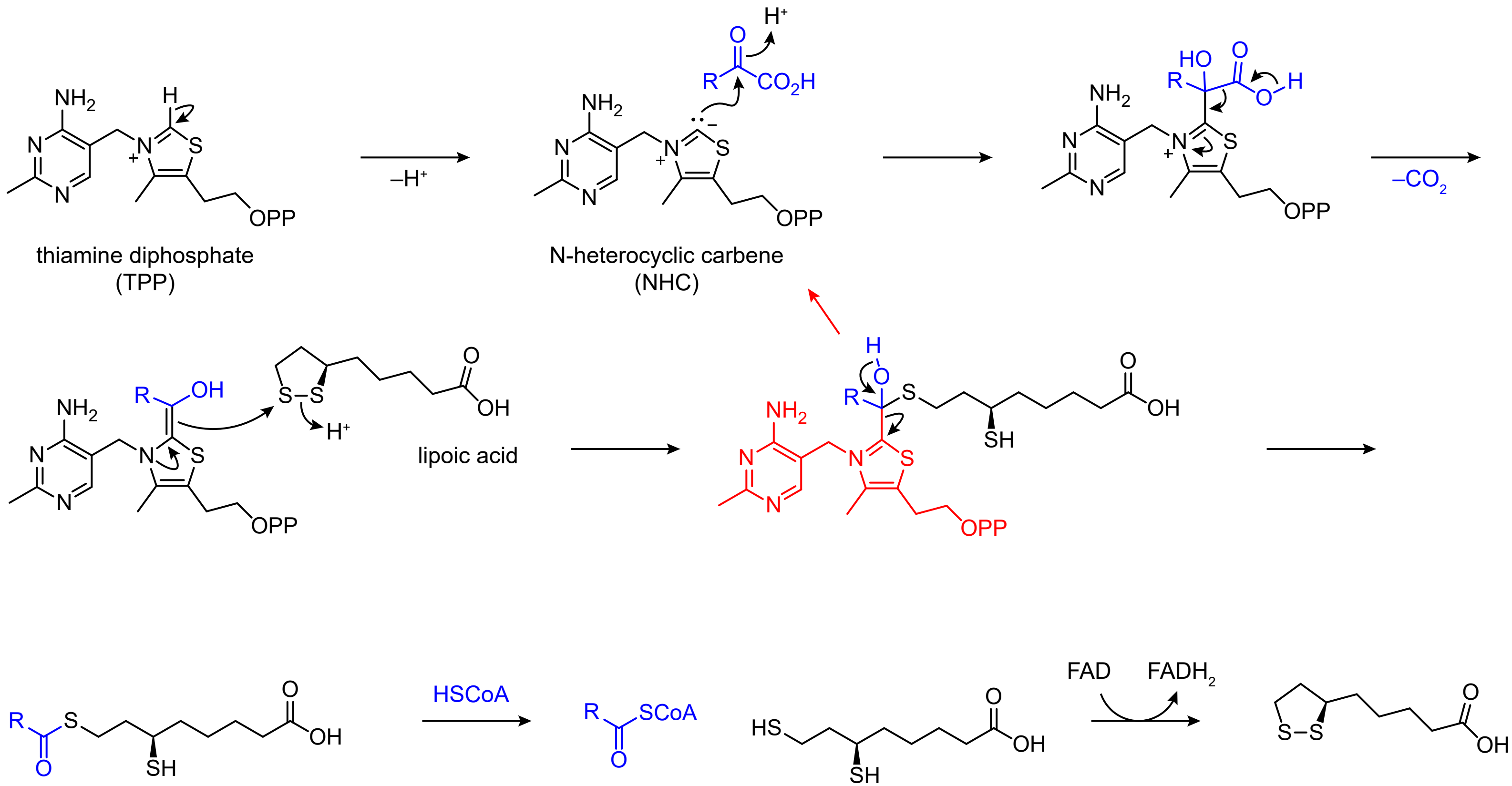
Biosynthesis of pyridoxal phosphate



1. Fatty acids and derivatives

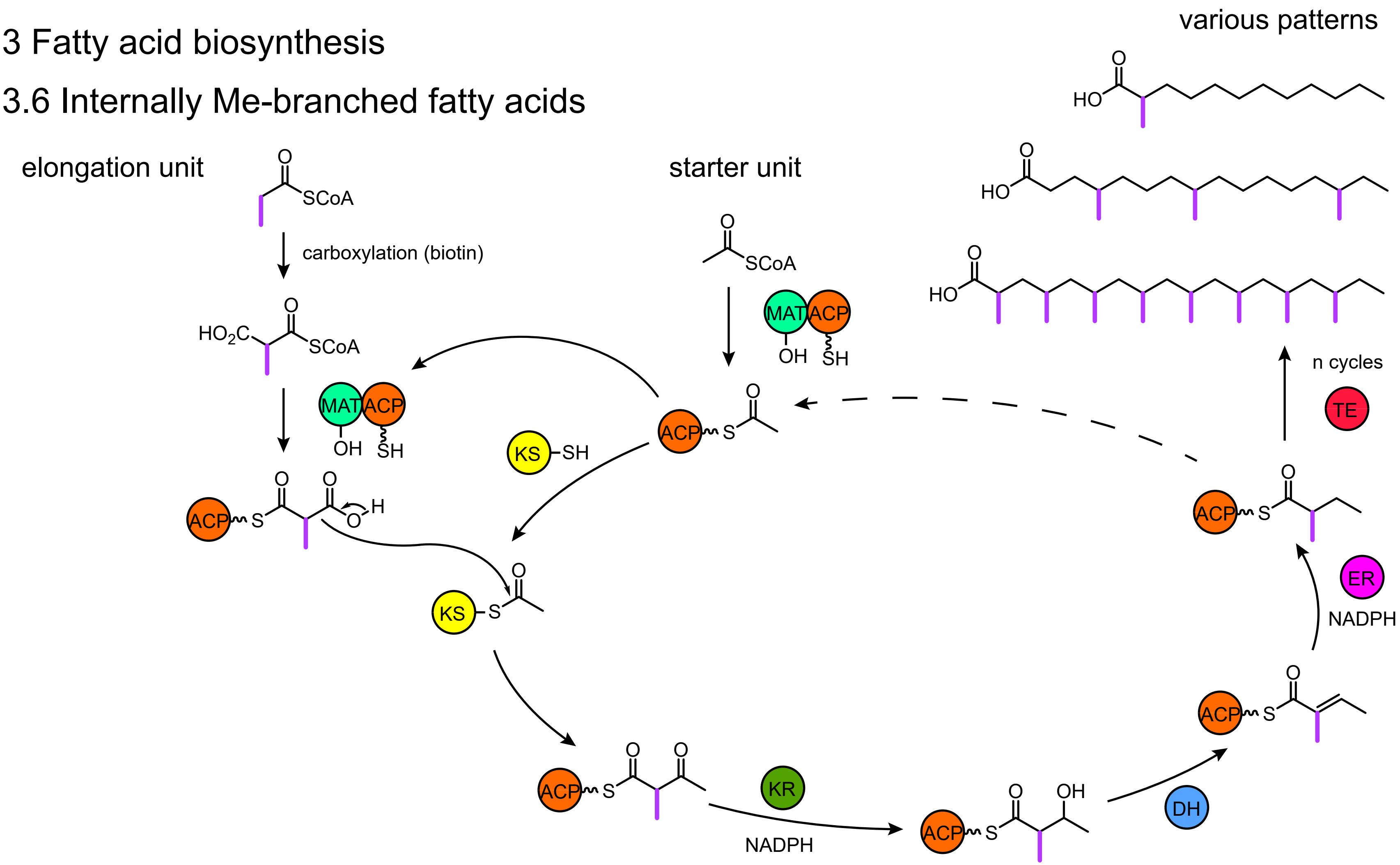
1.3 Fatty acid biosynthesis

Oxidative decarboxylation: thiamine diphosphate and lipoic acid



1.3 Fatty acid biosynthesis

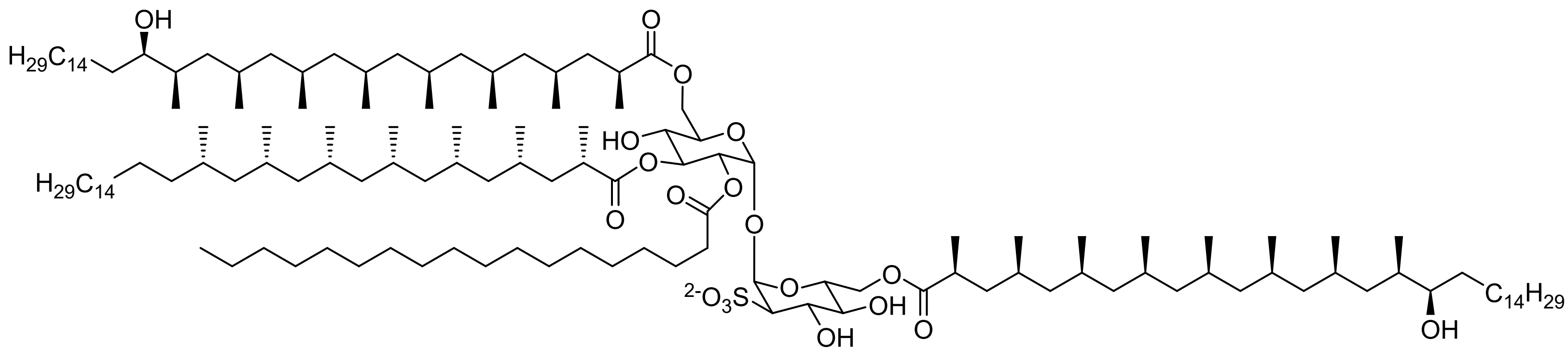
1.3.6 Internally Me-branched fatty acids



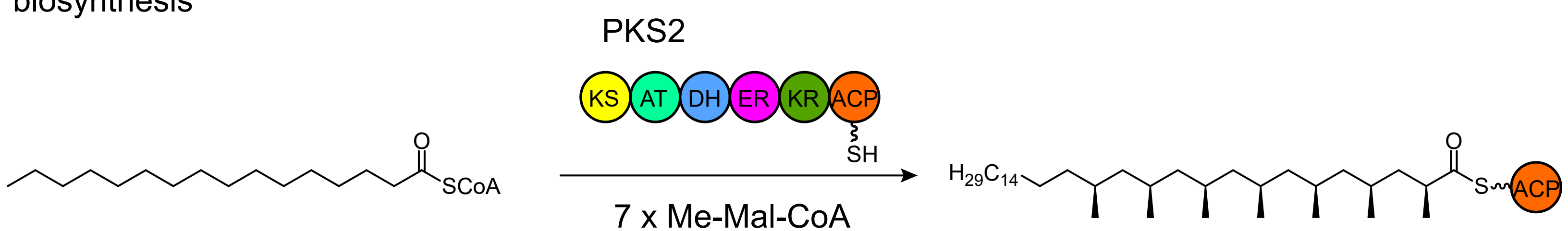
1.3 Fatty acid biosynthesis

1.3.6 Internally Me-branched fatty acids

sulfolipid-1 (*Mycobacterium tuberculosis*)



biosynthesis

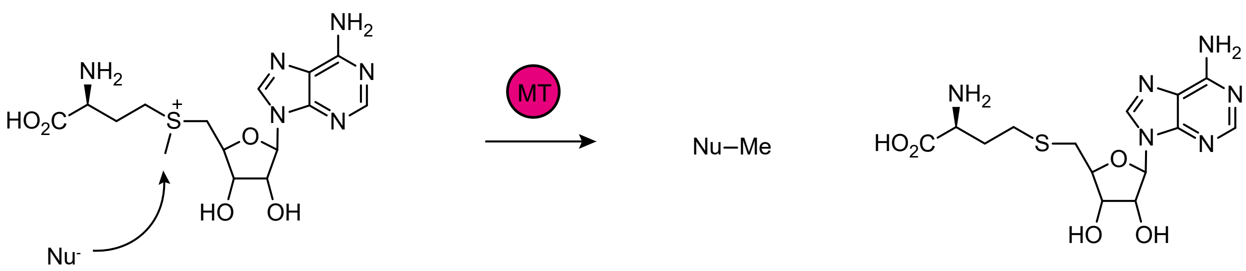


1. Fatty acids and derivatives

1.3 Fatty acid biosynthesis

1.3.6 Internally Me-branched fatty acids

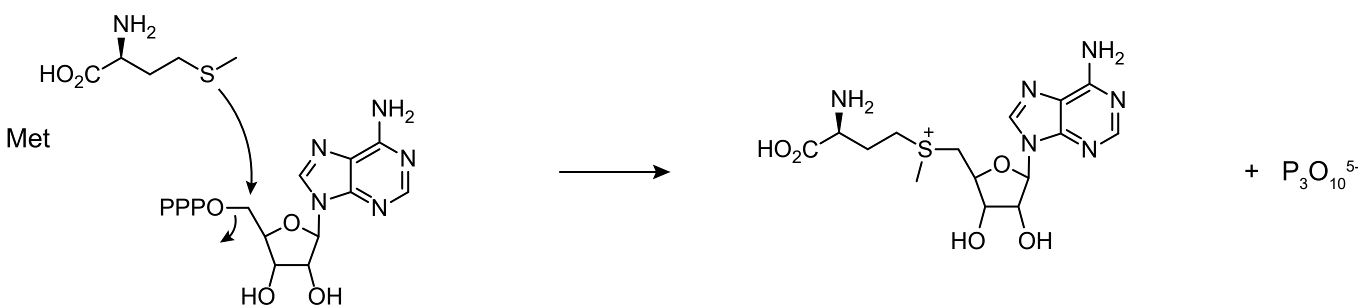
methylation of unsaturated fatty acids with SAM



S-adenosylmethionine (SAM)

S-adenosylhomocysteine (SAH)

biosynthesis

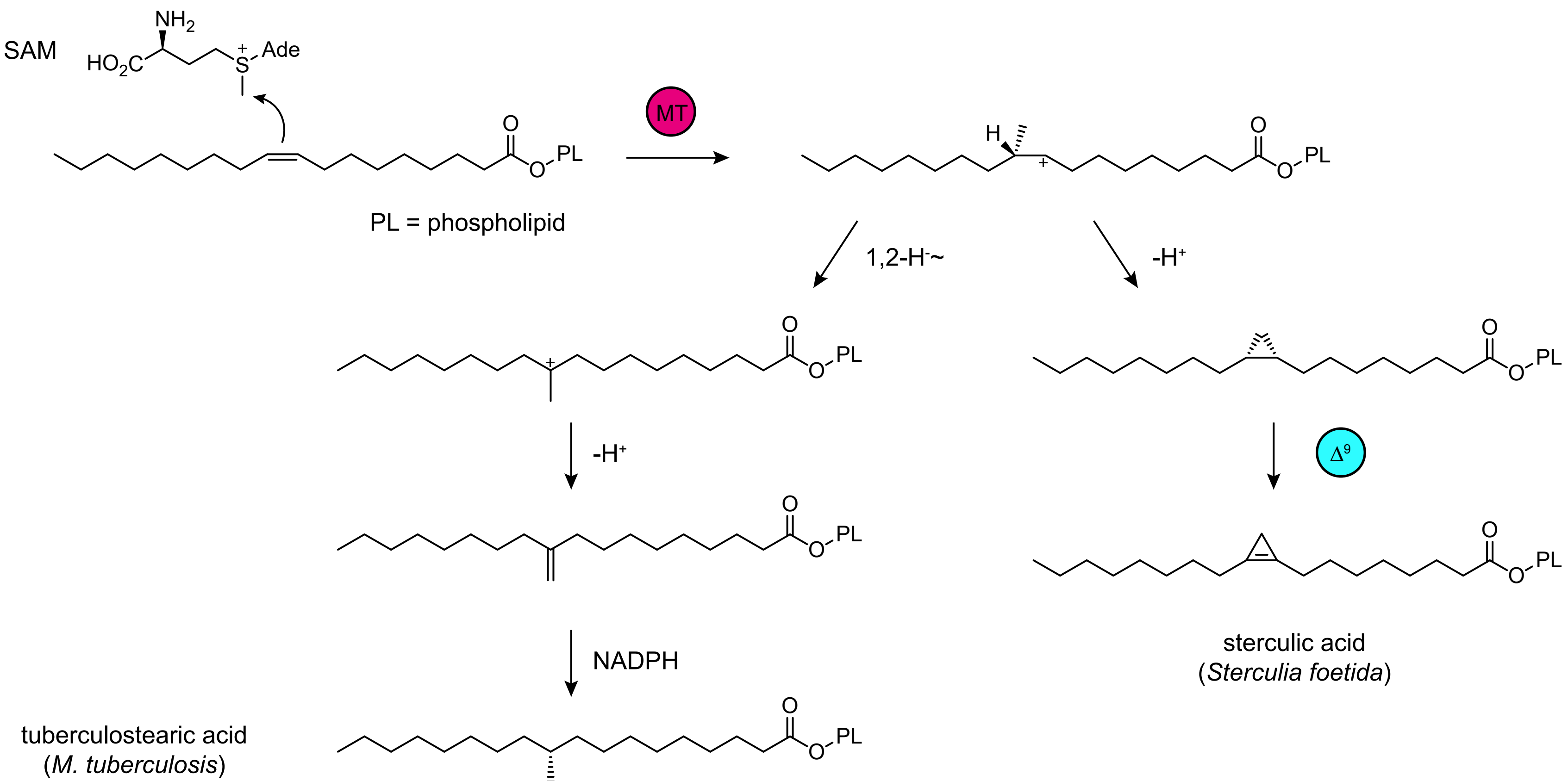


ATP

SAM

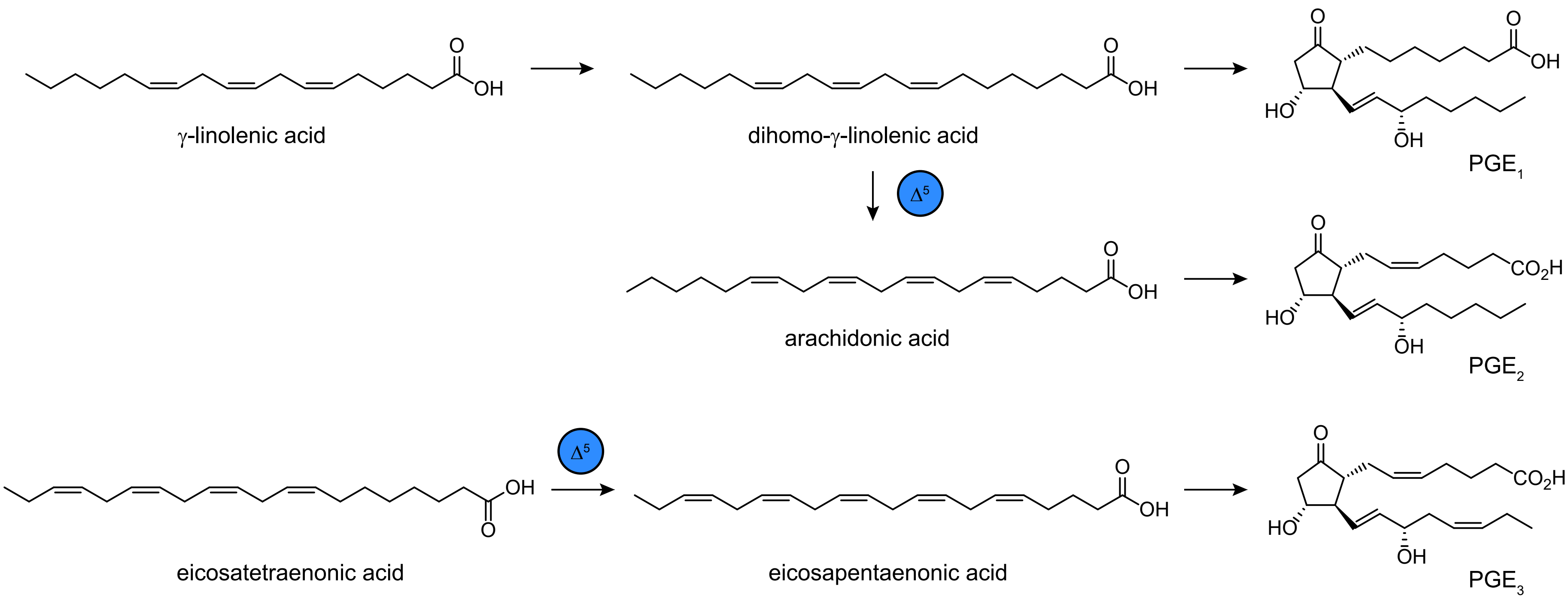
1.3 Fatty acid biosynthesis

1.3.6 Internally Me-branched fatty acids



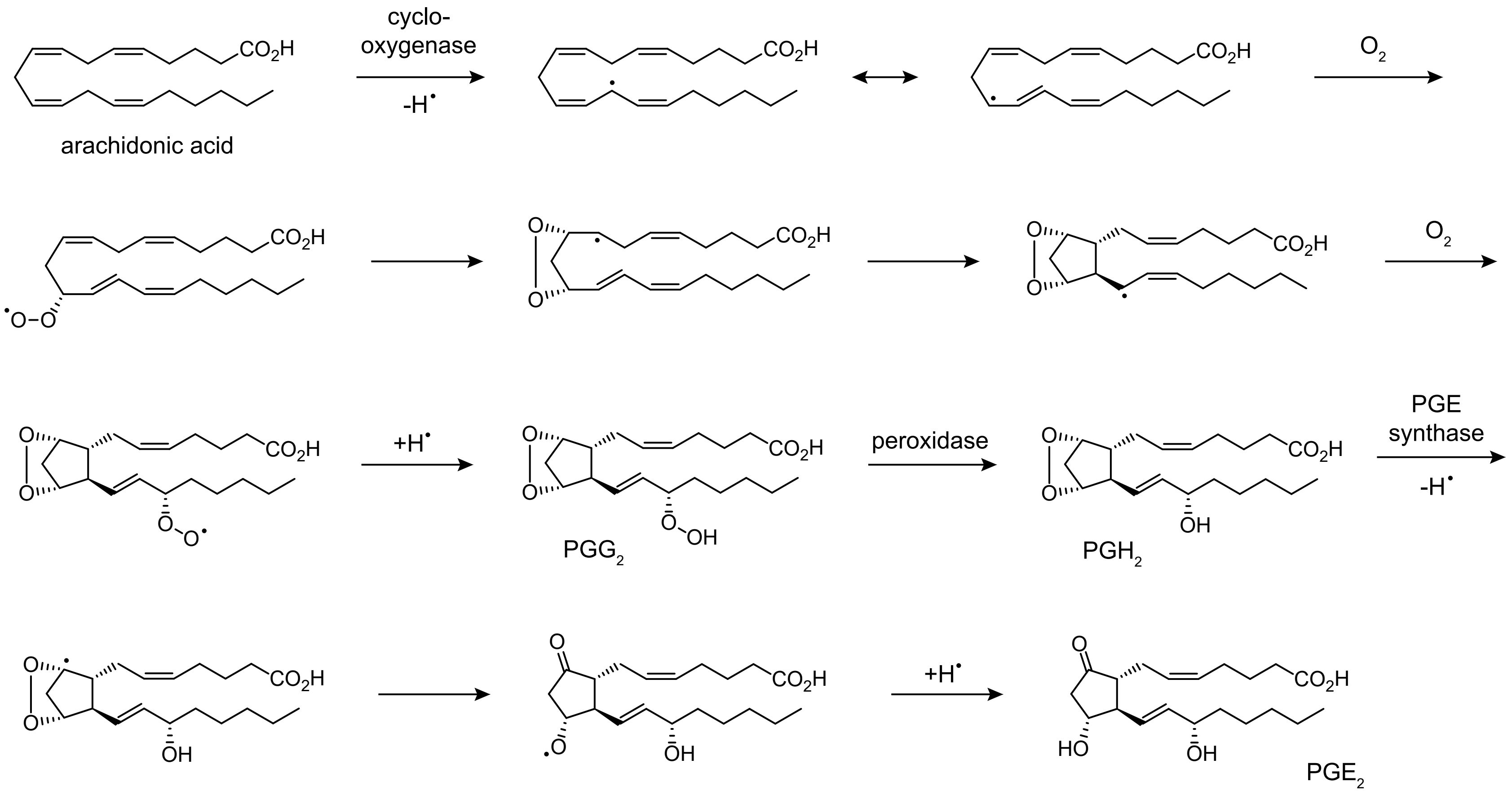
1.4 Prostaglandins

- eicosanoids (from C20 fatty acids)
- initially believed to be formed in the prostate gland (but actually made in various tissues)
- highest concentrations in sperm
- regulators of blood pressure, aggregation of blood platelets, inflammation, pain, muscle contraction



1.4 Prostaglandins

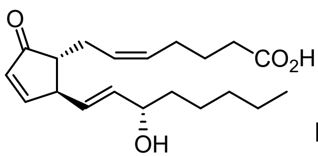
biosynthesis



1.4 Prostaglandins

nomenclature

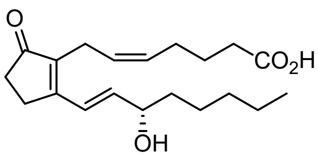
letter indicates functional group pattern



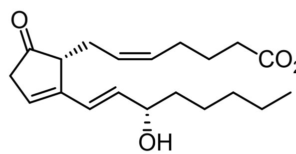
PGA$_2$

series number (fatty acid precursor)

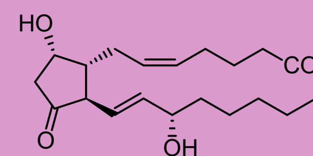
1: dihomo-γ-linolenic acid
2: arachidonic acid
3: eicosapentaenoic acid



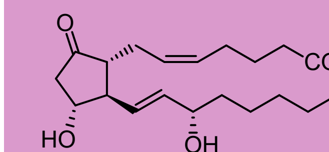
PGB$_2$



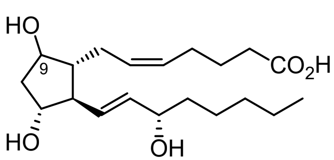
PGC$_2$



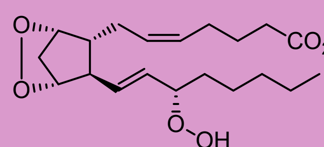
PGD$_2$



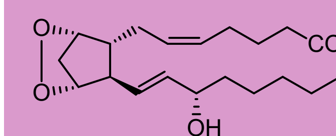
PGE$_2$



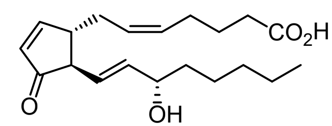
PGF$_{2\alpha}$ (9α-OH)
PGF$_{2\beta}$ (9β-OH)



PGG$_2$



PGH$_2$



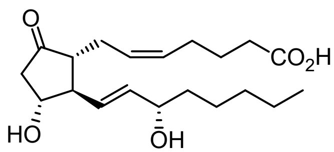
PGJ$_2$

boxed compounds physiologically most important

1. Fatty acids and derivatives

1.4 Prostaglandins

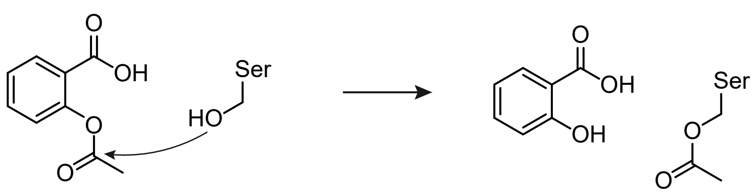
pain regulation

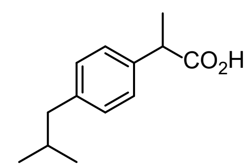


PGE$_2$

binds to pain receptors

painkillers





acetylsalicylic acid

inhibitor of cyclooxygenase
irreversible, covalent binding

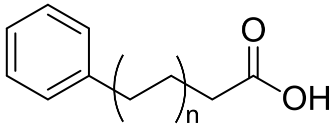
ibuprofen

inhibitor of cyclooxygenase
reversible, competitive inhibitor

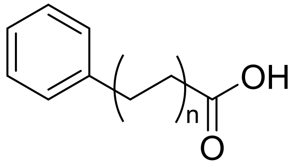
1. Fatty acids and derivatives

1.5 Fatty acid catabolism

first investigations by Franz Knoop (1904)



even



odd

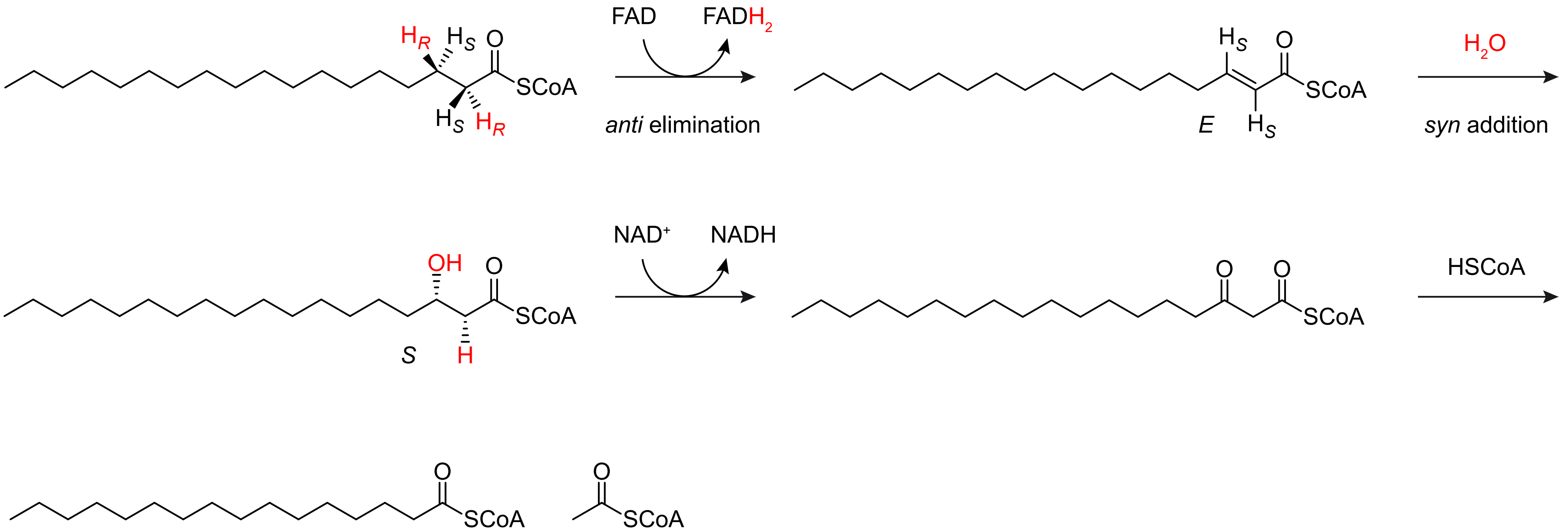
- feeding to dogs
- analysis of degradation products
- successive cleavage of C_2 units

Frederick Soddy, 1910: discovery of isotopes (Nobel Prize 1921)

1.5 Fatty acid catabolism

β-Oxidation

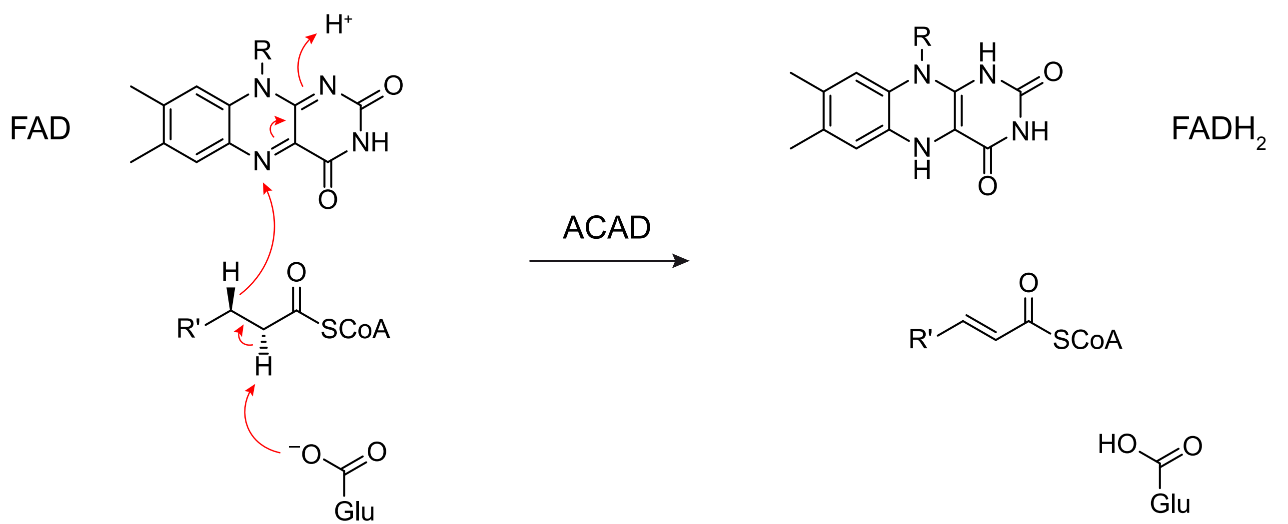
- inversion of fatty acid biosynthesis
- own enzymes
- substrates: SCoA esters (not ACP bound)
- stereochemistry different



1.5 Fatty acid catabolism

β-Oxidation

- acyl-CoA dehydrogenase (ACAD)



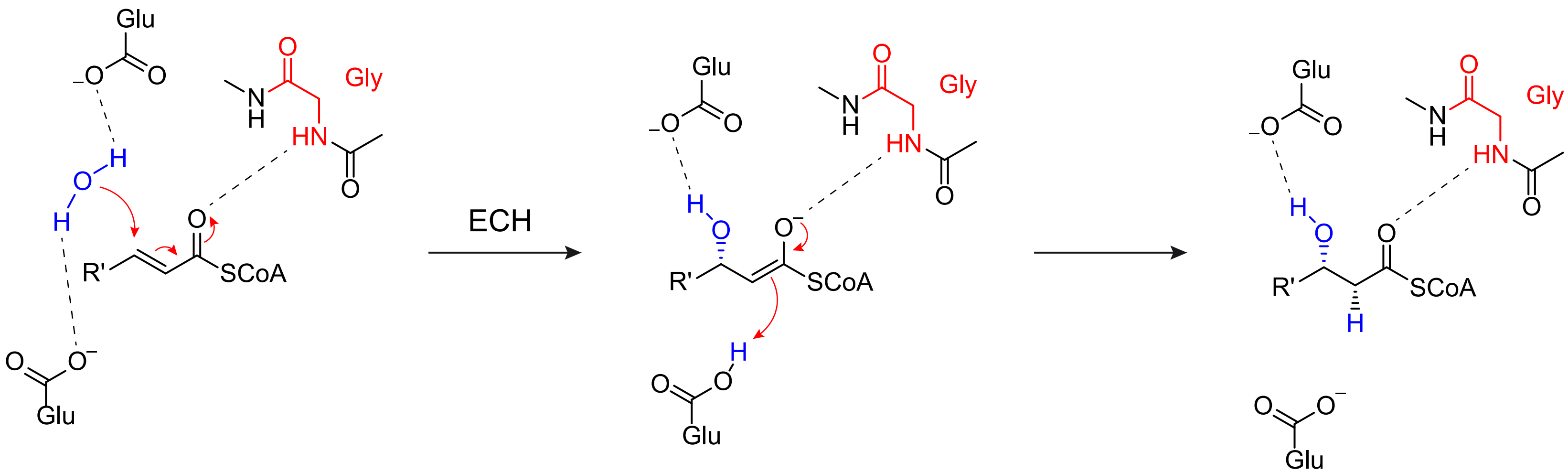
C. Thorpe, J.-J. P. Kim, *FASEB J.* **1995**, *9*, 718.

1.5 Fatty acid catabolism

β-Oxidation

- enoyl-CoA hydratase (ECH)

OH and proton from same water molecule

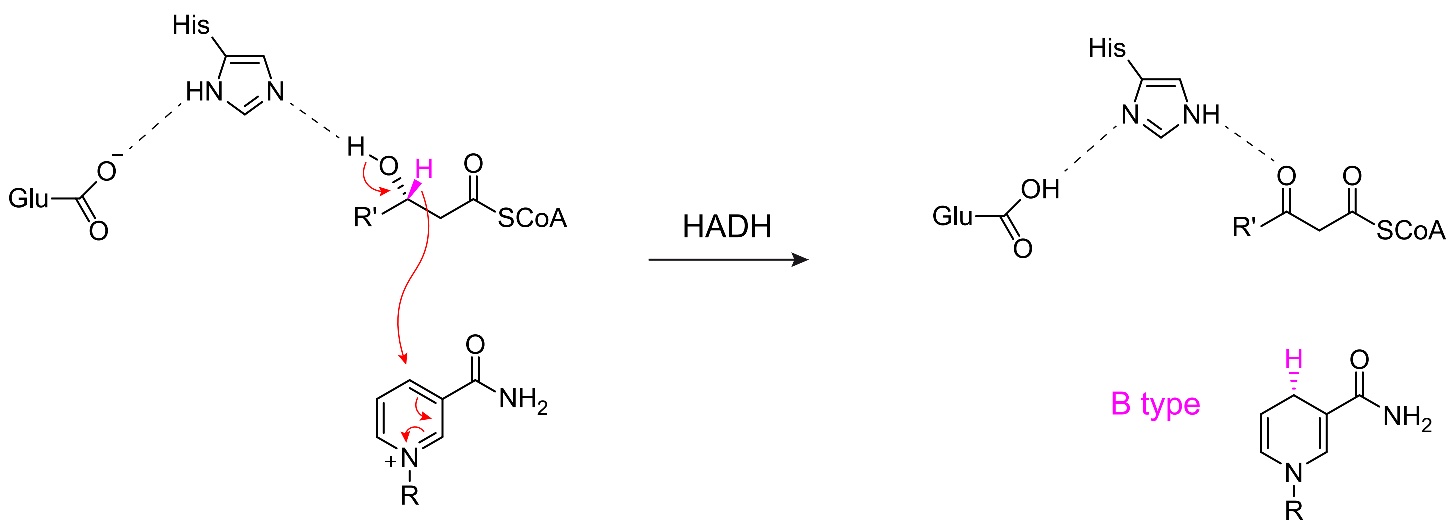


B. J. Bahnson, V. E. Anderson, G. A. Petsko, *Biochemistry* **2002**, *41*, 2621.

1.5 Fatty acid catabolism

β-Oxidation

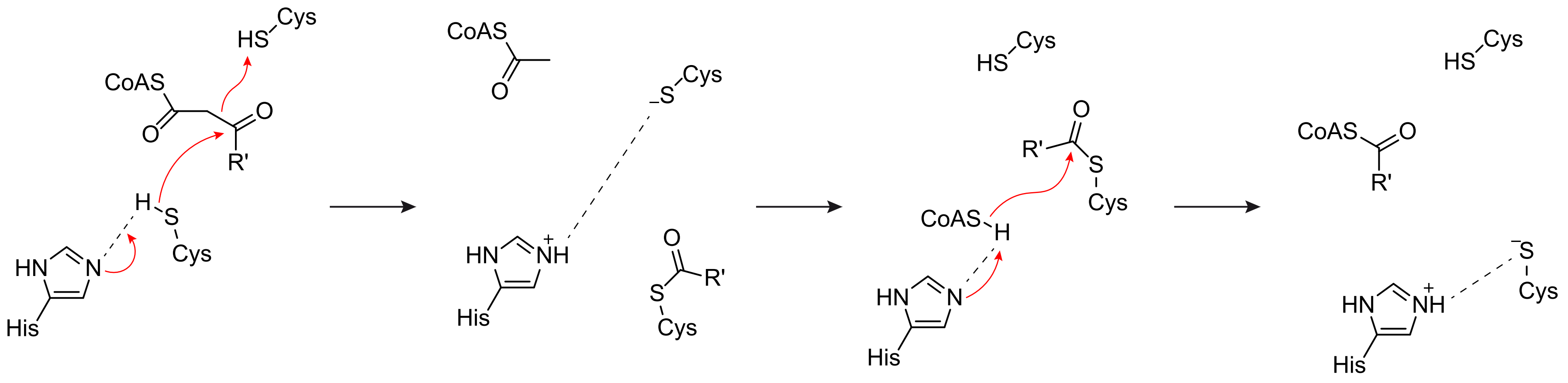
- β-hydroxyacyl-CoA dehydrogenase (HADH)



1.5 Fatty acid catabolism

β-Oxidation

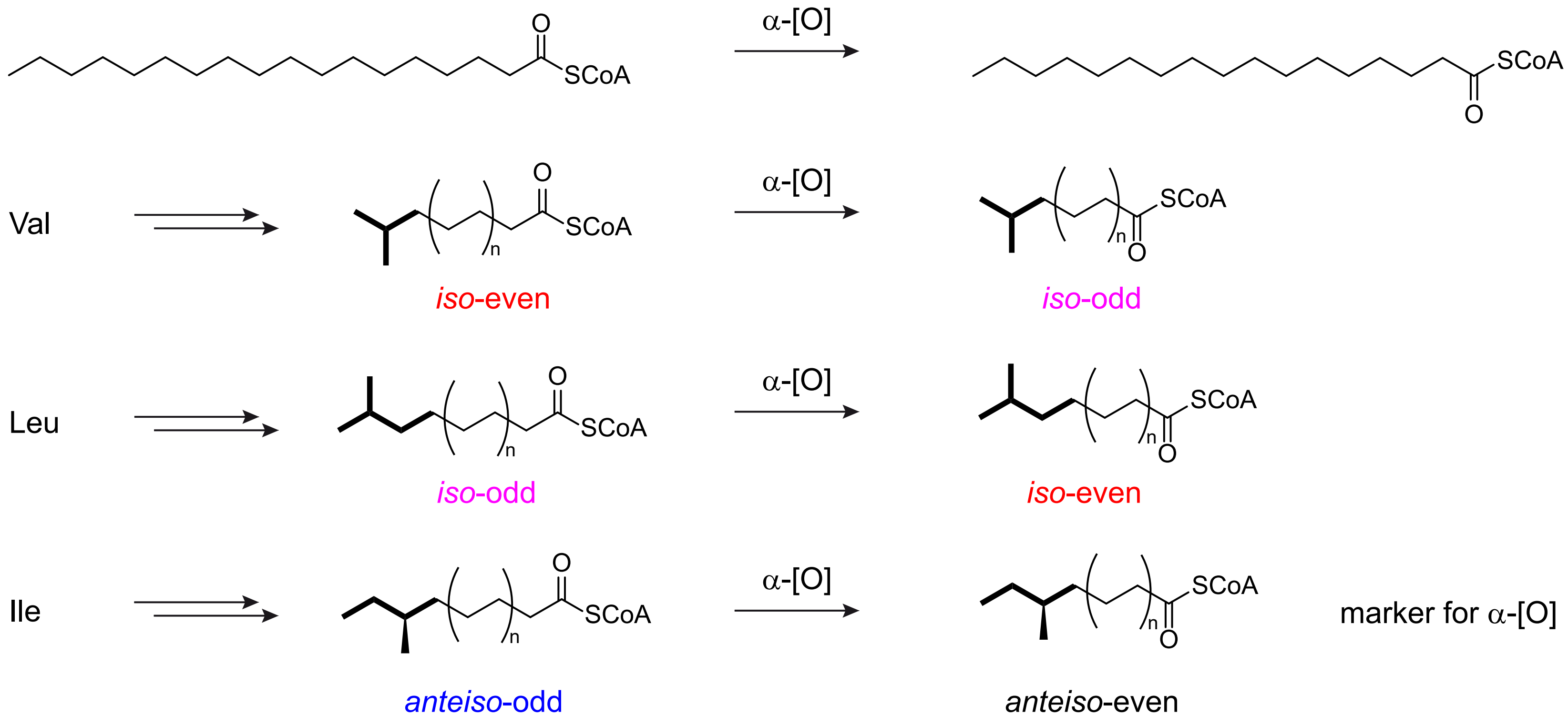
- β-ketothiolase



1.5 Fatty acid catabolism

α-Oxidation

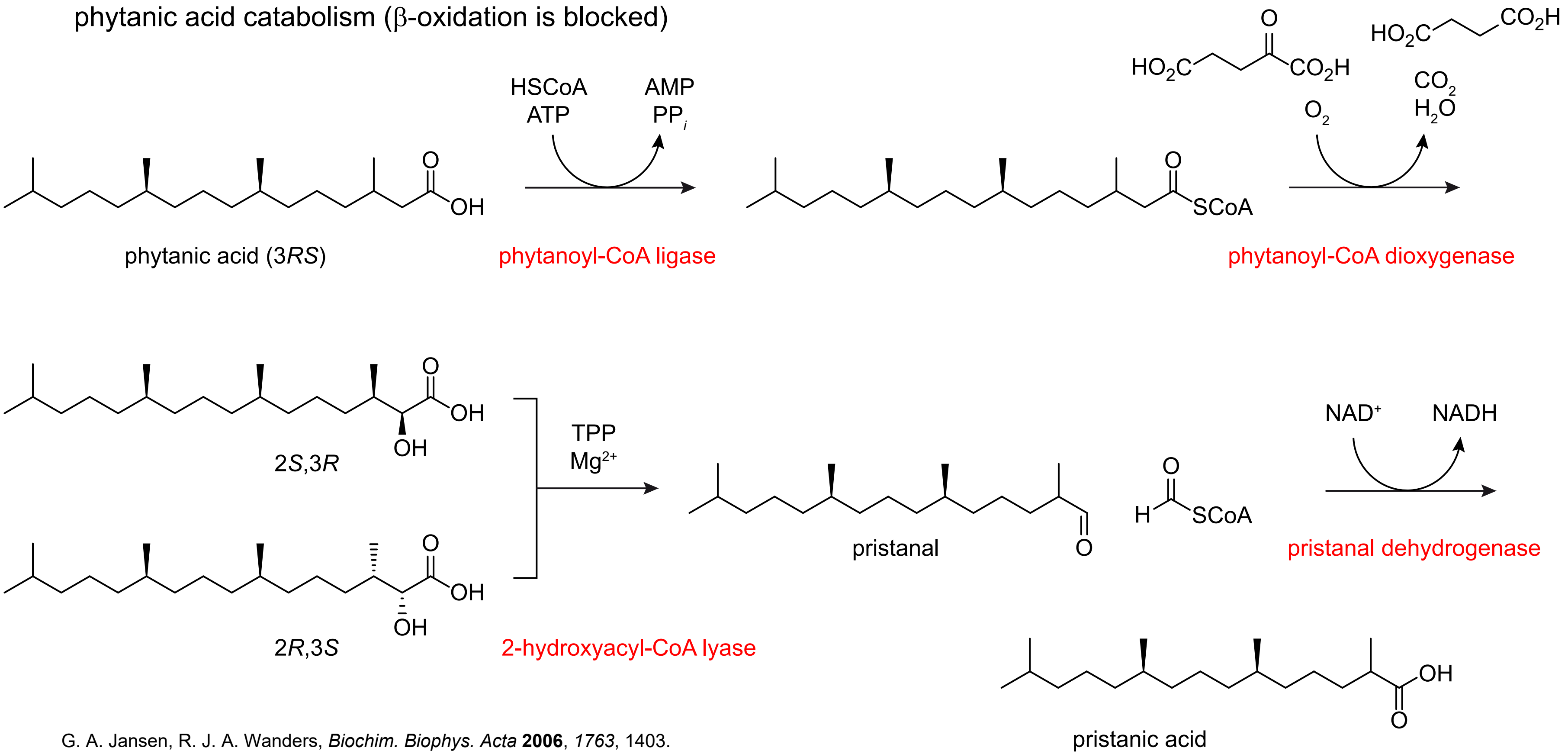
fatty acid degradation by one carbon



1.5 Fatty acid catabolism

α-Oxidation

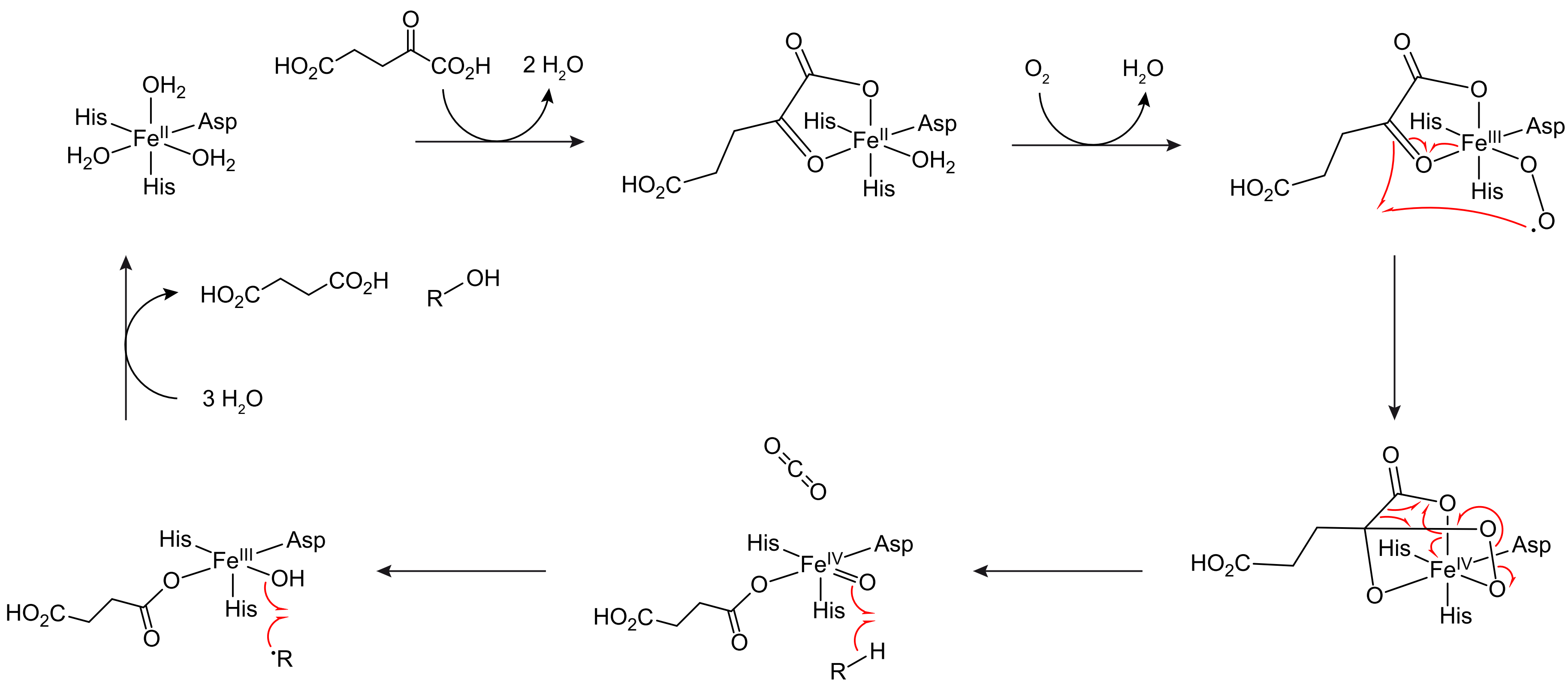
phytanic acid catabolism (β-oxidation is blocked)



G. A. Jansen, R. J. A. Wanders, *Biochim. Biophys. Acta* **2006**, *1763*, 1403.

1.5 Fatty acid catabolism

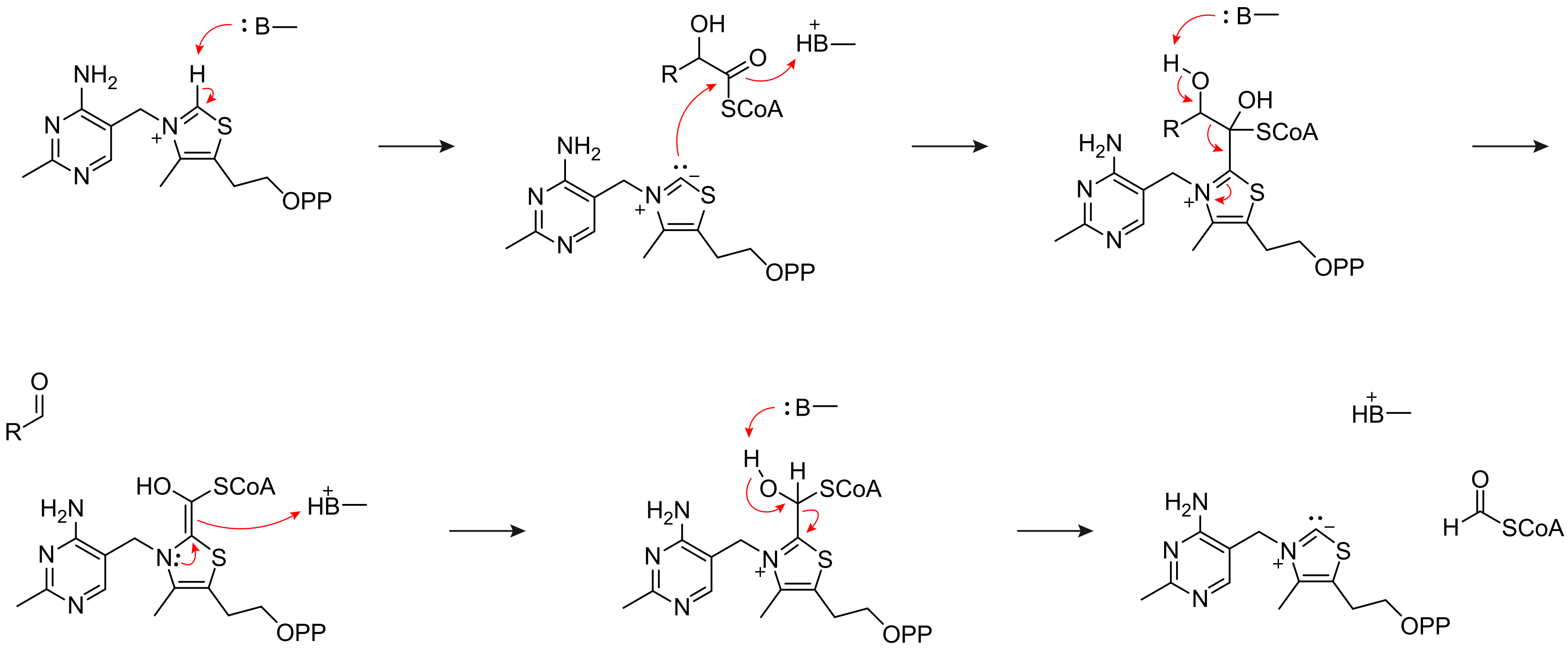
Mechanism of phytanoyl-CoA dioxygenase, a 2-ketoglutarate-dependent enzyme



S. Martinez, R. P. Hausinger, *J. Biol. Chem.* **2015**, *290*, 20702.

1.5 Fatty acid catabolism

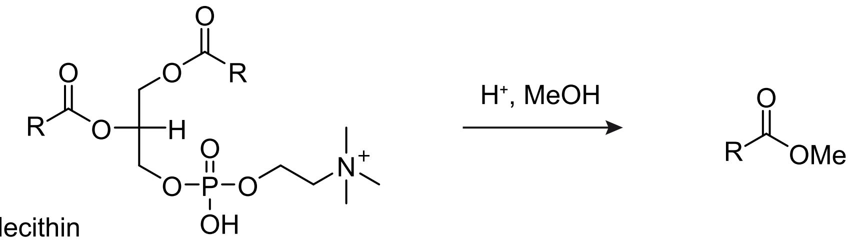
Mechanism of 2-hydroxyacyl-CoA lyase



V. Foulon, M. Sniekers, E. Huysmans, S. Asselberghs, V. Mahieu, G. P. Mannaerts, P. P. van Veldhoven, M. Casteels, *J. Biol. Chem.* **2005**, *280*, 9802.

1.6 Analysis of lipids

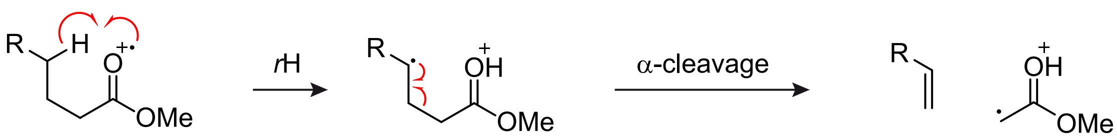
most suitable: GC/MS (after derivatisation)



lecithin

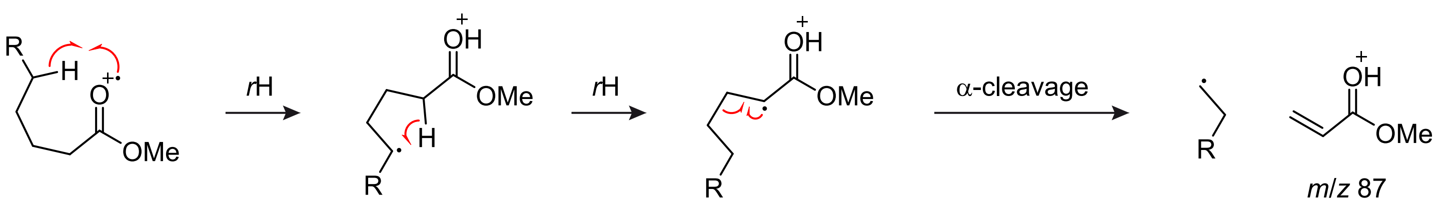
fatty acid methyl esters (FAMEs)

characteristic fragment ions



m/*z* 74

McLafferty rearrangement



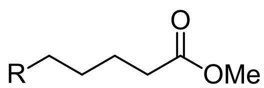
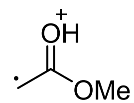
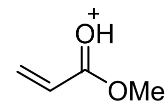
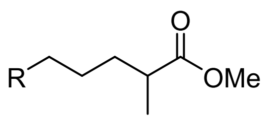
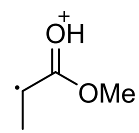
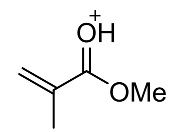
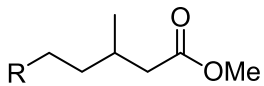
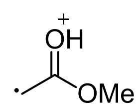
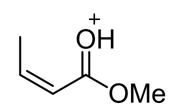
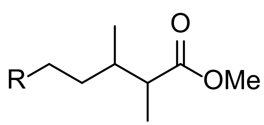
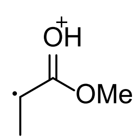
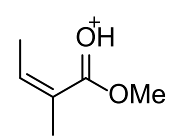
m/*z* 87

β-cleavage

J. S. Dickschat, *Nat. Prod. Rep.* **2014**, *31*, 838.

1.6 Analysis of lipids

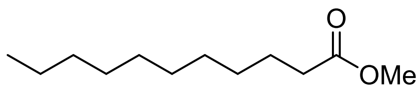
identification of Me-branched FAMEs

type	McLafferty	β-cleavage	
 unbranched	 *m*/*z* 74	 *m*/*z* 87	
 α-Me-branched	 *m*/*z* 88	 *m*/*z* 101	
 β-Me-branched	 *m*/*z* 74	 *m*/*z* 101	rare, biosynthesis needs α-oxidation
 α- and β-Me-branched	 *m*/*z* 88	 *m*/*z* 115	very rare

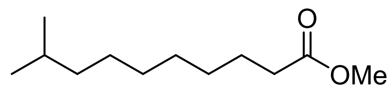
1. Fatty acids and derivatives

1.6 Analysis of lipids

Me branches near Me terminus



methyl undecanoate

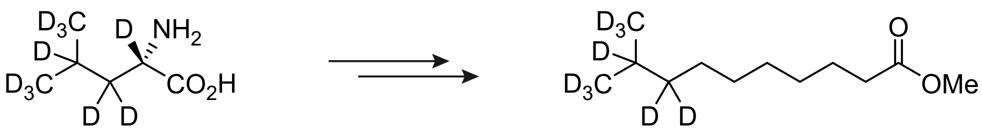


methyl 9-methyldecanoate

m/*z* 200 (unlabelled)

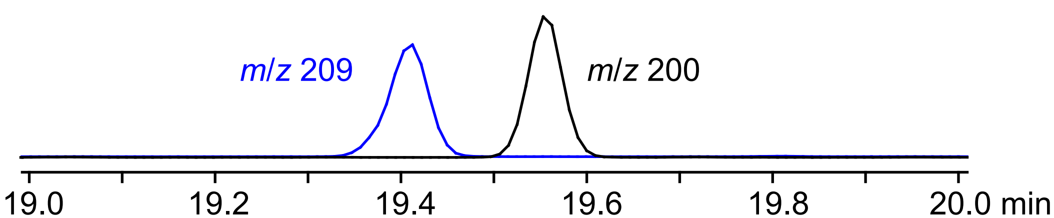
differentiation by MS difficult

feeding experiment with ($^2H_{10}$)leucine



m/*z* 209 (labelled)

gaschromatographic separation possible



deuterated compounds elute first

1.6 Analysis of lipids

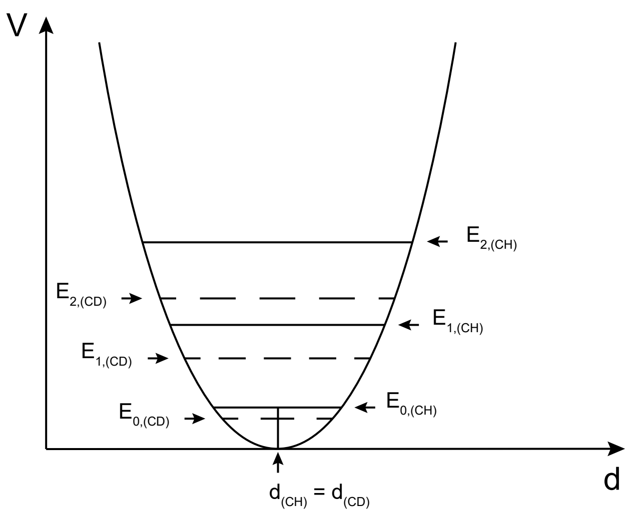
gaschromatographic separation of deuterated and non-deuterated compounds

$$E_n = \hbar\ \omega\ (n + 1/2) = \hbar \sqrt{\frac{k}{\mu}}\ (n + 1/2) = \hbar \sqrt{\frac{k\ m_1\ m_2}{m_1 + m_2}}\ (n + 1/2)$$

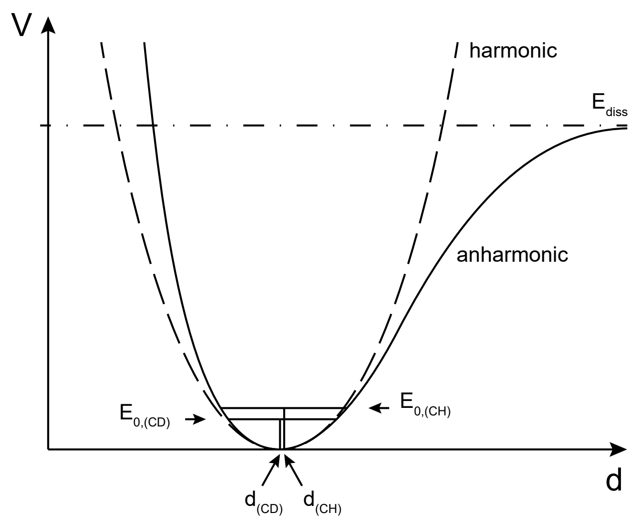
C-H $\quad \frac{1}{\mu} = \frac{m_1 + m_2}{m_1\ m_2} = \frac{13}{12} \approx 1{,}1$

C-D $\quad \frac{1}{\mu} = \frac{m_1 + m_2}{m_1\ m_2} = \frac{14}{24} \approx 0{,}6$

A) harmonic oscillator



B) anharmonic oscillator



C-D bond shorter

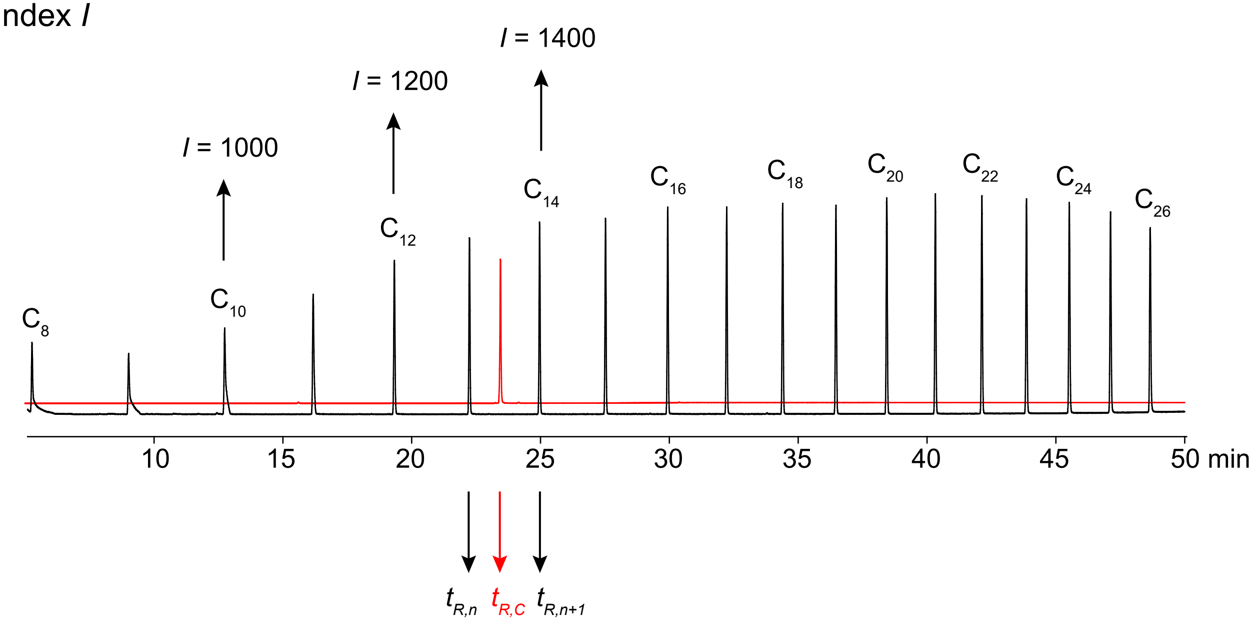
less polarisable

weaker interaction
with stationary phase

1. Fatty acids and derivatives

1.6 Analysis of lipids

Retention index *I*



$$I = 100 \times \frac{t_{R,C} - t_{R,n}}{t_{R,n+1} - t_{R,n}} + 100\,n$$

n: number of carbons of the n-alkane eluting before the analysed compound

1. Fatty acids and derivatives

1.6 Analysis of lipids

Retention index increment system

$I = 100\,n + FG + Me_i$

n	number of carbons in longest chain
FG	functional group increment
Me_i	increment for Me branch depending on position i

FG can be determined from *I* of unbranched compounds

$I = 1322 = 1000 + 322$

$I = 1421 = 1100 + 321$

$I = 1521 = 1200 + 321 \longrightarrow FG(ME) = 321$

1.6 Analysis of lipids

Retention index increment system

$I = 100\,n + FG + Me_i$

n	number of carbons in longest chain
FG	functional group increment
Me_i	increment for Me branch depending on position i

Me_i can then be determined from I of branched compounds

$I = 1384 = 1000 + 321 + 63$

$I = 1491 = 1100 + 321 + 70$

$I = 1556 = 1200 + 321 + 35$

$I = 1572 = 1200 + 321 + 51$

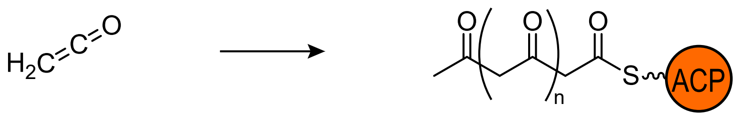
$\longrightarrow$

i	Me_i
α	35
γ	51
ω-2	70
ω-1	63

2. Polyketides

2.1 Definition

acetic acid derived natural products, formally „polymers of ketene"



2.2 Polyketide synthases (PKS)

2.2.1 Type I PKS

megasynthases (multidomain enzymes, cf. FAS from plants and fungi)

a) iterative (iPKS) - mostly fungal, but also known from bacteria

aa) non-reducing (NR-iPKS)

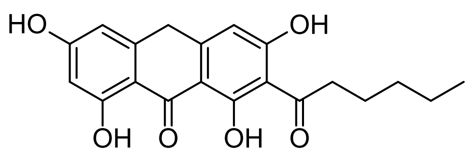
ab) partially reducing (PR-iPKS)

ac) highly reducing (HR-iPKS)

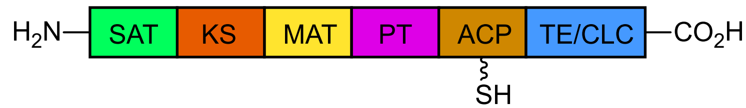
b) modular PKS - bacterial

2.2.1 Type I PKS

aa) non-reducing iterative PKS (NR-iPKS)



norsolorinic acid anthrone (*Aspergillus flavus*)

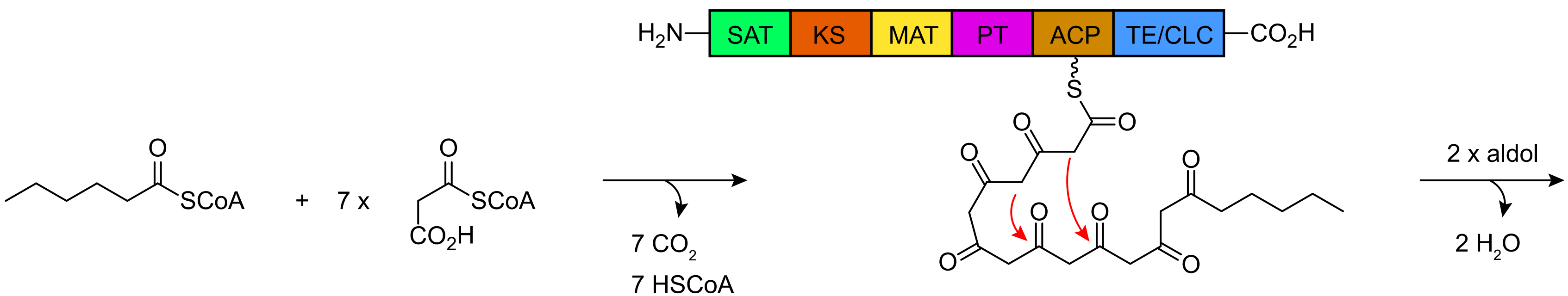


SAT: starter unit:ACP transacylase

MAT: malonyl-CoA:ACP transacylase

PT: product template (folds and protects growing polyketide chain)

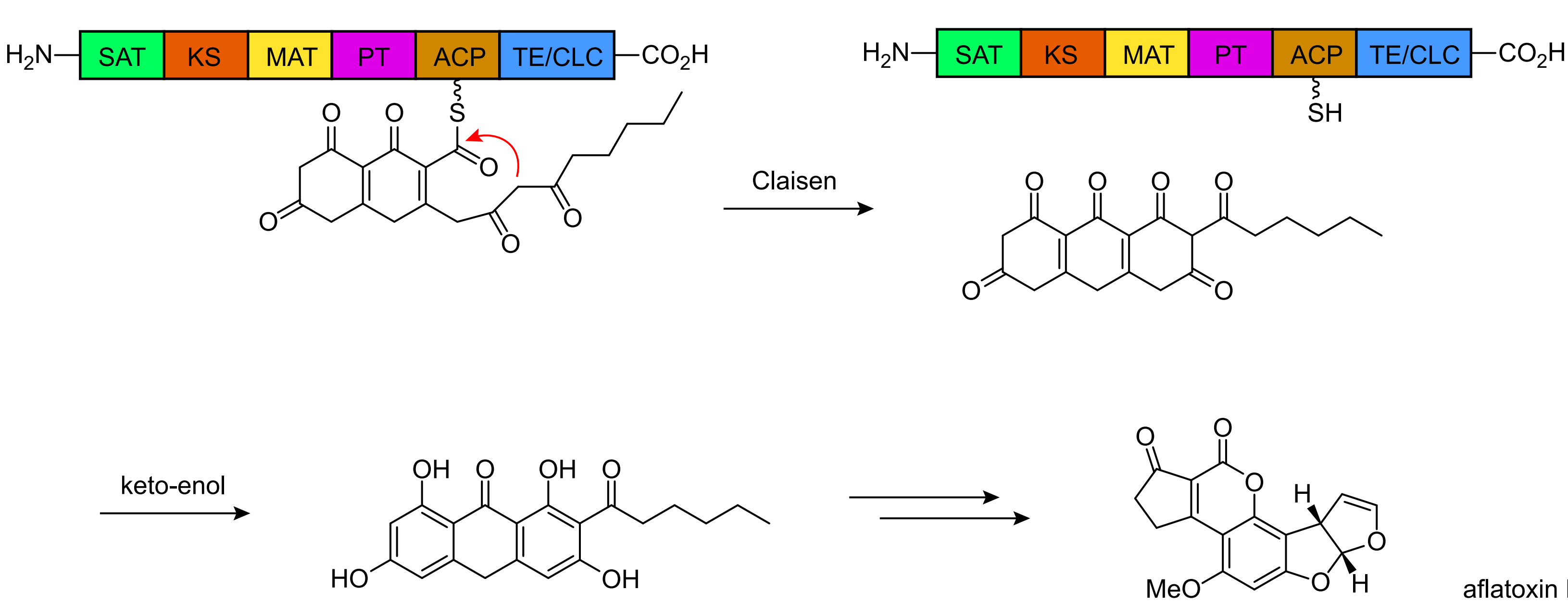
TE/CLC: thioesterase/Claisen condensation domain



J. M. Crawford, T. P. Korman, J. W. Labonte, A. L. Vagstad, E. A. Hill, O. Kamari-Bidkorpeh, S.-C. Tsai, C. A. Townsend, *Nature* **2009**, *461*, 1139.

2.2.1 Type I PKS

aa) non-reducing iterative PKS (NR-iPKS)



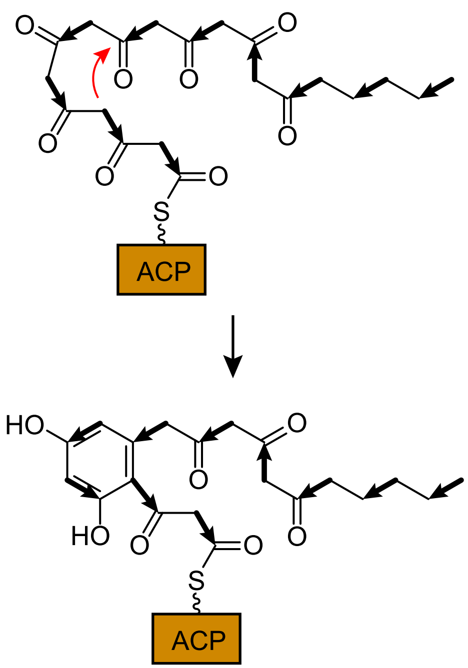
aflatoxin B_1

J. M. Crawford, T. P. Korman, J. W. Labonte, A. L. Vagstad, E. A. Hill, O. Kamari-Bidkorpeh, S.-C. Tsai, C. A. Townsend, *Nature* **2009**, *461*, 1139.

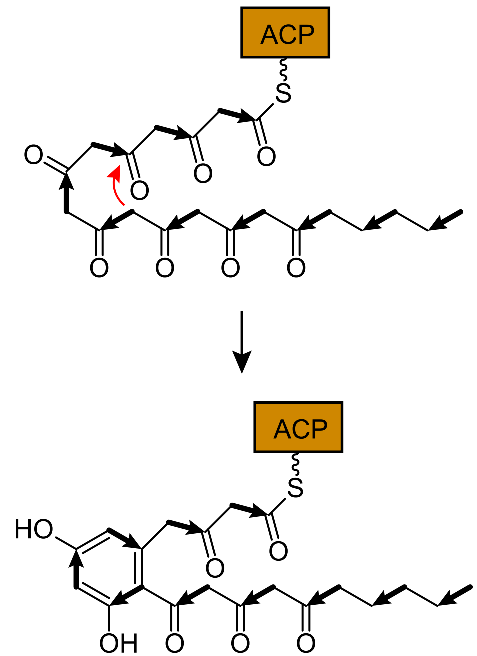
2.2.1 Type I PKS

aa) non-reducing iterative PKS (NR-iPKS)

fungal type (F-type)
first ring from 2 full and 2 half acetate units



Streptomyces type (S-type)
first ring from 3 full acetate units



exceptions known!

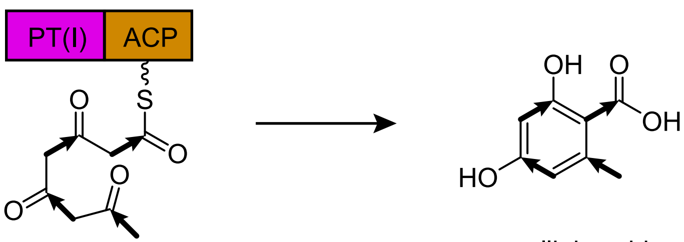
R. Thomas, *ChemBioChem* **2001**, 2, 612.

2. Polyketides

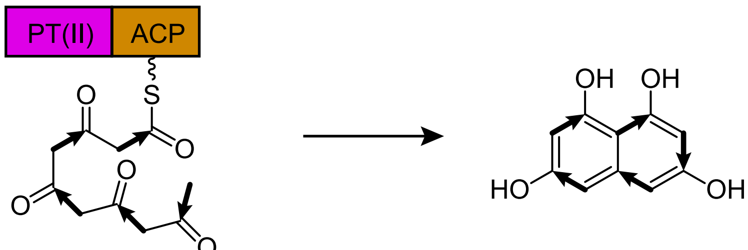
2.2.1 Type I PKS

aa) non-reducing iterative PKS (NR-iPKS)

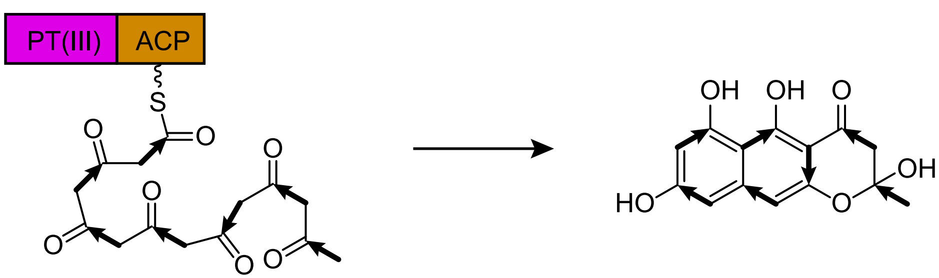
cyclisation mode is determined by PT domain
phylogenetic analysis reveals five different PT types



orsellinic acid



1,3,6,8-tetrahydroxynapthalene



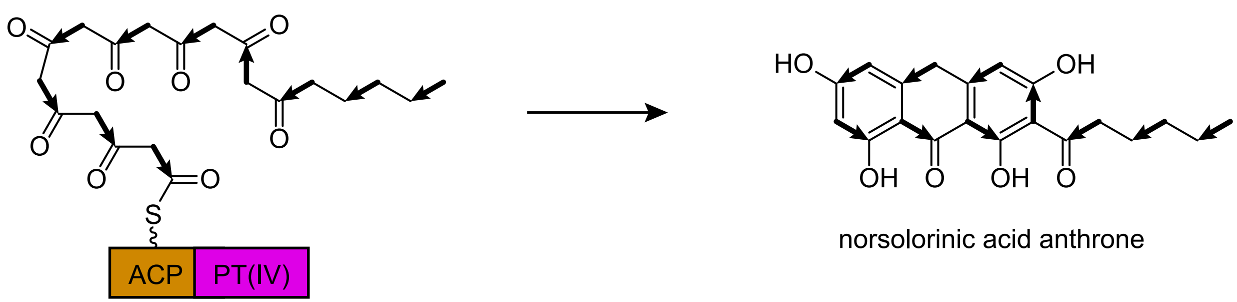
2,3-dihydro-4*H*-benzo[g]chromen-4-one

Y. Li, W. Xu, Y. Tang, *J. Biol. Chem.* **2010**, *285*, 22764.

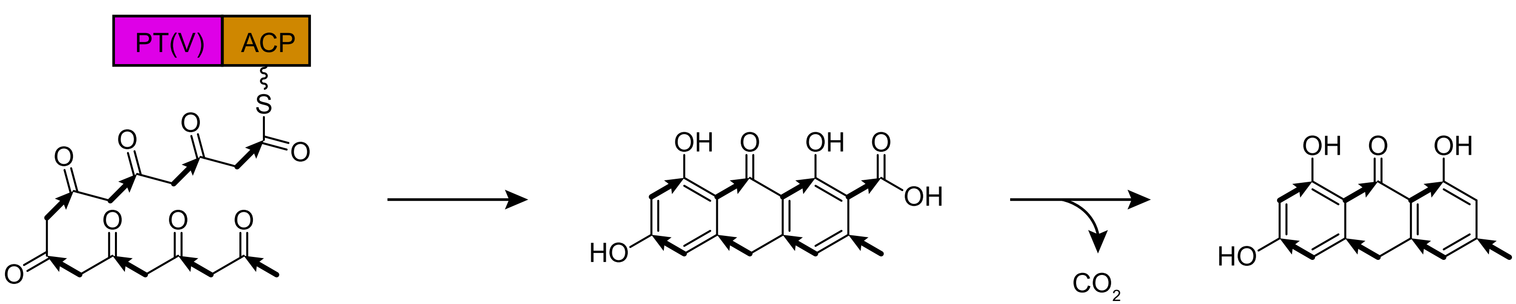
2.2.1 Type I PKS

aa) non-reducing iterative PKS (NR-iPKS)

cyclisation mode is determined by PT domain
phylogenetic analysis reveals different PT types



norsolorinic acid anthrone

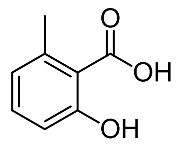


emodin anthrone

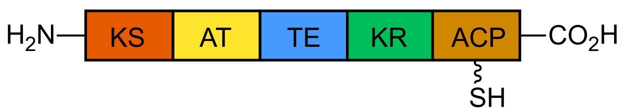
Y. Li, W. Xu, Y. Tang, *J. Biol. Chem.* **2010**, *285*, 22764.

2.2.1 Type I PKS

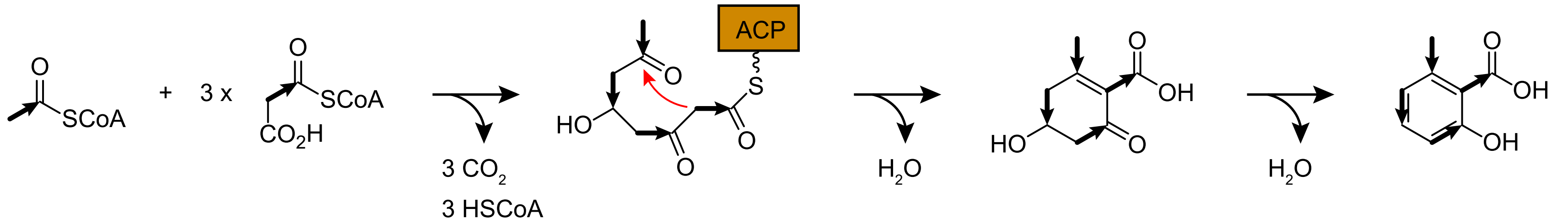
ab) partially reducing iterative PKS (PR-iPKS)



6-methylsalicylic acid



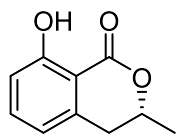
6-methylsalicylic acid synthase (6-MSAS)



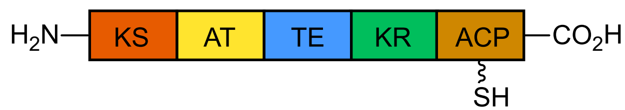
2. Polyketides

2.2.1 Type I PKS

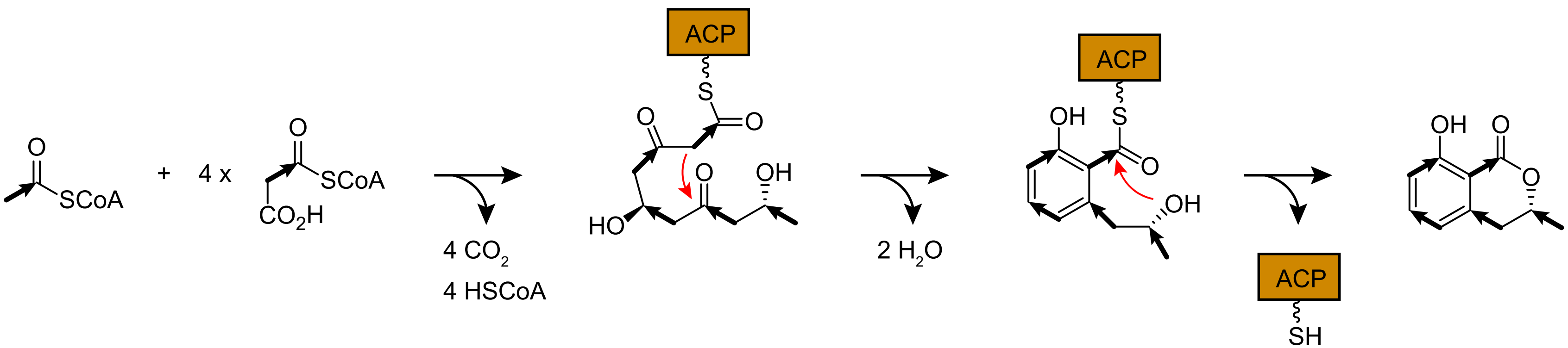
ab) partially reducing iterative PKS (PR-iPKS)



(R)-mellein



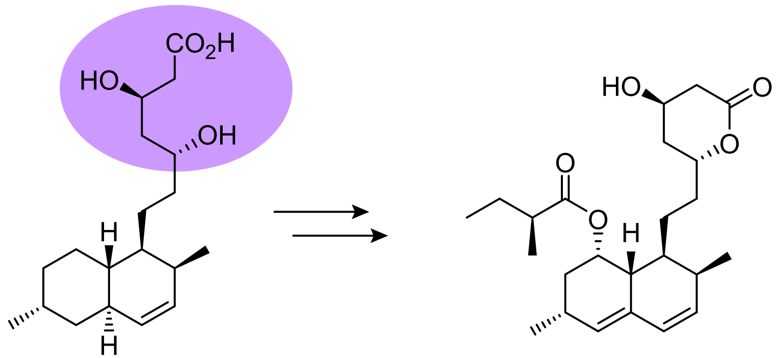
mellein synthase (*Saccharopolyspora erythraea*)



H. Sun, C. L. Ho, F. Ding, I. Soehano, X.-W. Liu, Z.-X. Liang, *J. Am. Chem. Soc.* **2012**, *134*, 11924.

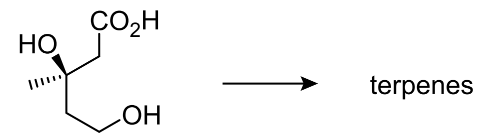
2.2.1 Type I PKS

ac) highly reducing iterative PKS (PR-iPKS)



dihydromonacolinic acid

lovastatin



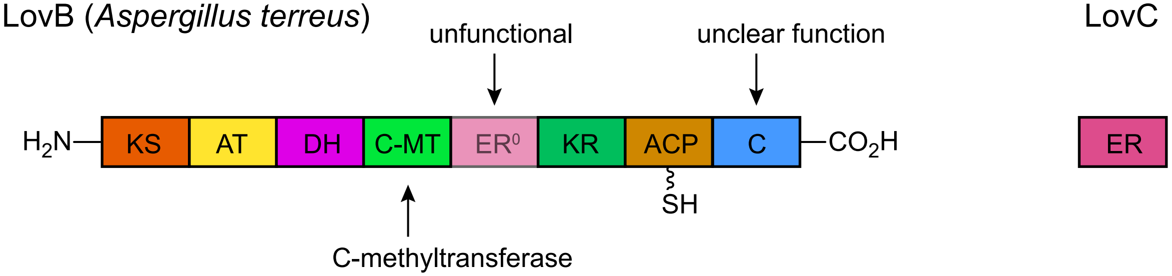
structurally related to mevalonic acid

statin (cholesterol-lowering drug)

inhibitor of HMG-CoA reductase

LovB (*Aspergillus terreus*)

LovC

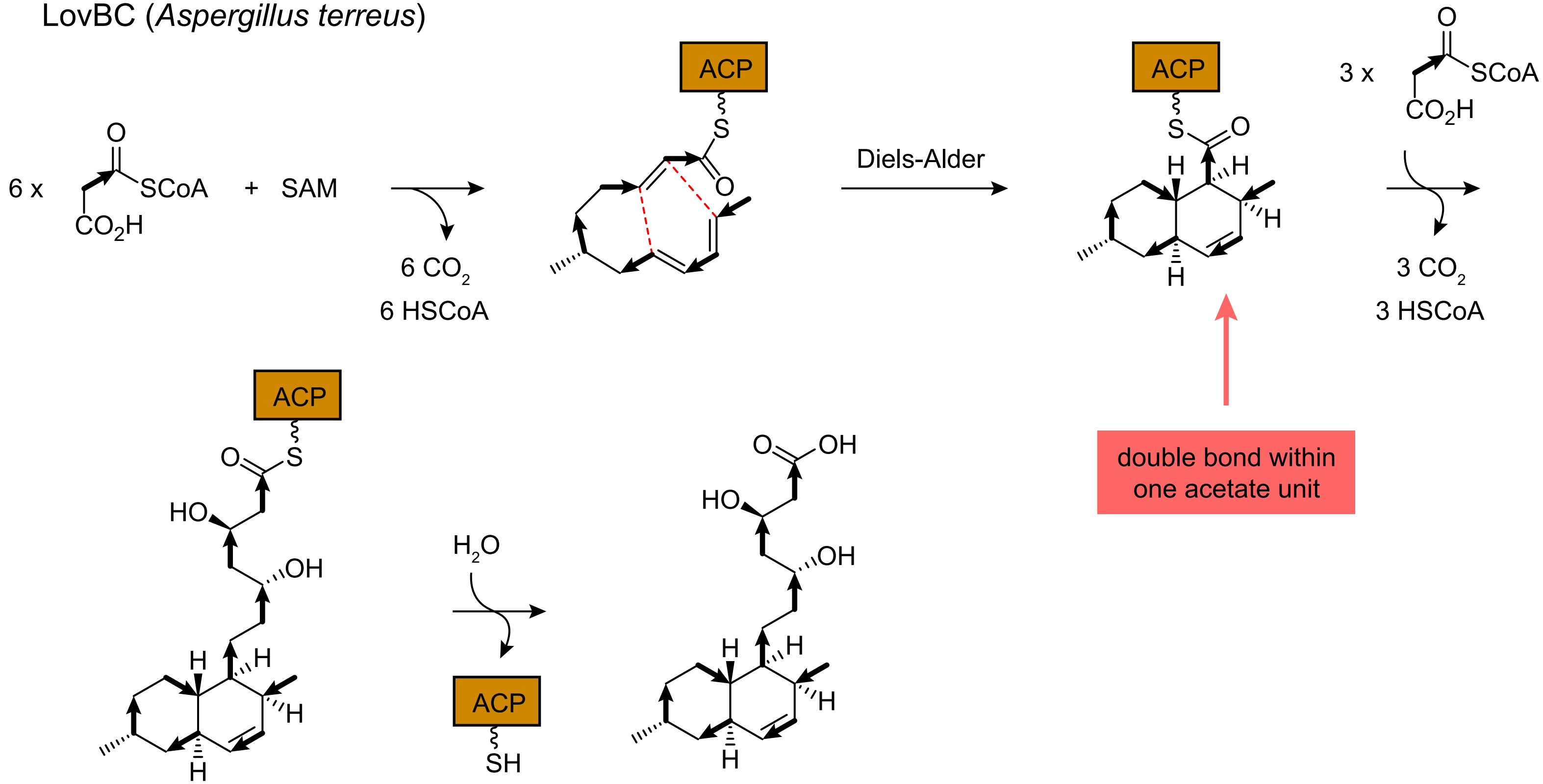


S. M. Ma, J. W. H. Li, J. W. Choi, H. Zhou, K. K. M. Lee, V. A. Moorthie, X. Xie, J. T. Kealey, N. A. Da Silva, J. C. Vederas, Y. Tang, *Science* **2009**, *326*, 589.

2.2.1 Type I PKS

ac) highly reducing iterative PKS (PR-iPKS)

LovBC (*Aspergillus terreus*)



S. M. Ma, J. W. H. Li, J. W. Choi, H. Zhou, K. K. M. Lee, V. A. Moorthie, X. Xie, J. T. Kealey, N. A. Da Silva, J. C. Vederas, Y. Tang, *Science* **2009**, *326*, 589.

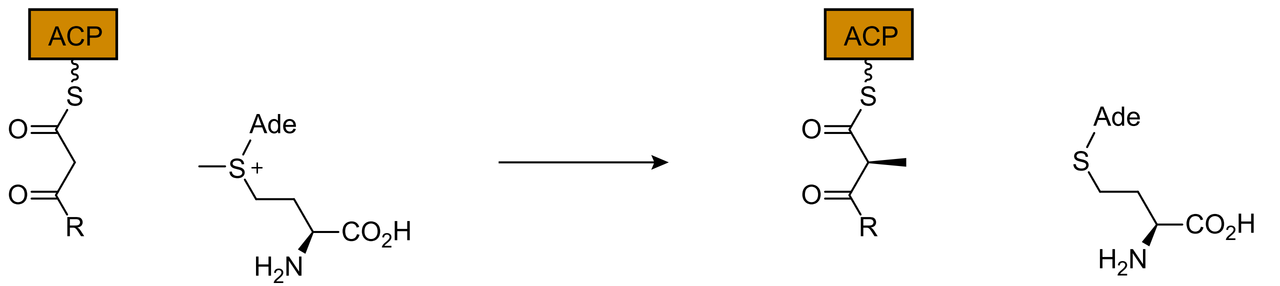
2. Polyketides

2.2.1 Type I PKS

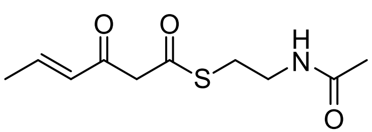
ac) highly reducing iterative PKS (PR-iPKS)

LovB - Timing of C-methyltransferase

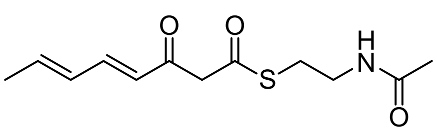
Me branches in iPKS are generally SAM-derived



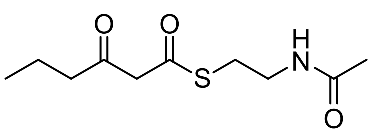
methylation of
β-keto intermediate



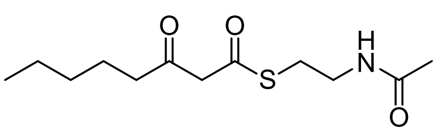
0.04%



100%



0.02%



0.3%

relative in vitro reactivity
of substrate analogs

R. A. Cacho, J. Thuss, W. Xu, R. Sanichar, Z. Gao, A. Nguyen, J. C. Vederas, Y. Tang, *J. Am. Chem. Soc.* **2015**, *137*, 15688.

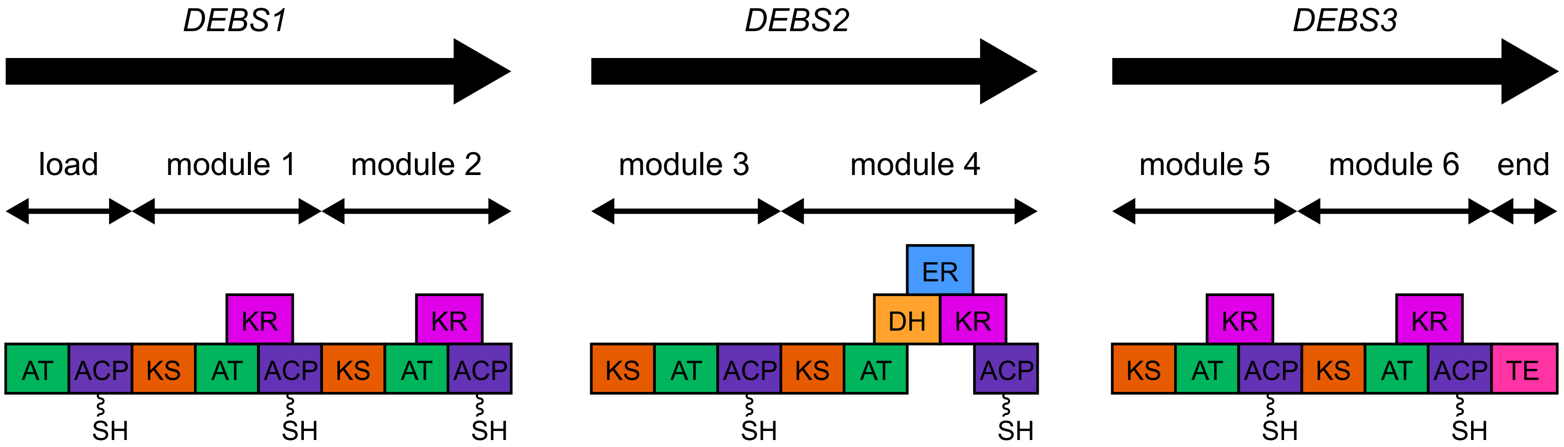
2.2.1 Type I PKS

b) modular PKS

widespread in bacteria

first example: erythromycin (*Saccharopolyspora erythraea*)

macrolide antibiotic, binds to the bacterial ribosome and inhibits protein biosynthesis



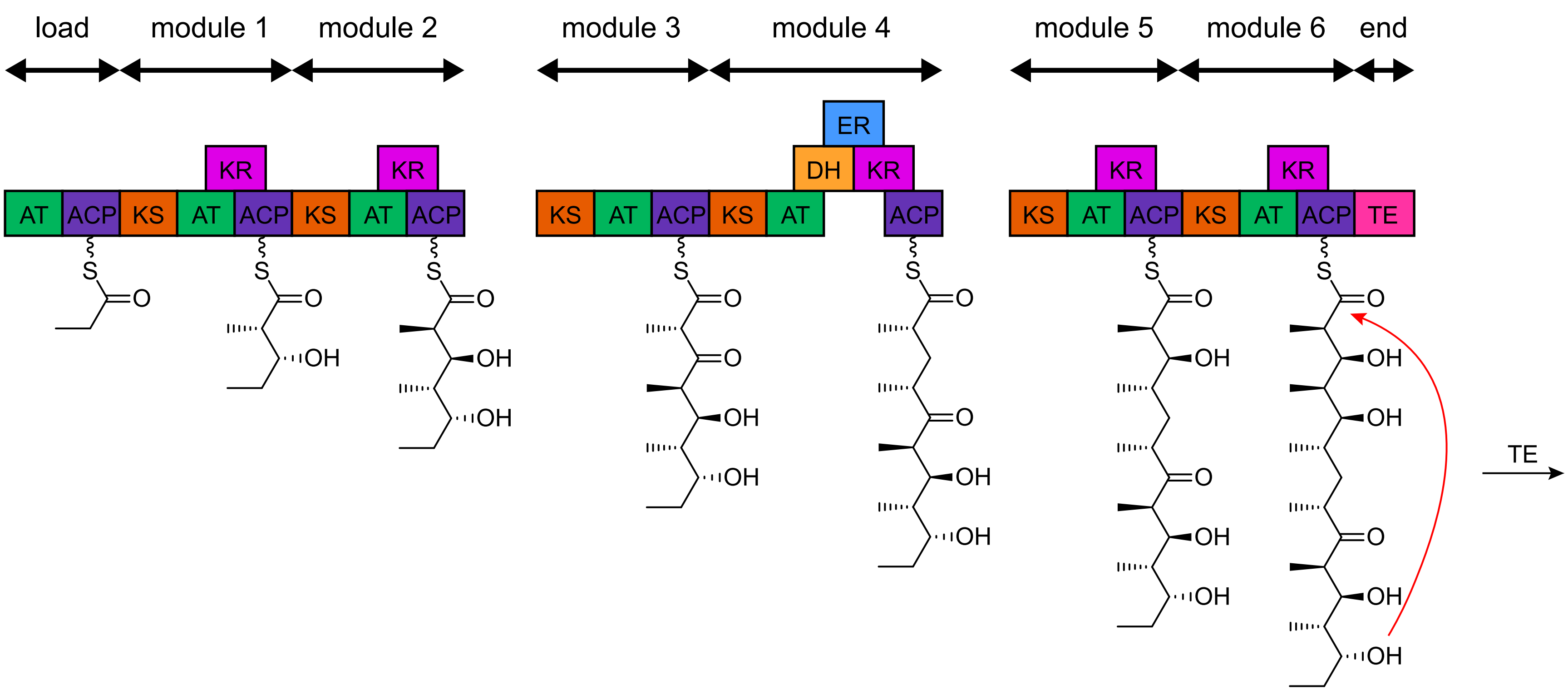
each module incorporates one unit, processing can be predicted from presence of optional domains

J. Cortez, S. F. Haydock, G. A. Roberts, D. J. Bevitt, P. F. Leadlay, *Nature* **1990**, *348*, 176.
S. Donadio, M. J. Staver, J. B. McAlpine, S. J. Swanson, L. Katz, *Science* **1991**, *252*, 675.

2.2.1 Type I PKS

b) modular PKS

erythromycin PKS (*Saccharopolyspora erythraea*)

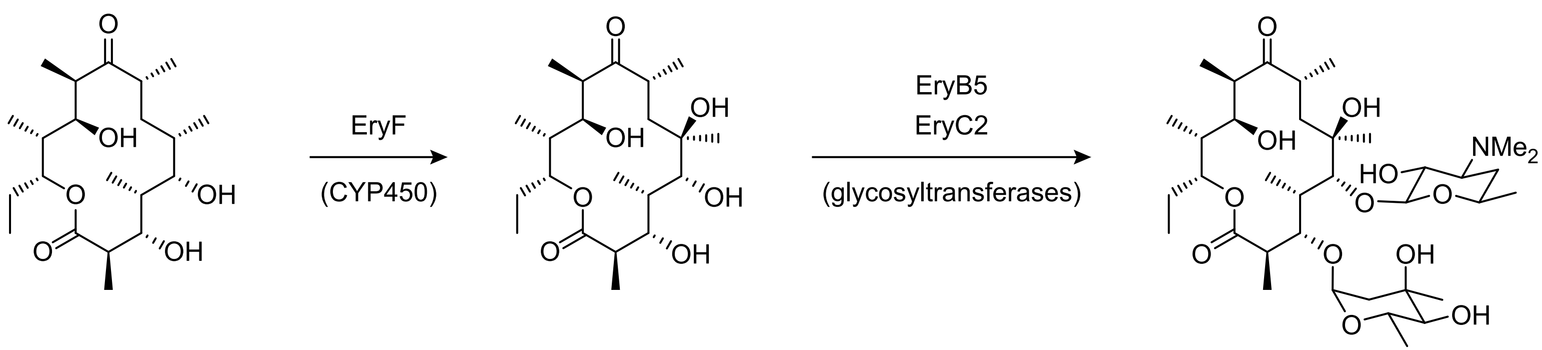


J. Cortez, S. F. Haydock, G. A. Roberts, D. J. Bevitt, P. F. Leadlay, *Nature* **1990**, *348*, 176.
S. Donadio, M. J. Staver, J. B. McAlpine, S. J. Swanson, L. Katz, *Science* **1991**, *252*, 675.

2.2.1 Type I PKS

b) modular PKS

tayloring steps of erythromycin biosynthesis



6-deoxyerythronolide B

erythronolide B

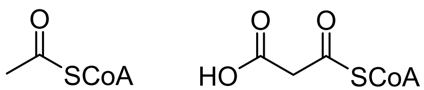
erythromycin D

J. M. Weber, J. O. Leung, S. J. Swanson, K. B. Idler, J. B. McAlpine, *Science* **1991**, *252*, 114.
J. Staunton, B. Wilkinson, *Chem. Rev.* **1997**, *97*, 2611.

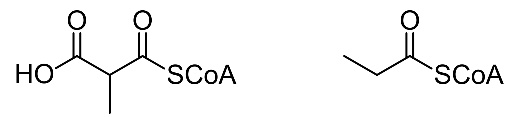
2.2.1 Type I PKS

b) modular PKS

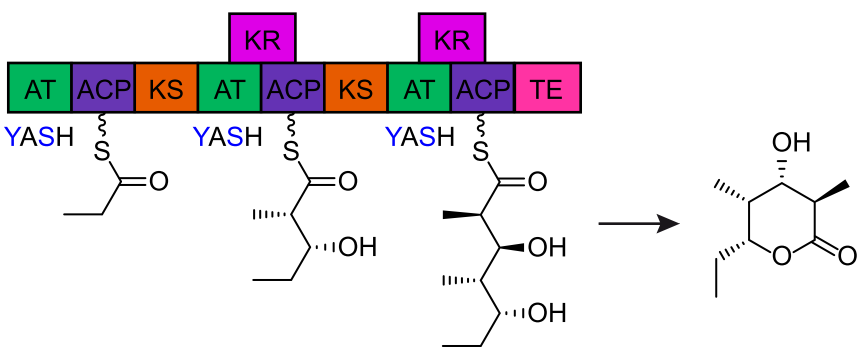
AT domains: substrate specificity indicated by highly conserved motifs



HAFH

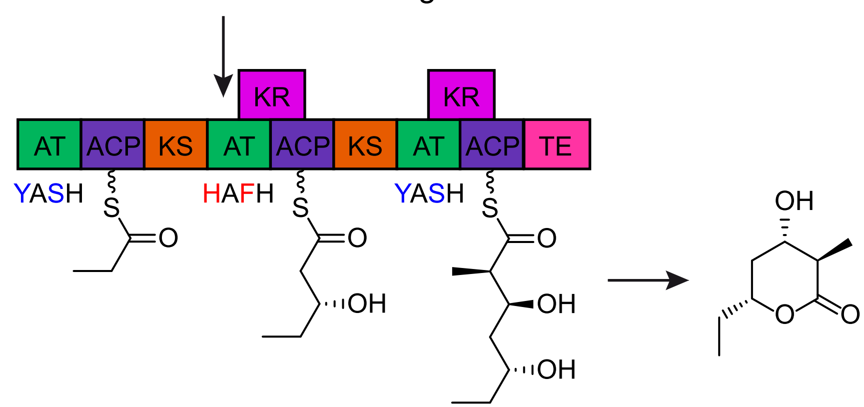


YASH



DEBS1-TE

site-directed mutagenesis



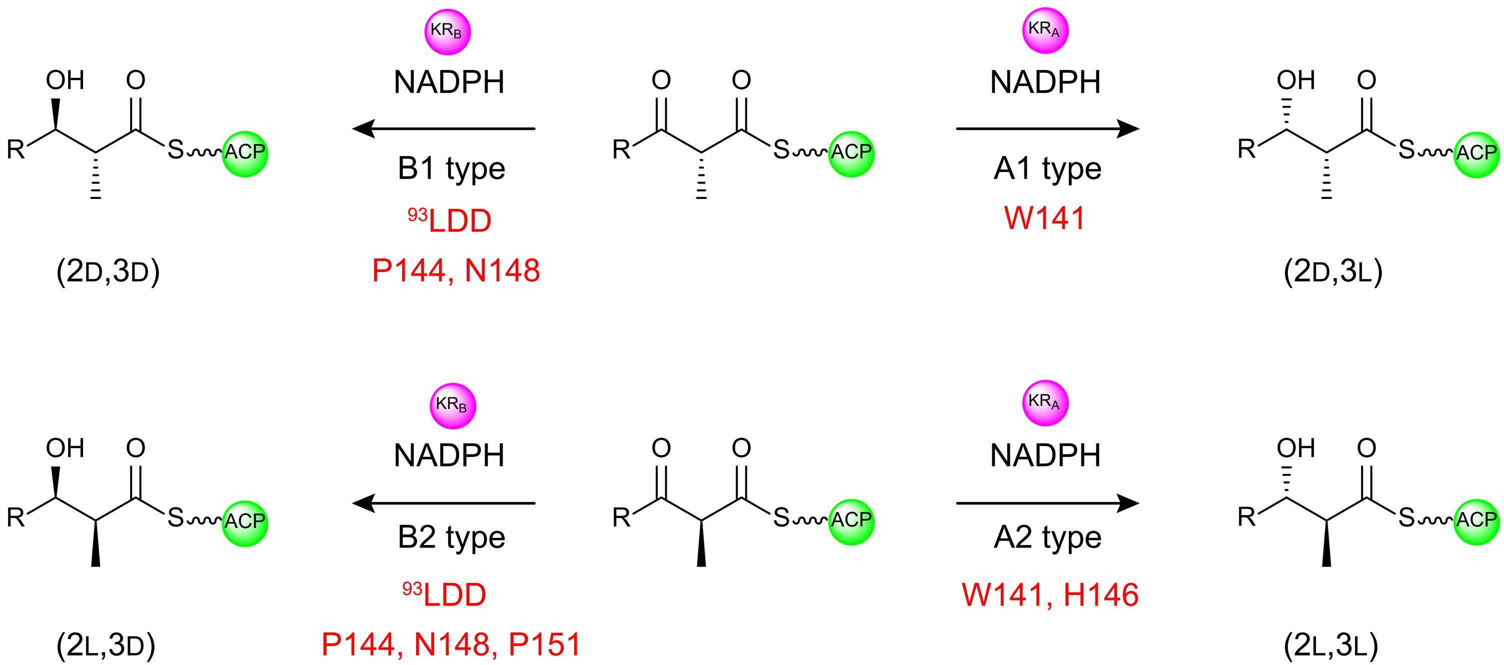
DEBS1-TE

F. Del Vecchio, H. Petkovic, S. G. Kendrew, L. Low, B. Wilkinson, R. Lill, J. Cortes, B. A. M. Rudd, J. Staunton, P. F. Leadlay, *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 489.

2.2.1 Type I PKS

b) modular PKS

KR domains: stereospecificity is indicated by highly conserved motifs

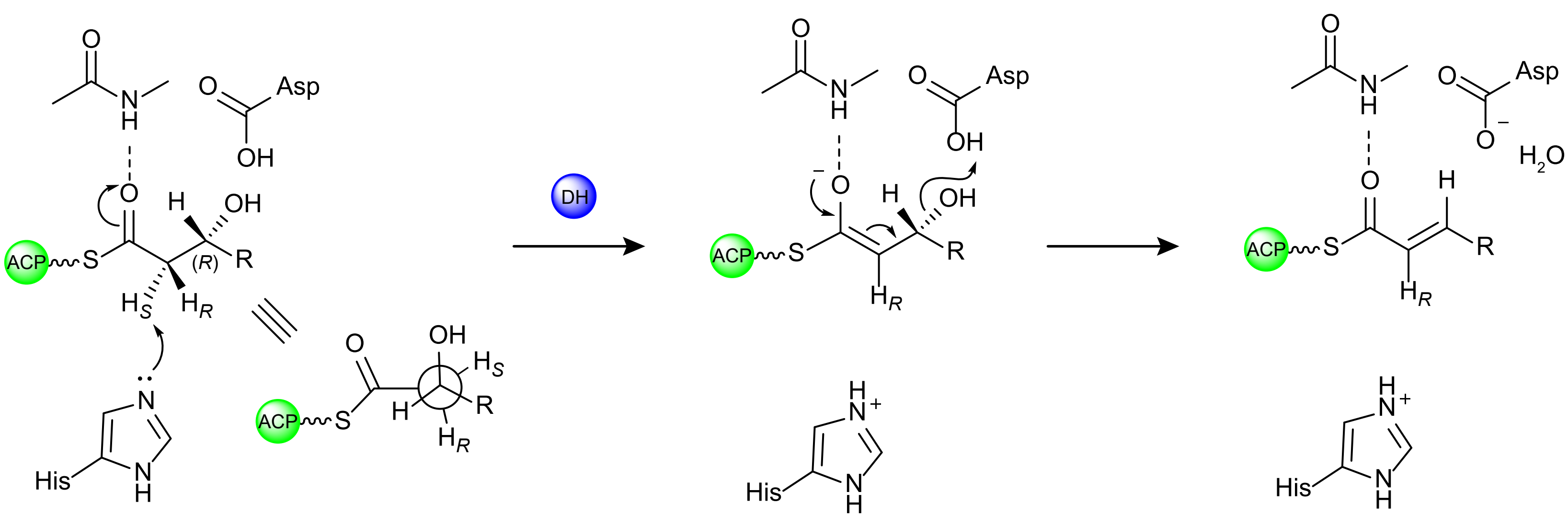


P. Caffrey, *ChemBioChem* **2003**, *4*, 654.

2.2.1 Type I PKS

b) modular PKS

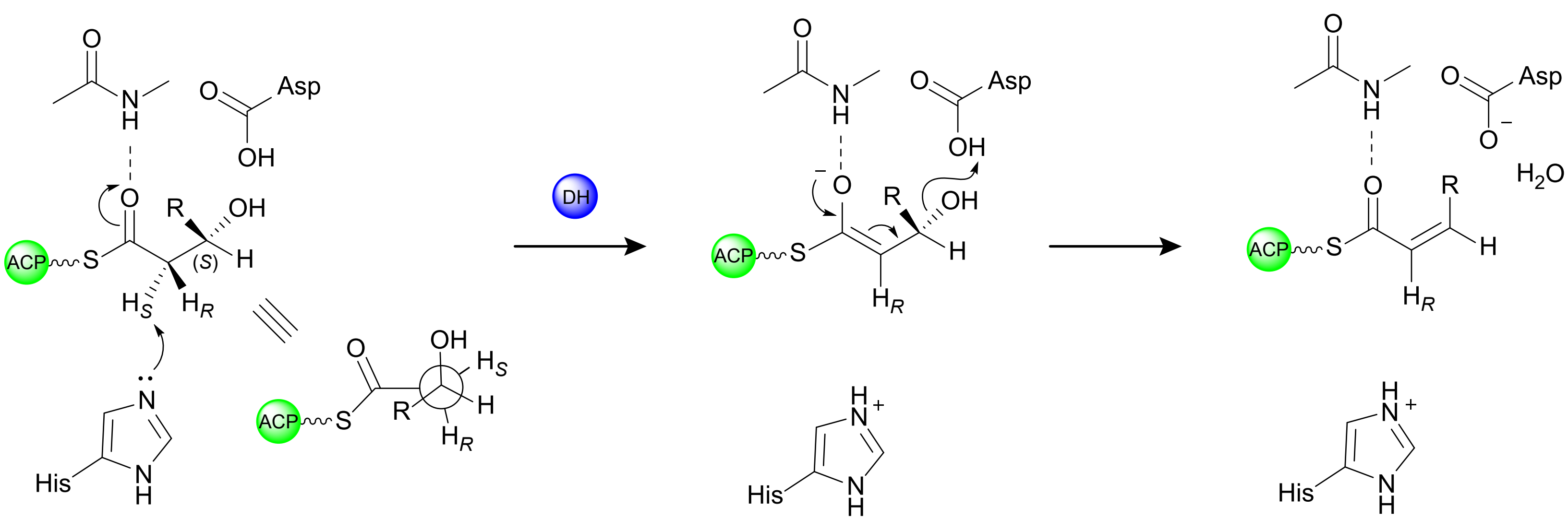
DH domains catalyse *syn* eliminations 3D alcohol ⟶ *E* alkene



2.2.1 Type I PKS

b) modular PKS

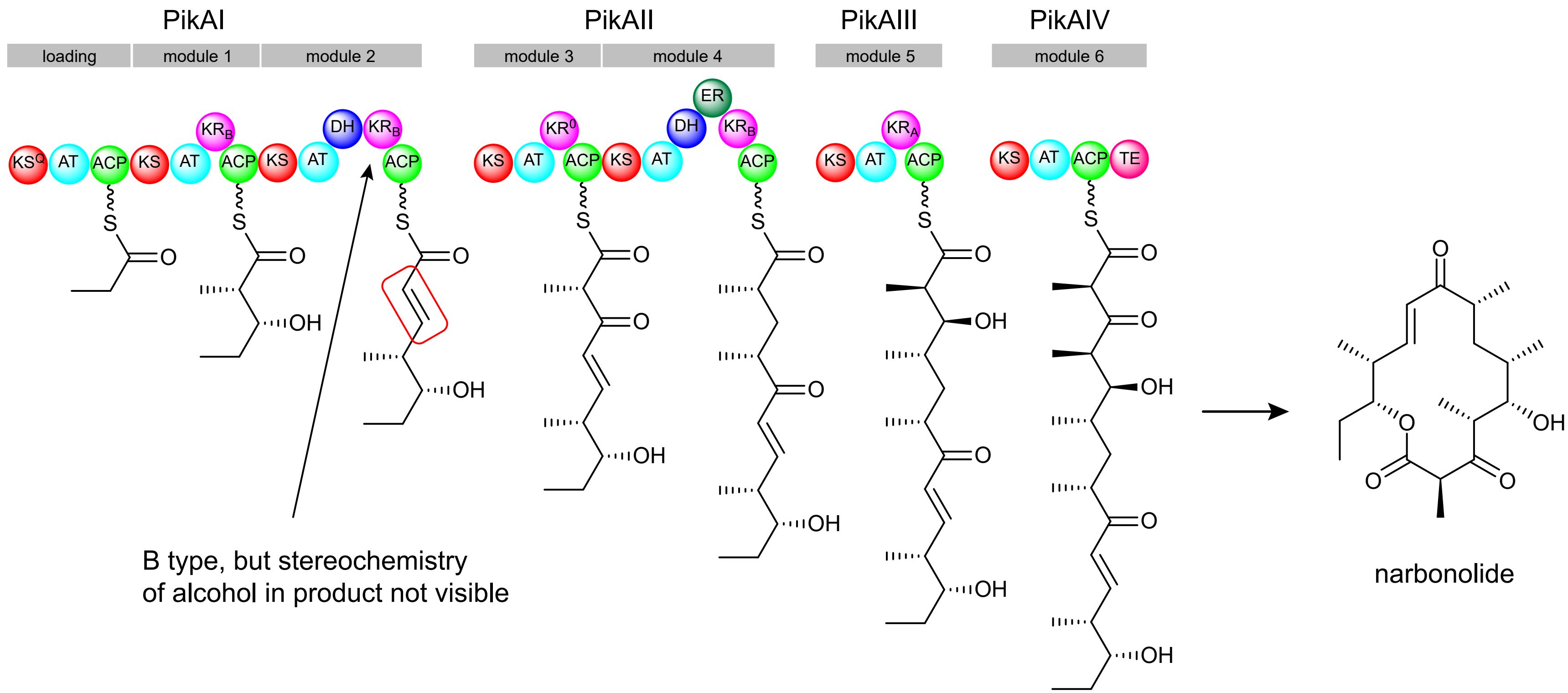
DH domains catalyse *syn* eliminations 3L alcohol ⟶ *Z* alkene (rare)



2.2.1 Type I PKS

b) modular PKS

DH domains: double bond formation by the pikromycin PKS

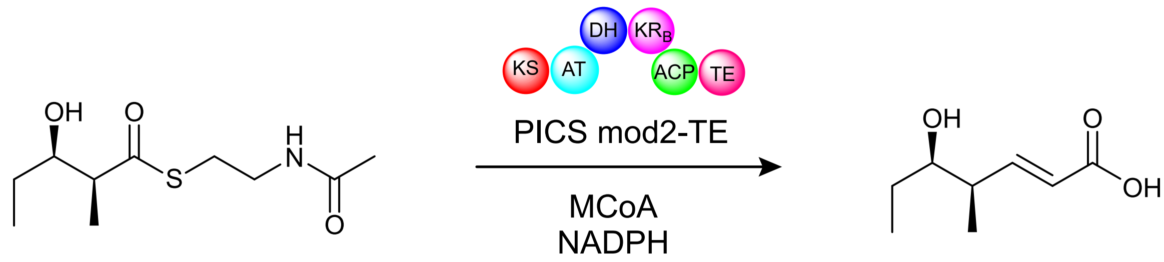


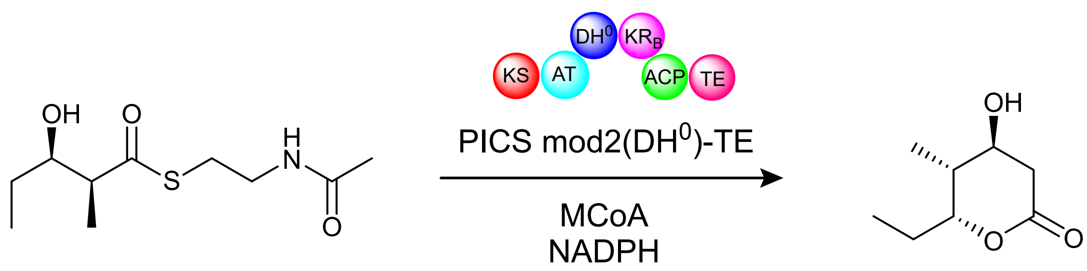
J. Wu, T. J. Zaleski, C. Valenzano, C. Khosla, D. E. Cane, *J. Am. Chem. Soc.* **2005**, *127*, 17393.

2.2.1 Type I PKS

b) modular PKS

DH domains: double bond formation by the pikromycin PKS (PICS)





inactivation of DH by
site-directed mutagenesis
(H3611F)

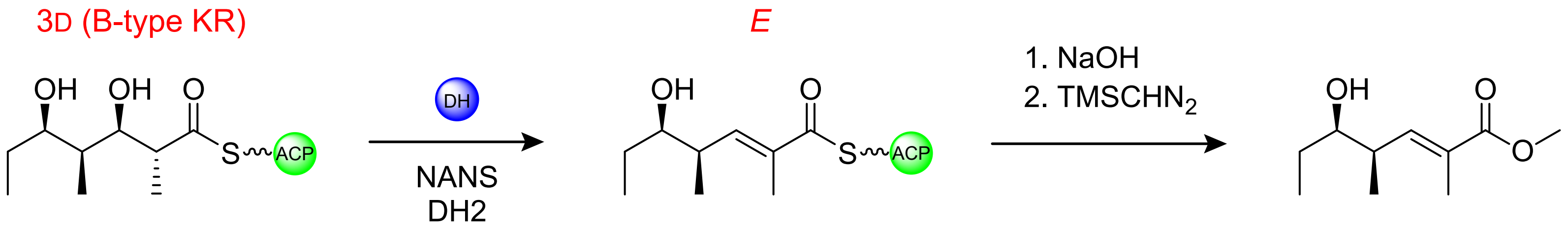
J. Wu, T. J. Zaleski, C. Valenzano, C. Khosla, D. E. Cane, *J. Am. Chem. Soc.* **2005**, *127*, 17393.

2.2.1 Type I PKS

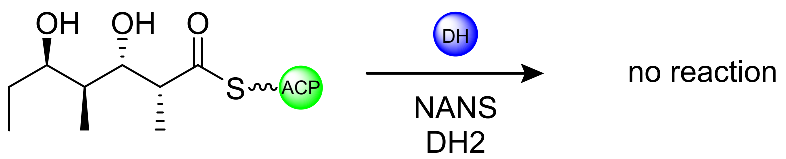
b) modular PKS

DH domains: DH2 of nanchangmycin PKS (NANS)

3D (B-type KR)



3L (A-type KR)

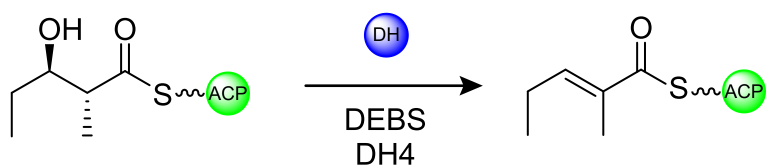


X. Guo, T. Liu, C. R. Valenzano, Z. Deng and D. E. Cane, *J. Am. Chem. Soc.* **2010**, *132*, 14694.

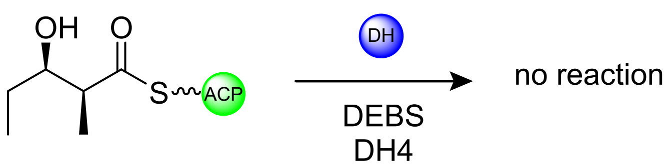
2.2.1 Type I PKS

b) modular PKS DH domains: DH4 of erythromycin PKS (DEBS)

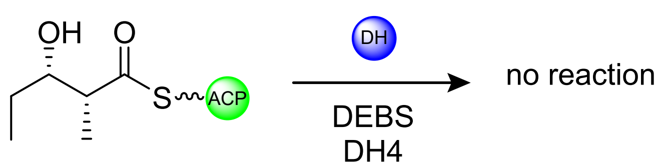
2D,3D (B1-type KR) *E*



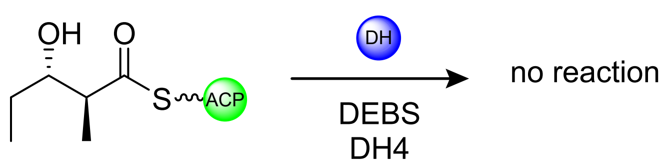
2L,3D (B2-type KR)



2D,3L (A1-type KR)



2L,3L (A2-type KR)

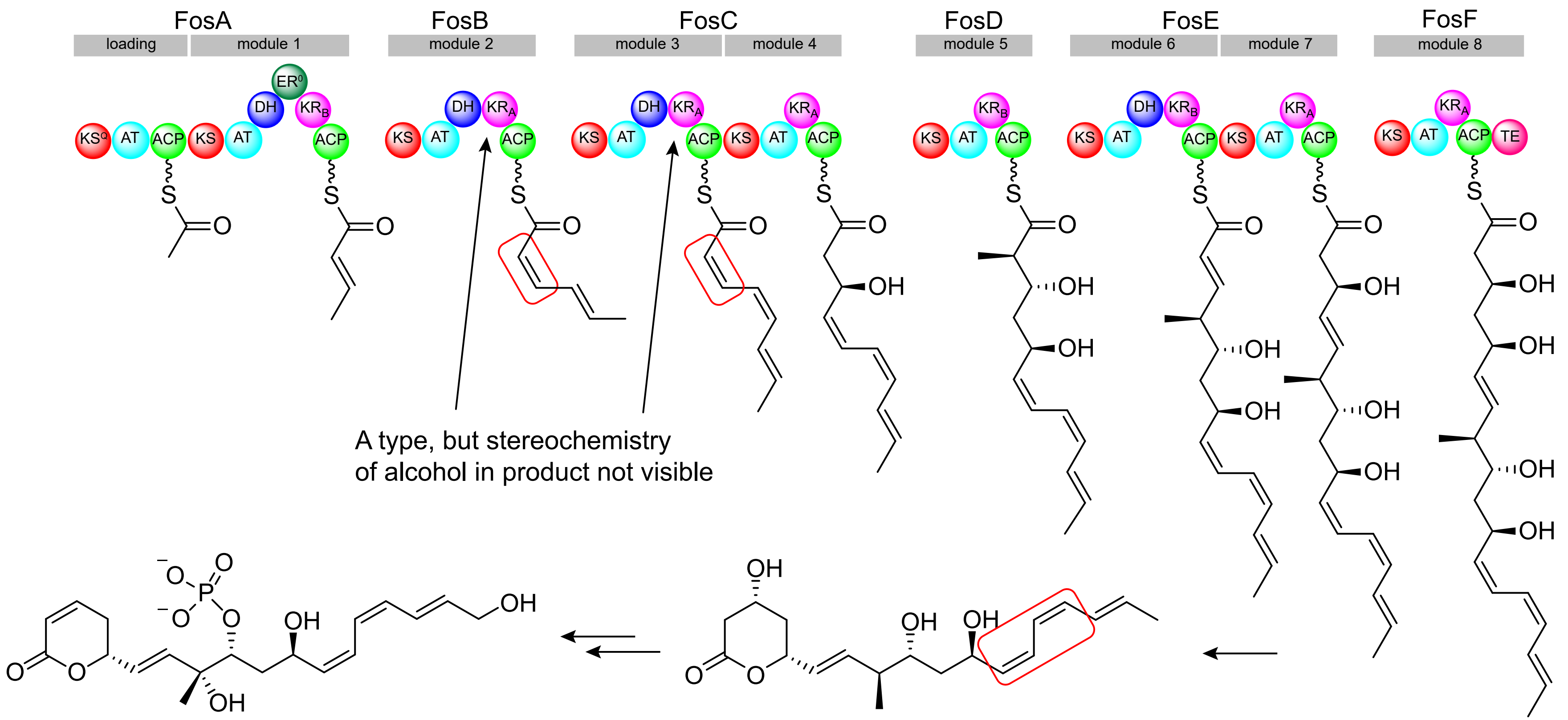


C. R. Valenzano, Y.-O. You, A. Garg, A. Keatinge-Clay, C. Khosla and D. E. Cane, *J. Am. Chem. Soc.* **2010**, *132*, 14697.

2.2.1 Type I PKS

b) modular PKS

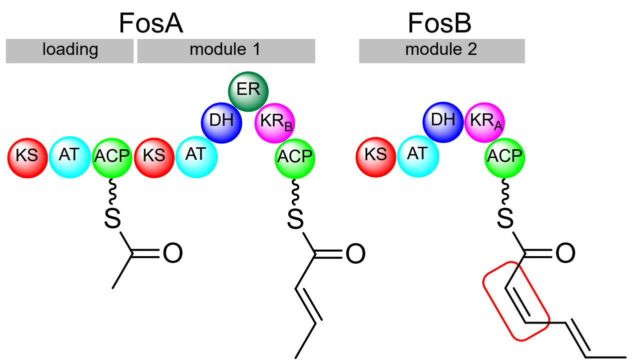
DH domains in fostriecin PKS: *Z* double bonds



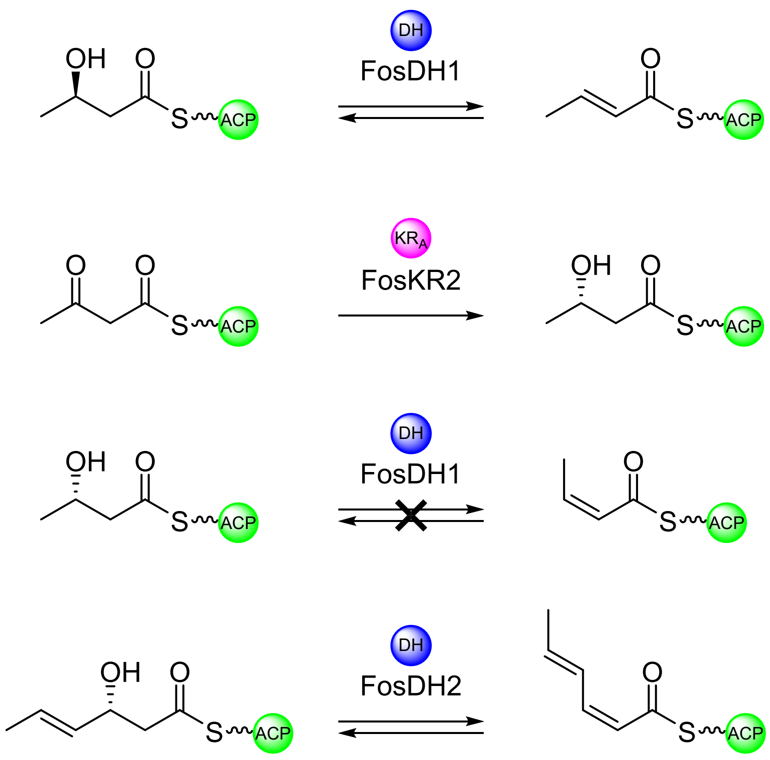
R. Kong, X. Liu, C. Su, C. Ma, R. Qiu and L. Tang, *Chem. Biol.* **2013**, *20*, 45.

2.2.1 Type I PKS

b) modular PKS



DH domains in fostriecin PKS: *Z* double bonds



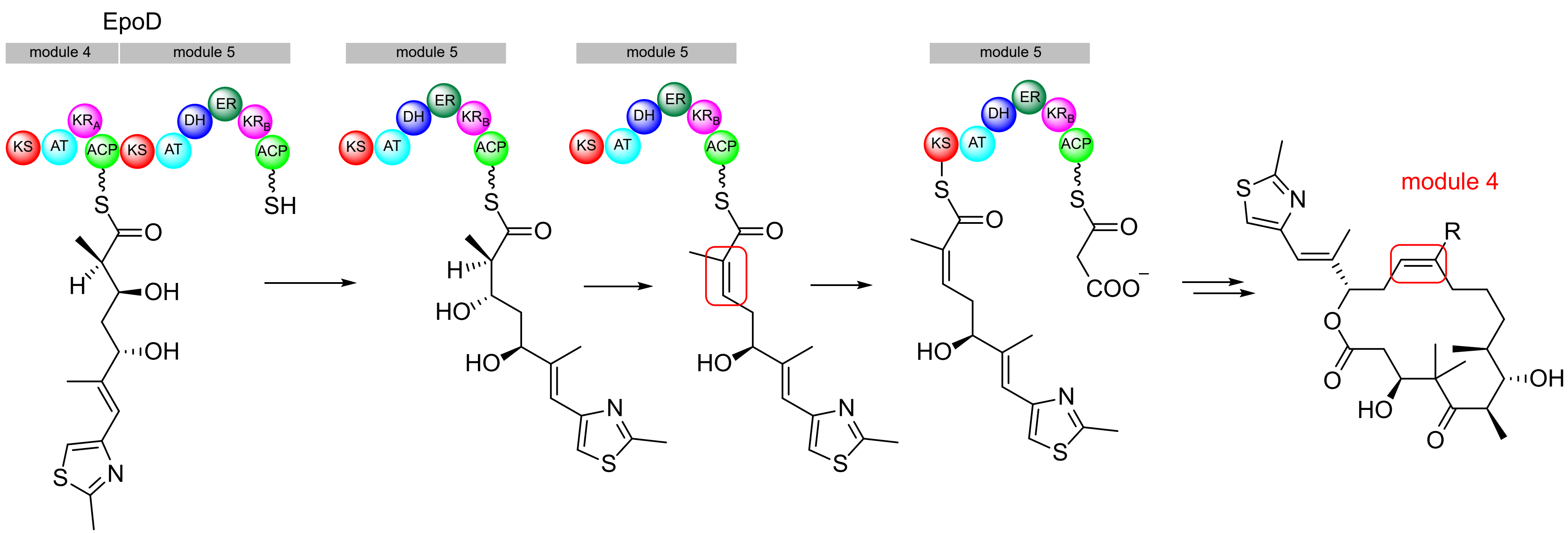
D. D. Shah, Y. O. You and D. E. Cane, *J. Am. Chem. Soc.* **2017**, *139*, 14322.

2. Polyketides

2.2.1 Type I PKS

b) modular PKS

Epothilon PKS: module stuttering mechanism



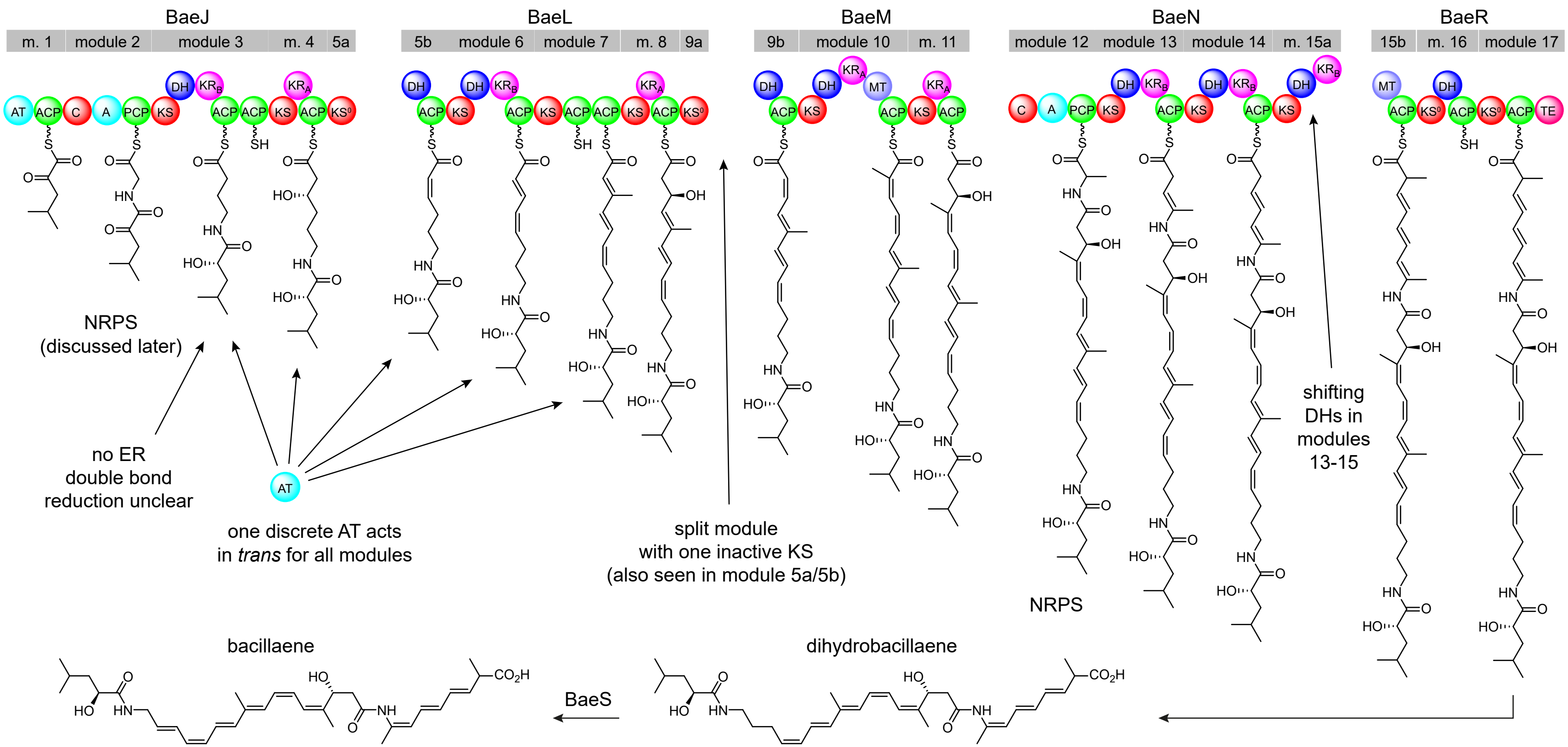
no DH in module 4

L. Tang, S. Shah, L. Chung, J. Carney, L. Katz, C. Khosla and B. Julien, *Science* **2000**, *287*, 640.
L. Tang, S. Ward, L. Chung, J. R. Carney, Y. Li, R. Reid and L. Katz, *J. Am. Chem. Soc.* **2004**, *126*, 46.

2. Polyketides

2.2.1 Type I PKS

b) modular PKS *trans*-AT PKS



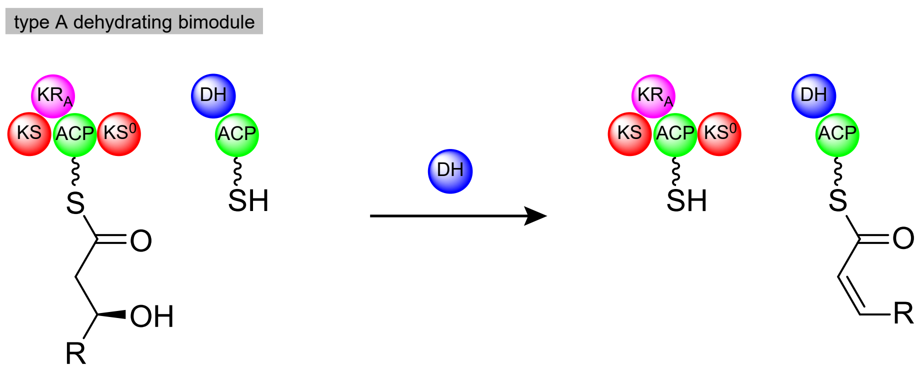
J. Moldenhauer, X. H. Chen, R. Borriss and J. Piel, *Angew. Chem. Int. Ed.* **2007**, *46*, 8195.
J. Moldenhauer, D. C. G. Götz, C. R. Albert, S. K. Bischof, K. Schneider, R. D. Süssmuth, M. Engeser, H. Gross, G. Bringmann, J. Piel, *Angew. Chem. Int. Ed.* **2010**, *49*, 1465.

2.2.1 Type I PKS

b) modular PKS

trans-AT PKS: dihydrating bimodules

type A is split after inactive KS



D. T. Wagner, J. Zeng, C. B. Bailey, D. C. Gay, F. Yuan, H. R. Manion and A. T. Keatinge-Clay, *Structure* **2017**, *25*, 1045.

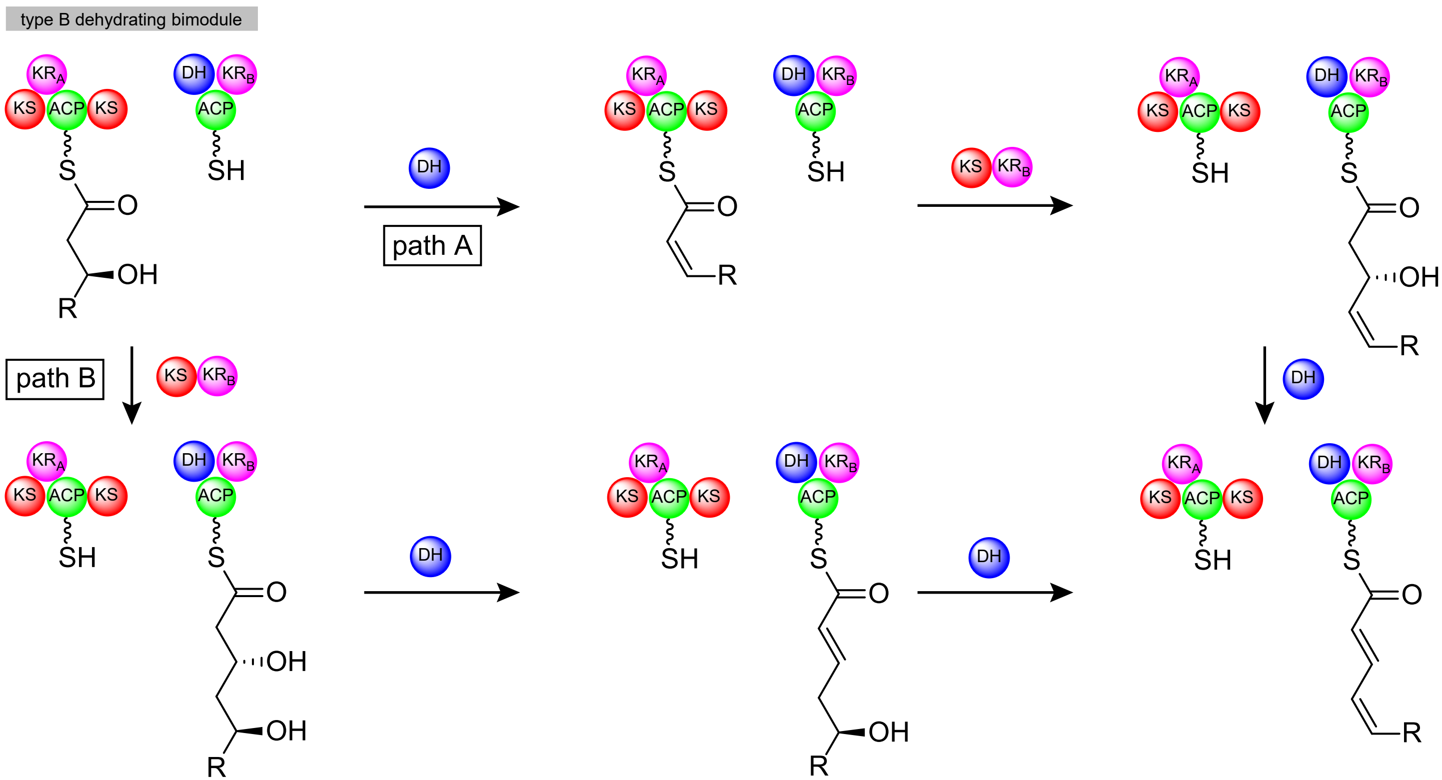
2. Polyketides

2.2.1 Type I PKS

b) modular PKS

trans-AT PKS: dihydrating bimodules

type B is split after active KS and contains an A-type and a B-type KR, DH acts twice

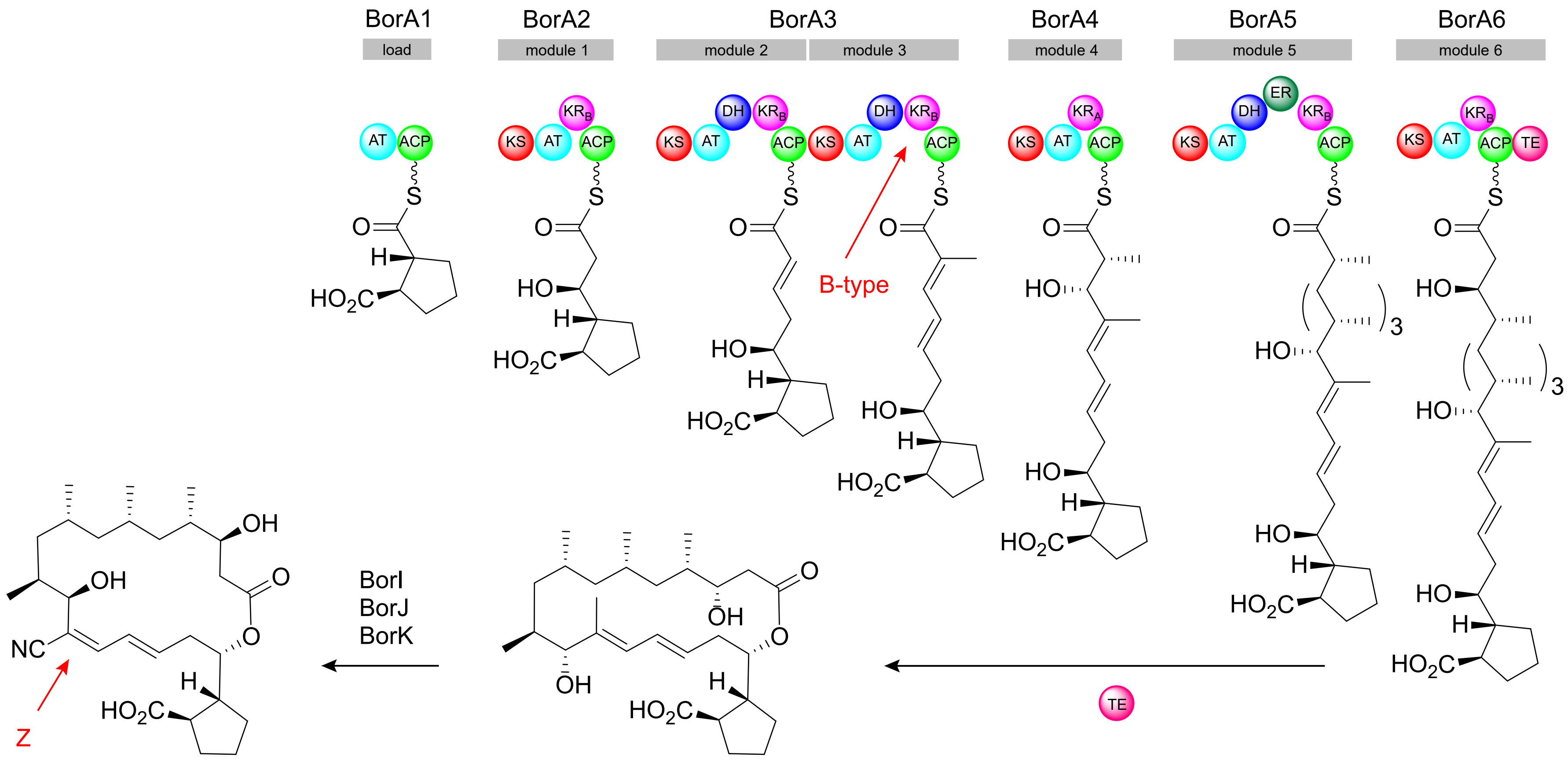


D. T. Wagner, J. Zeng, C. B. Bailey, D. C. Gay, F. Yuan, H. R. Manion and A. T. Keatinge-Clay, *Structure* **2017**, *25*, 1045.

2.2.1 Type I PKS

b) modular PKS

Borrelidin PKS: module iteration and post-PKS double bond isomerisation

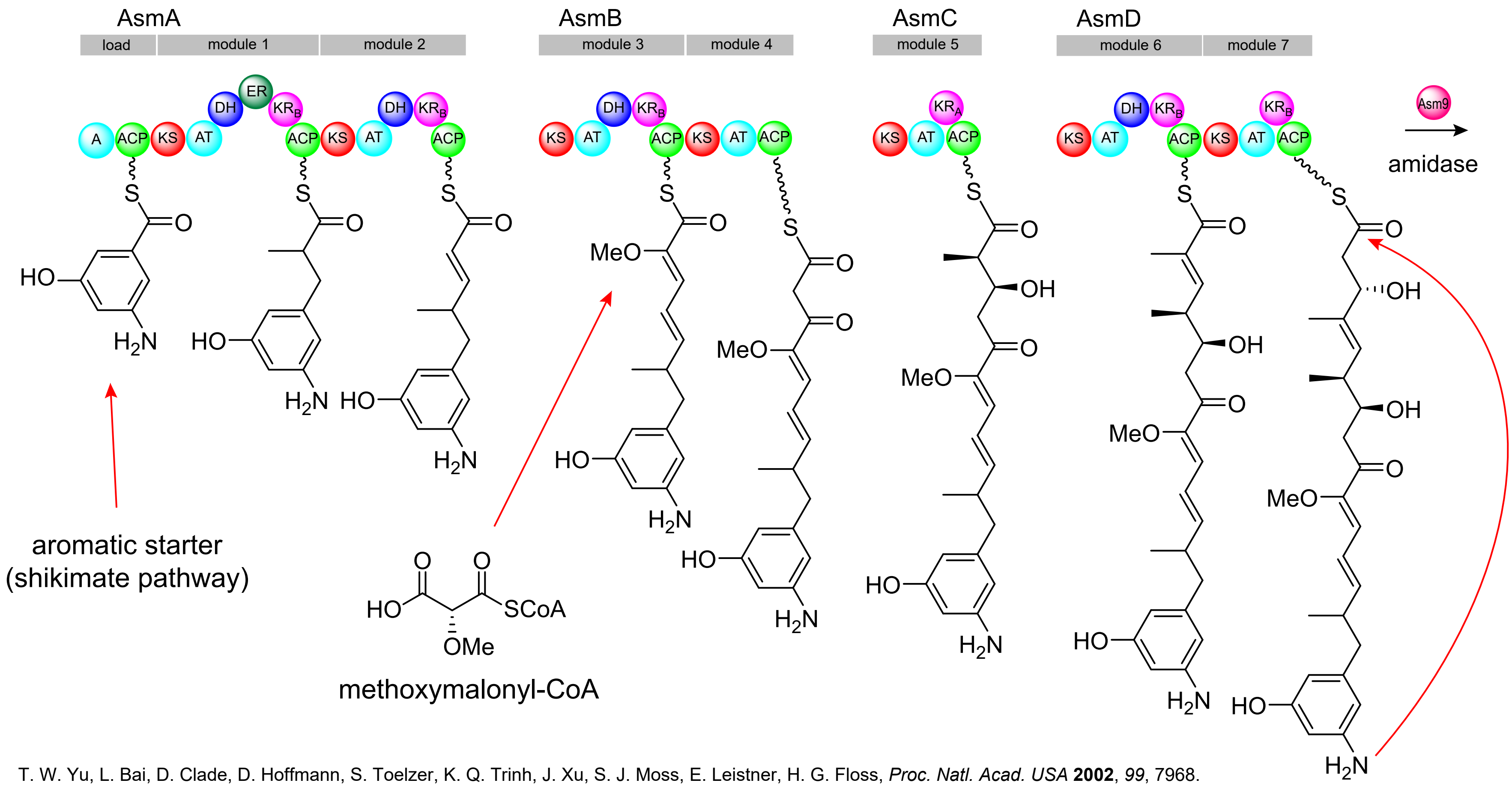


N. Kandziora, J. N. Andexer, S. J. Moss, B. Wilkinson, P. F. Leadlay and F. Hahn, *Chem. Sci.* **2014**, *5*, 3563.

2.2.1 Type I PKS

b) modular PKS

Ansamitocin PKS: unusual starter and extender unit

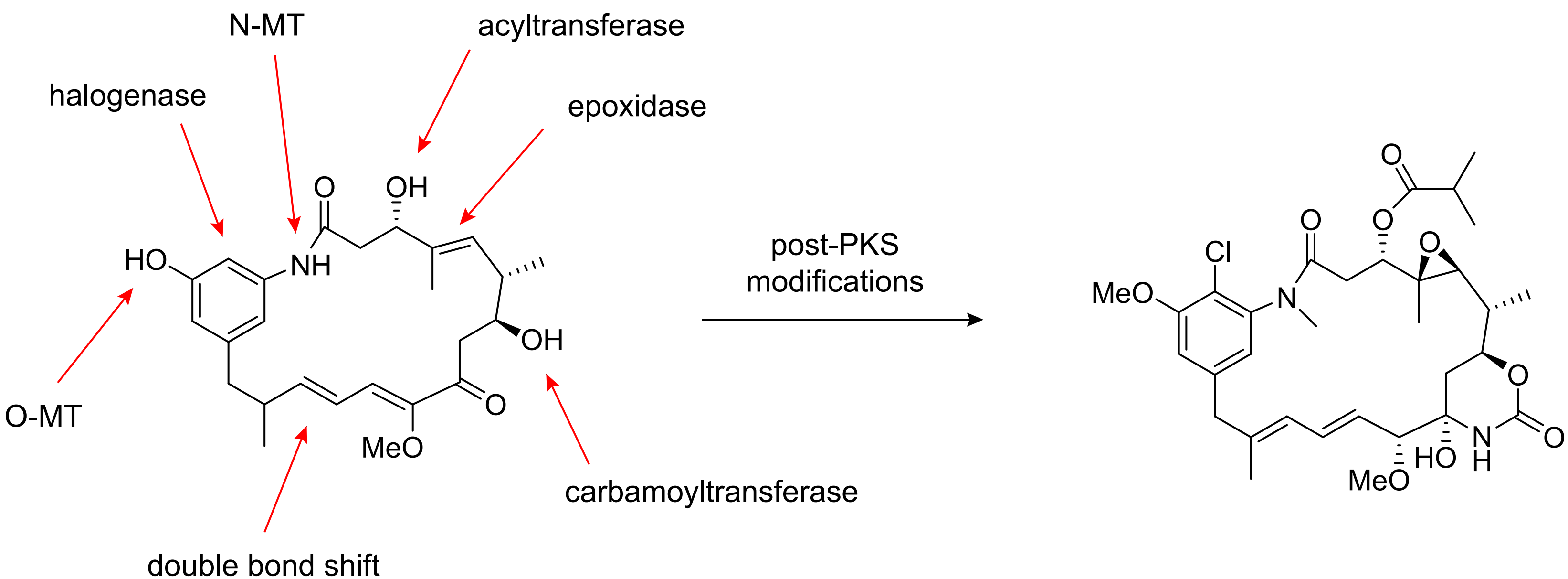


T. W. Yu, L. Bai, D. Clade, D. Hoffmann, S. Toelzer, K. Q. Trinh, J. Xu, S. J. Moss, E. Leistner, H. G. Floss, *Proc. Natl. Acad. USA* **2002**, *99*, 7968.

2.2.1 Type I PKS

b) modular PKS

Ansamitocin PKS: unusual starter and extender unit



preansamitocin

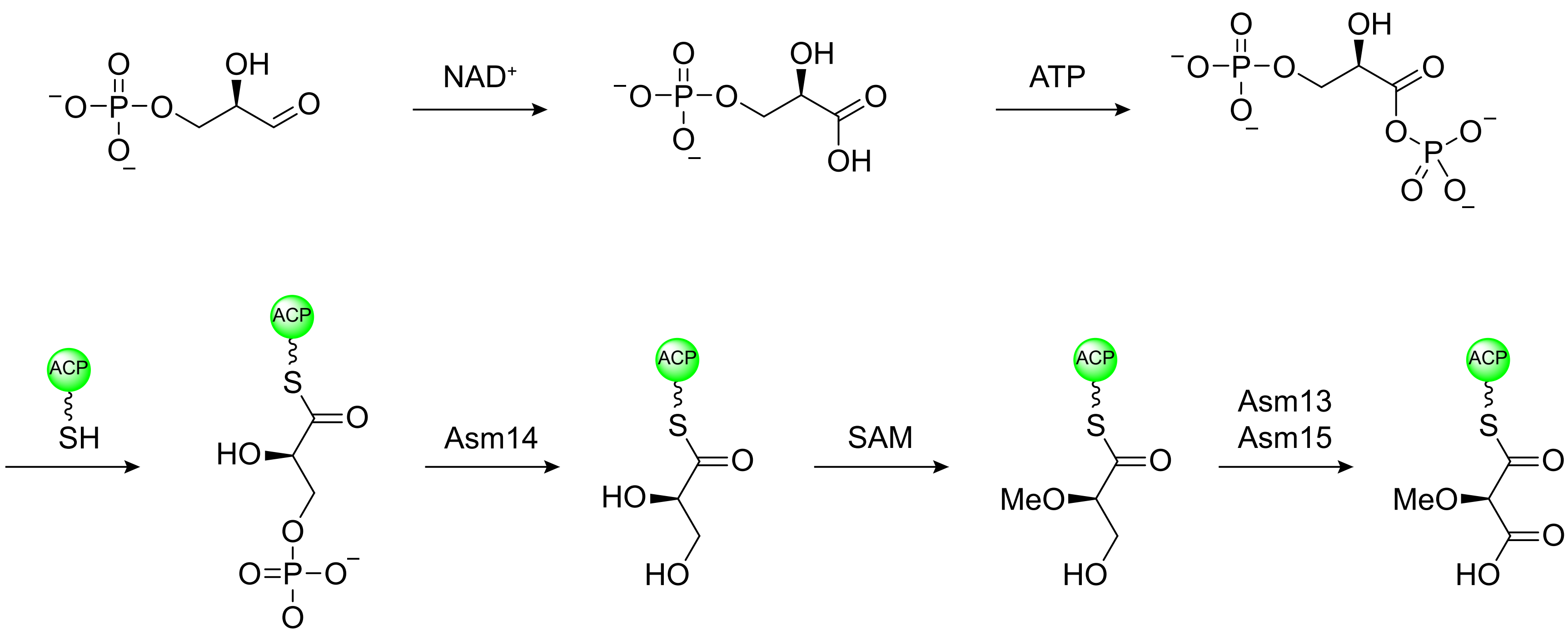
ansamitocin
(*Actinosynnema pretiosum*)

P. Spiteller, L. Bai, G. Shang, B. J. Carroll, T.-W. Yu, H. G. Floss, *J. Am. Chem. Soc.* **2003**, *125*, 14236.

2.2.1 Type I PKS

b) modular PKS Ansamitocin PKS: unusual starter and extender unit

Biosynthesis of methoxymalonyl-CoA

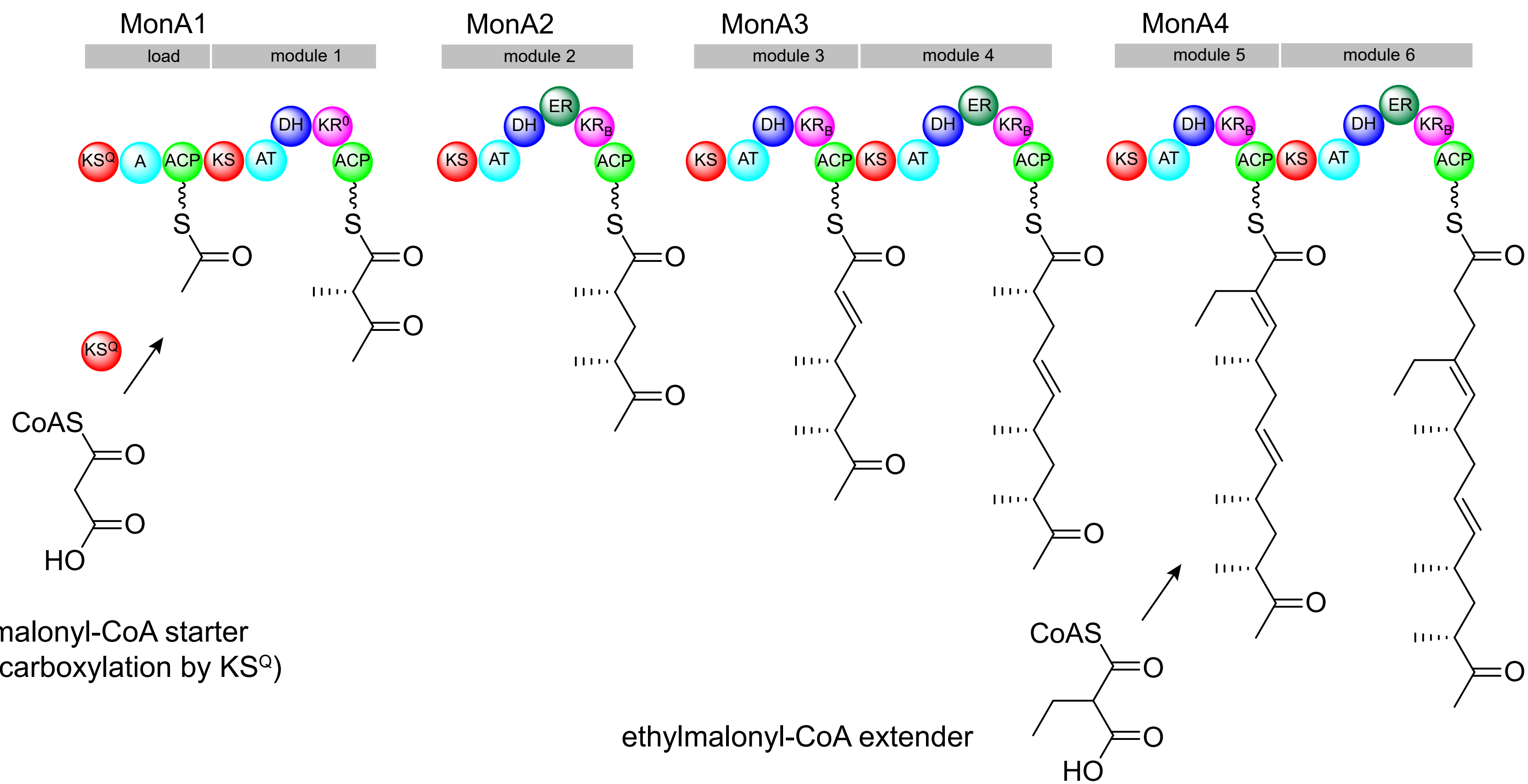


B. J. Carroll, S. J. Moss, L. Bai, Y. Kato, S. Toelzer, T.-W. Yu, H. G. Floss, *J. Am. Chem. Soc.* **2002**, *124*, 4176.

2.2.1 Type I PKS

b) modular PKS

Monensin PKS: polyether ionophores



malonyl-CoA starter
(decarboxylation by KS^Q)

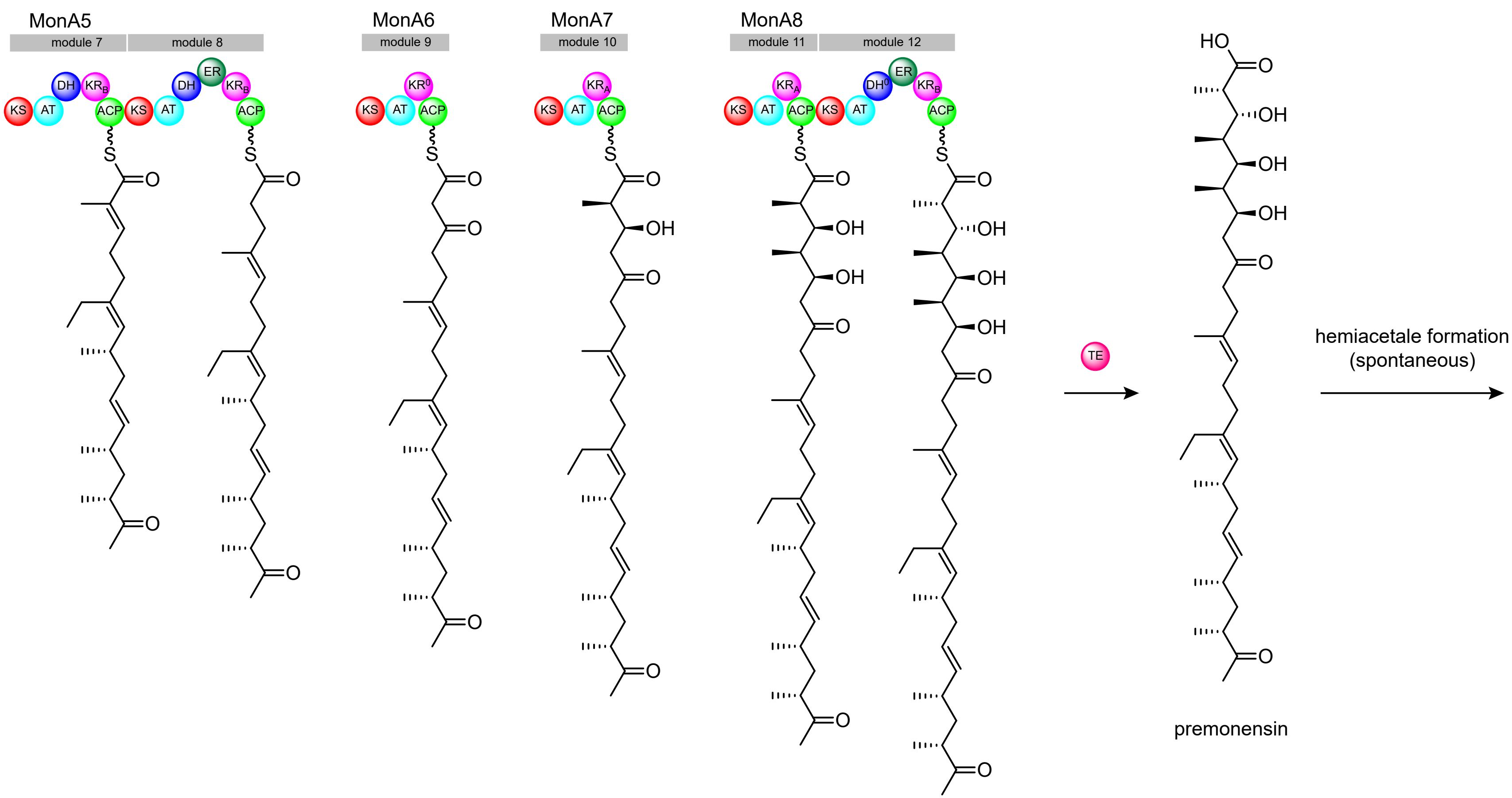
ethylmalonyl-CoA extender

M. Oliynyk, C. B. W. Stark, A. Bhatt, M. A. Jones, Z. A. Hughes-Thomas, C. Wilkinson, Z. Oliynyk, Y. Demydchuk, J. Staunton, P. F. Leadlay, *Mol. Microbiol.* **2003**, *49*, 1179.

2.2.1 Type I PKS

b) modular PKS

Monensin PKS: polyether ionophores

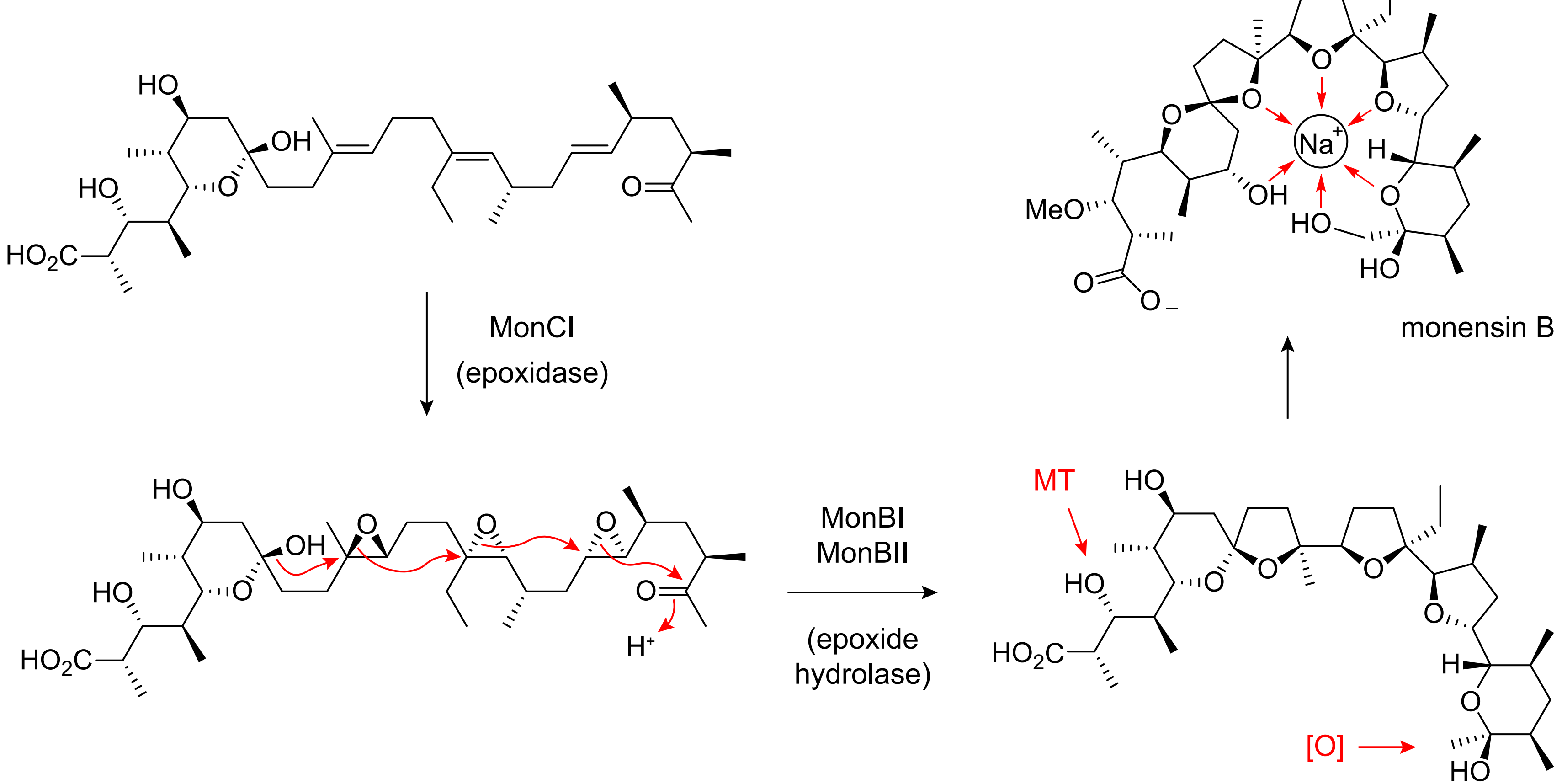


M. Oliynyk, C. B. W. Stark, A. Bhatt, M. A. Jones, Z. A. Hughes-Thomas, C. Wilkinson, Z. Oliynyk, Y. Demydchuk, J. Staunton, P. F. Leadlay, *Mol. Microbiol.* **2003**, *49*, 1179.

2.2.1 Type I PKS

b) modular PKS

Monensin PKS: polyether ionophores

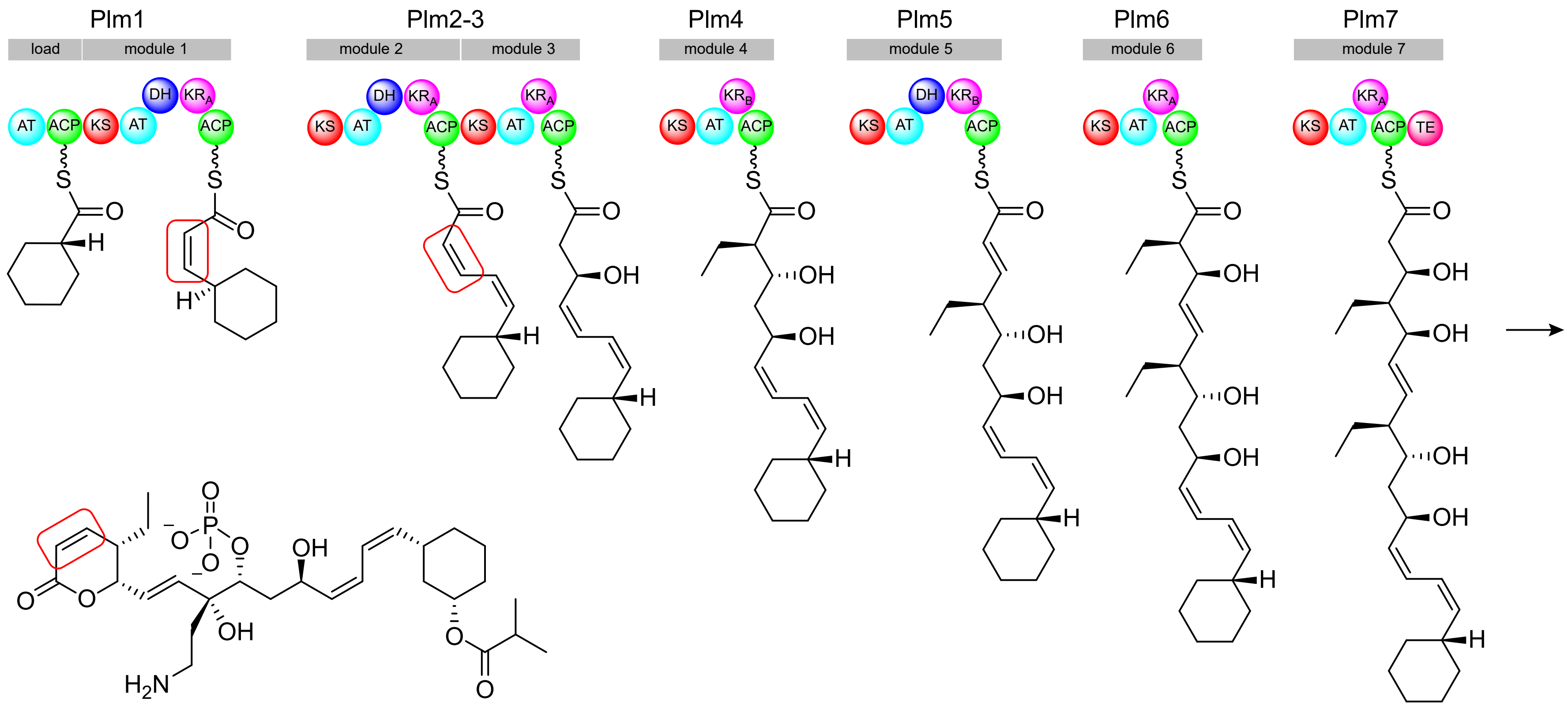


A. R. Gallimore, C. B. W. Stark, A. Bhatt, B. M. Harvey, Y. Demydchuk, V. Bolanos-Garcia, D. J. Fowler, J. Staunton, P. F. Leadlay, J. B. Spencer, *Chem. Biol.* **2006**, *13*, 453.
M. Oliynyk, C. B. W. Stark, A. Bhatt, M. A. Jones, Z. A. Hughes-Thomas, C. Wilkinson, Z. Oliynyk, Y. Demydchuk, J. Staunton, P. F. Leadlay, *Mol. Microbiol.* **2003**, *49*, 1179.

2.2.1 Type I PKS

b) modular PKS

Phoslactomycin: post-PKS *Z*-double bond formation



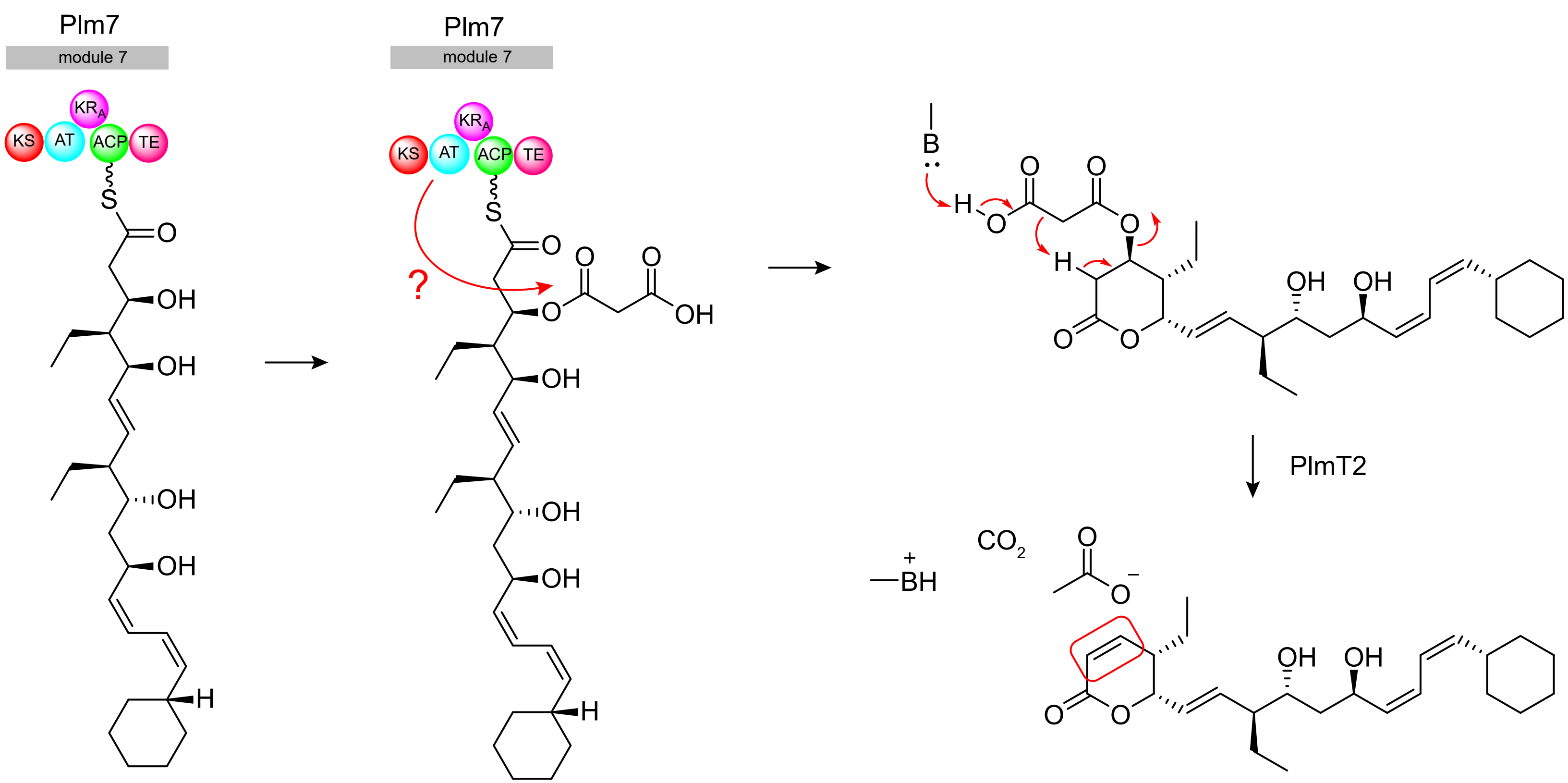
phoslactomycin

Palaniappan, N. Alhamadsheh, M. M. Reynolds, K. A. *J. Am. Chem. Soc.*, **2008**, *130*, 12236.

2.2.1 Type I PKS

b) modular PKS

Phoslactomycin: post-PKS *Z*-double bond formation



N. Palaniappan, M. M. Alhamadsheh, K. A. Reynolds, *J. Am. Chem. Soc.*, **2008**, *130*, 12236.

2. Polyketides

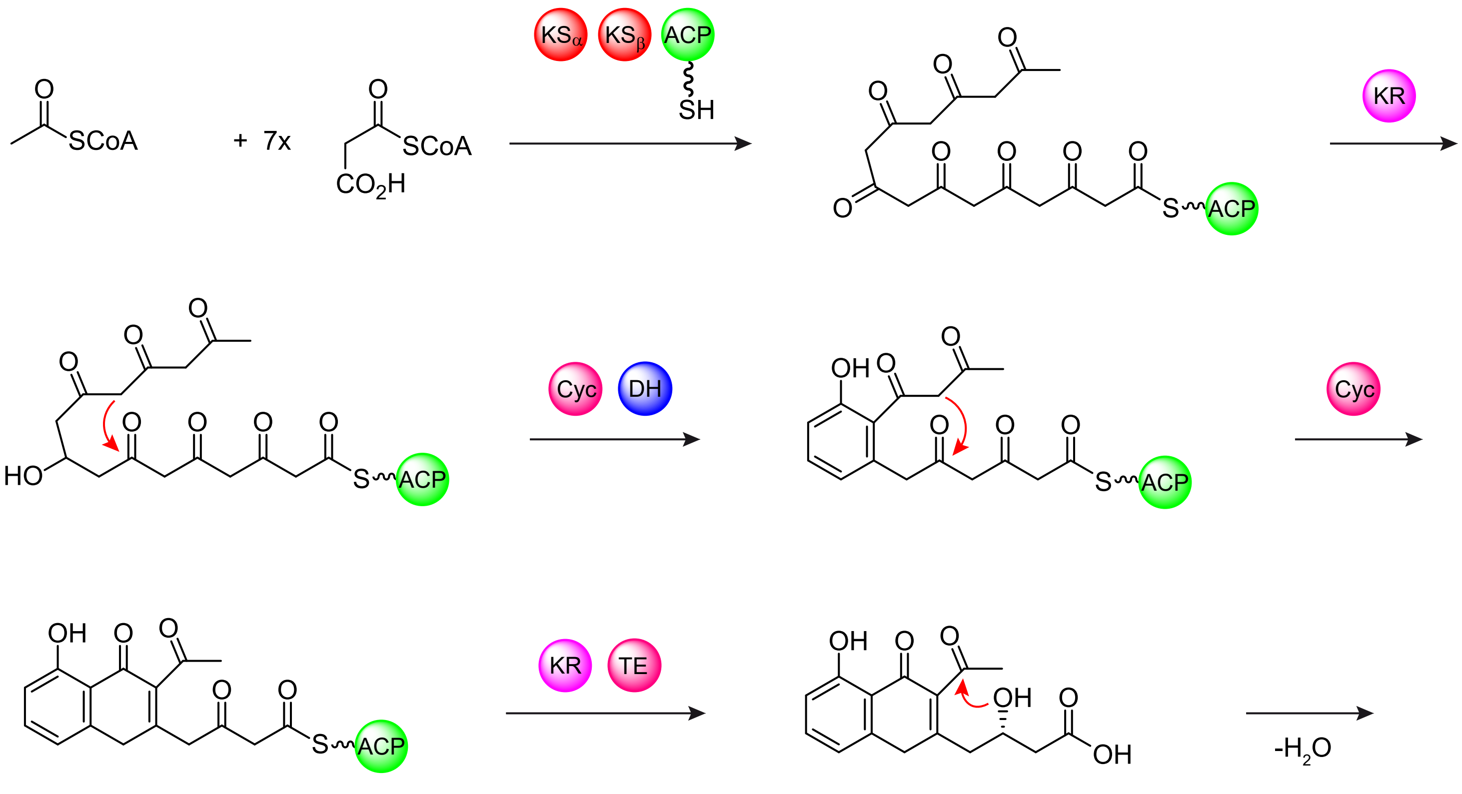
2.2.2 Type II PKS

occur in bacteria (mainly Gram-positives, actinomycetes)

discrete enzymes (cf. FAS in bacteria)

2.2.2 Type II PKS

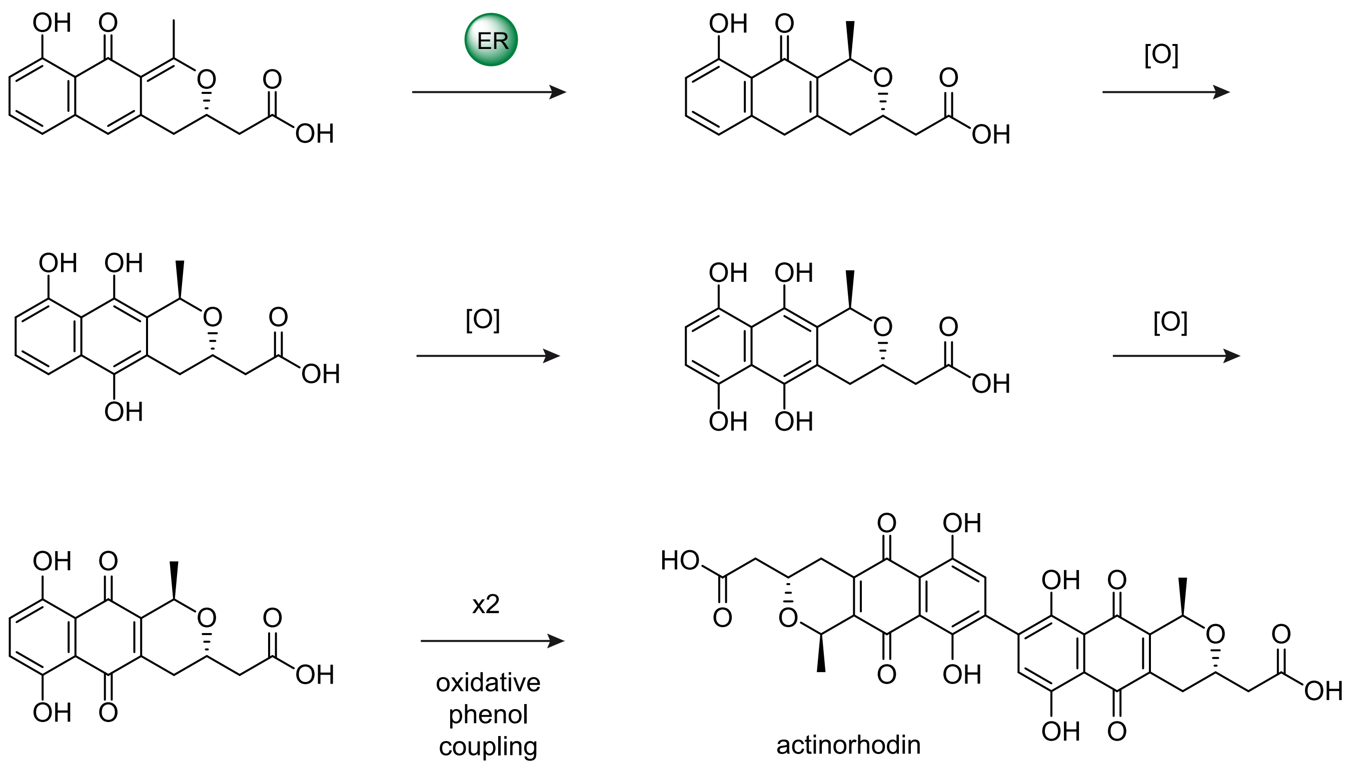
first example: actinorhodin



M. A. Fernandez-Moreno, E. Martinez, J. L. Caballero, K. Ichinose, D. A. Hopwood, F. Malpartida, *J. Biol. Chem.* **1994**, *269*, 24854.

2.2.2 Type II PKS

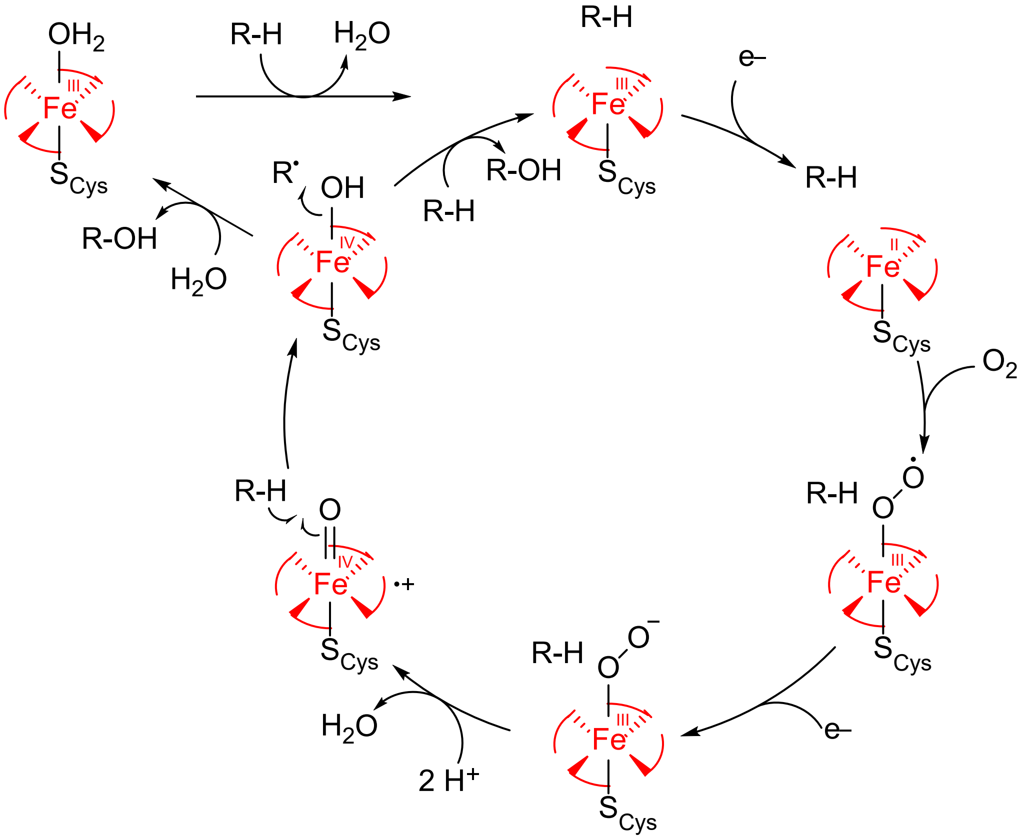
first example: actinorhodin

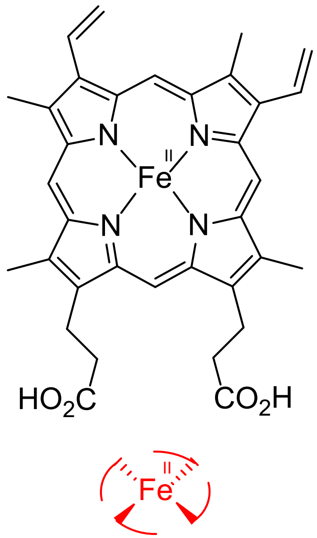


M. A. Fernandez-Moreno, E. Martinez, J. L. Caballero, K. Ichinose, D. A. Hopwood, F. Malpartida, *J. Biol. Chem.* **1994**, *269*, 24854.

2.2.2 Type II PKS

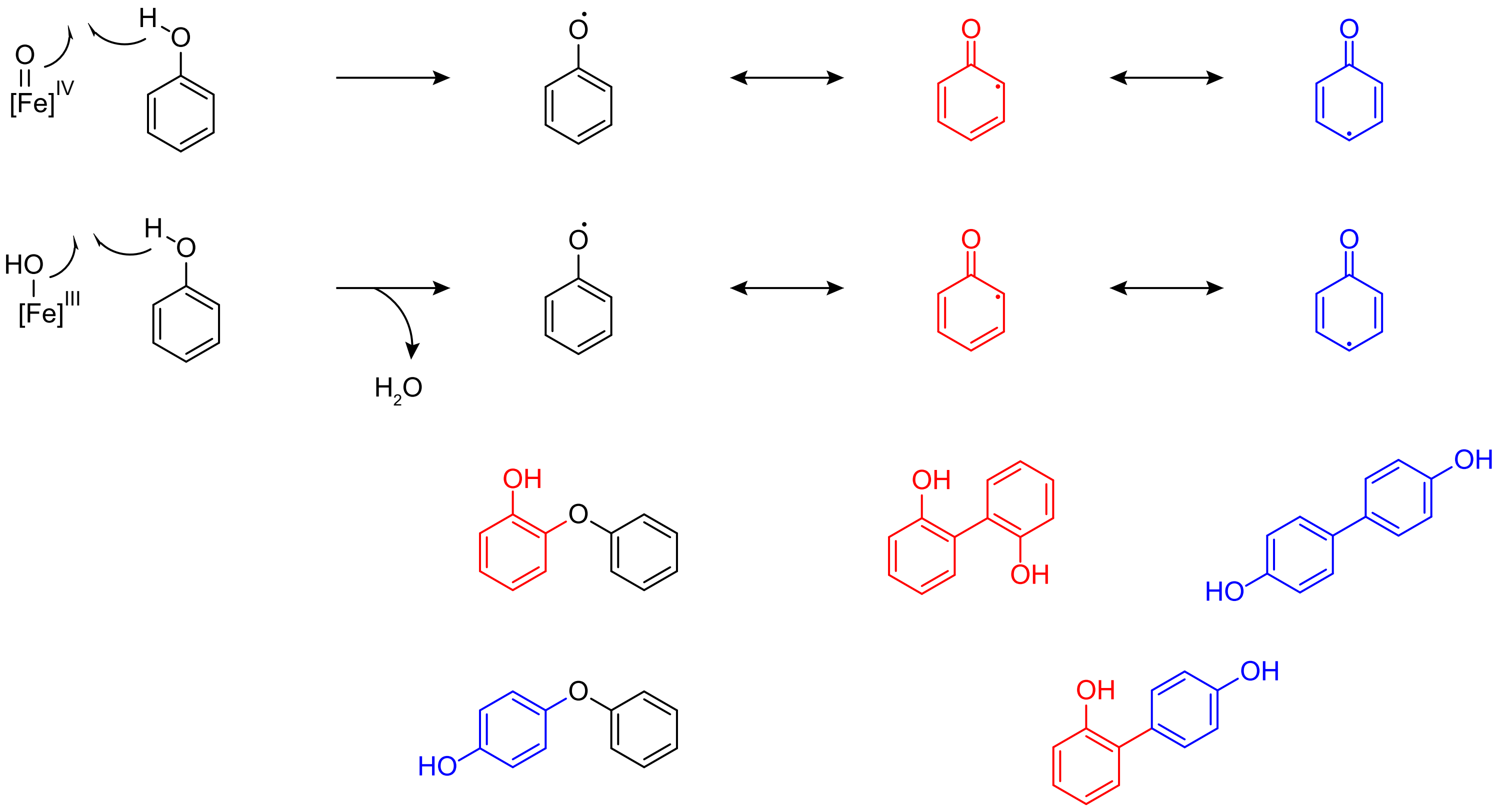
Cytochrome P450 monooxygenases (CYP450)





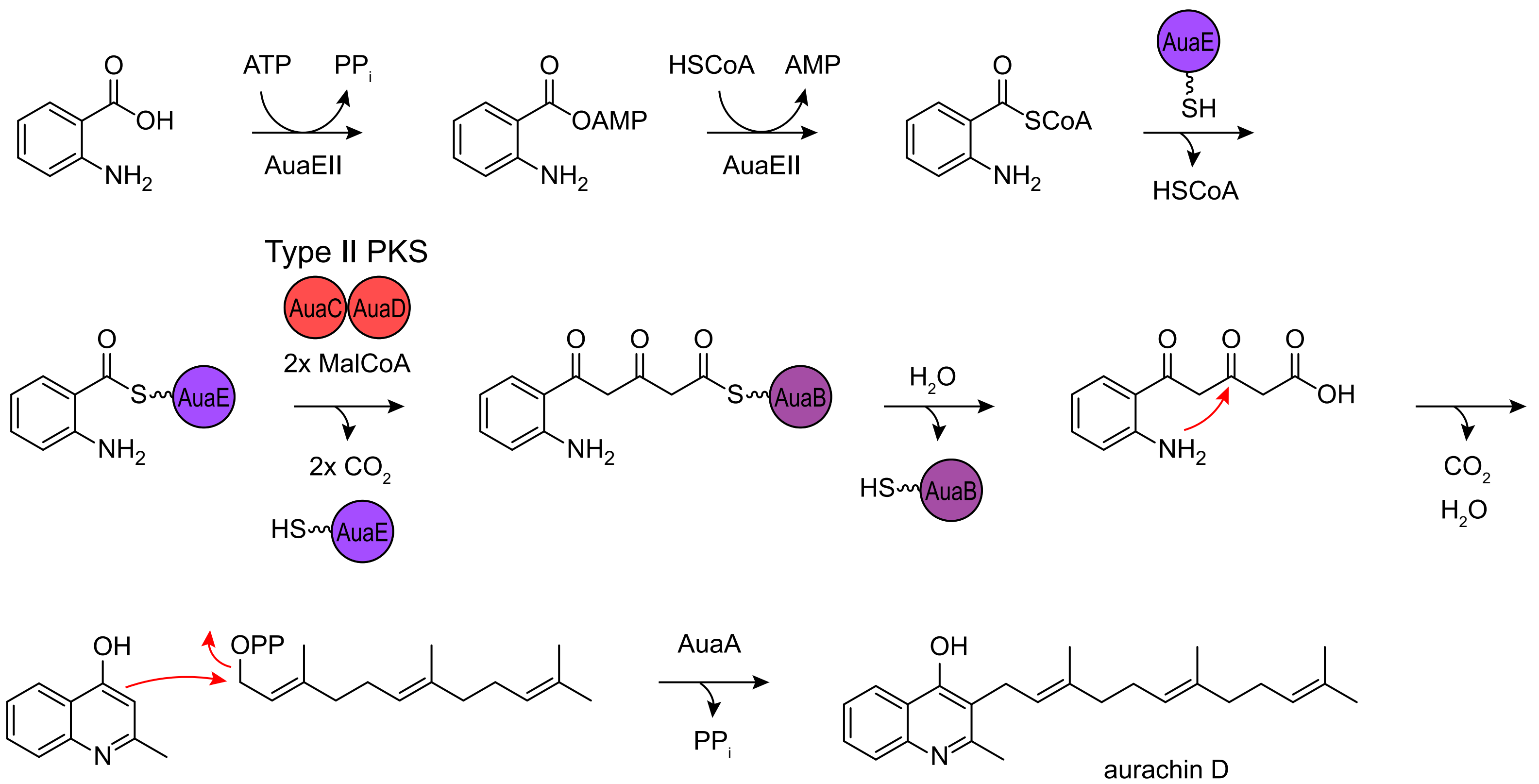
2.2.2 Type II PKS

Oxidative phenol couplings by CYP450s proceed without incorporation of oxygen from O_2



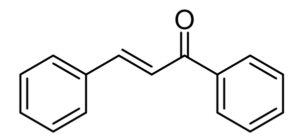
2.2.2 Type II PKS

Aurachin: first example of a type II PKS from a Gram negative bacterium (*Stigmatella aurantiaca*)



A. Sandmann, J. S. Dickschat, H. Jenke-Kodama, B. Kunze, E. Dittmann, R. Müller, *Angew. Chem. Int. Ed.* **2007**, *46*, 2712.
D. Pistorius, Y. Li, S. Mann, R. Müller, *J. Am. Chem. Soc.* **2011**, *133*, 12362.

2.2.3 Type III PKS

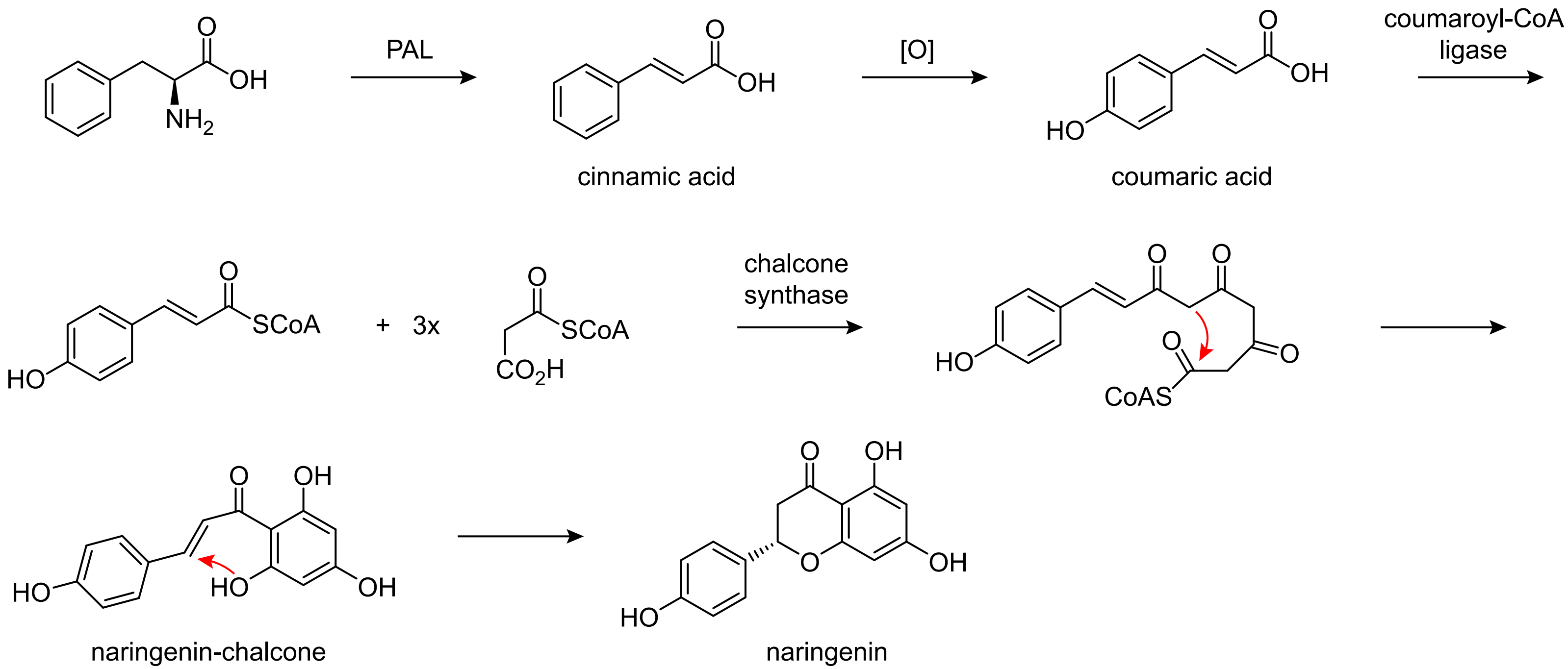


chalcone

chalcone and stilbene synthases (in plants and bacteria)

similar to FabH (= KSIII) of FAS in plants and bacteria

broad starter unit diversity, intermediates are not ACP bound, but CoA thioesters



J. Schröder, *Trends Plant Sci.* **1997**, *2*, 373.

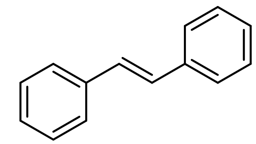
2. Polyketides

2.2.3 Type III PKS

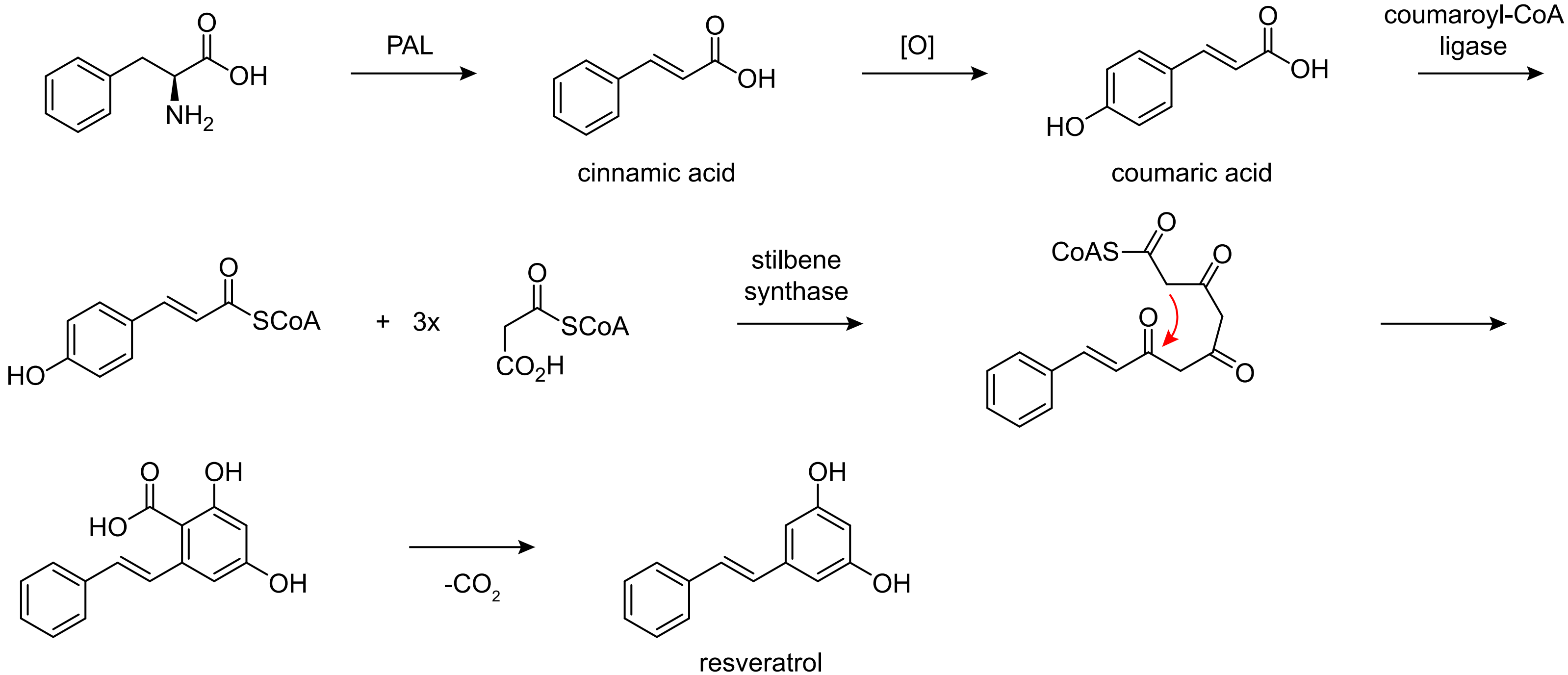
chalcone and stilbene synthases (in plants and bacteria)

similar to FabH (= KSIII) of FAS in plants and bacterial

broad starter unit diversity, intermediates are not ACP bound, but CoA thioesters



stilbene



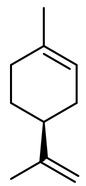
J. Schröder, *Trends Plant Sci.* **1997**, *2*, 373.

3. Terpenes

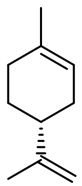
3.1 Occurrence, bioactivity and classification

>80,000 known compounds, largest class of natural products

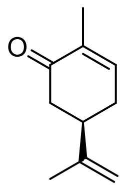
aroma compounds



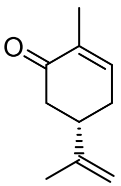
(*S*)-limonene
(*Abies nobilis*)



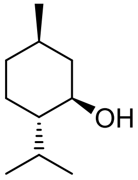
(*R*)-limonene
(*Citrus sinensis*)



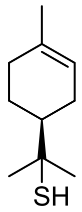
(+)-carvone
(*Carum carvi*)



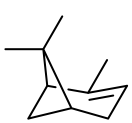
(–)-carvone
(*Mentha spicata*)



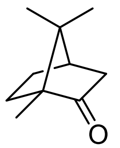
(–)-menthol
(*Mentha piperita*)



(–)-α-thioterpineol
(*Citrus paradisi*)



(+)-α-pinene
(*Pinus sylvestris*)



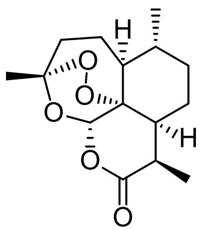
(+)-camphor
(*Cinamomum camphora*)

G. F. Russell, J. I. Hills, *Science* **1971**, *172*, 1043.

3. Terpenes

3.1 Occurrence, bioactivity and classification

drugs

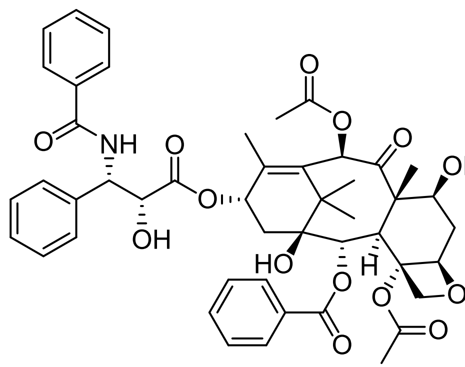


artemisinin (*Artemisia annua*)

against malaria
(*Plasmodium falciparum*)

isolation, structure elucidation
by Tu Youyou (Nobel Price 2015)

EMA approved (2011)



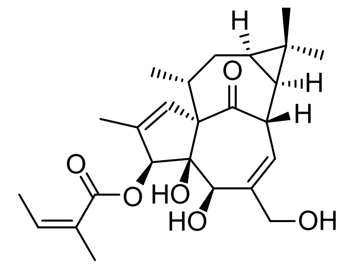
taxol (*Taxus brevifolia*)

against ovarian, breast, lung and
postate cancer, Karposi‘s sarcoma

often in combination with cisplatin

EMA approved (2008)

FDA approved (2018)



ingenol 3-angelate (*Euphorbia peplus*)

against actinic keratosis

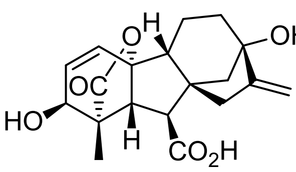
FDA approved (2012)

EMA approved (2012 - 2020)

approval withdrawn because of strong
side effects (development of skin cancer)

3.1 Occurrence, bioactivity and classification

hormones and pheromones

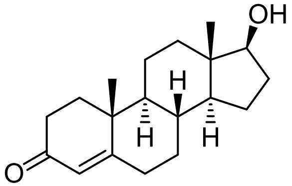


gibberellic acid GA_3

phytohormone in plants
regulates growth and development

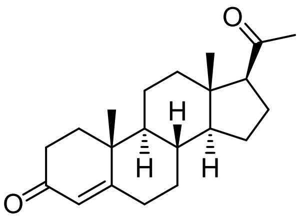
fungi: *Fusarium fujikuroi*
large amounts, phytopathogen

bacteria: *Bradyrhizobium japonicum*
small amounts, plant symbionts



testosterone

male sex hormone

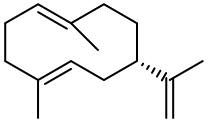


progesterone

female sex hormone

regulate development of sexual characteristics

regulate female menstrual cycle and pregnancy



(–)-germacrene A

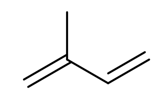
alarm pheromone in
Therioaphis maculata

W. S. Bowers, C. Nishino, M. E. Montgomery, L. R. Nault, M. W. Nielson, *Science* **1977**, *196*, 680.

3.1 Occurrence, bioactivity and classification

classification

terpenes are built from Me branched C_5 units (isoprene rule, Wallach 1885, Bonn)



isoprene

C_5H_8	hemiterpenes
$C_{10}H_{16}$	monoterpenes
$C_{15}H_{24}$	sesquiterpenes
$C_{20}H_{32}$	diterpenes
$C_{25}H_{40}$	sesterterpenes
$C_{30}H_{48}$	triterpenes
$C_{35}H_{56}$	sesquarterpenes
$C_{40}H_{64}$	tetraterpenes

$$C_{5n}H_{8n} \xrightarrow{+H_2O} C_{5n}H_{8n+2}O$$

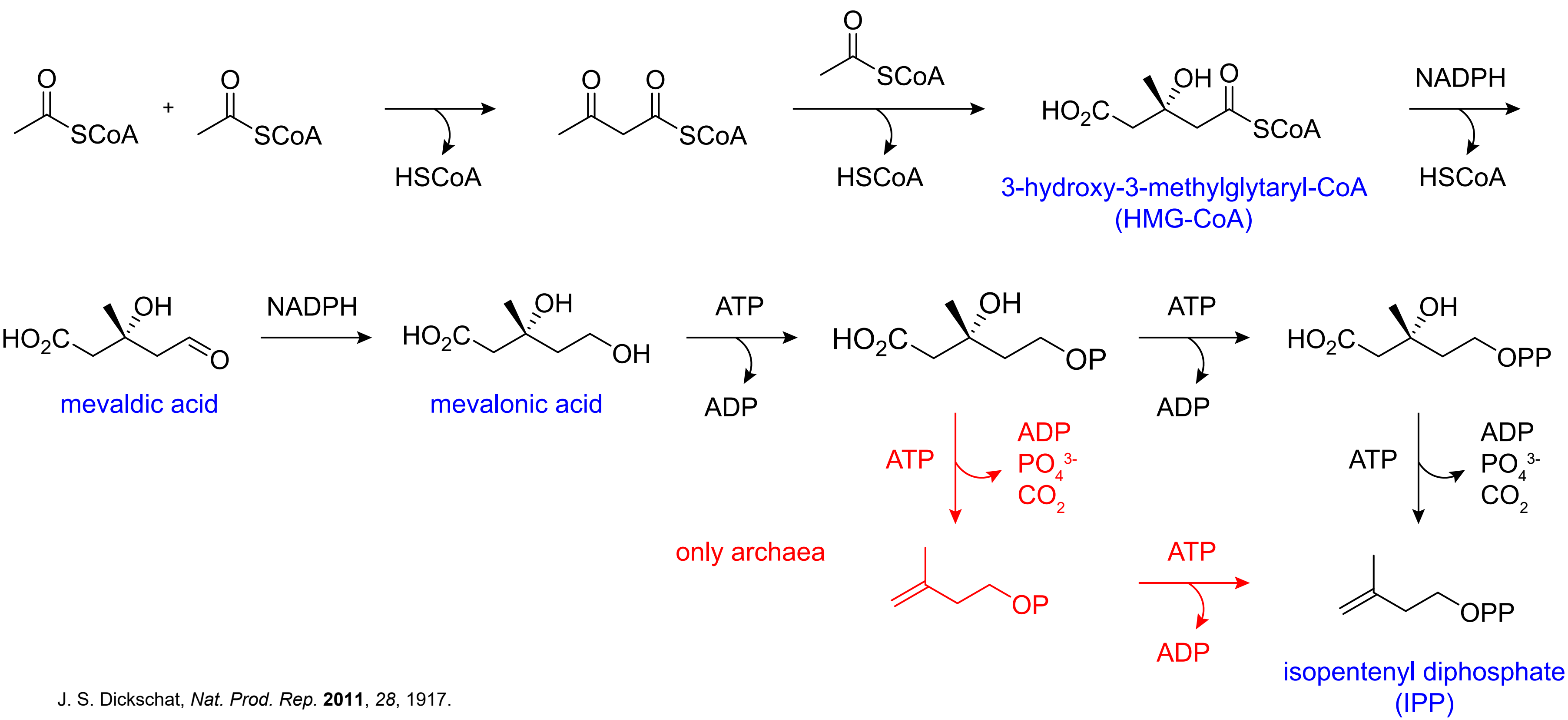
terpene hydrocarbon → terpene alcohol

deviations from these formulae point to oxidations, reductions, other modifications

3.2. Biosynthesis of monomers and acyclic precursors

3.2.1. Mevalonate pathway

in fungi and animals, in the cytosol of plants, in bacteria (rare), in archaea (modified)

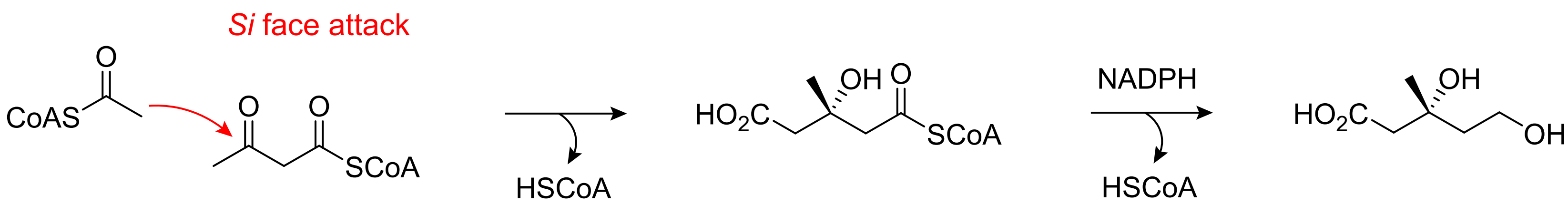


J. S. Dickschat, *Nat. Prod. Rep.* **2011**, *28*, 1917.

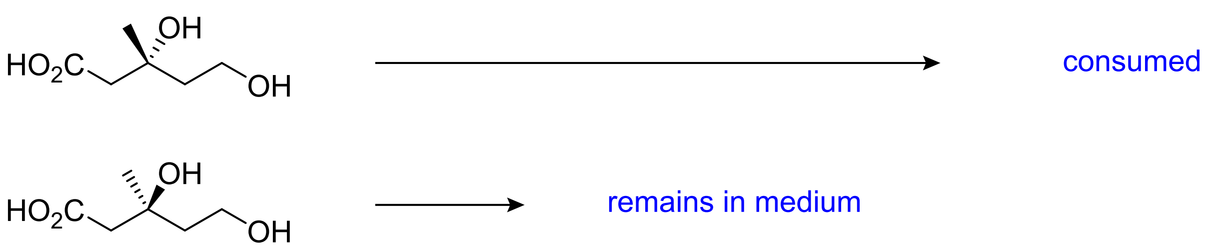
3.2. Biosynthesis of monomers and acyclic precursors

3.2.1. Mevalonate pathway

Stereochemistry: HMG-CoA synthase



feeding of (*rac*)-mevalonic acid
to *Mycobacterium*



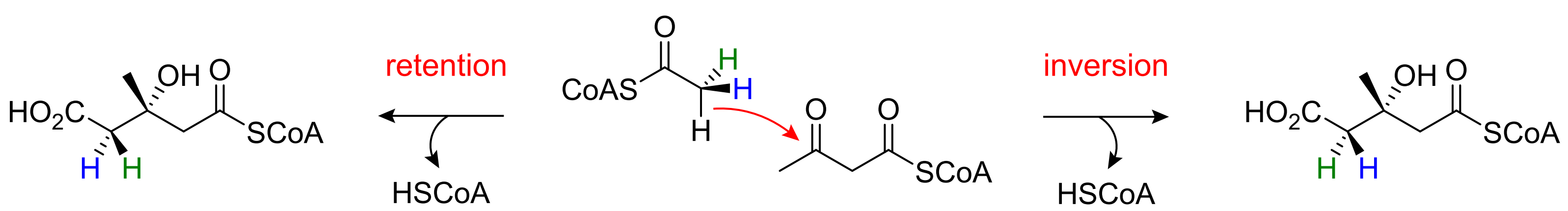
F. Lynen, M. Grassl, *Hoppe Seyler's Z. Physiol. Chem.* **1958**, *313*, 291.

3.2. Biosynthesis of monomers and acyclic precursors

3.2.1. Mevalonate pathway

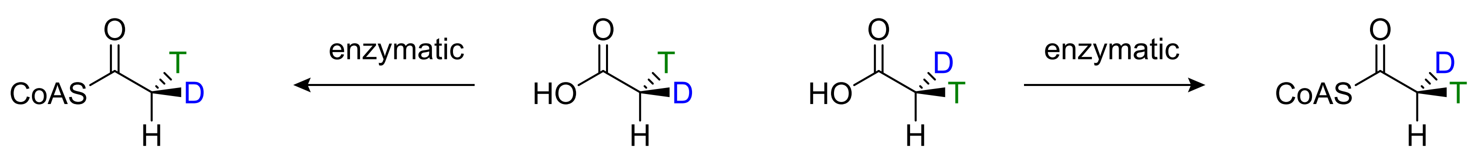
Stereochemistry: HMG-CoA synthase

less obvious question: retention or inversion of configuration at the Me group of acetyl-CoA?



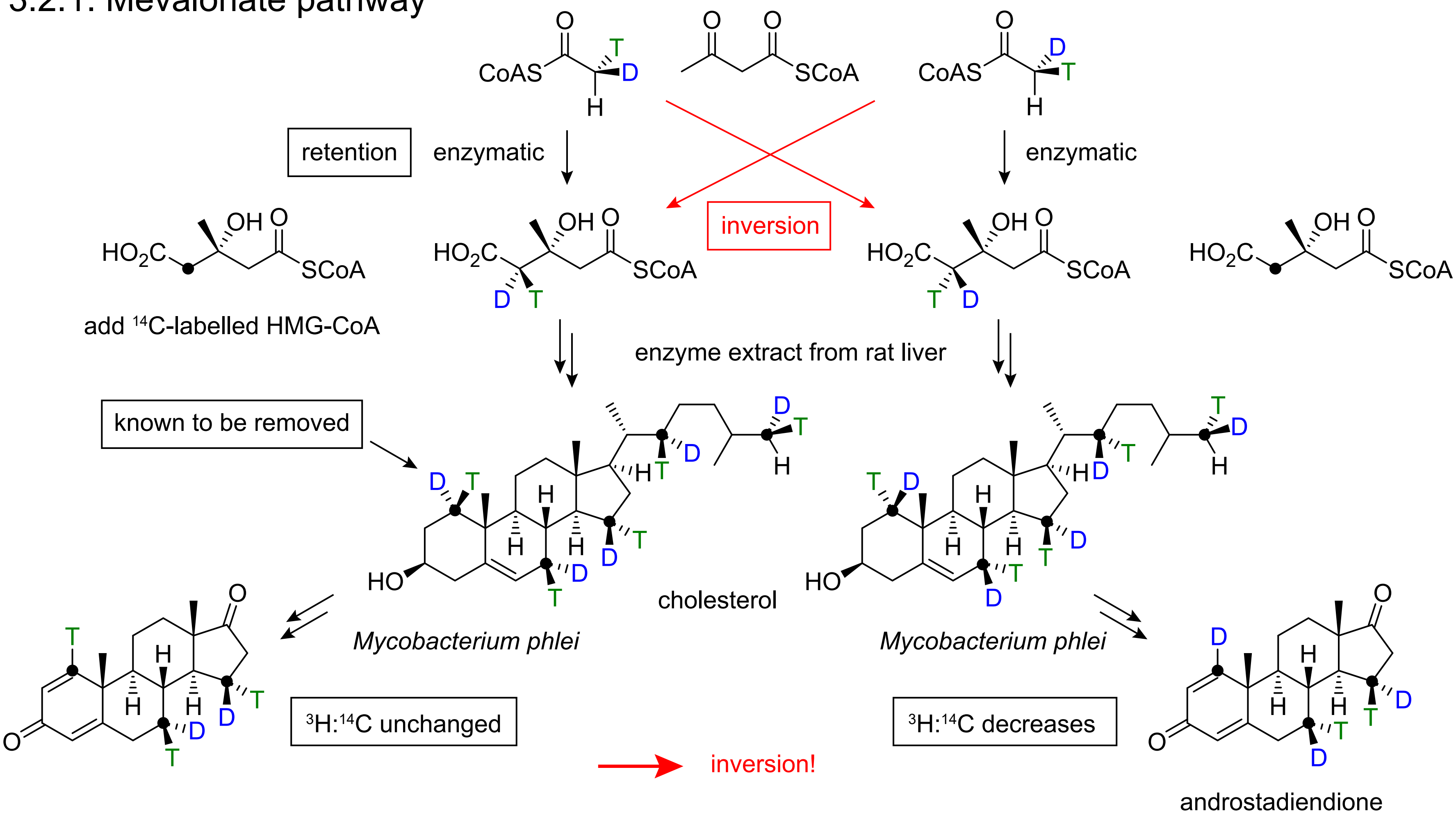
synthesis of chiral acetic acid

problems: high enantiomeric purity required, T is radioactive, which enantiomer is formed?



J. W. Cornforth, G. T. Phillips, *Eur. J. Biochem.* **1974**, *42*, 591.

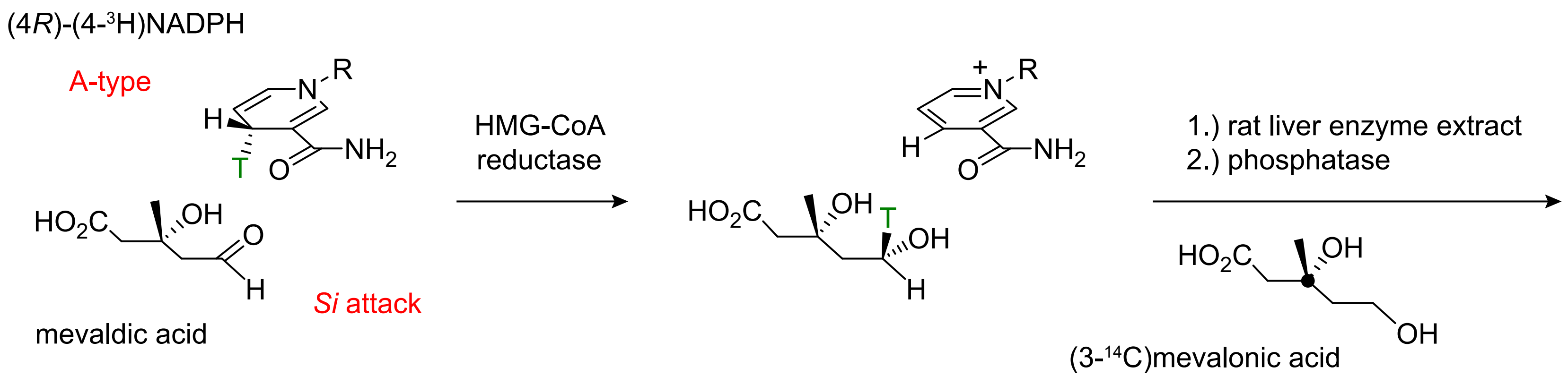
3.2.1. Mevalonate pathway

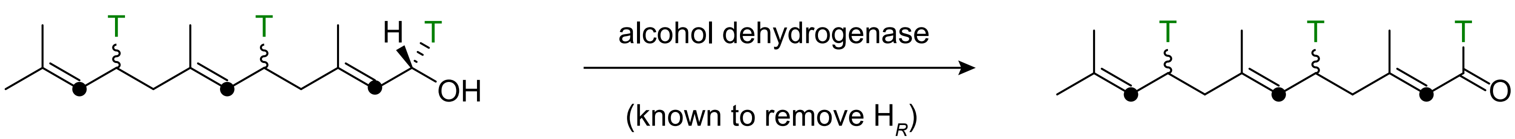


J. W. Cornforth, G. T. Phillips, *Eur. J. Biochem.* **1974**, *42*, 591.

3.2.1. Mevalonate pathway

HMG-CoA reductase



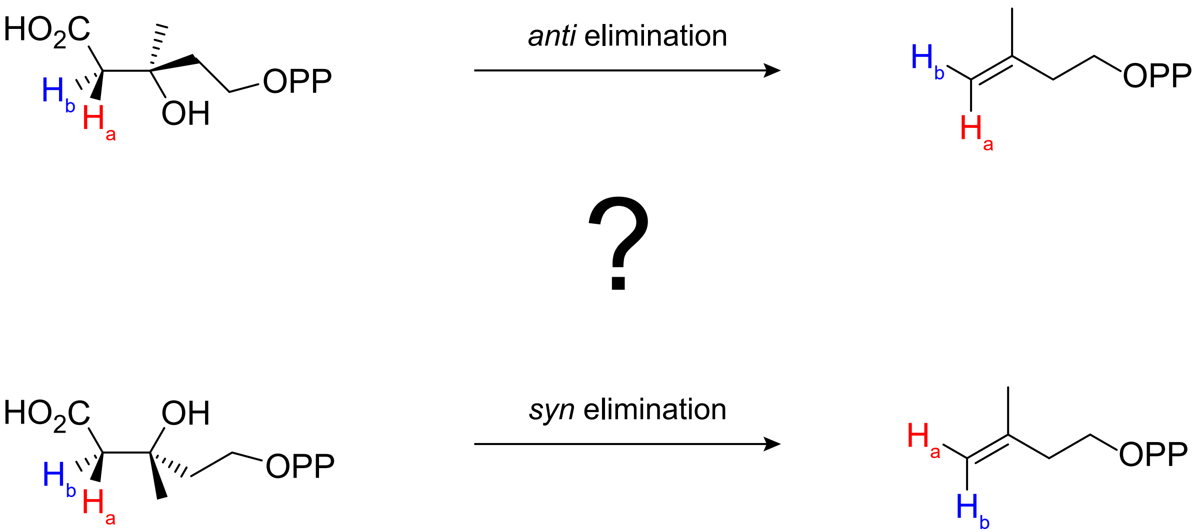


$\boxed{^{3}\text{H}:^{14}\text{C unchanged}}$

A. S. Beedle, K. A. Munday, D. C. Wilton, *FEBS Lett.* **1972**, *28*, 13.

3.2.1. Mevalonate pathway

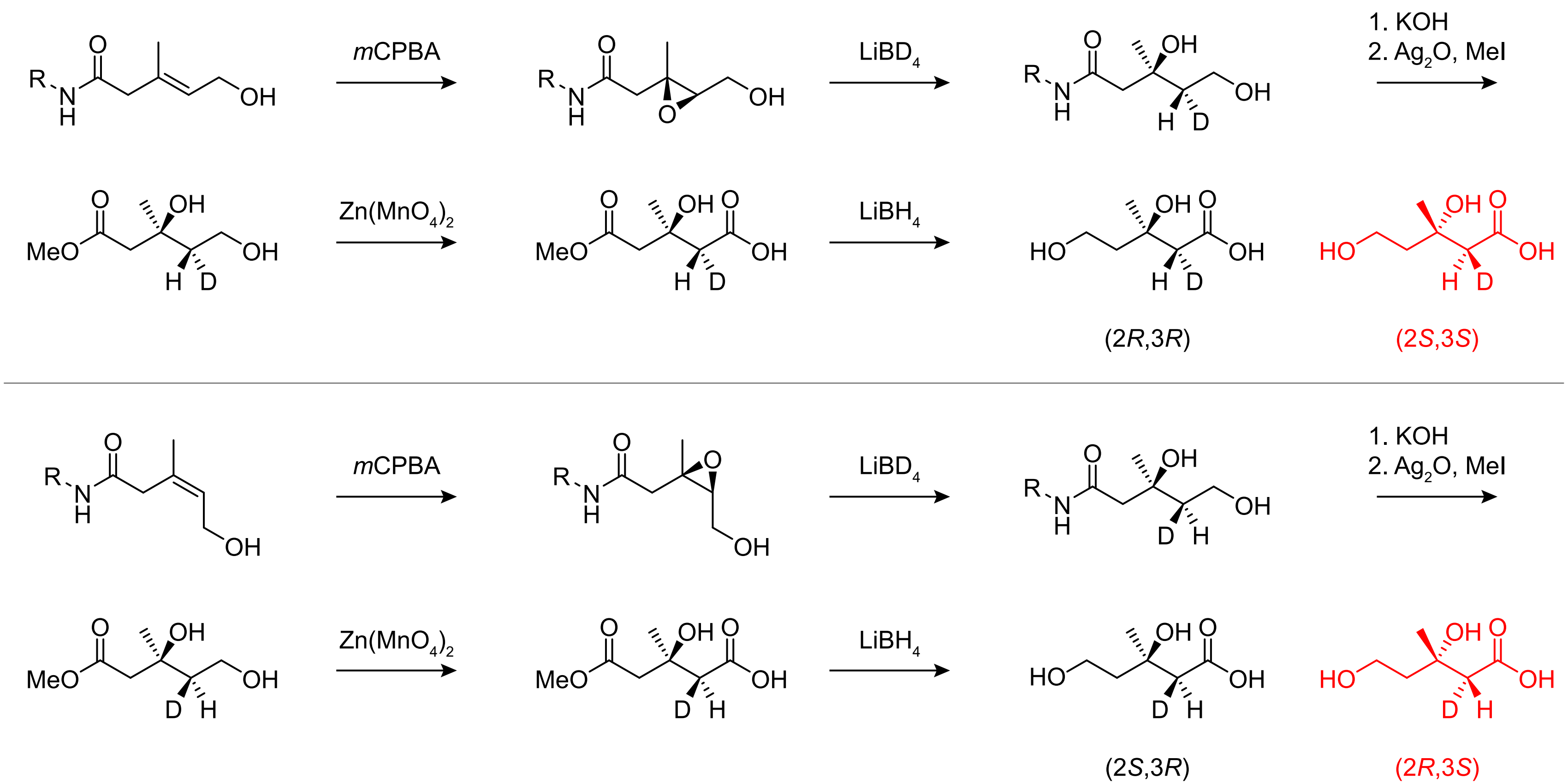
Diphosphomevalonate decarboxylase



J. W. Cornforth, R. H. Cornforth, G. Popjak, L. Yengoyan, *J. Biol. Chem.* **1966**, *241*, 3970.

3.2.1. Mevalonate pathway

Diphosphomevalonate decarboxylase

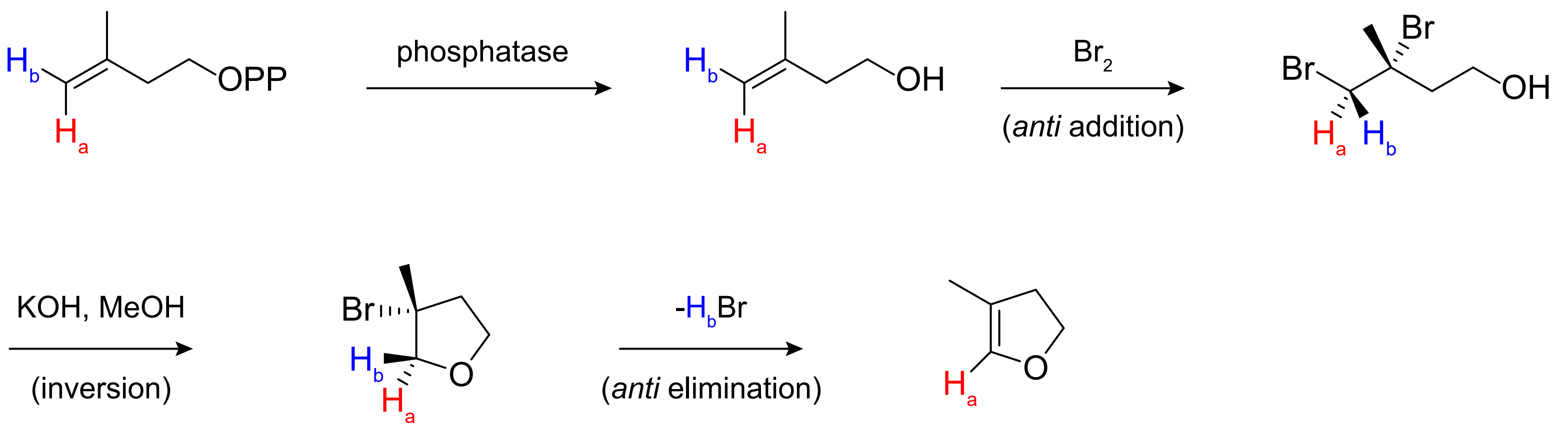


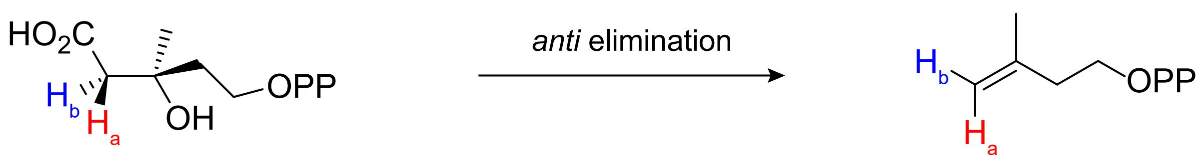
J. W. Cornforth, R. H. Cornforth, G. Popjak, L. Yengoyan, *J. Biol. Chem.* **1966**, *241*, 3970.

3.2.1. Mevalonate pathway

Diphosphomevalonate decarboxylase

Analysis of result:

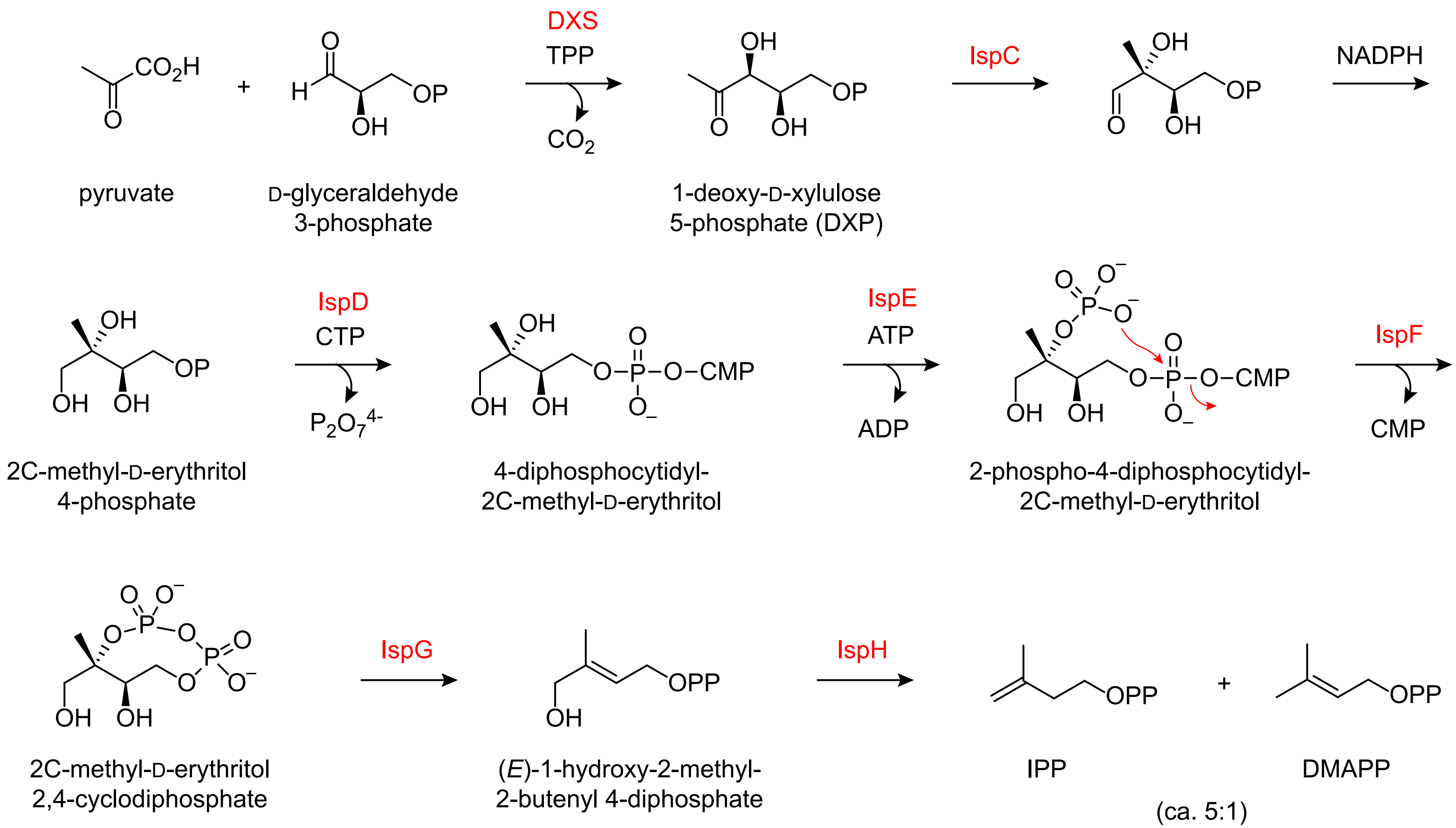




J. W. Cornforth, R. H. Cornforth, G. Popjak, L. Yengoyan, *J. Biol. Chem.* **1966**, *241*, 3970.

3.2.2. Deoxyxylulose phosphate pathway

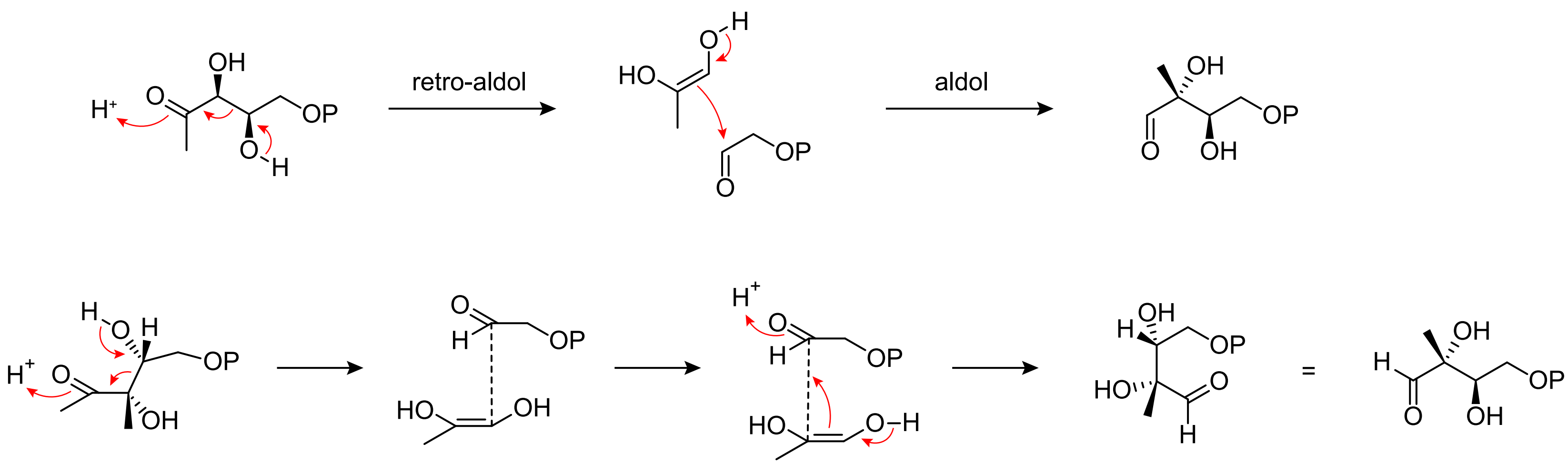
in plants (in the plastids), in most bacteria



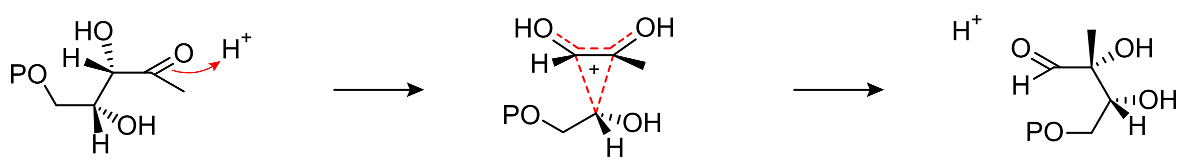
3.2.2. Deoxyxylulose phosphate pathway

mechanism of IspC

a) retro-aldol / aldol

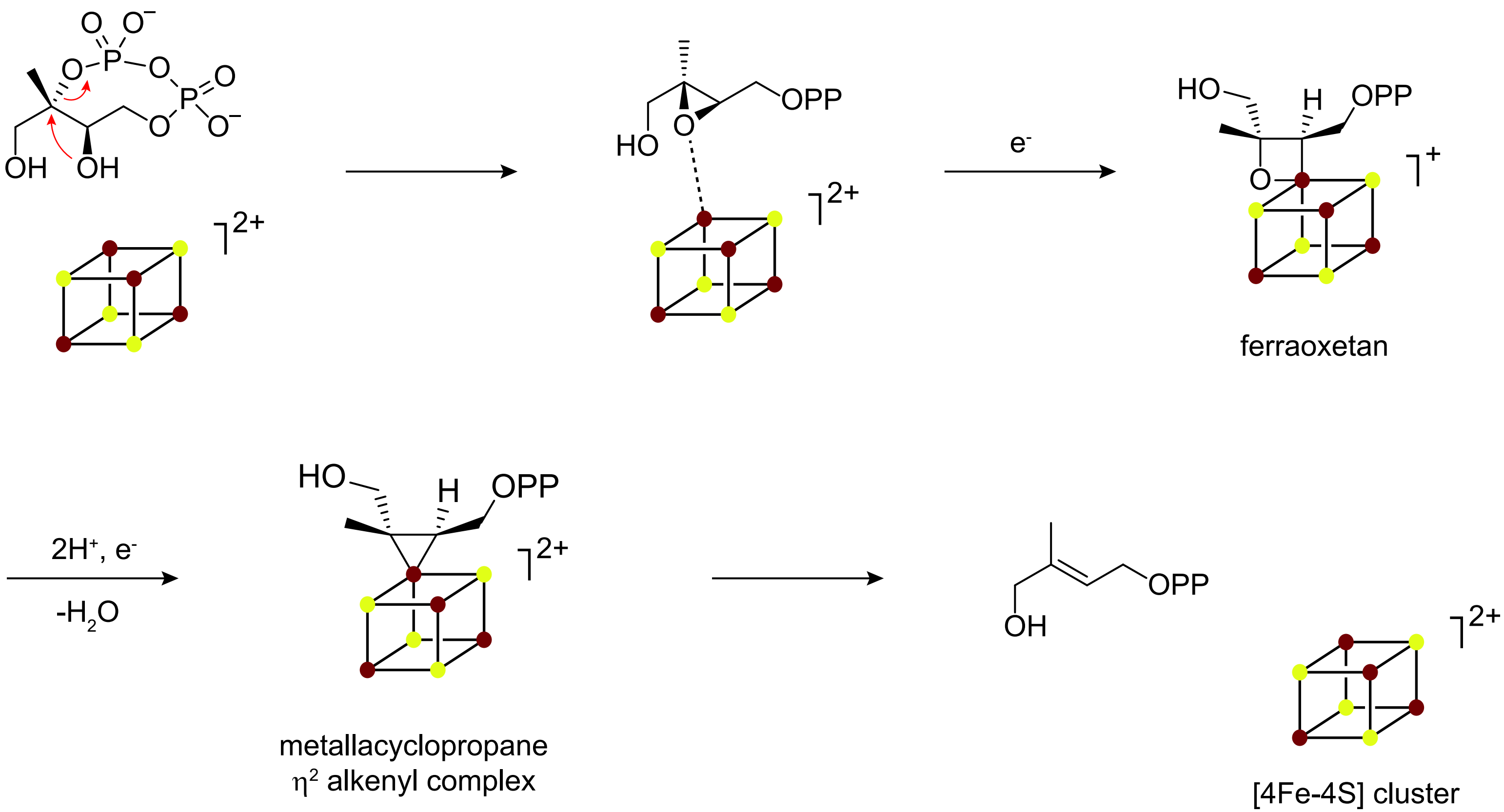


b) concerted: α-ketol rearrangement



3.2.2. Deoxyxylulose phosphate pathway

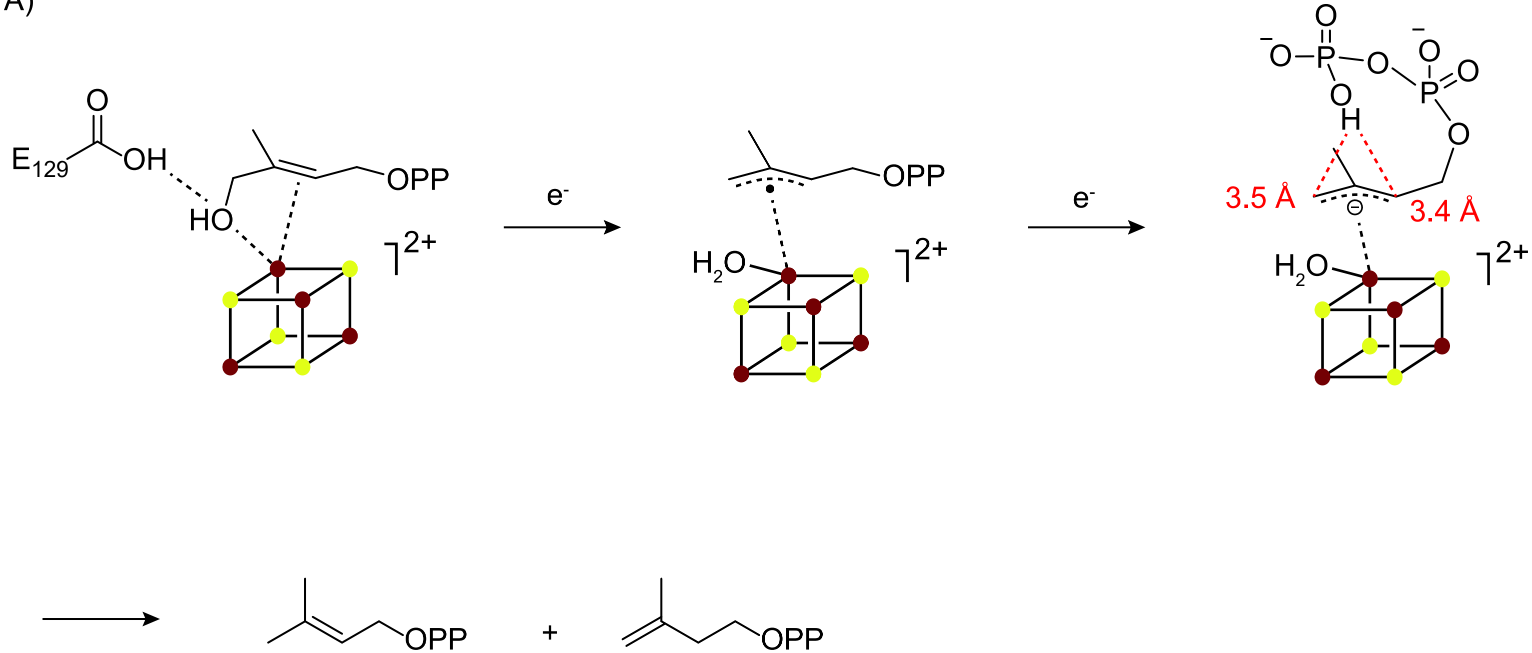
mechanism of IspG



3.2.2. Deoxyxylulose phosphate pathway

two alternative mechanisms for IspH

A)

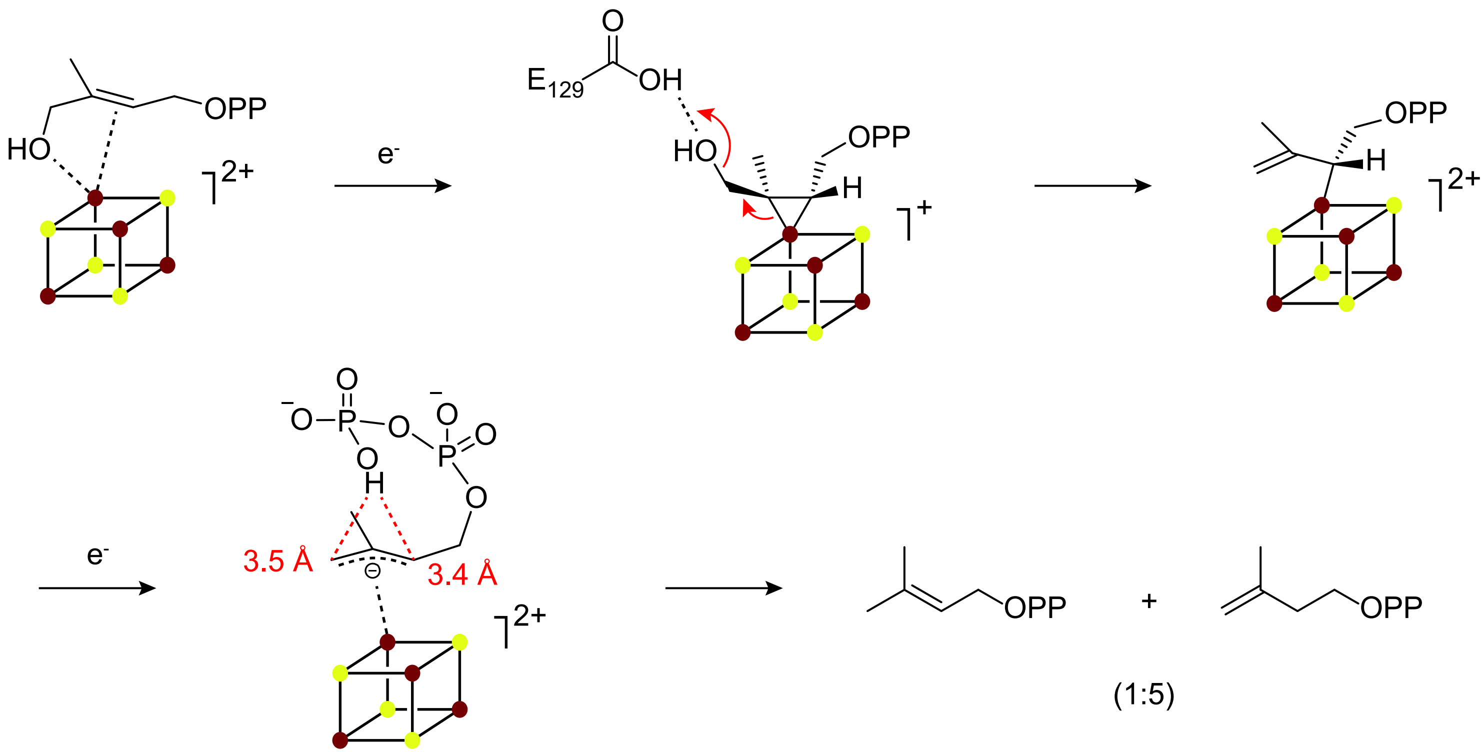


(1:5)

3.2.2. Deoxyxylulose phosphate pathway

two alternative mechanisms for IspH

B)

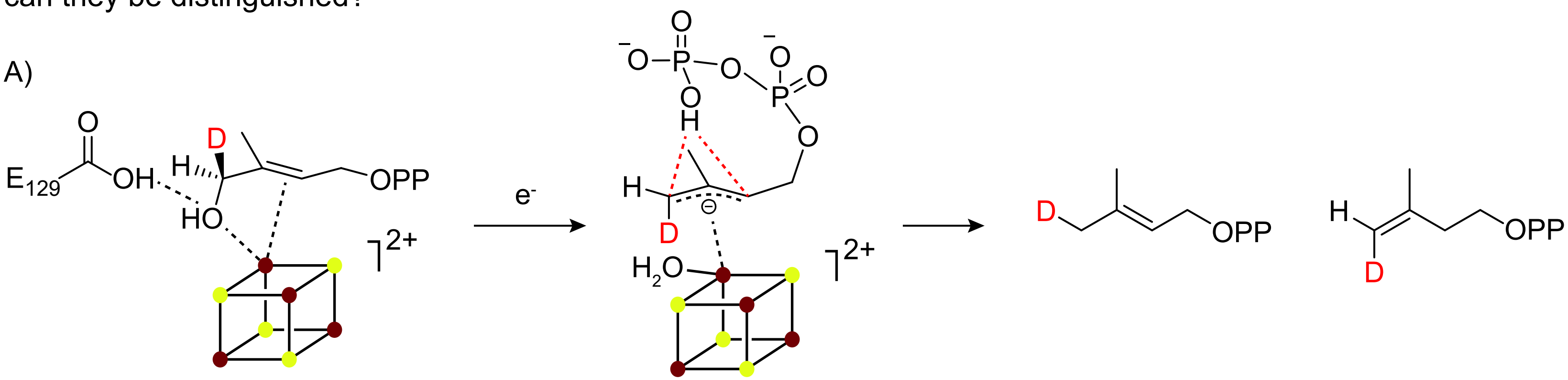


3.2.2. Deoxyxylulose phosphate pathway

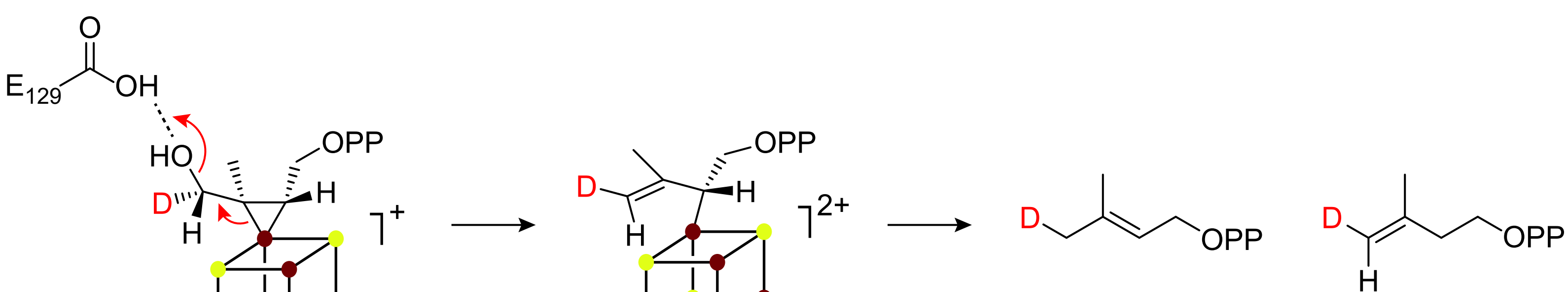
two alternative mechanisms for IspH

can they be distinguished?

A)



B)



observed experimentally

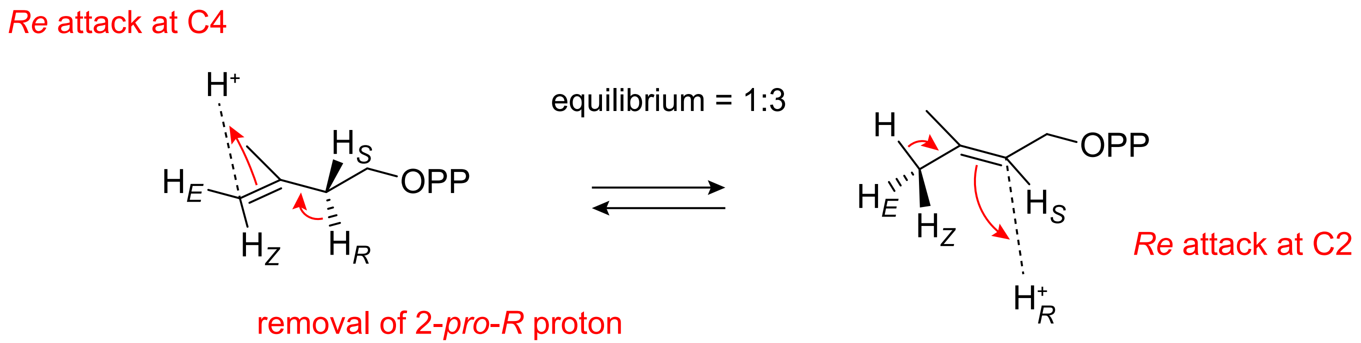
C. A. Citron, N. L. Brock, P. Rabe, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2012**, *51*, 4053.

3.2.3. Polyisoprenoid biosynthesis

3.2.3.1. Isopentenyl diphosphate isomerase (IDI)

a) type I IDI

Mg^{2+} and Zn^{2+} dependent



reversible 1,3-antarafacial addition / elimination of a proton

side reactions:

- deprotonation of *Z* methyl group of DMAPP (2%)
- abstraction of 2-*pro*-*S* proton of IPP (0.5%)

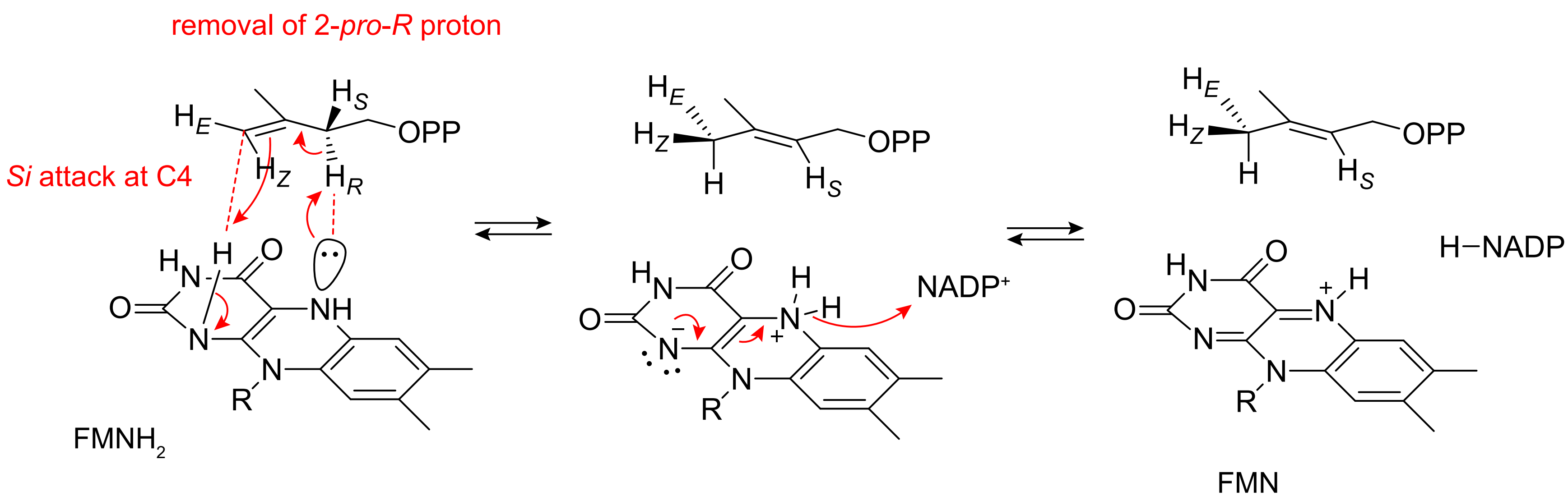
J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, *Proc. R. Soc. London Ser. B* **1966**, *163*, 492.
K. Clifford, J. W. Cornforth, R. Mallaby, G. T. Phillips, *J. Chem. Soc. Chem. Commun.* **1971**, 1599.

3.2.3. Polyisoprenoid biosynthesis

3.2.3.1. Isopentenyl diphosphate isomerase (IDI)

b) type II IDI

FMN, NADPH and Mg^{2+} dependent



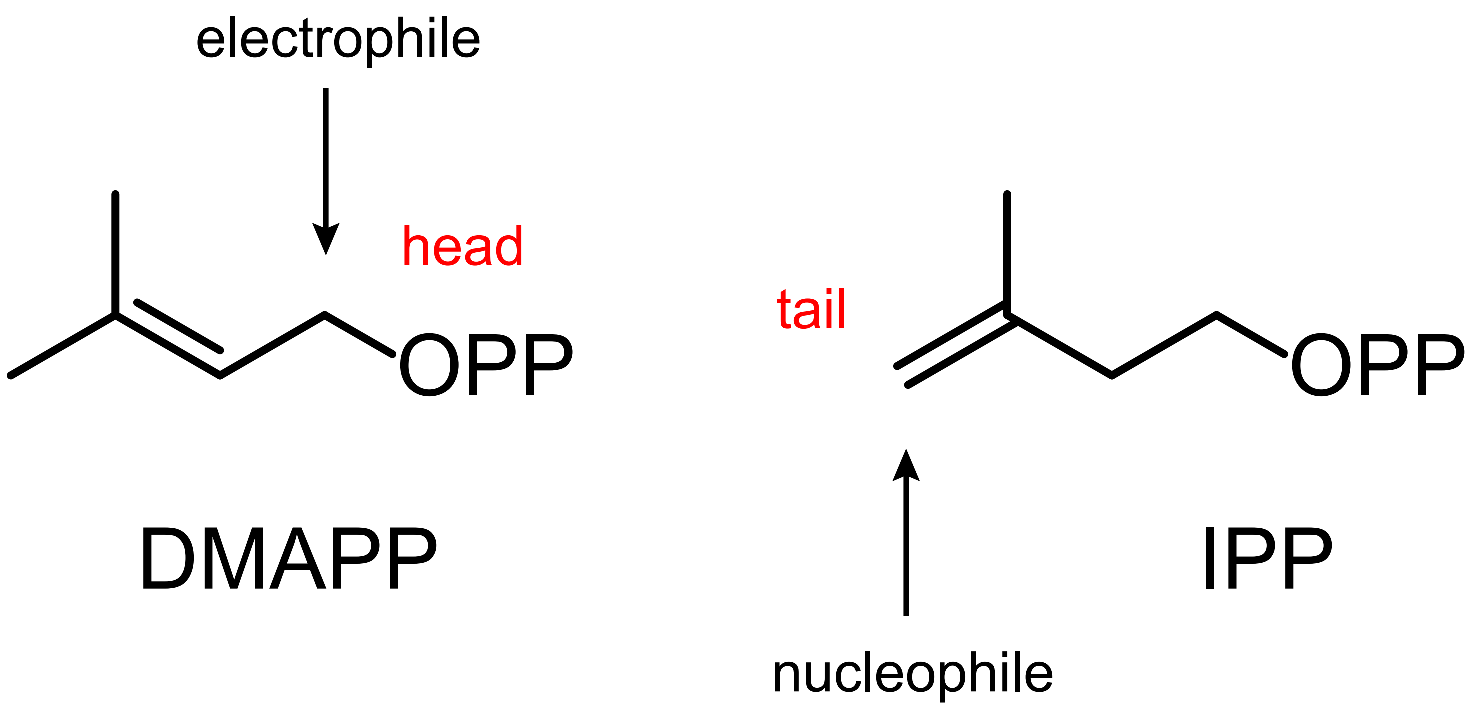
reversible 1,3-suprafacial addition / elimination of a proton

strict stereochemical course

K. Kaneda, T. Kuzuyama, M. Takagi, Y. Hayakawa, H. Seto, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 932.

3.2.3. Polyisoprenoid biosynthesis

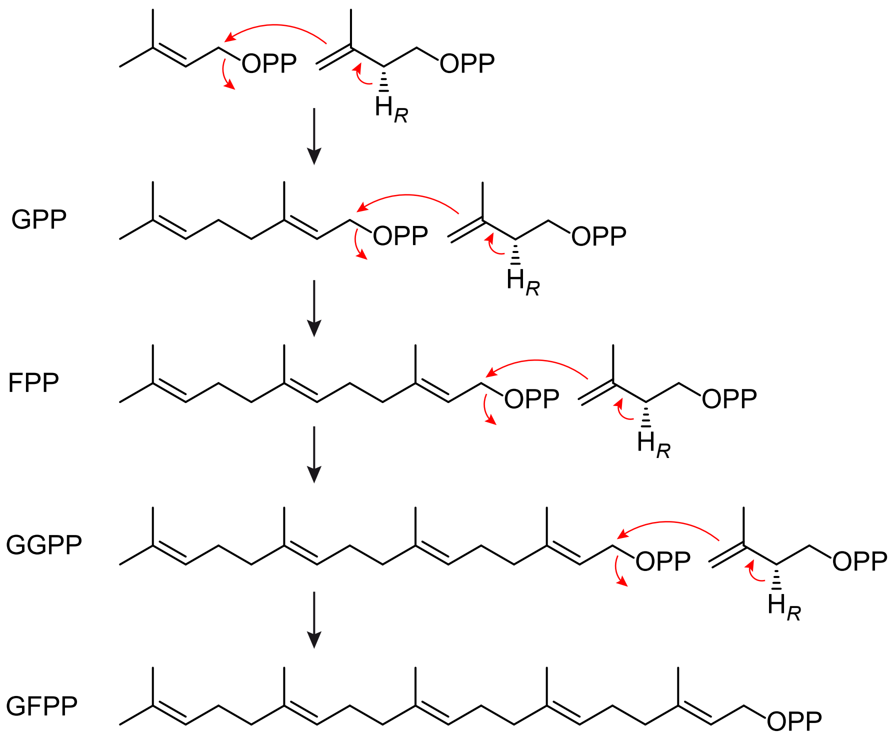
3.2.3.2. Polyisoprenoid synthases

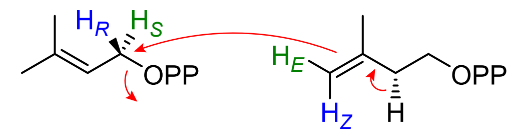


3.2.3. Polyisoprenoid biosynthesis

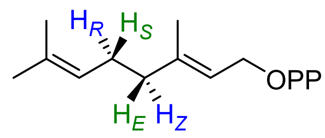
3.2.3.2. Polyisoprenoid synthases

a) type I: *E* double bonds, homodimers, Mg^{2+} dependent, highly conserved DDXXD motif





inversion at C1 *Si* attack at C4

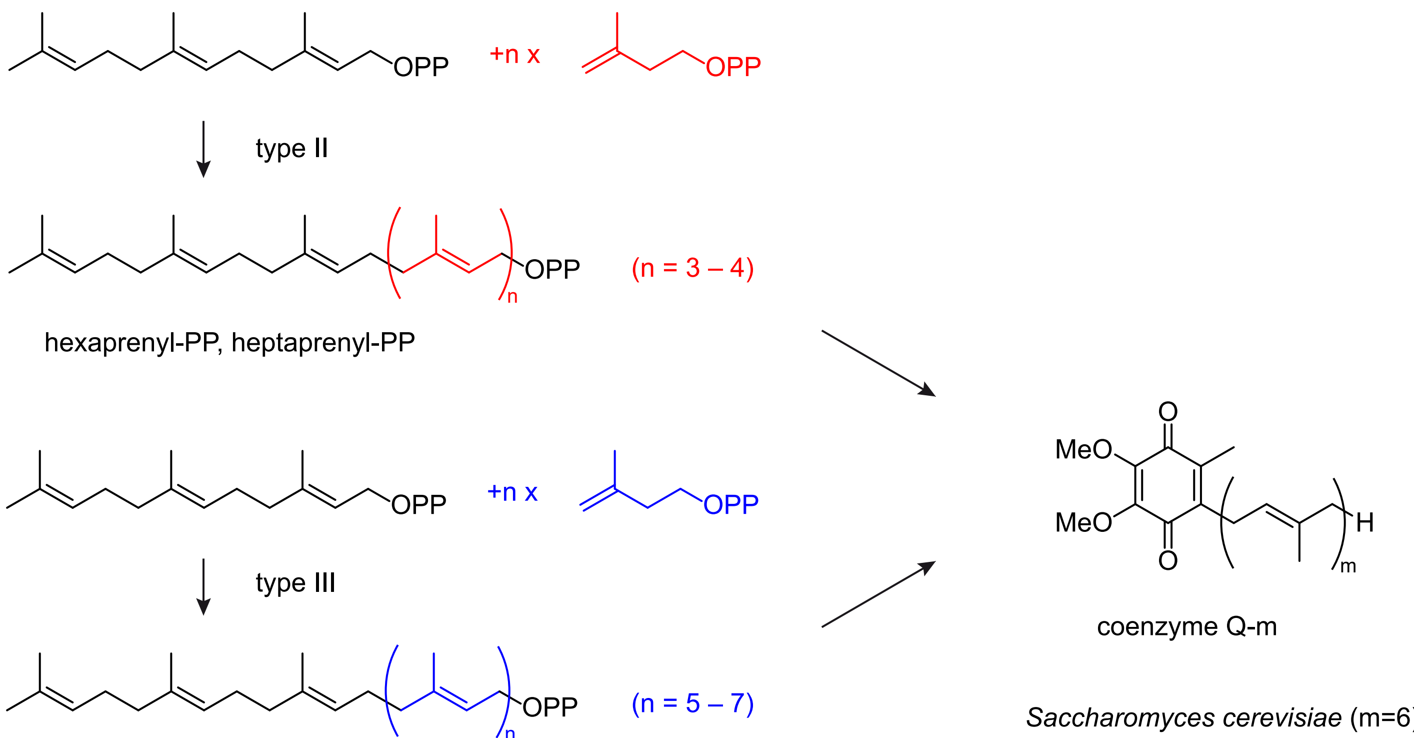


J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, *Proc. R. Soc. London Ser. B* **1966**, *163*, 492.
J. W. Cornforth, R. H. Cornforth, G. Popjak, L. Yengoyan, *J. Biol. Chem.* **1966**, *241*, 3970.

3.2.3. Polyisoprenoid biosynthesis

3.2.3.2. Polyisoprenoid synthases

b) type II and type III: *E*-selective, different chain lengths



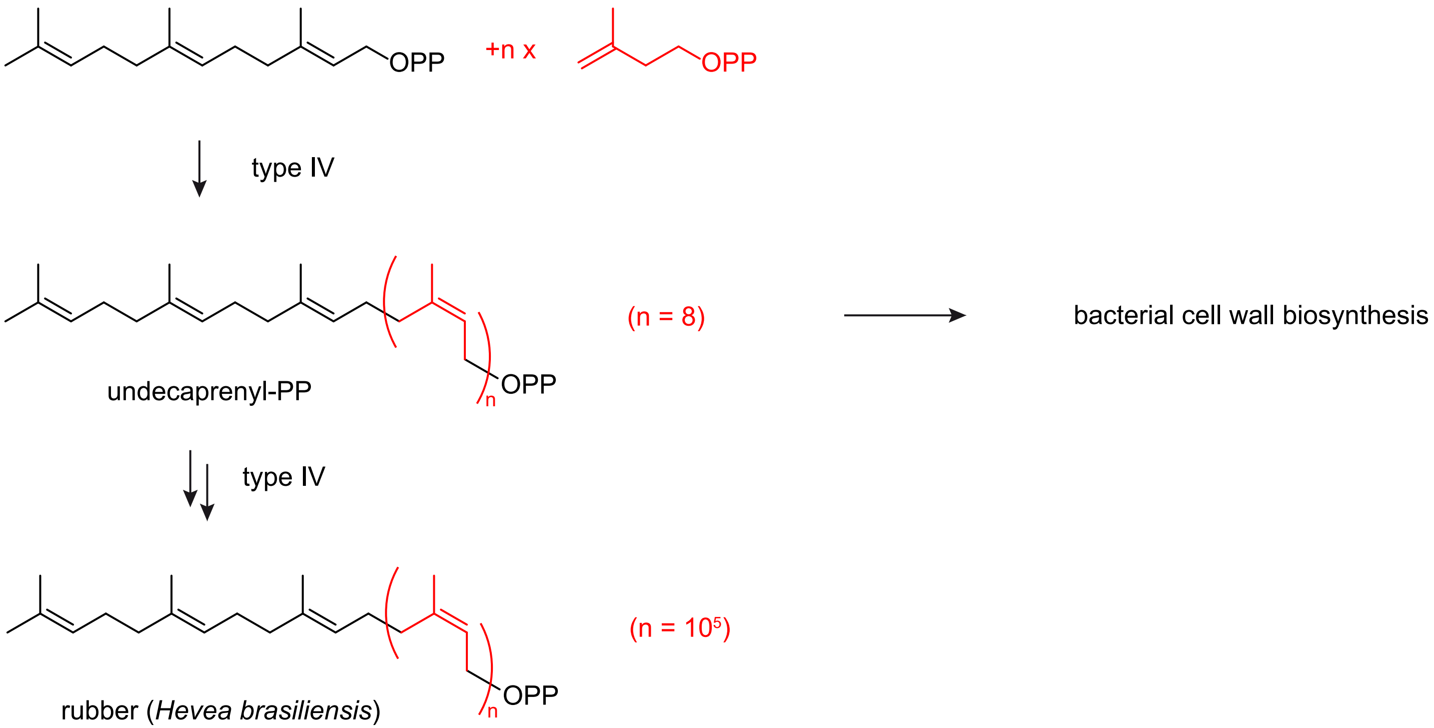
octaprenyl-PP, nonaprenyl-PP, decaprenyl-PP

Saccharomyces cerevisiae (m=6)
Escherichia coli (m=8)
Homo sapiens (m=10)

3.2.3. Polyisoprenoid biosynthesis

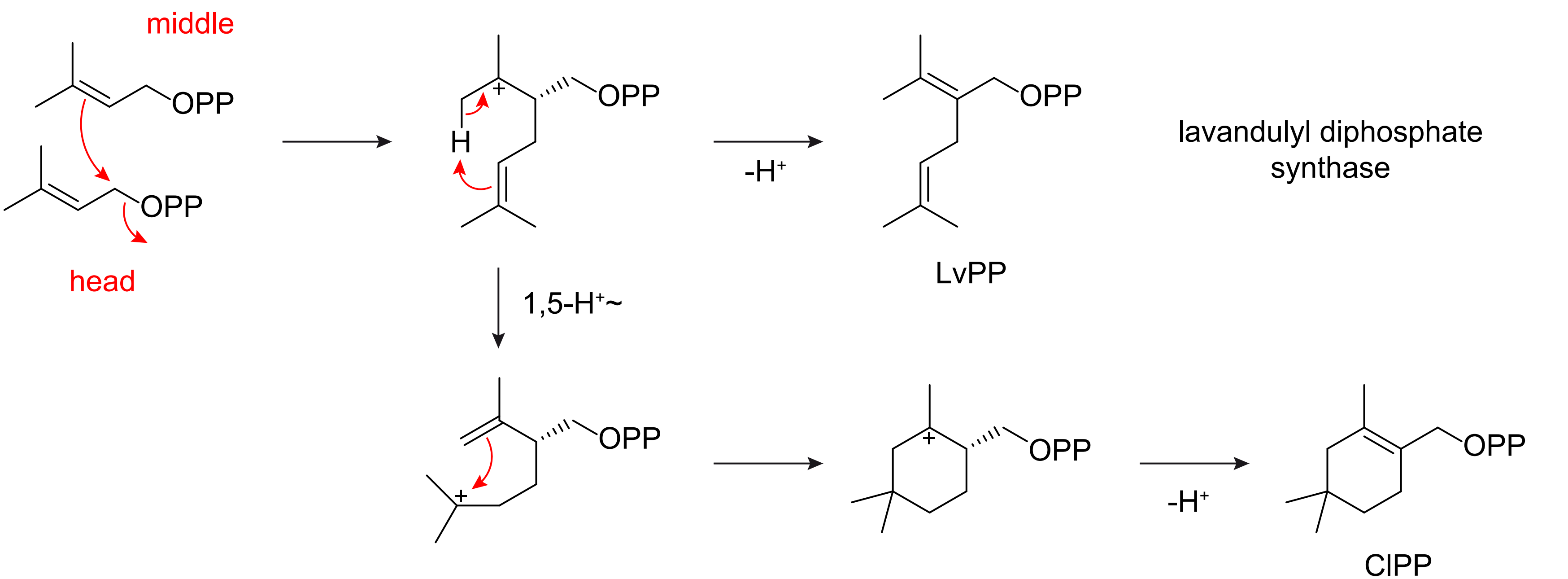
3.2.3.2. Polyisoprenoid synthases

b) type IV: *Z*-selective, from FPP starter



3.2.3. Polyisoprenoid biosynthesis

3.2.3.3. Head-to-middle prenyltransferases



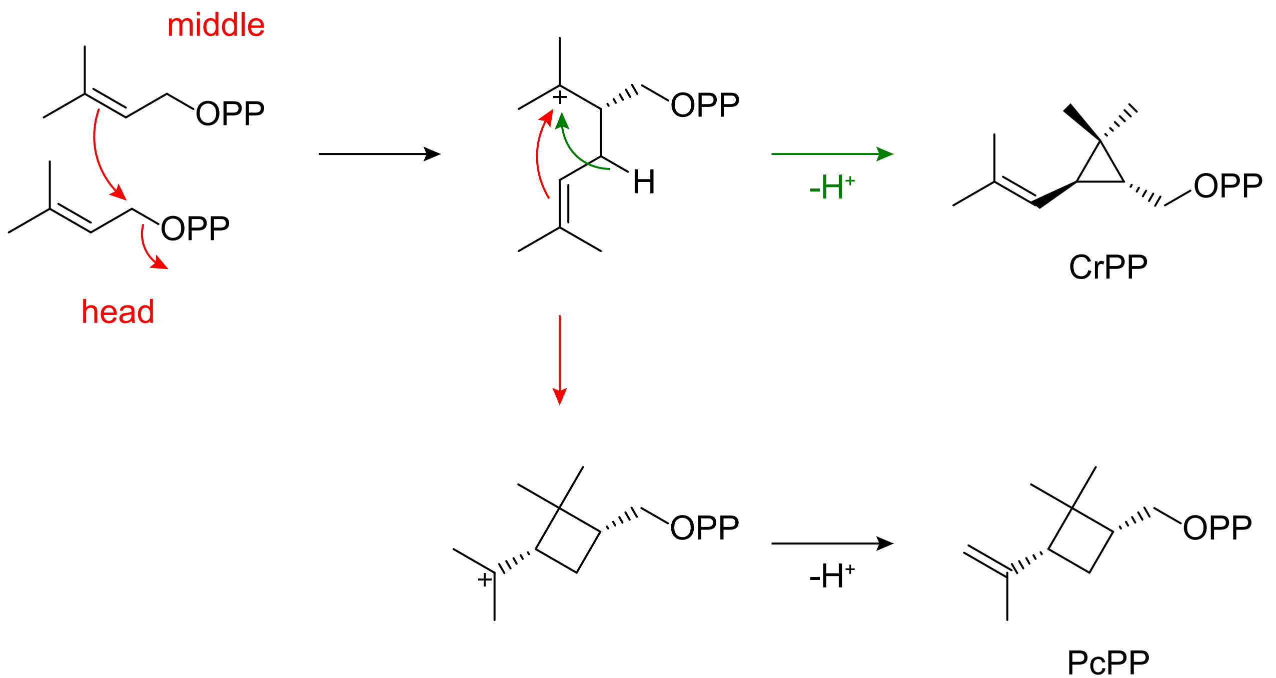
cyclolavandulyl diphosphate synthase

T. Tomita, M. Kobayashi, Y. Karita, Y. Yasuno, T. Shinada, M. Nishiyama, T. Kuzuyama, *Angew. Chem. Int. Ed.* **2017**, *56*, 14913.

M. Liu, C.-C. Chen, L. Chen, X. Xiao, Y. Zheng, J.-W. Huang, W. Liu, T.-P. Ko, Y.-S. Cheng, X. Feng, E. Oldfield, R.-T. Guo, Y. Ma, *Angew. Chem. Int. Ed.* **2016**, *55*, 4613.

3.2.3. Polyisoprenoid biosynthesis

3.2.3.3. Head-to-middle prenyltransferases

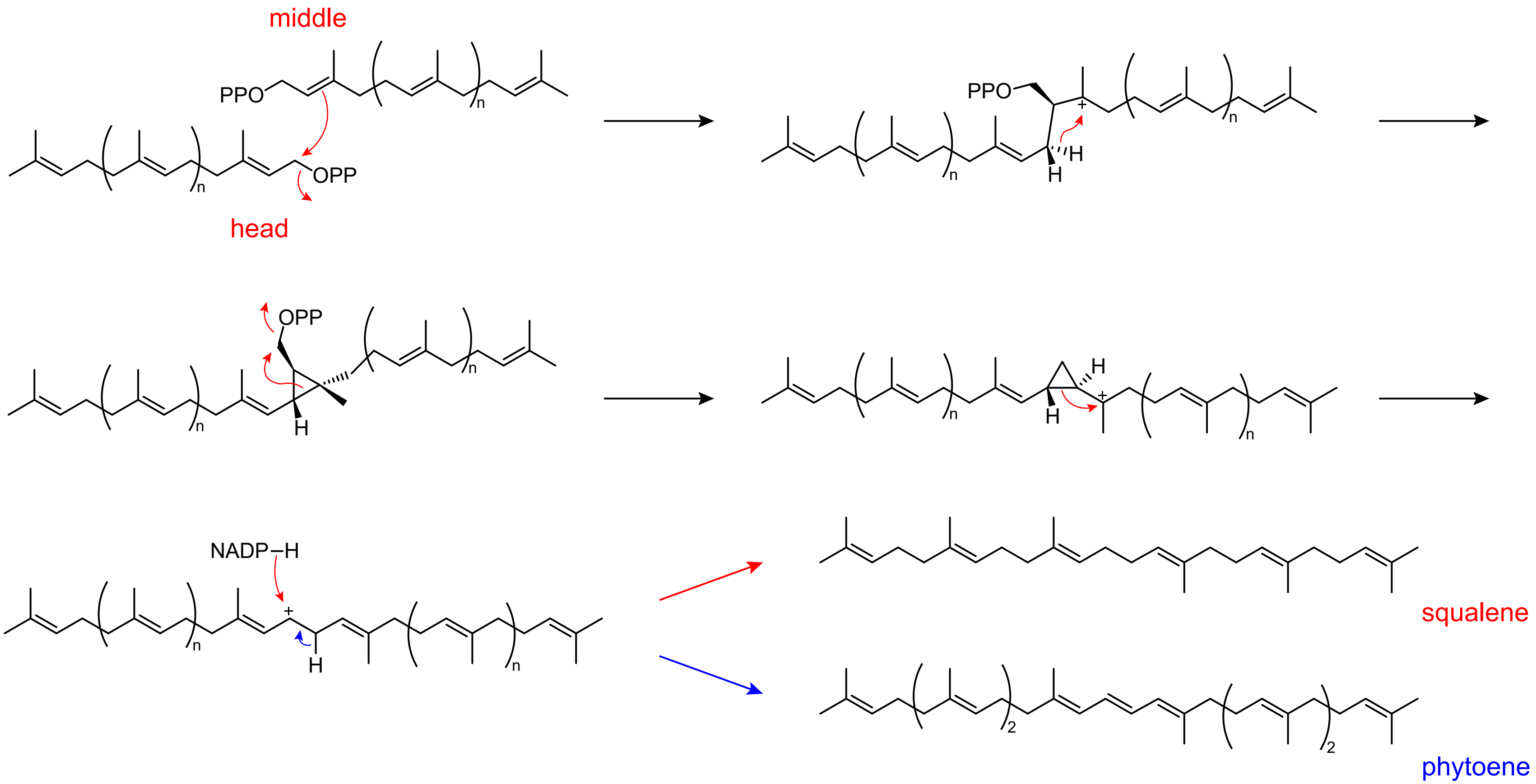


chrysanthemyl diphosphate synthase

planococcyl diphosphate synthase

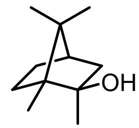
3.2.3. Polyisoprenoid biosynthesis

3.2.3.4. Squalene synthase and phytoene synthase: head-to-middle prenyltransferases

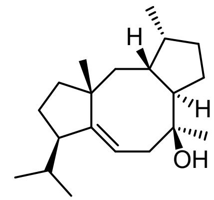


3.2.4. Terpene Synthases

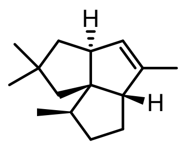
3.2.4.1. Type I



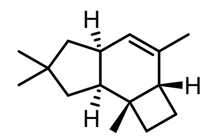
Streptomyces
(Cane & Ikeda, 2008)



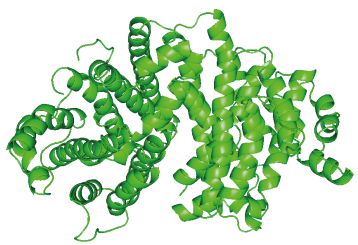
Streptomyces melanosporofaciens
(Kuzuyama, 2009)

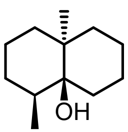


Streptomyces exfoliatus
(Cane, 1994)

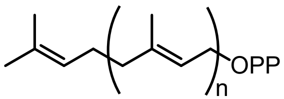


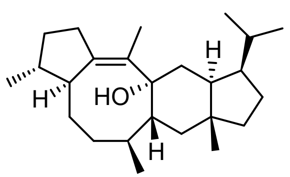
Dictyostelium discoideum
(Chen & Dickschat, 2016)



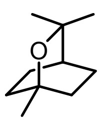


Streptomyces coelicolor
(Cane, 2007)

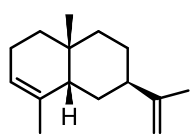




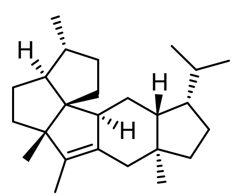
Neosartorya fischeri
(Oikawa, 2015)



Streptomyces clavuligerus
(Ohnishi, 2011)



Nostoc punctiforme
(Schmidt-Dannert, 2008)



Emericella variecolor
(Abe, 2016)

J. S. Dickschat, *Nat. Prod. Rep.* **2016**, *33*, 87.
J. S. Dickschat, *Angew. Chem. Int. Ed.* **2019**, *58*, 15964.
D. W. Christianson, *Chem. Rev.* **2017**, *117*, 11570.

3.2.4. Terpene Synthases

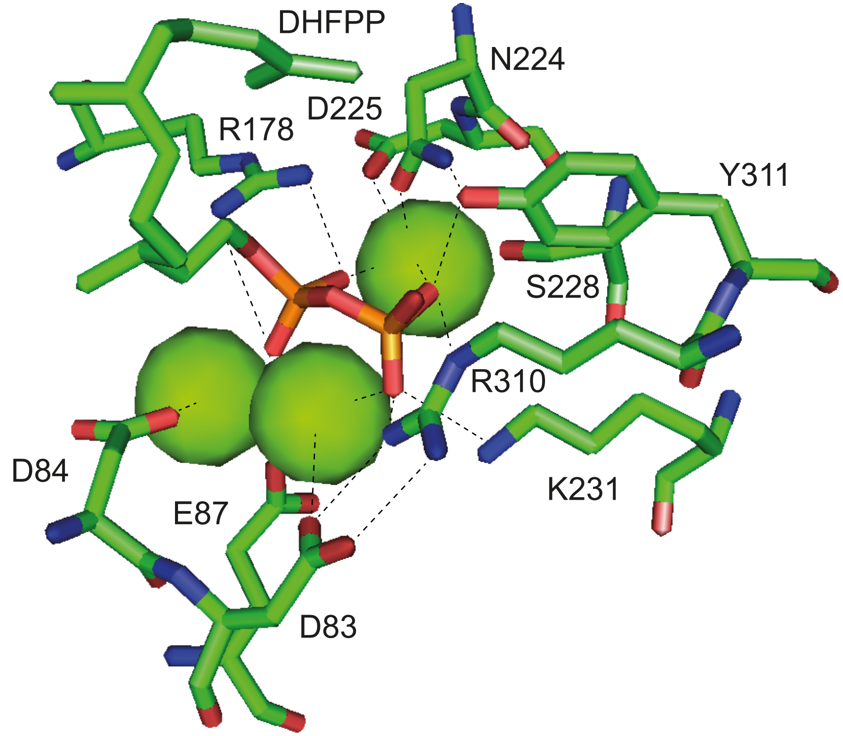
3.2.4.1. Type I

highly conserved motifs

DDXXD
R
NDLXSXXXE
RY

Mg^{2+}-dependent

ionisation by abstraction of diphosphate

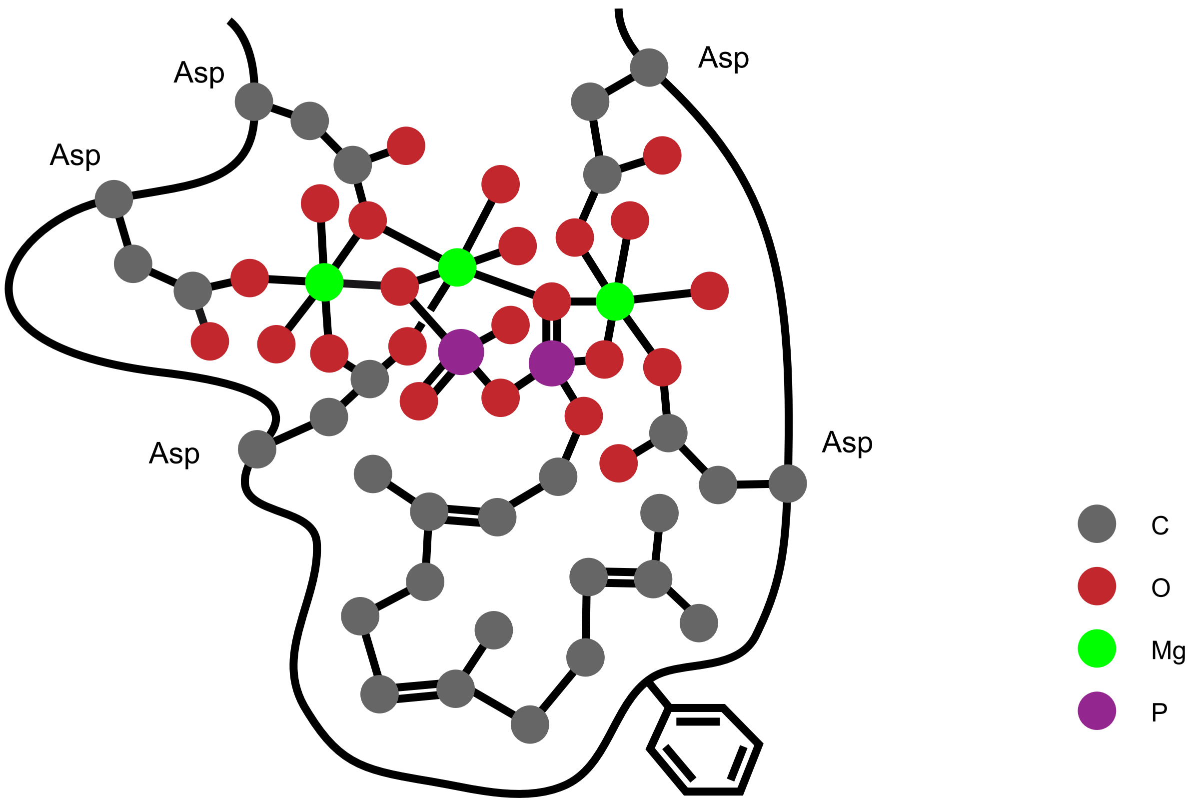


crystal structure of selina-4(15),7(11)-diene synthase (*Streptomyces pristinaespiralis*)

P. Baer, P. Rabe, K. Fischer, C. A. Citron, T. A. Klapschinski, M. Groll, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2014**, *53*, 7652.

3.2.4. Terpene Synthases

3.2.4.1. Type I



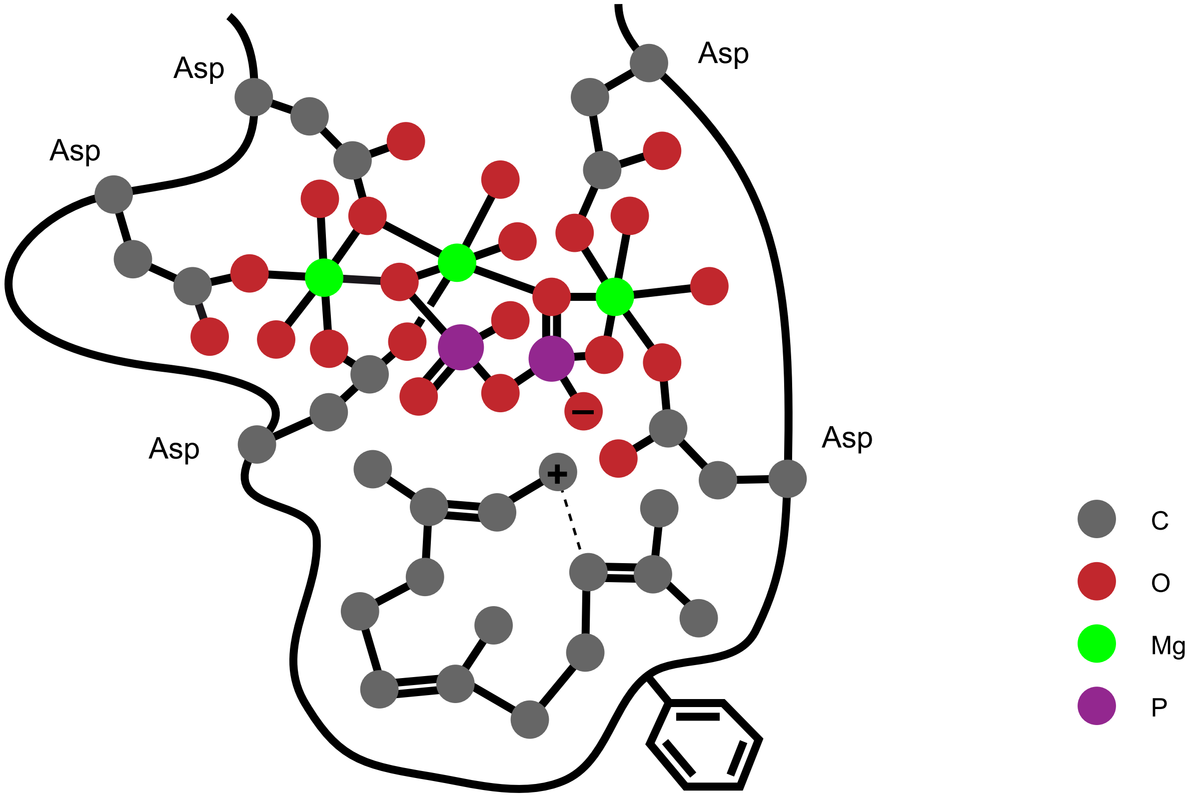
hydrophobic pocket

exclusion of water

substrate folding

3.2.4. Terpene Synthases

3.2.4.1. Type I



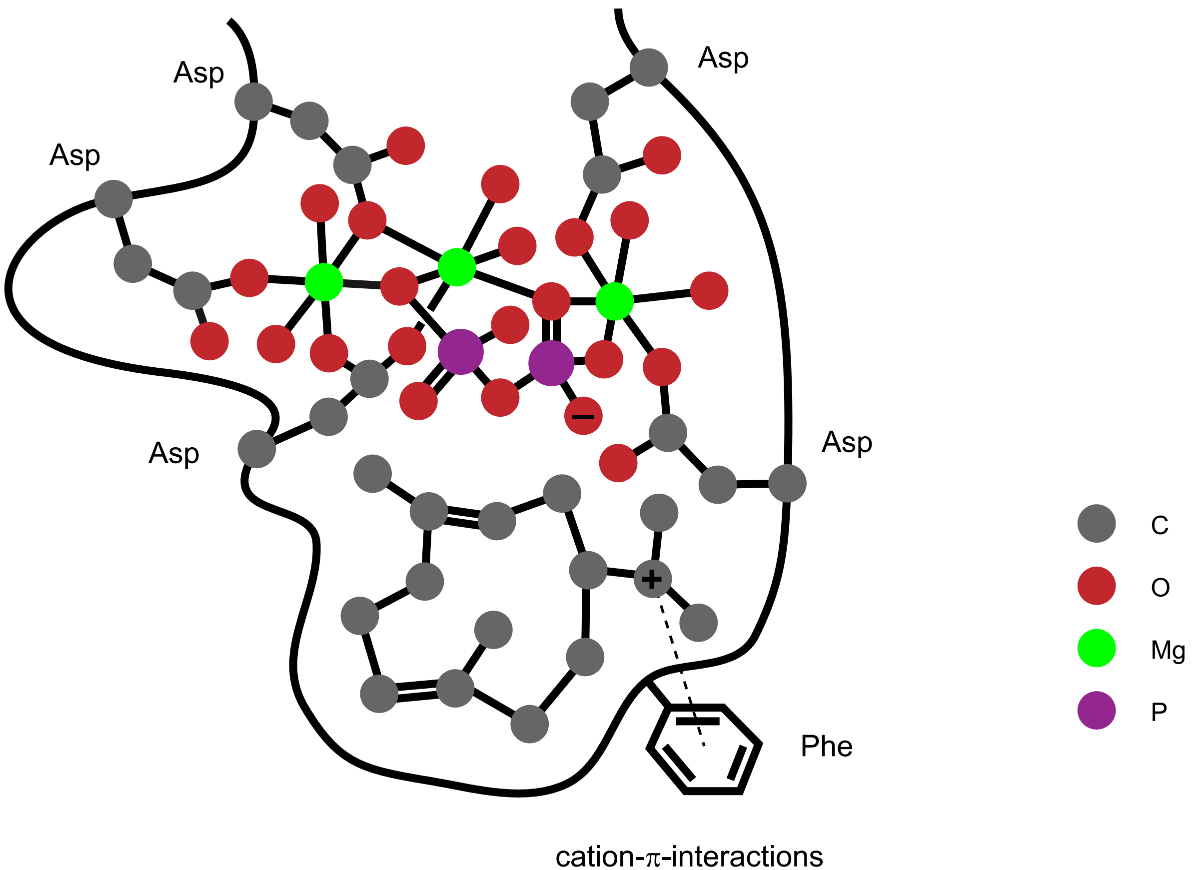
hydrophobic pocket

exclusion of water

substrate folding

3.2.4. Terpene Synthases

3.2.4.1. Type I



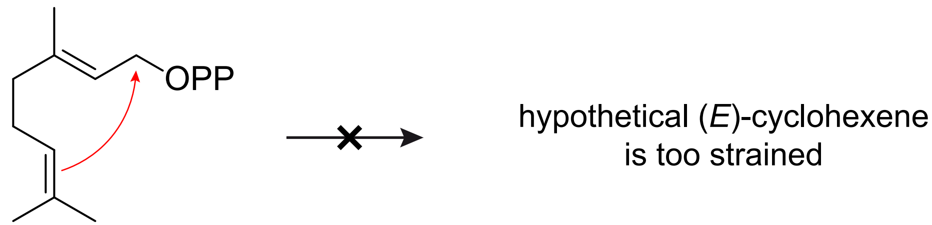
hydrophobic pocket

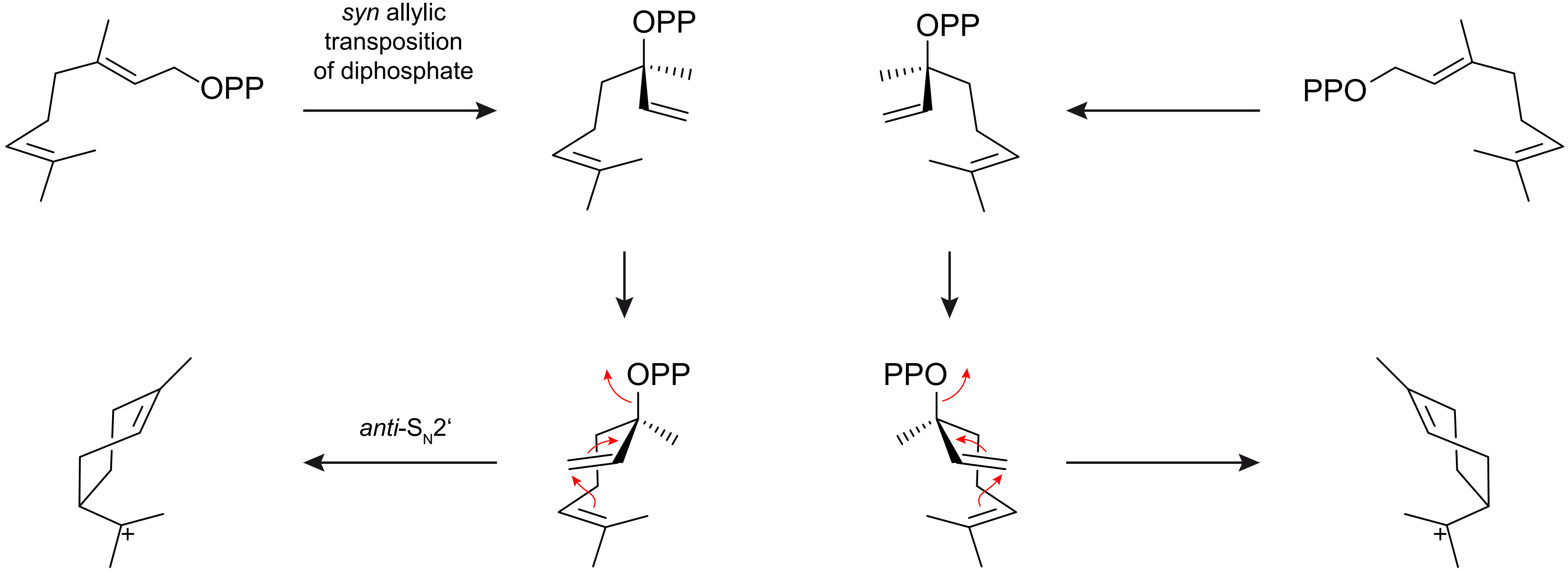
exclusion of water

substrate folding

3.2.4. Terpene Synthases

3.2.4.2. Type I monoterpene synthases

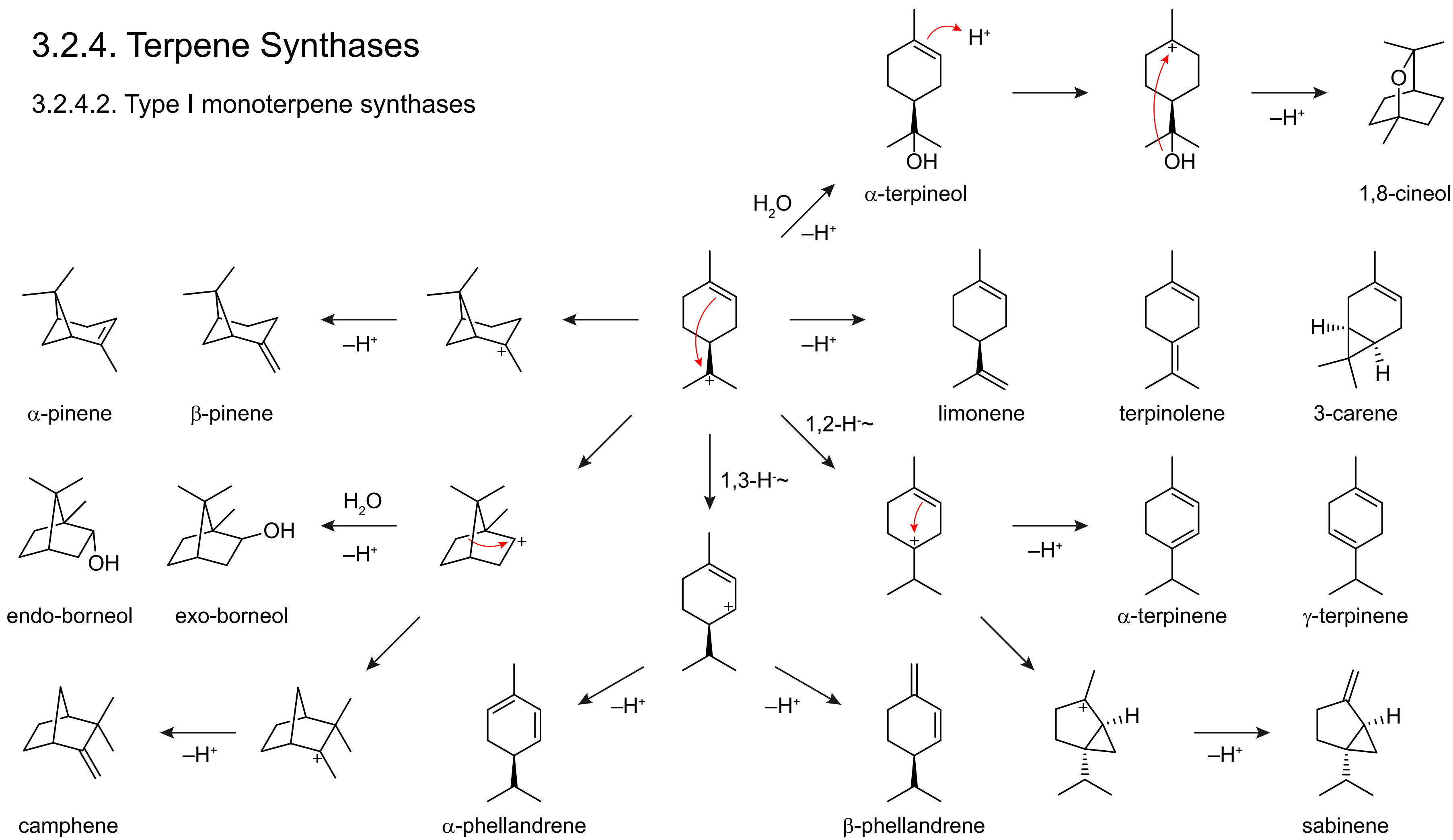




(*R*)-terpinyl cation (*R*)-linalyl diphosphate (*S*)-linalyl diphosphate (*S*)-terpinyl cation

3.2.4. Terpene Synthases

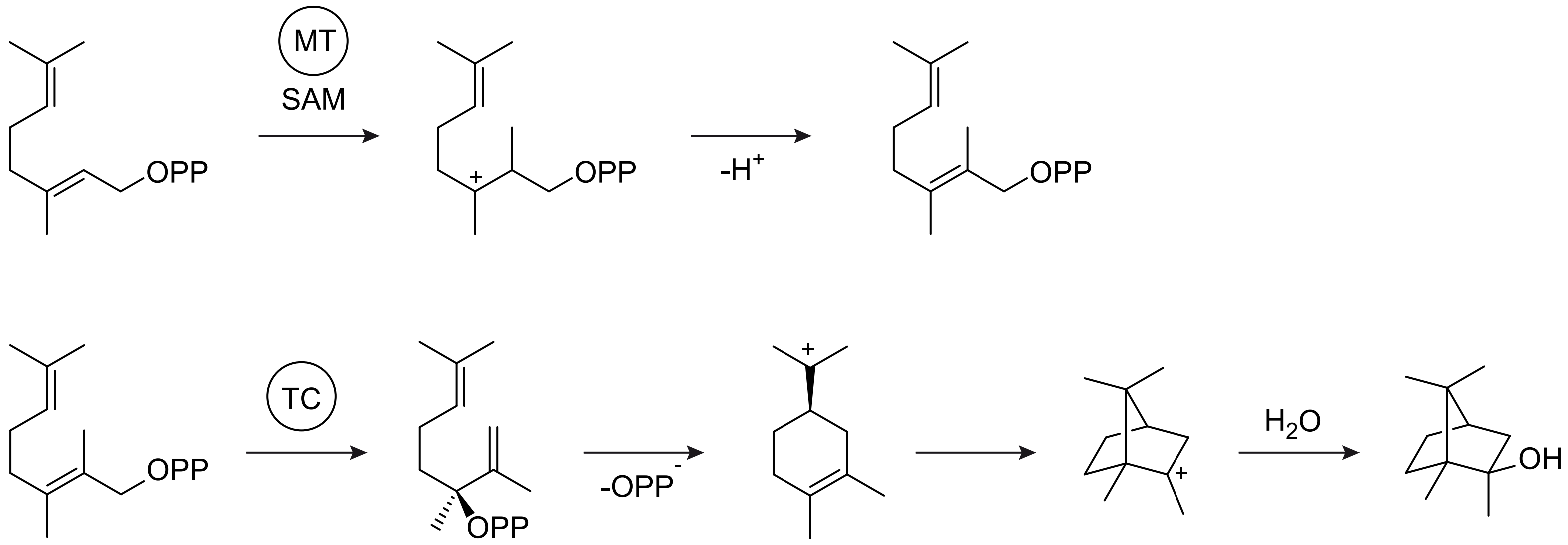
3.2.4.2. Type I monoterpene synthases



3.2.4. Terpene Synthases

3.2.4.3. 2-Methylisoborneol - a methylated monoterpene

musty odorant produced by bacteria (actinomycetes, myxobacteria, cyanobacteria)



J. S. Dickschat, T. Nawrath, V. Thiel, B. Kunze, R. Müller, S. Schulz, *Angew. Chem. Int. Ed.* **2007**, *46*, 8287.

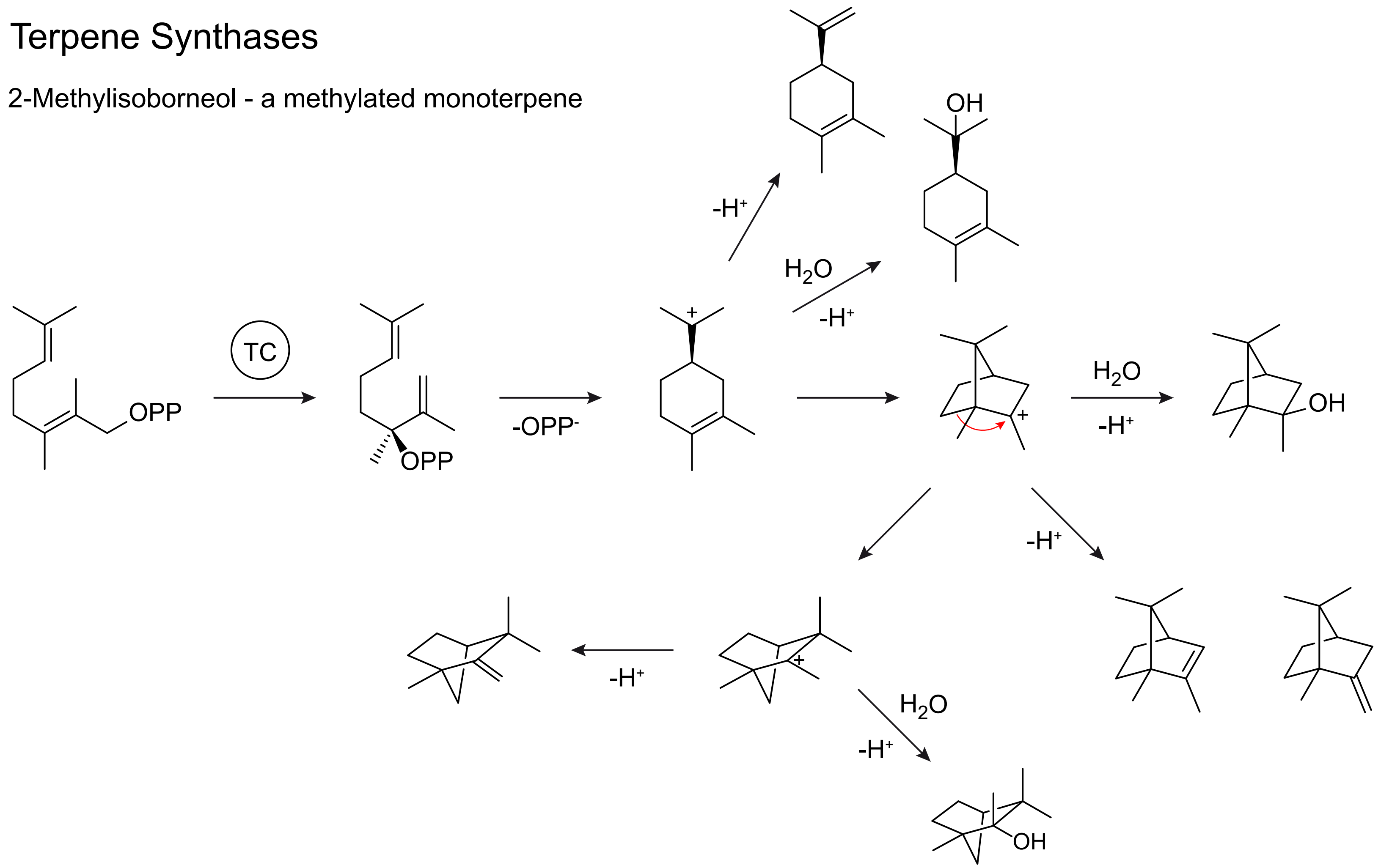
M. Komatsu, M. Tsuda, S. Omura, H. Oikawa, H. Ikeda, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 7422.

C.-M. Wang, D. E. Cane, *J. Am. Chem. Soc.* **2008**, *130*, 8908.

N. L. Brock, S. R. Ravella, S. Schulz, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2013**, *52*, 2100.

3.2.4. Terpene Synthases

3.2.4.3. 2-Methylisoborneol - a methylated monoterpene

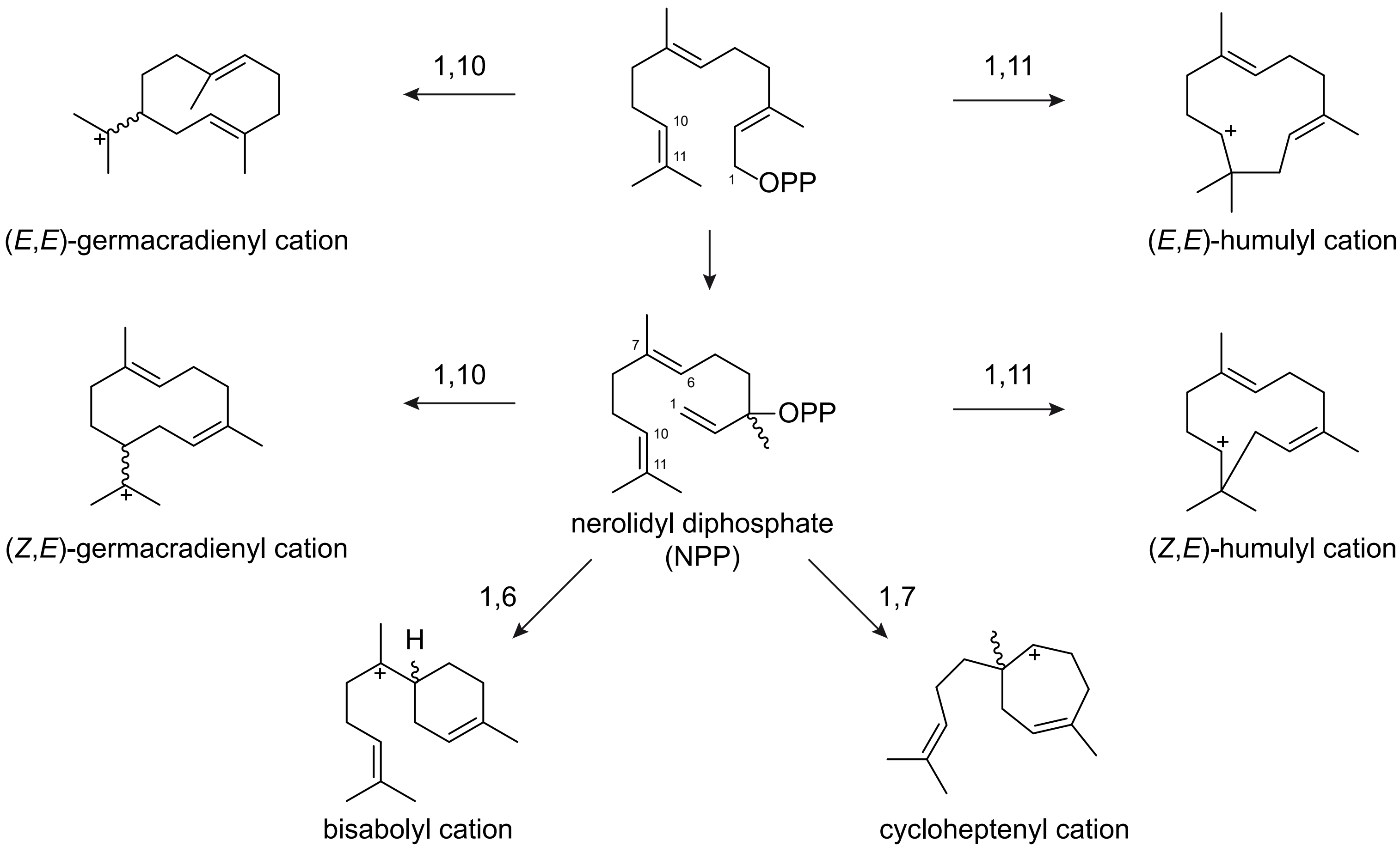


N. L. Brock, S. R. Ravella, S. Schulz, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2013**, *52*, 2100.

3. Terpenes

3.2.4. Terpene Synthases

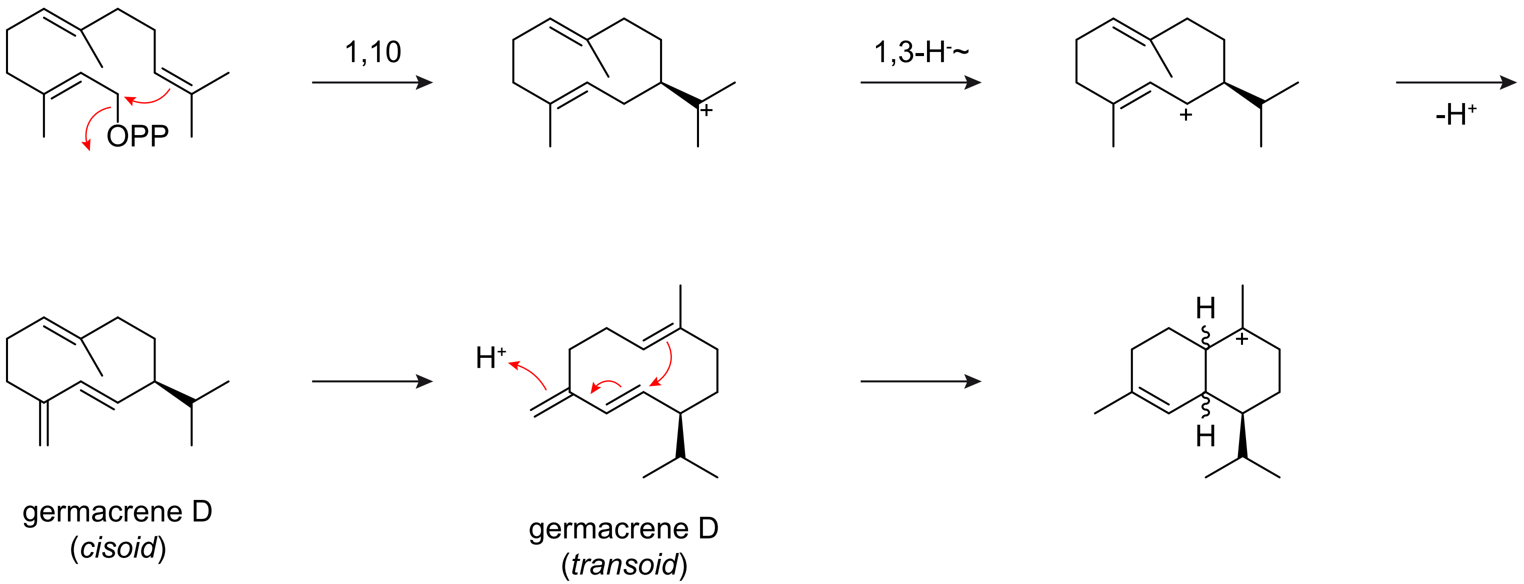
3.2.4.4. Type I sesquiterpene synthases



3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

alternative mechanism for installation of *Z* double bonds

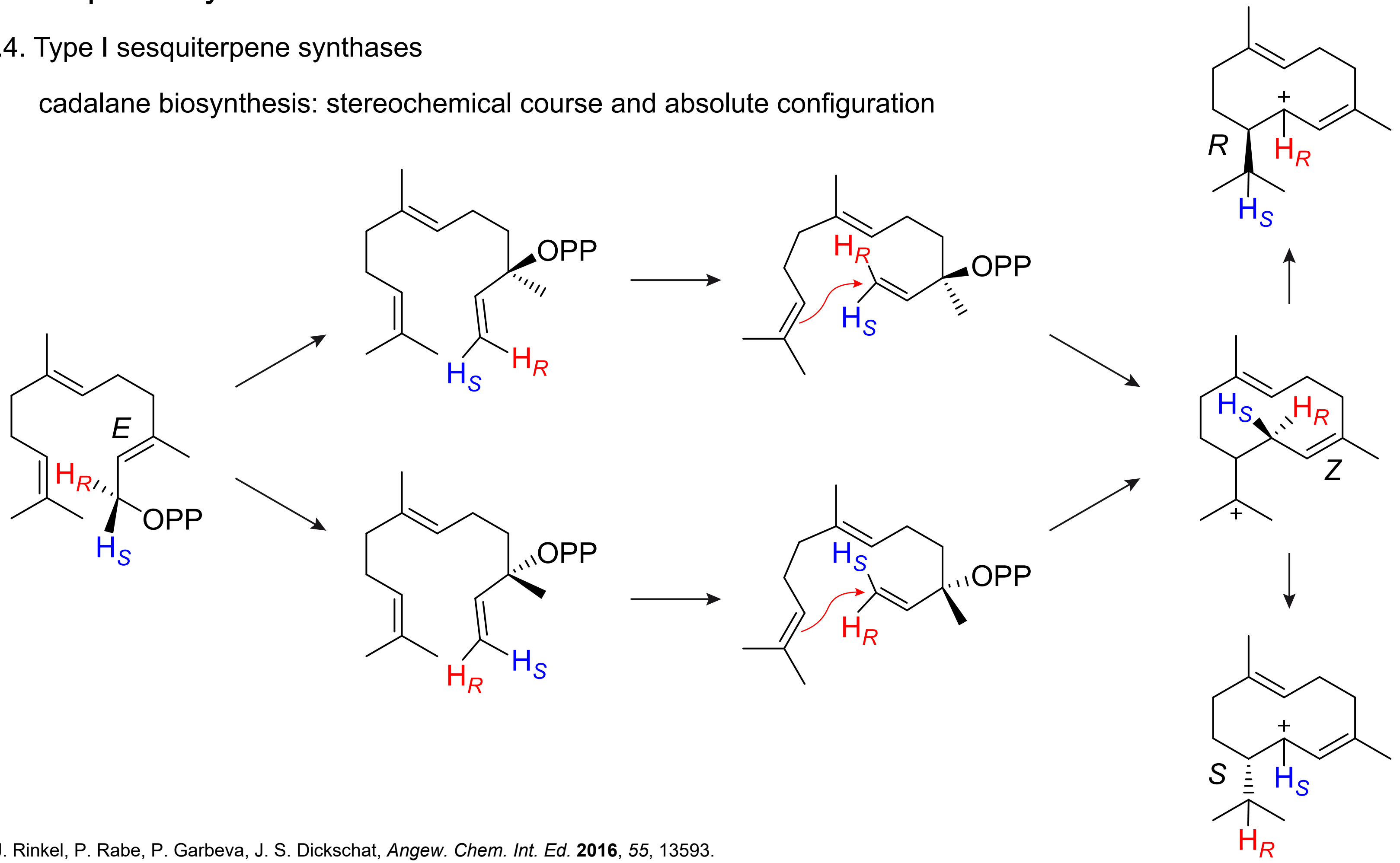


D. Arigoni, *Pure Appl. Chem.* **1975**, *41*, 219.

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

cadalane biosynthesis: stereochemical course and absolute configuration

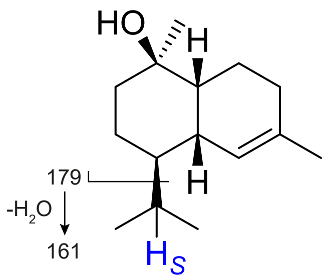


J. Rinkel, P. Rabe, P. Garbeva, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2016**, *55*, 13593.

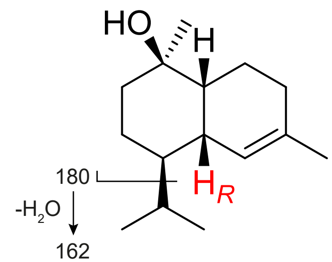
3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

cadalane biosynthesis: stereochemical course and absolute configuration

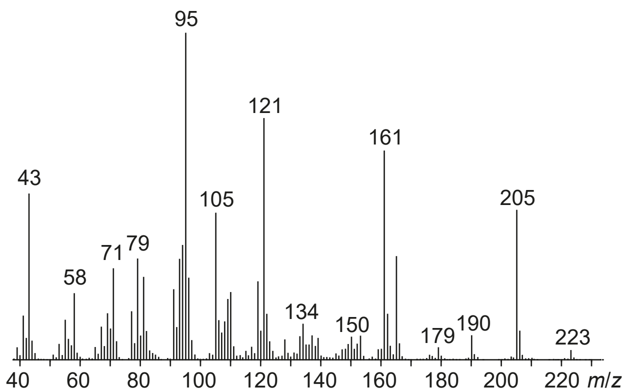


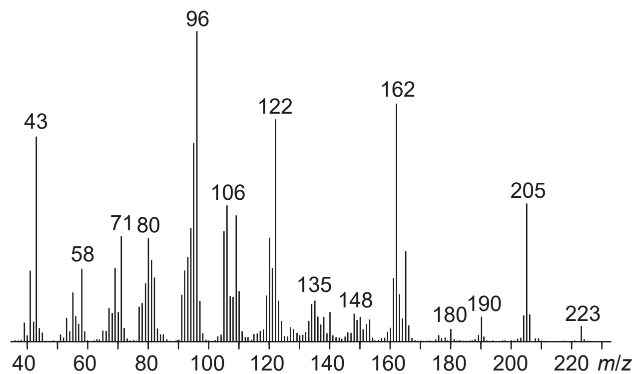
T-muurolol

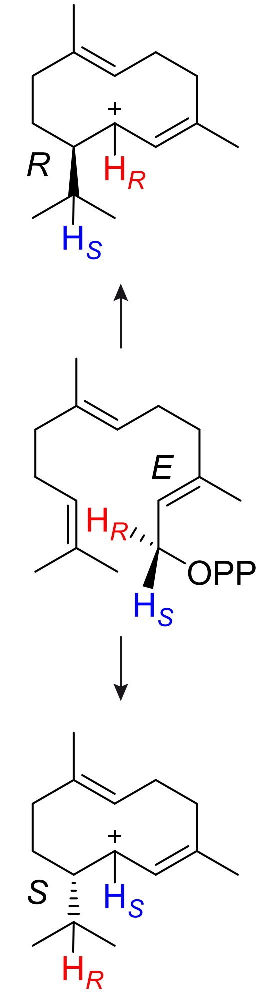


H_S = ^{2}H

H_R = ^{2}H







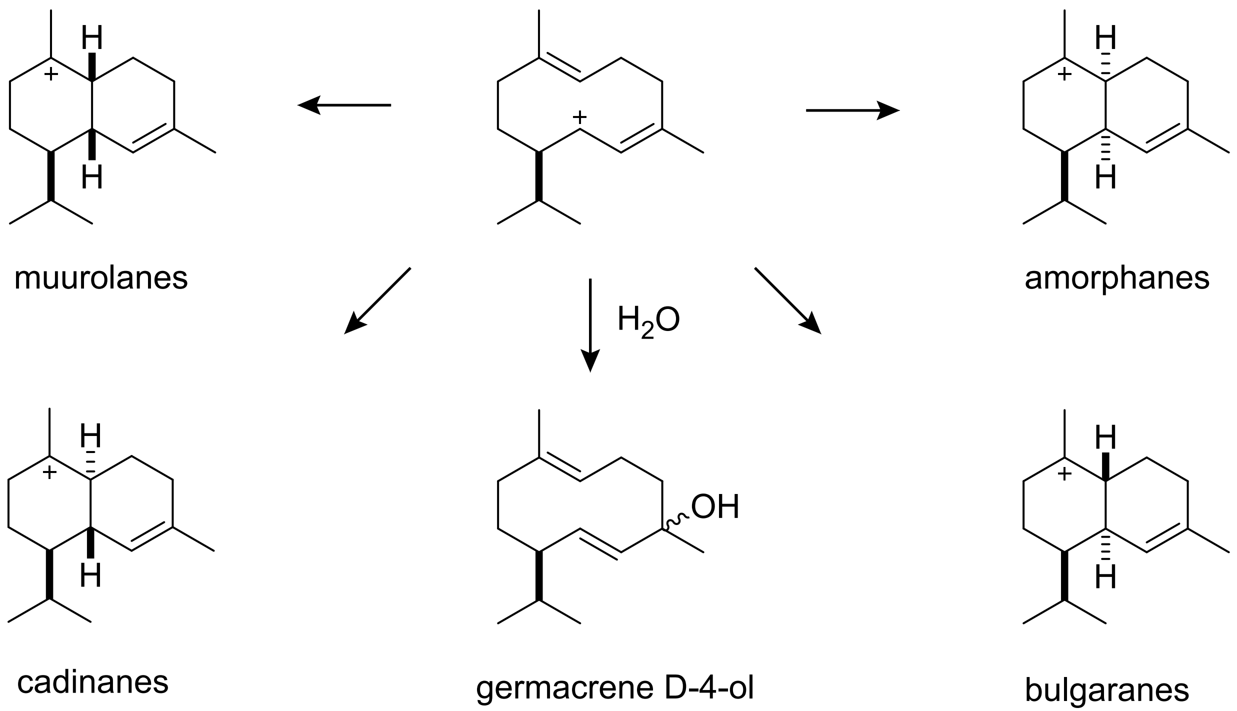
J. Rinkel, P. Rabe, P. Garbeva, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2016**, *55*, 13593.

3. Terpenes

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

cadalane biosynthesis: stereochemical course and absolute configuration

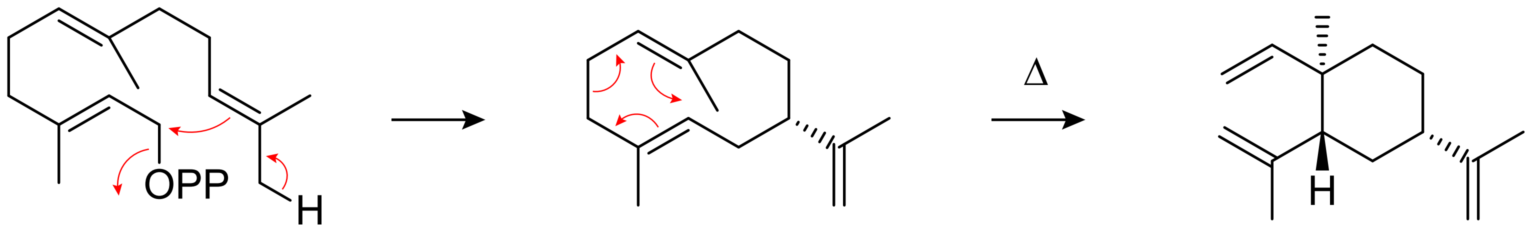


J. Rinkel, P. Rabe, P. Garbeva, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2016**, *55*, 13593.

3.2.4. Terpene Synthases

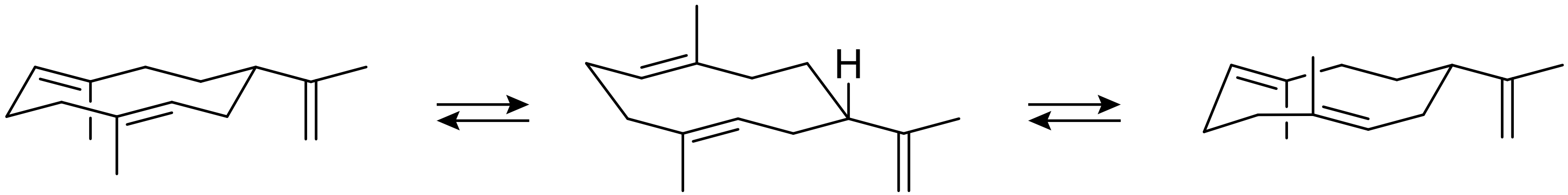
3.2.4.4. Type I sesquiterpene synthases

germacrene A synthase (*Micromonospora marina*)



germacrene A

β-elemene



DD conformer
(Me groups down-down)

DU (down-up)

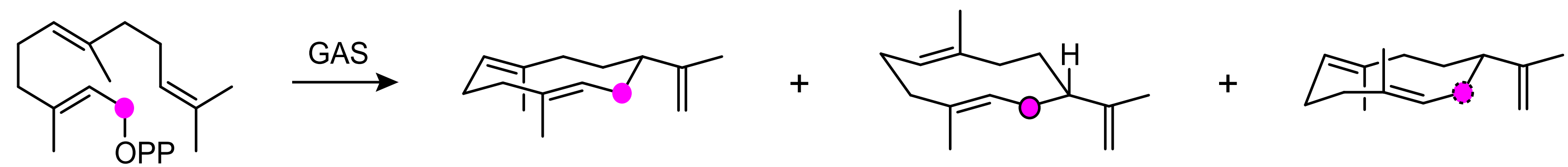
UD

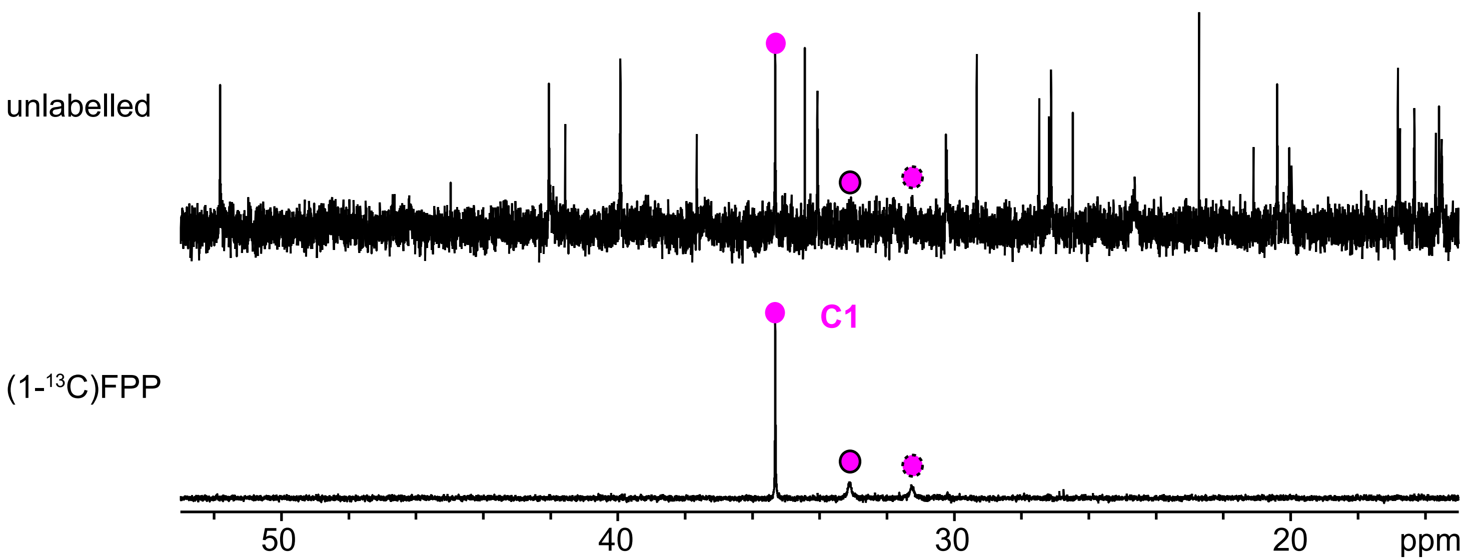
J. Rinkel, J. S. Dickschat, *Org. Lett.* **2019**, *21*, 2426.

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

^{13}C-NMR data of germacrene A



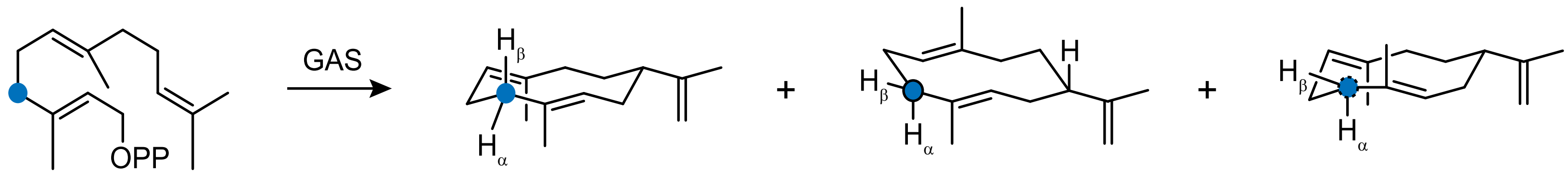


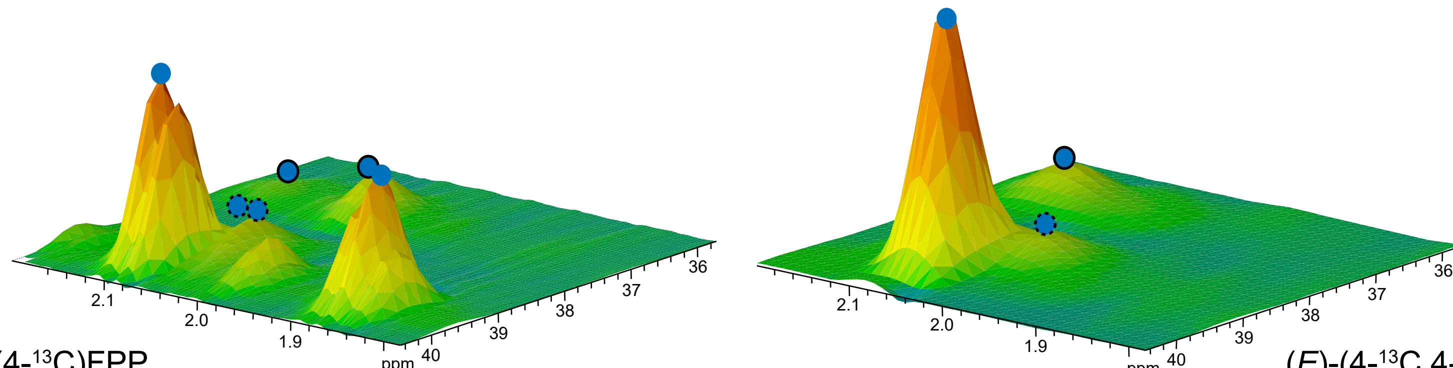
J. Rinkel, J. S. Dickschat, *Org. Lett.* **2019**, *21*, 2426.

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

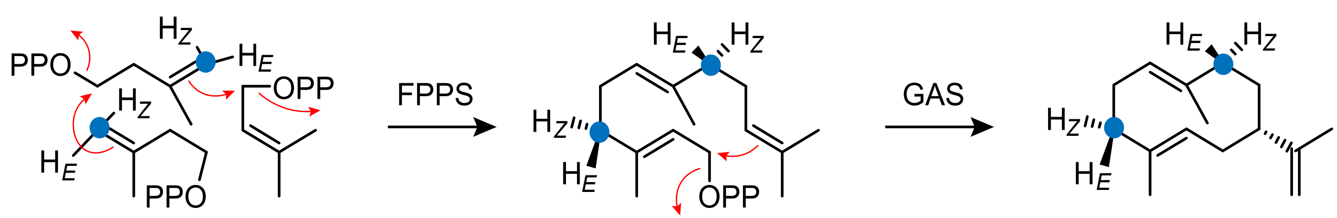
^{1}H-NMR data of germacrene A by HSQC





(4-^{13}C)FPP

(*E*)-(4-^{13}C,4-^{2}H)IPP

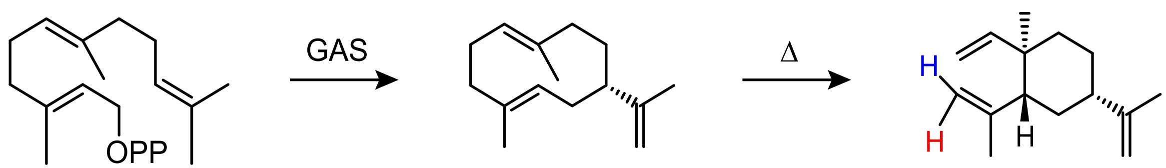


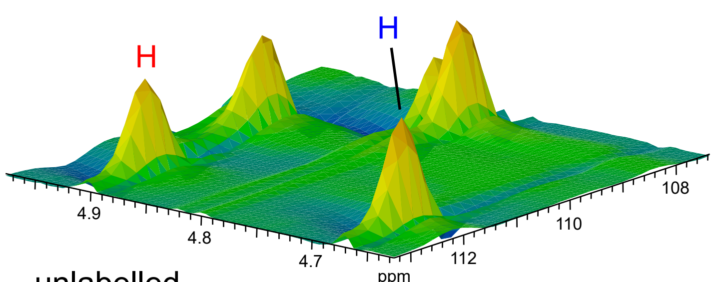
J. Rinkel, J. S. Dickschat, *Org. Lett.* **2019**, *21*, 2426.

3.2.4. Terpene Synthases

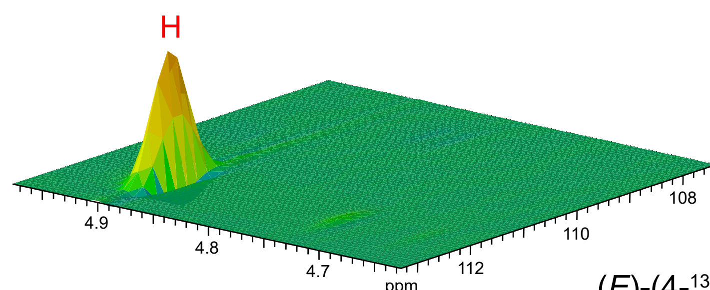
3.2.4.4. Type I sesquiterpene synthases

stereochemical course of Cope rearrangement

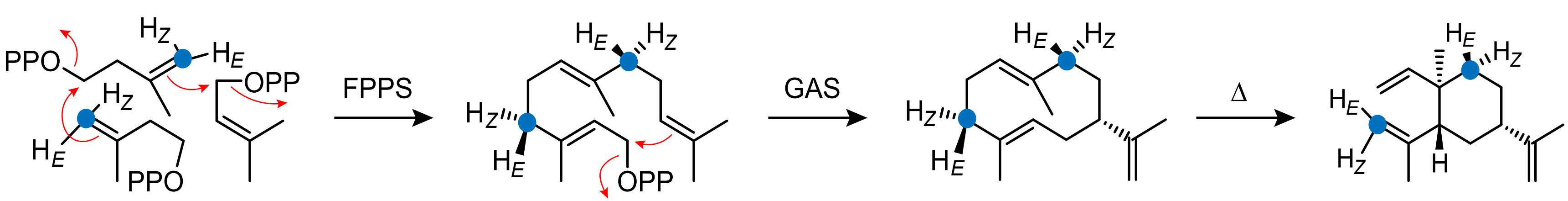




unlabelled



(*E*)-(4-^{13}C,4-^{2}H)IPP



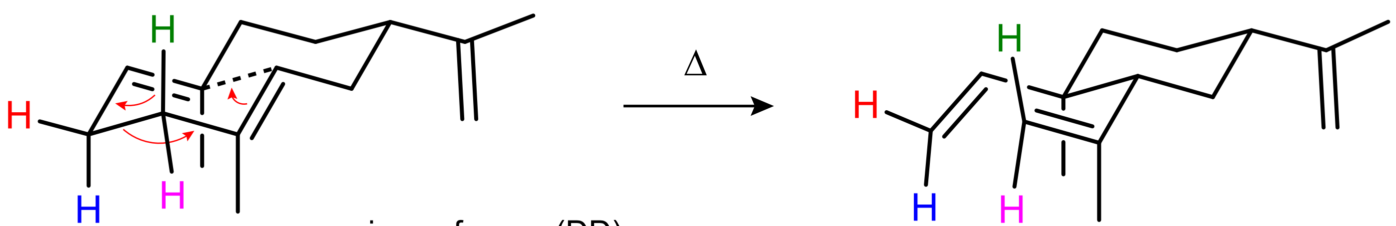
J. Rinkel, J. S. Dickschat, *Org. Lett.* **2019**, *21*, 2426.

3. Terpenes

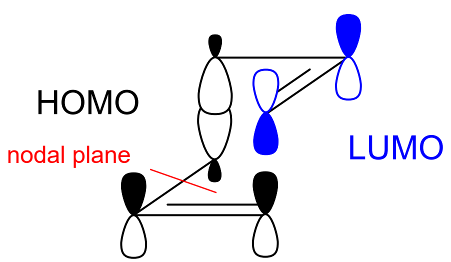
3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

stereochemical course of Cope rearrangement

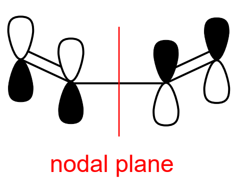


main conformer (DD)
chair-like transition state



can be understood as [4+2]-cycloaddition

HOMO has equal symmetry properties to HOMO of butadiene

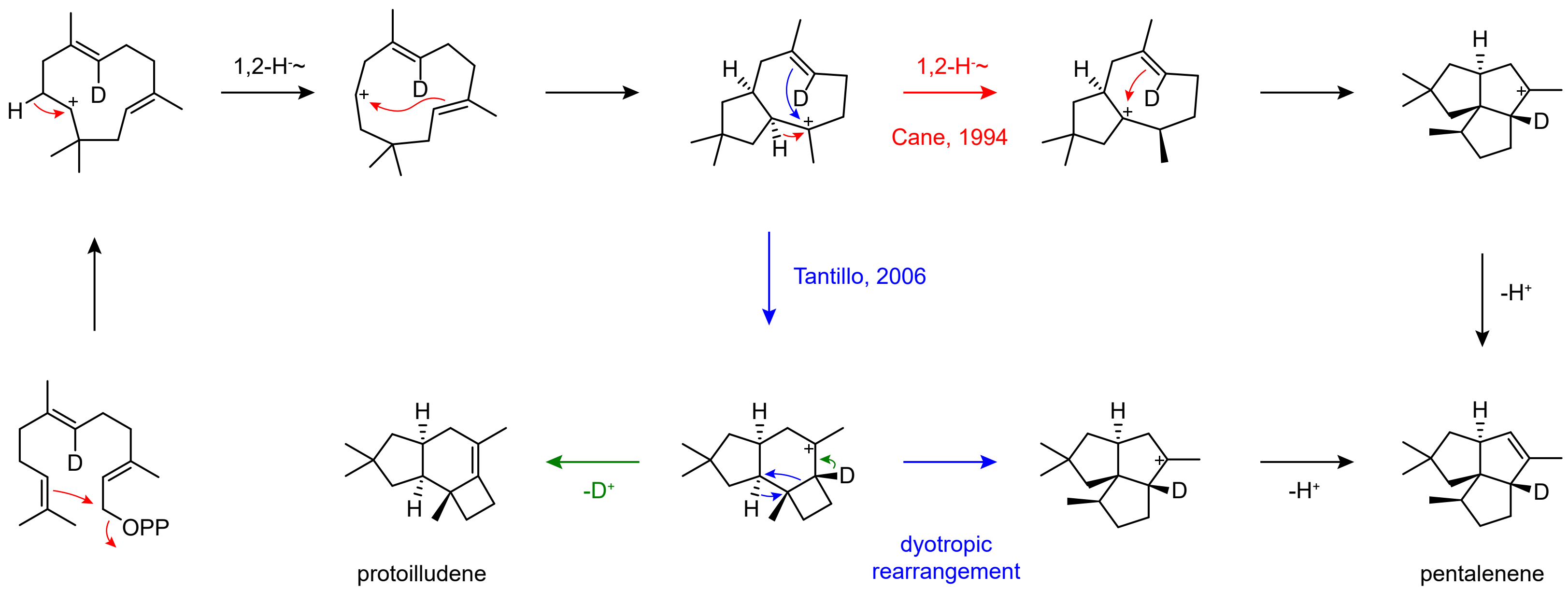


J. Rinkel, J. S. Dickschat, *Org. Lett.* **2019**, *21*, 2426.

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

pentalenene synthase (*Streptomyces exfoliatus*)

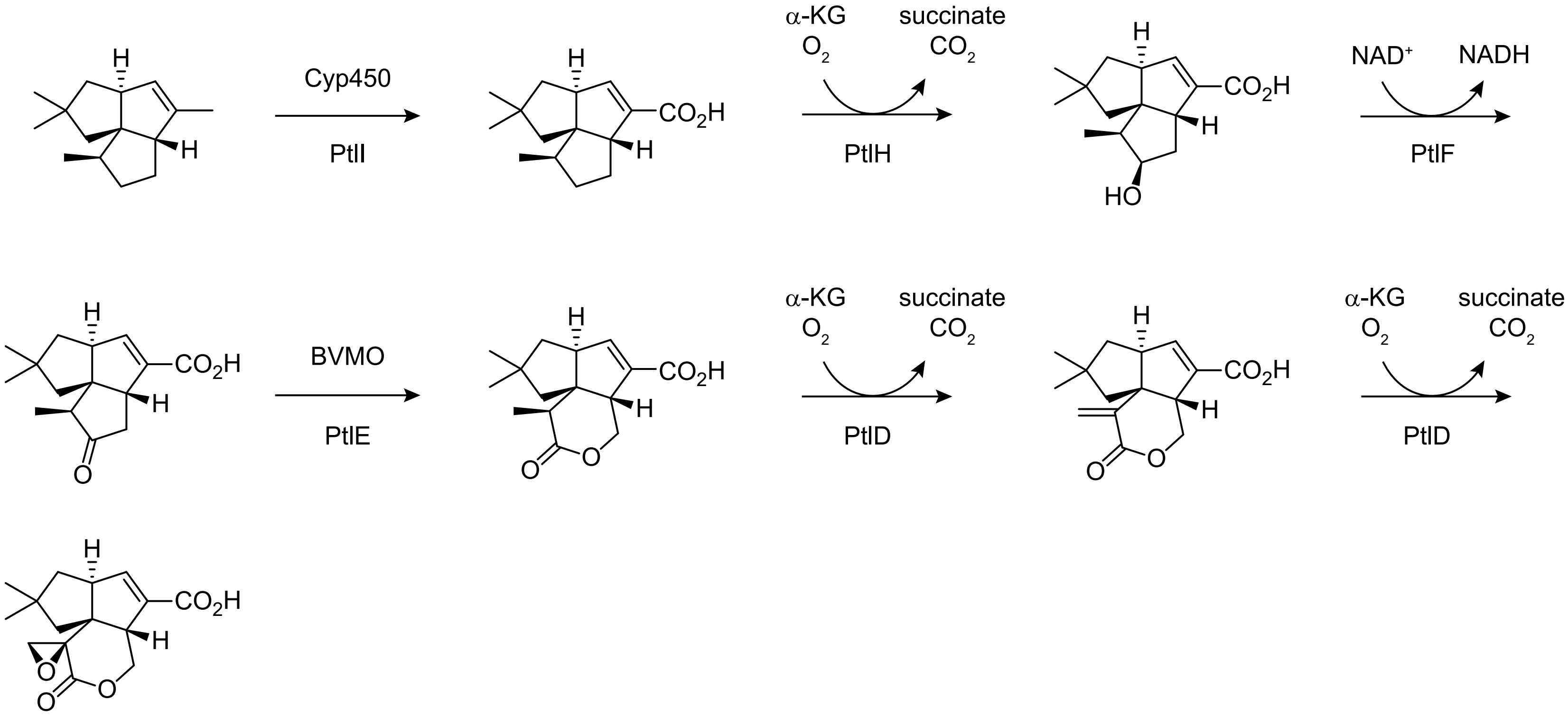


D. E. Cane, J.-K. Sohng, C. R. Lamberson, S. M. Rudnicki, Z. Wu, M. D. Lloyd, J. S. Oliver, B. R. Hubbard, *Biochemistry* **1994**, *33*, 5846.
P. Gutta, D. J. Tantillo, *J. Am. Chem. Soc.* **2006**, *128*, 6172.
L. Zu, M. Xu, M. W. Lodewyk, D. E. Cane, R. J. Peters, D. J. Tantillo, *J. Am. Chem. Soc.* **2012**, *134*, 11369.

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

pentalenolactone biosynthesis (*Streptomyces exfoliatus*)

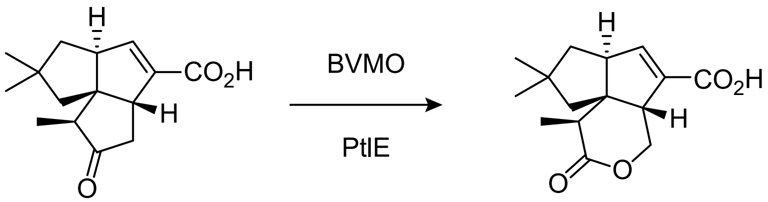


M.-J. Seo, D. Zhu, S. Endo, H. Ikeda, D. E. Cane, *Biochemistry* **2011**, *50*, 1739.

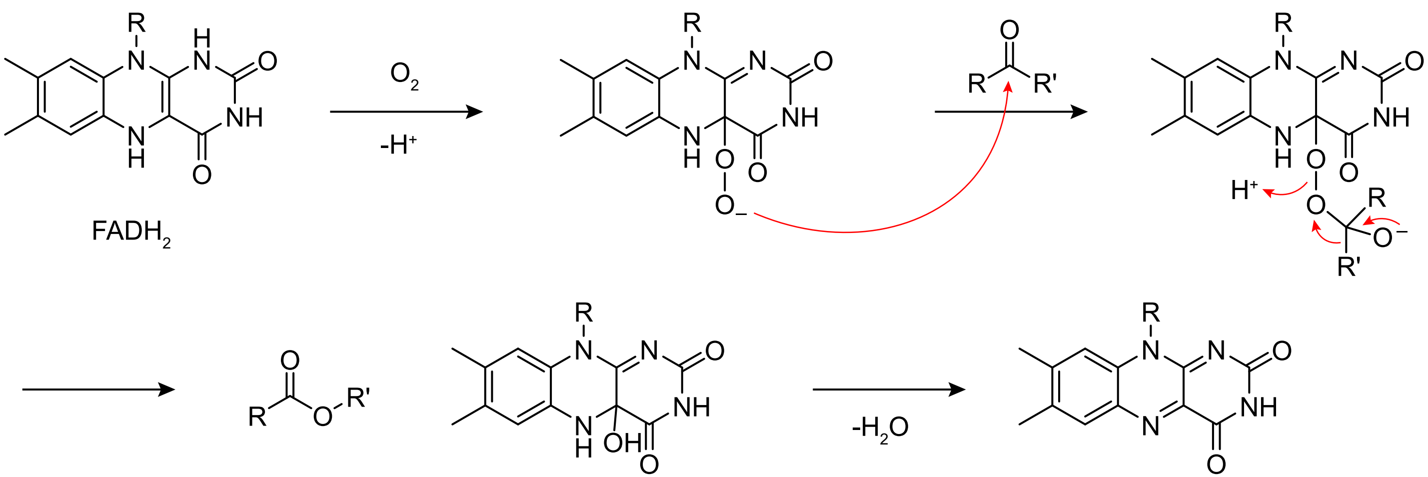
3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

pentalenolactone biosynthesis (*Streptomyces exfoliatus*)



Baeyer-Villiger Monooxygenase



$FADH_2$

Criegee intermediate

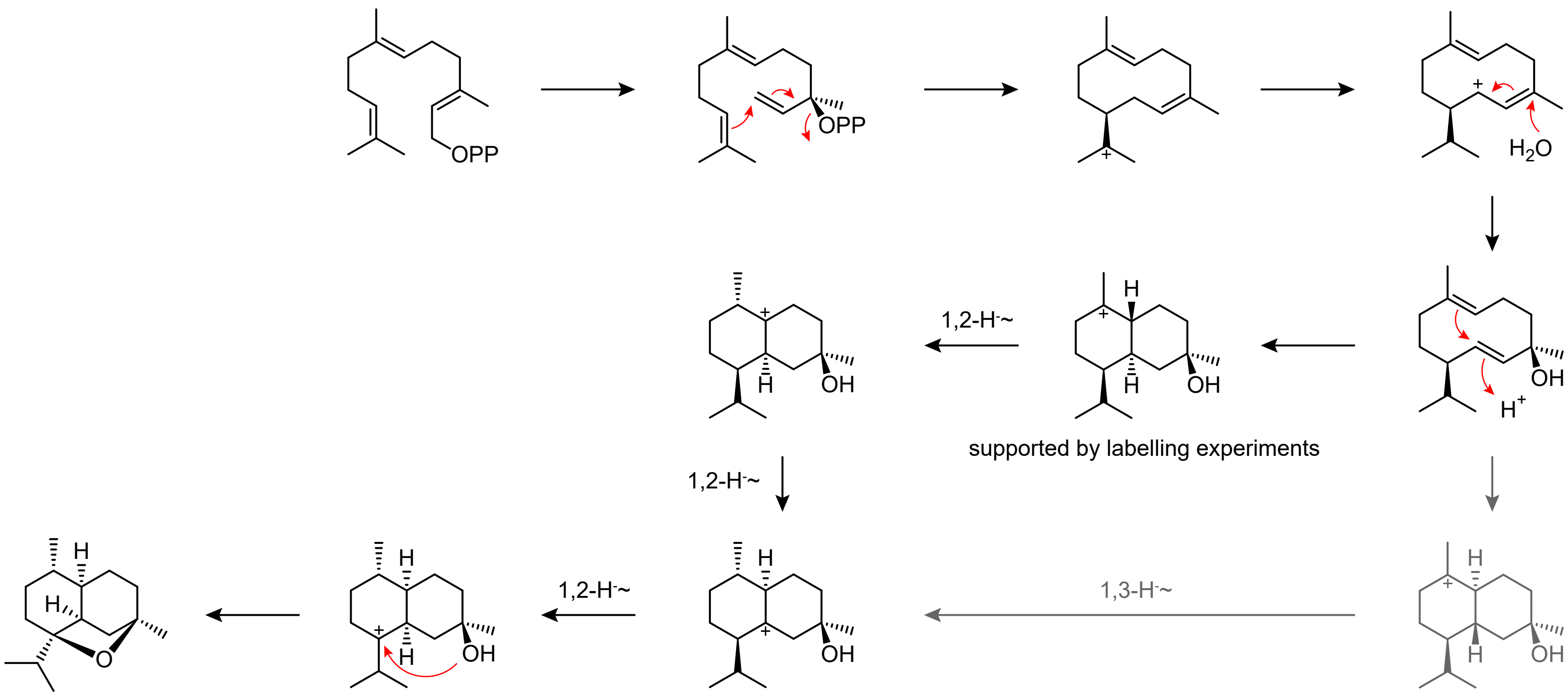
FAD

C. T. Walsh, Y.-C. Jack Chen, *Angew. Chem. Int. Ed.* **1988**, *27*, 333.

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

corvol ether synthase (*Kitasatospora setae*)



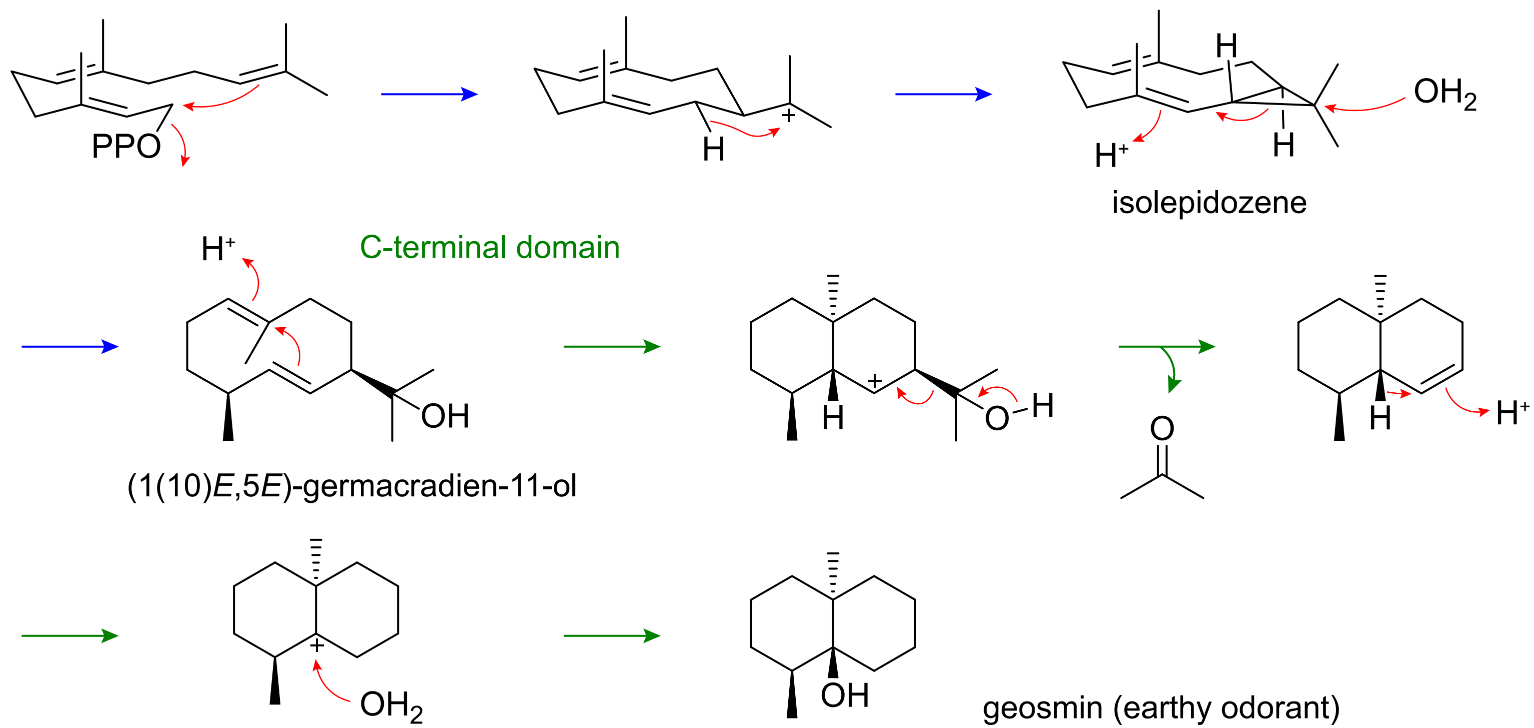
P. Rabe, K. A. K. Pahirulzaman, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2015**, *54*, 6041.

3.2.4. Terpene Synthases

3.2.4.4. Type I sesquiterpene synthases

geosmin synthase (*Streptomyces coelicolor*) = terpene synthase with two domains

N-terminal domain



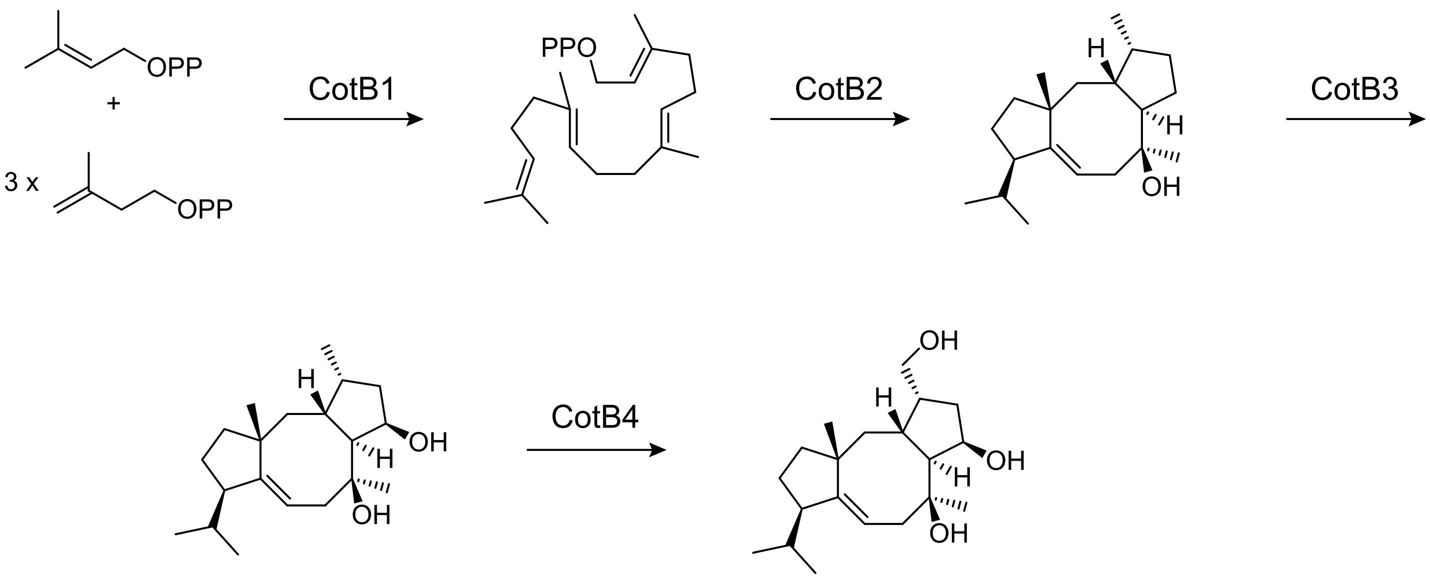
J. Jiang, X. He, D. E. Cane, *Nat. Chem. Biol.* **2007**, *3*, 711.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

Cyclooctat-9-en-7-ol synthase (*Streptomyces melanosporofaciens*)

first characterised bacterial type I diterpene synthase, 4 clustered genes *cotB1-4*

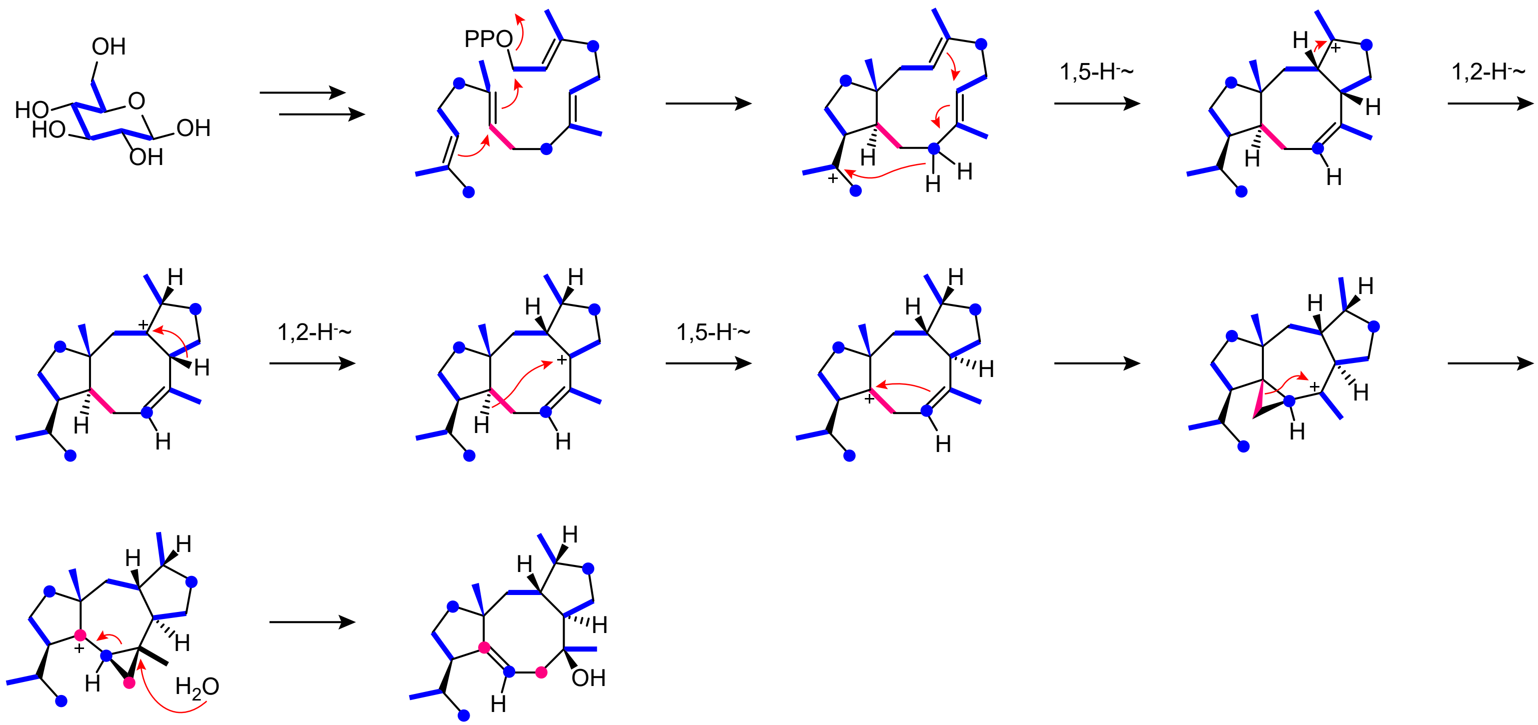


S.-Y. Kim, P. Zhao, M. Igarashi, R. Sawa, T. Tomita, M. Nishiyama, T. Kuzuyama, *Chem. Biol.* **2009**, *16*, 736.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

Cyclooctat-9-en-7-ol synthase (*Streptomyces melanosporofaciens*)

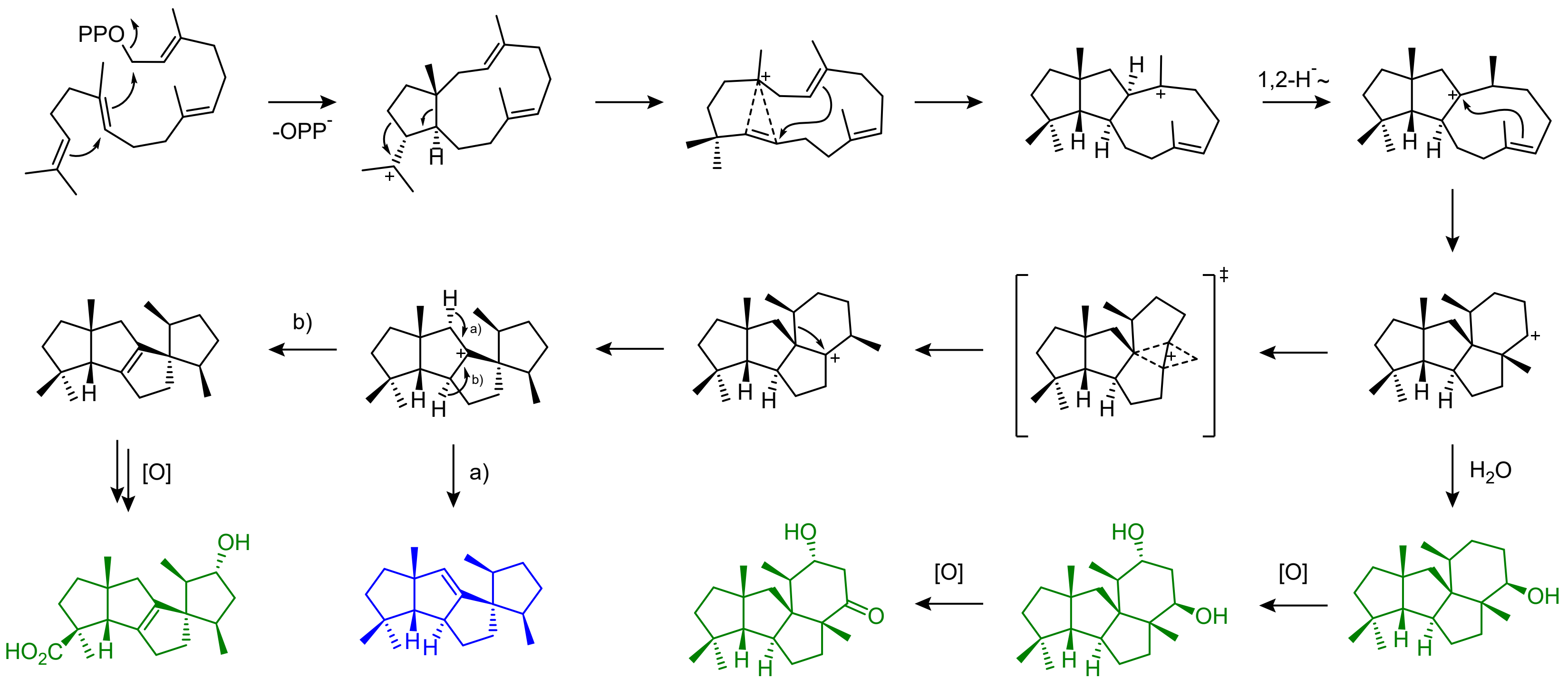


A. Meguro, Y. Motoyoshi, K. Teramoto, S. Ueda, Y. Totsuka, Y. Ando, T. Tomita, ..., T. Kuzuyama, *Angew. Chem. Int. Ed.* **2015**, *54*, 4353.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

Spiroviolene synthase (*Streptomyces violens*) and its connection to fungal diterpenes



H. Xu, J. S. Dickschat, *ChemBioChem* **2020**, online.

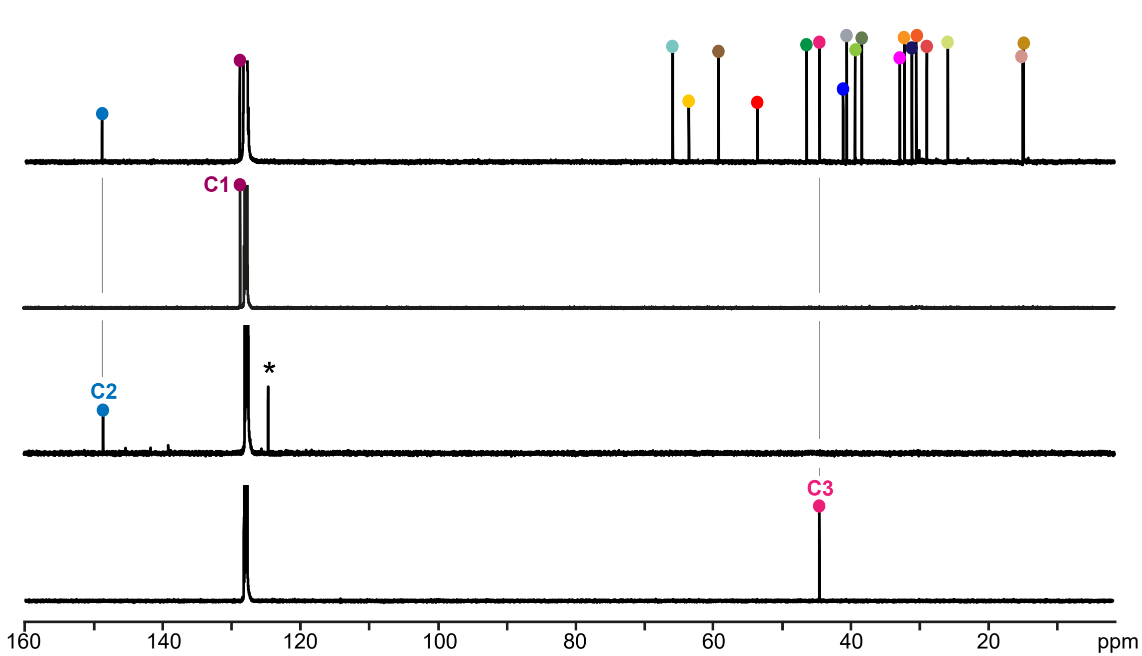
P. Rabe, J. Rinkel, E. Dolja, T. Schmitz, B. Nubbemeyer, T. H. Luu, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2017**, *56*, 2776.

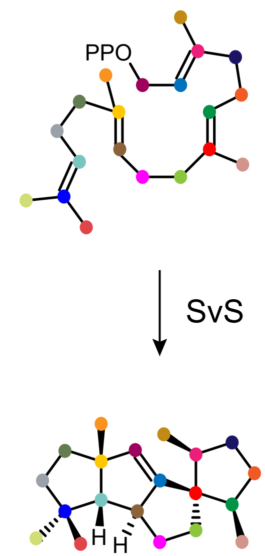
3. Terpenes

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

Spiroviolene synthase (*Streptomyces violens*)



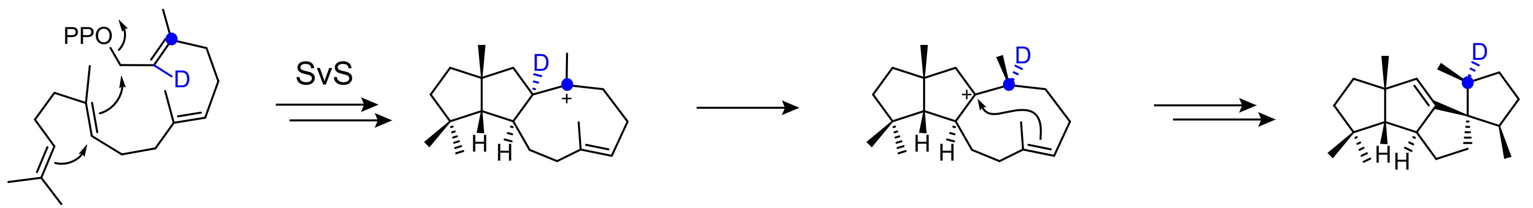


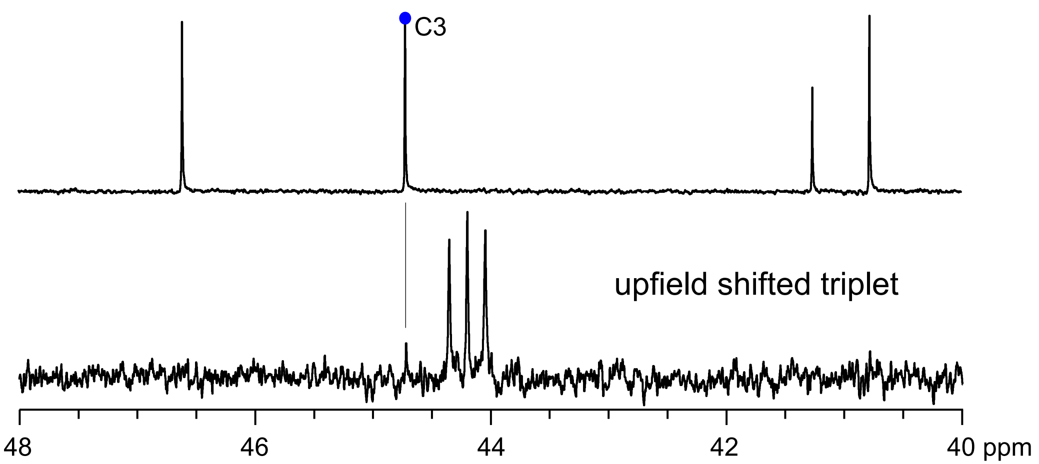
P. Rabe, J. Rinkel, E. Dolja, T. Schmitz, B. Nubbemeyer, T. H. Luu, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2017**, *56*, 2776.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

Spiroviolene synthase (*Streptomyces violens*)



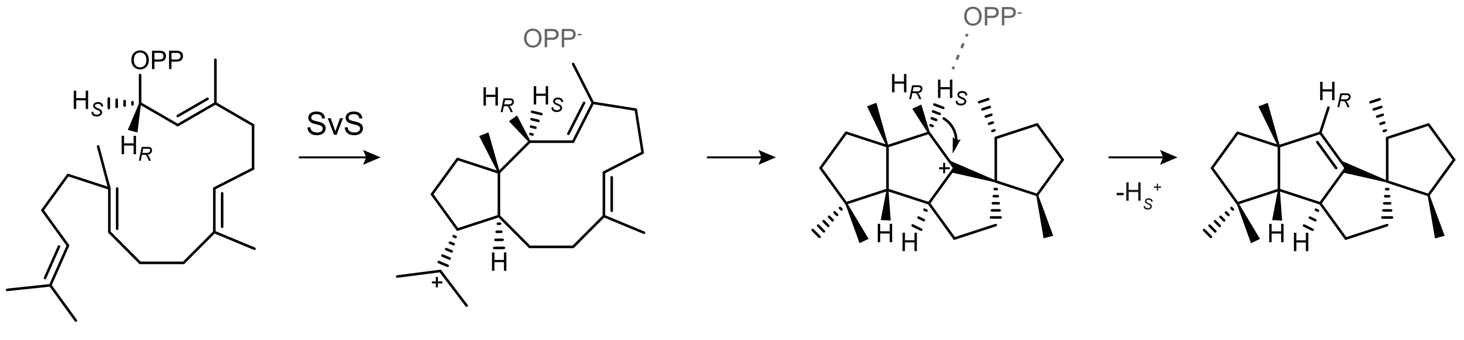


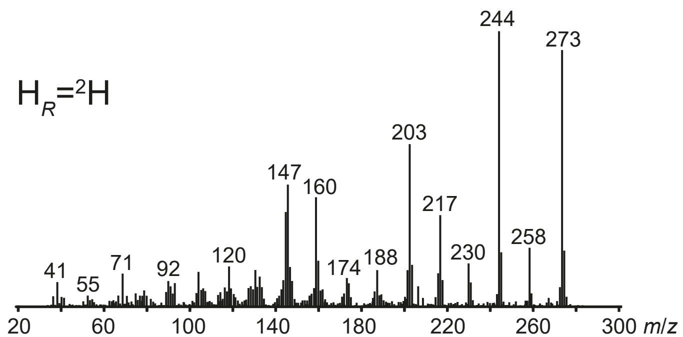
P. Rabe, J. Rinkel, E. Dolja, T. Schmitz, B. Nubbemeyer, T. H. Luu, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2017**, *56*, 2776.

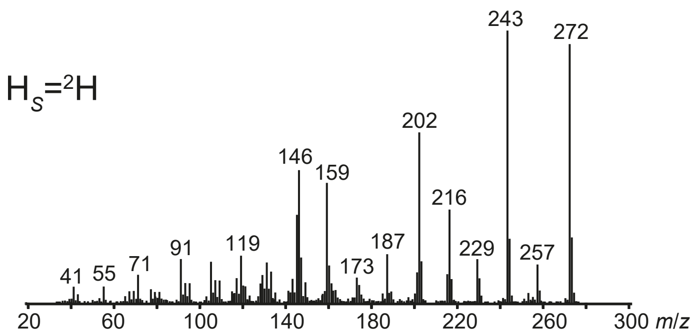
3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

Spiroviolene synthase (*Streptomyces violens*)





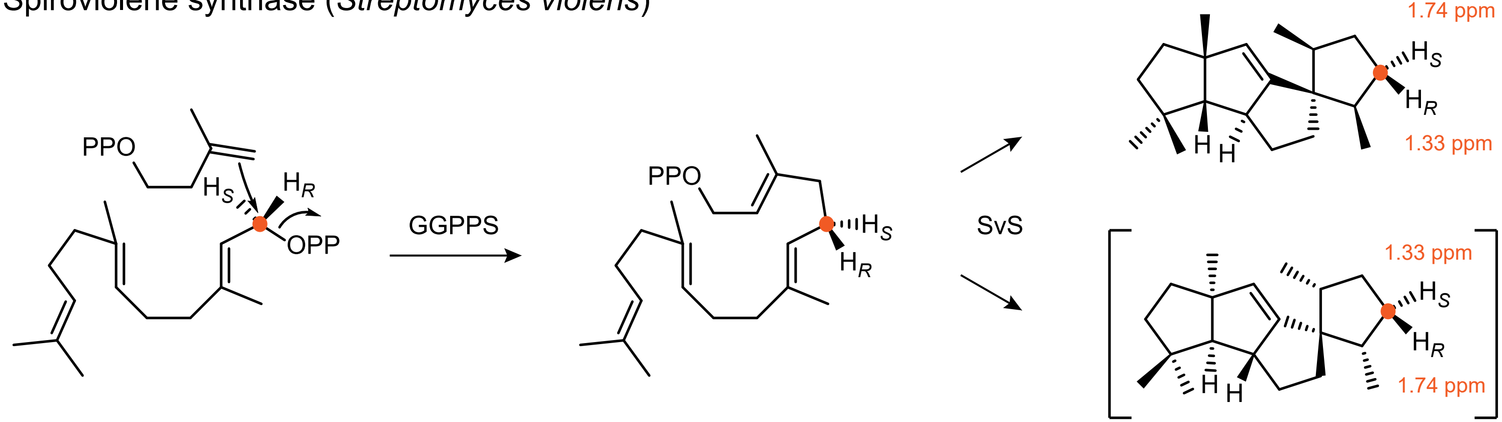


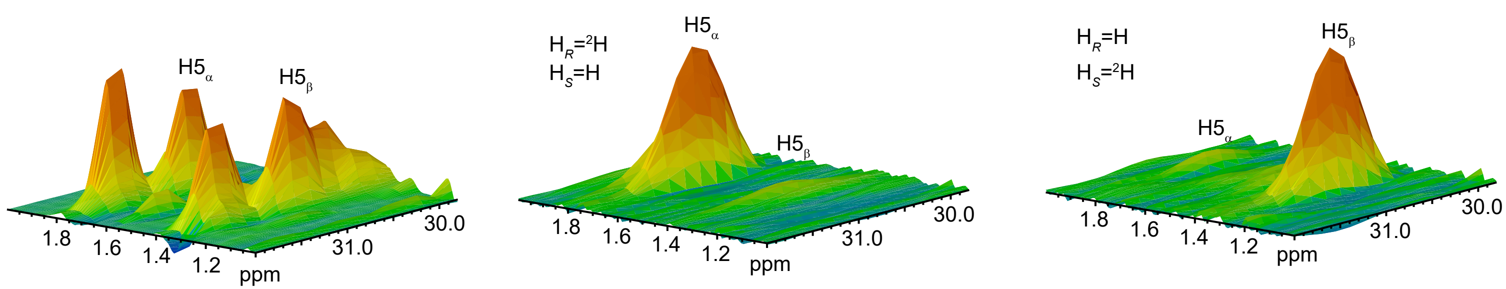
P. Rabe, J. Rinkel, E. Dolja, T. Schmitz, B. Nubbemeyer, T. H. Luu, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2017**, *56*, 2776.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

Spiroviolene synthase (*Streptomyces violens*)





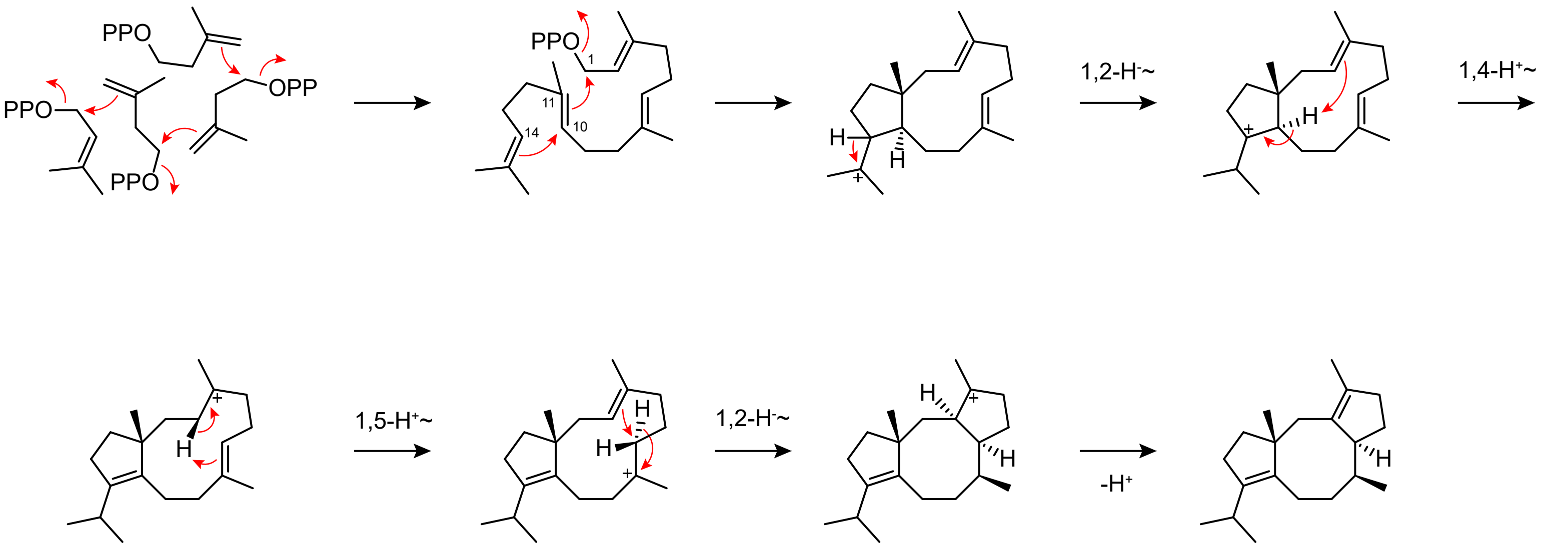
P. Rabe, J. Rinkel, E. Dolja, T. Schmitz, B. Nubbemeyer, T. H. Luu, J. S. Dickschat, *Angew. Chem. Int. Ed.* **2017**, *56*, 2776.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

in fungi: chimeric enzymes with a GGPPS and a DTS domain

first example: fusicoccadiene synthase (*Phomopsis amygdali*)



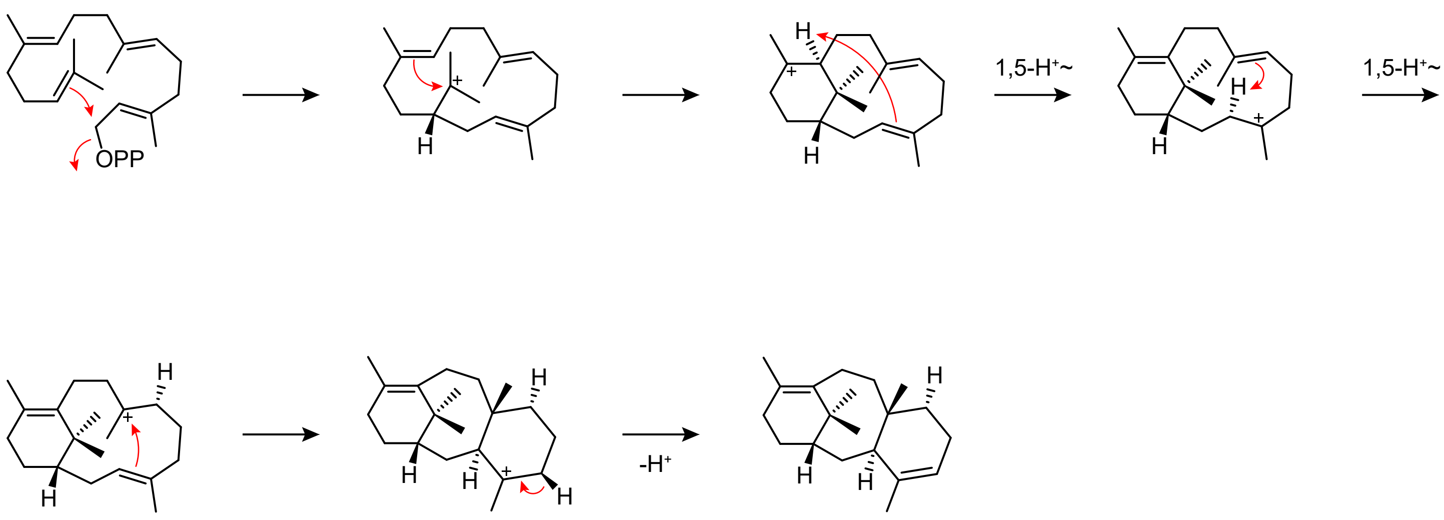
T. Toyomasu, M. Tsukahara, A. Kaneko, R. Niida, W. Mitsuhashi, T. Dairi, N. Kato, T. Sassa, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 3084.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases

in plants: enzymes with three domains (αβγ), but only α is catalytically active, βγ have structural role

taxadiene synthase (*Taxus brevifolia*)

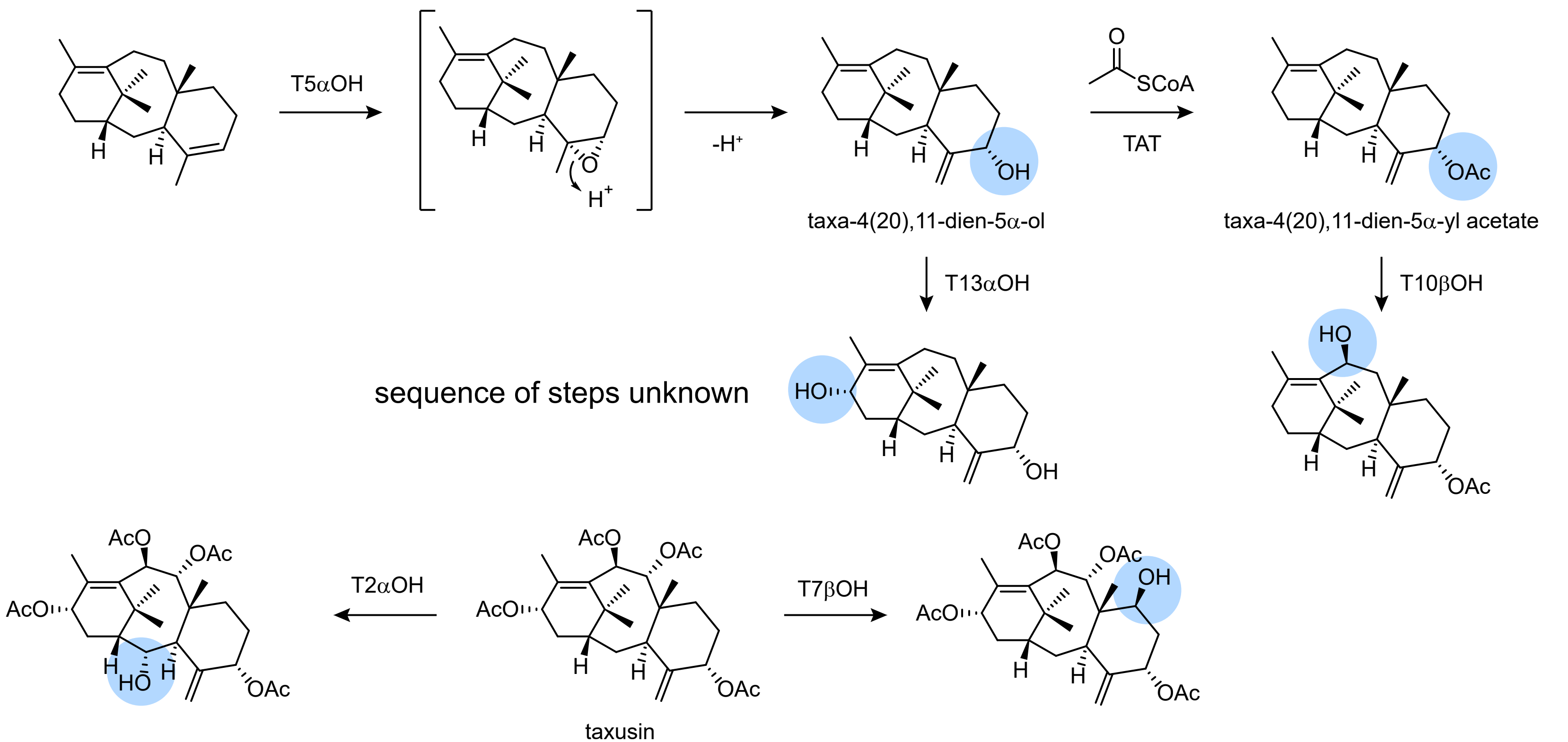


taxa-4,11-diene

M. Köksal, J. Yin, R. M. Coates, R. Croteau, D. W. Christianson, *Nature* **2011**, *469*, 116.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases: taxol biosynthesis

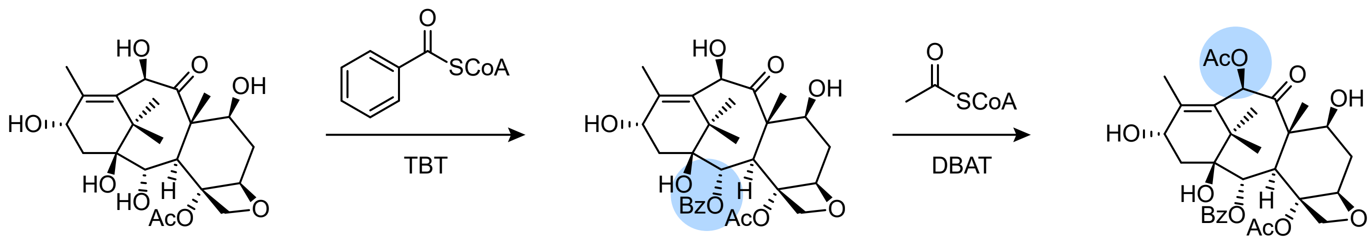


taxusin is not a pathway intermediate, natural substrate unknown

J. S. Dickschat, in: *Comprehensive Natural Products III* **2020**, *1*, 506.

3.2.4. Terpene Synthases

3.2.4.5. Type I diterpene synthases: taxol biosynthesis

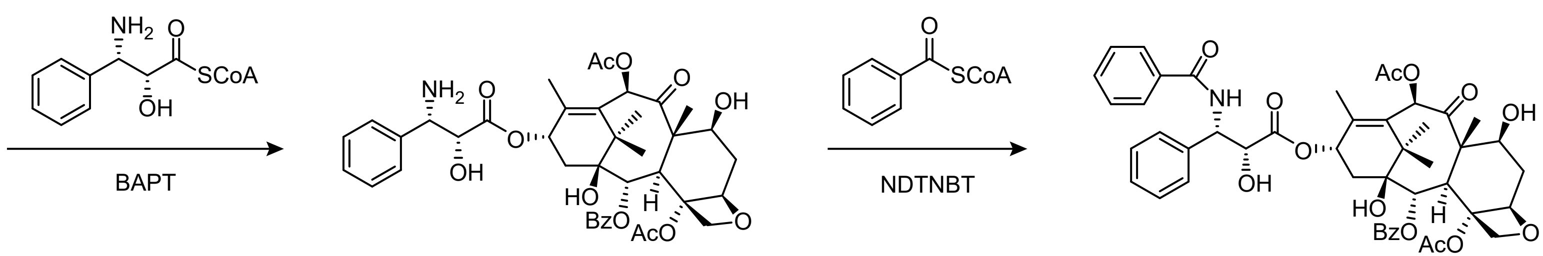


2-*O*-debenzoyl-10-*O*-deacetylbaccatin III

10-*O*-deacetylbaccatin III

baccatin III

can be isolated from *Taxus baccata*
biotechnological or semisynthetic conversion into taxol



N-debenzoyltaxol

taxol

J. S. Dickschat, in: *Comprehensive Natural Products III* **2020**, *1*, 506.

3.2.4. Terpene Synthases

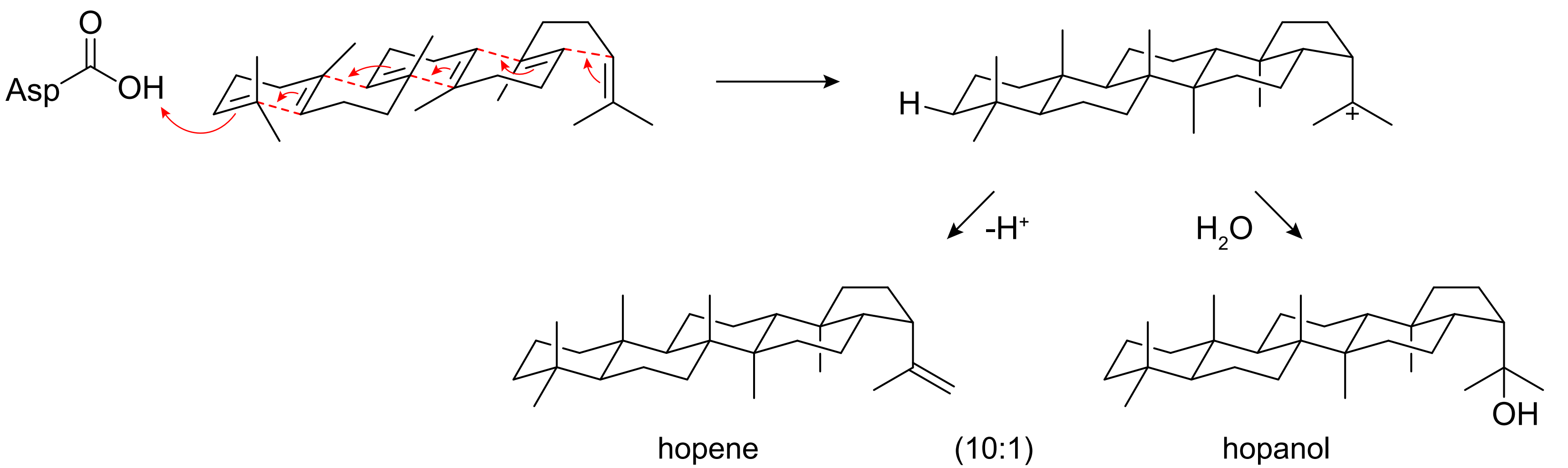
3.2.4.6. Type II terpene synthases

substrate activation by protonation

highly conserved motif DXDD

proton donor

squalene-hopene cyclase

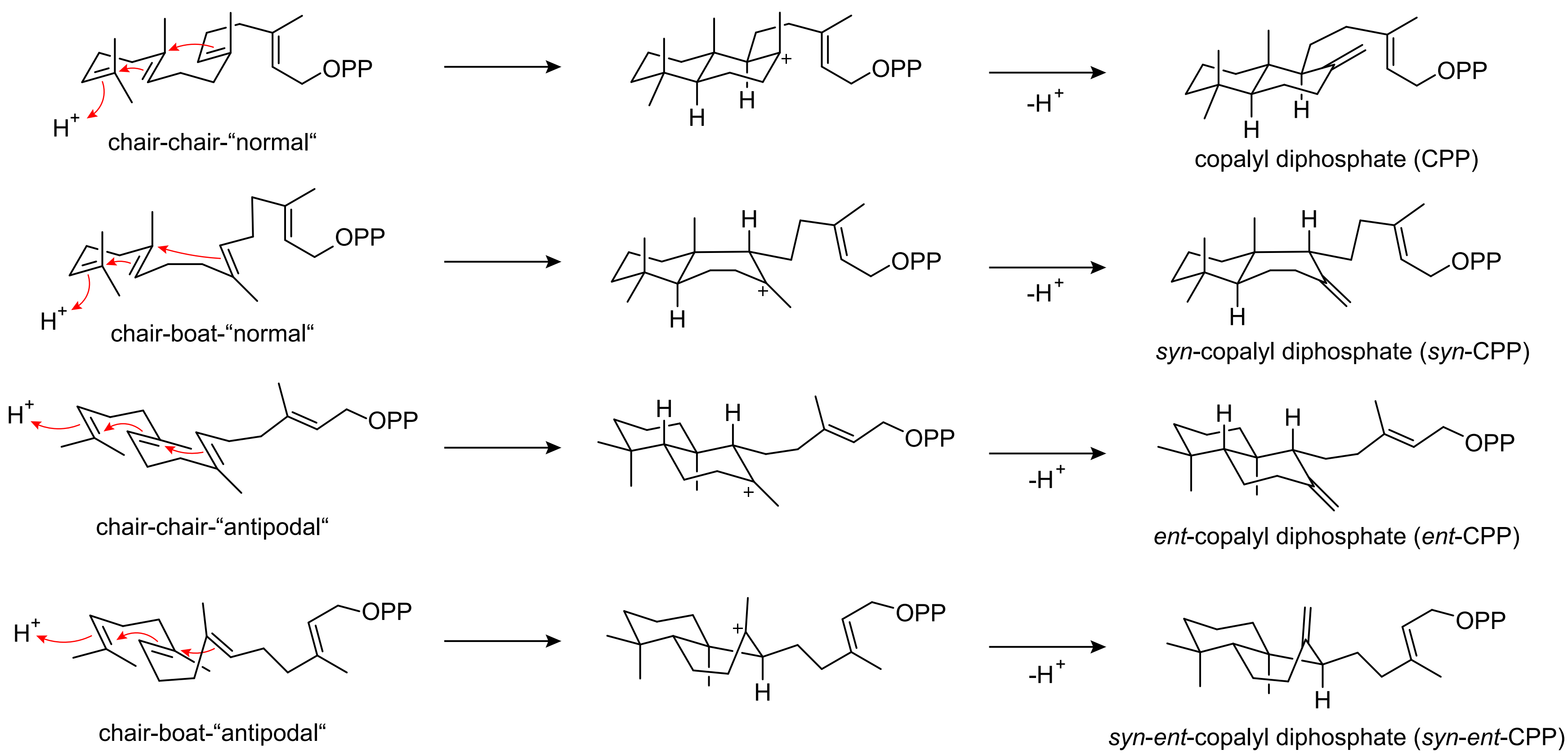


K. U. Wendt, K. Poralla, G. E. Schulz, *Science* **1997**, *277*, 1811.

3. Terpenes

3.2.4. Terpene Synthases

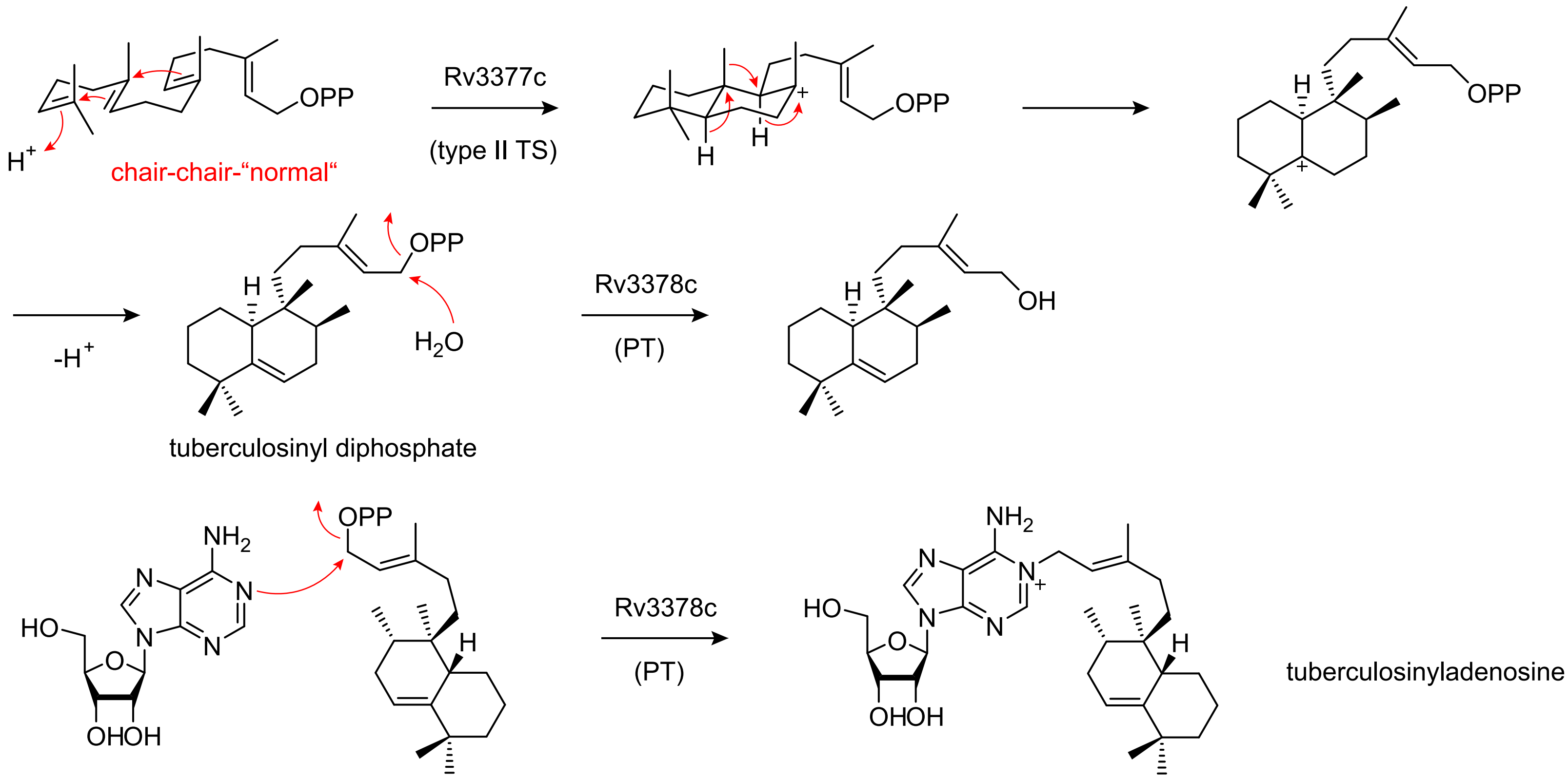
3.2.4.6. Type II diterpene synthases: labdane related diterpenes



R. J. Peters, *Nat. Prod. Rep.* **2010**, *27*, 1521.

3.2.4. Terpene Synthases

3.2.4.6. Type II diterpene synthases: tuberculosinyladenosine biosynthesis in *Mycobacterium tuberculosis*

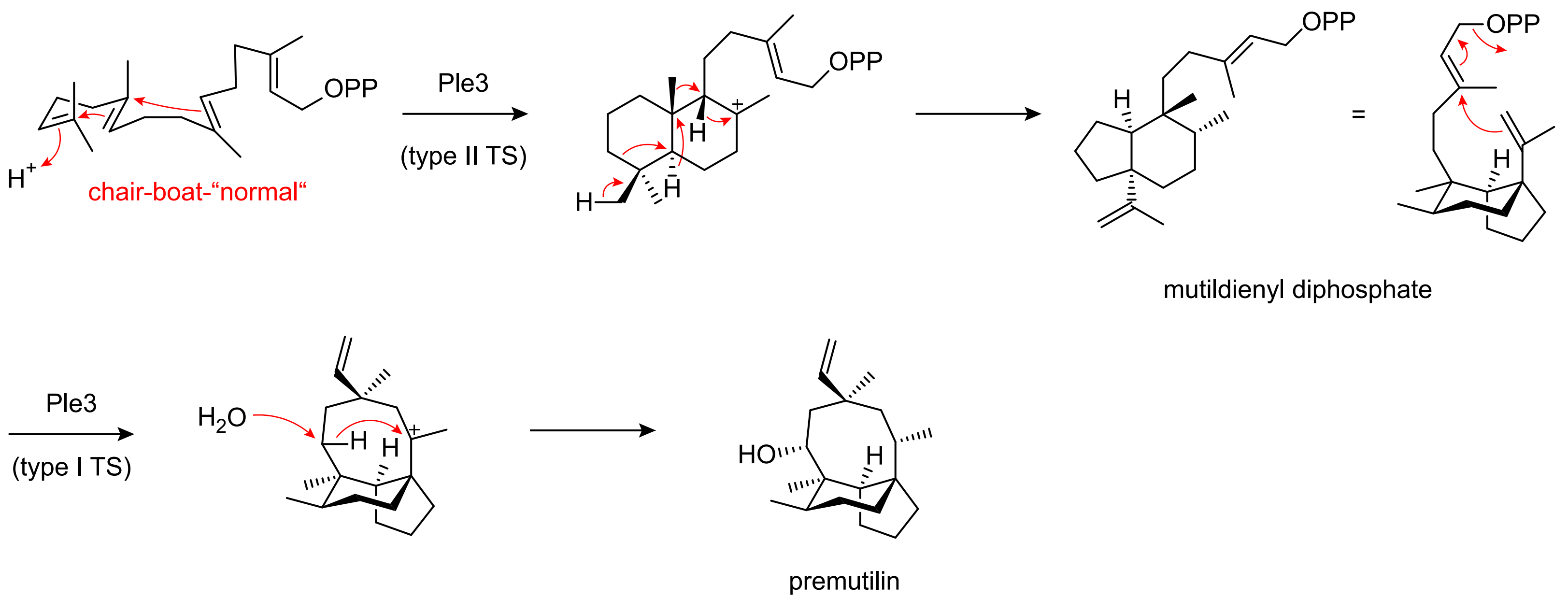


C. Nakano, T. Ootsuka, K. Takayama, T. Mitsui, T. Sato, T. Hoshino, *Biosci. Biotechnol. Biochem.* **2011**, *75*, 75.
E. Layre, H. J. Lee, D. C. Young, A. J. Martinot, J. Buter, A. J. Minnaard, ..., D. B. Moody, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 2978.

3.2.4. Terpene Synthases

3.2.4.6. Type II diterpene synthases: pleuromutilin biosynthesis in *Clitopilus passeckerianus*

Ple3: bifunctional terpene synthase composed of type II and type I domain

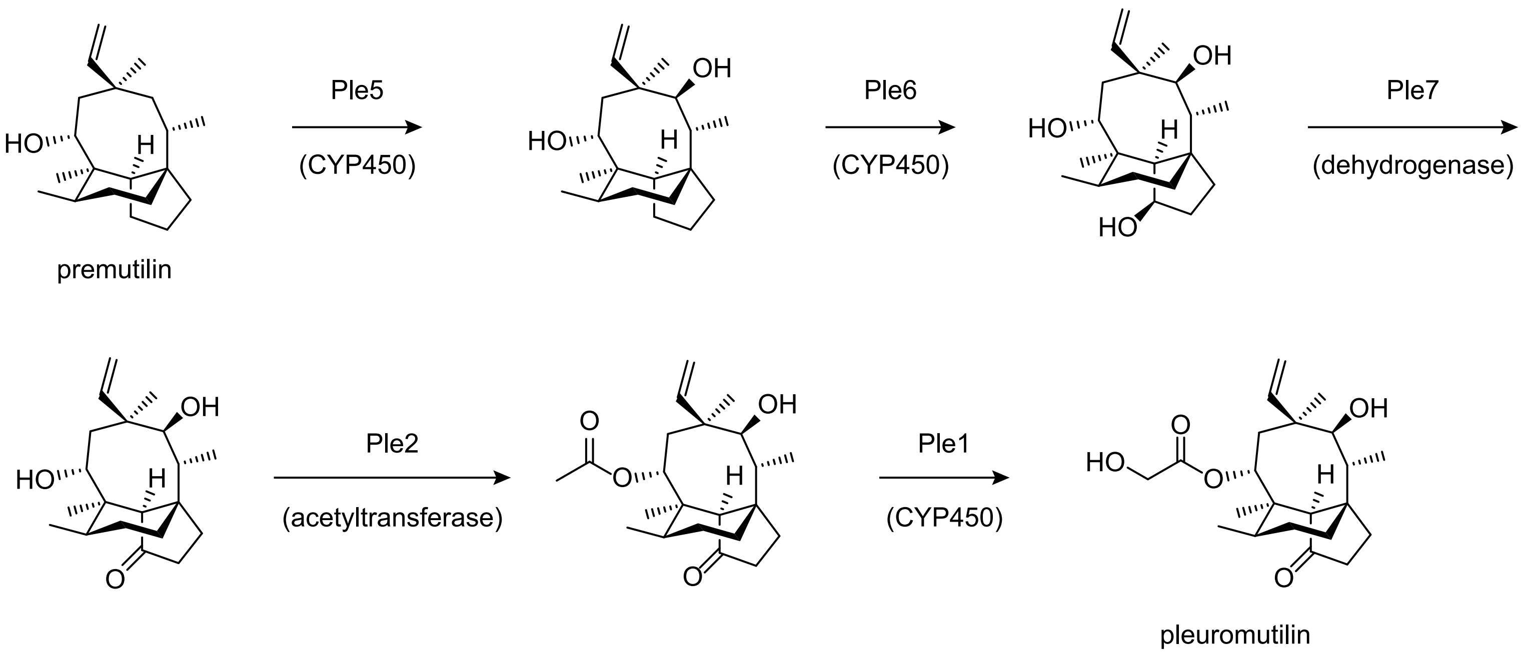


M. Yamane, A. Minami, C. Liu, T. Ozaki, I. Takeuchi, T. Tsukagoshi, T. Tokiwano, K. Gomi, H. Oikawa, *ChemBioChem* **2017**, *18*, 2317.
F. Alberti, K. Khairudin, E. Rodriguez Venegas, J. A. Davies, P. M. Hayes, C. L. Willis, A. M. Mailey, G. D. Foster, *Nat. Commun.* **2017**, *8*, 1831.

3.2.4. Terpene Synthases

3.2.4.6. Type II diterpene synthases: pleuromutilin biosynthesis in *Clitopilus passeckerianus*

downstream steps

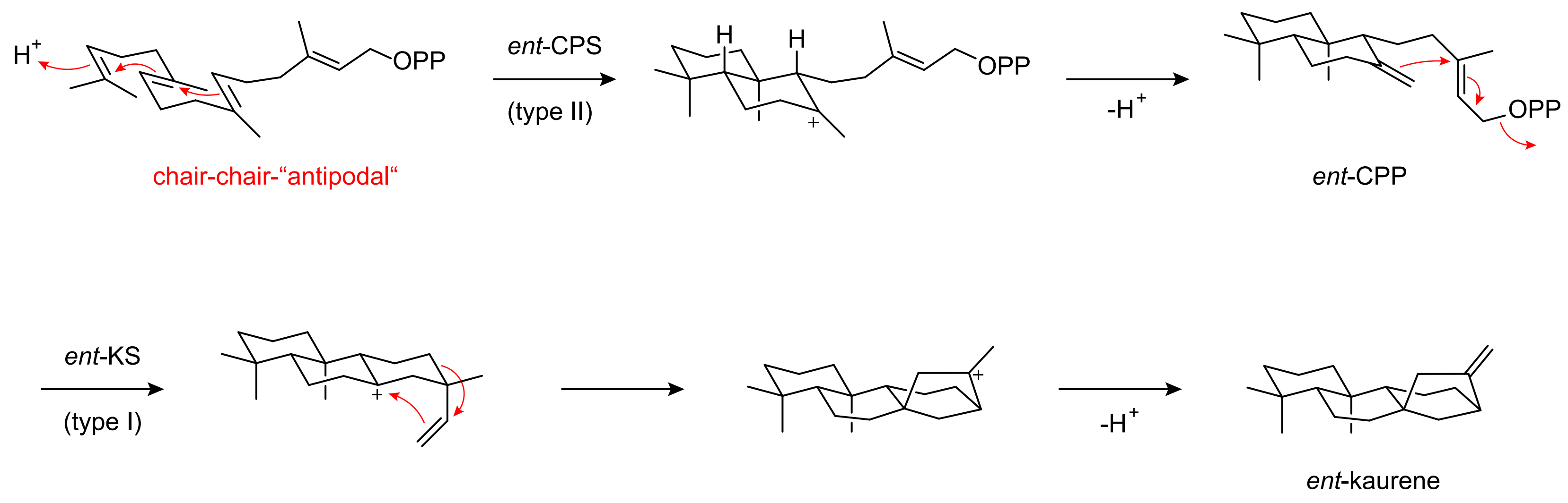


M. Yamane, A. Minami, C. Liu, T. Ozaki, I. Takeuchi, T. Tsukagoshi, T. Tokiwano, K. Gomi, H. Oikawa, *ChemBioChem* **2017**, *18*, 2317.
F. Alberti, K. Khairudin, E. Rodriguez Venegas, J. A. Davies, P. M. Hayes, C. L. Willis, A. M. Mailey, G. D. Foster, *Nat. Commun.* **2017**, *8*, 1831.

3.2.4. Terpene Synthases

3.2.4.6. Type II diterpene synthases: gibberellin biosynthesis in *Bradyrhizobium japonicum*

discrete type II and type I terpene synthases



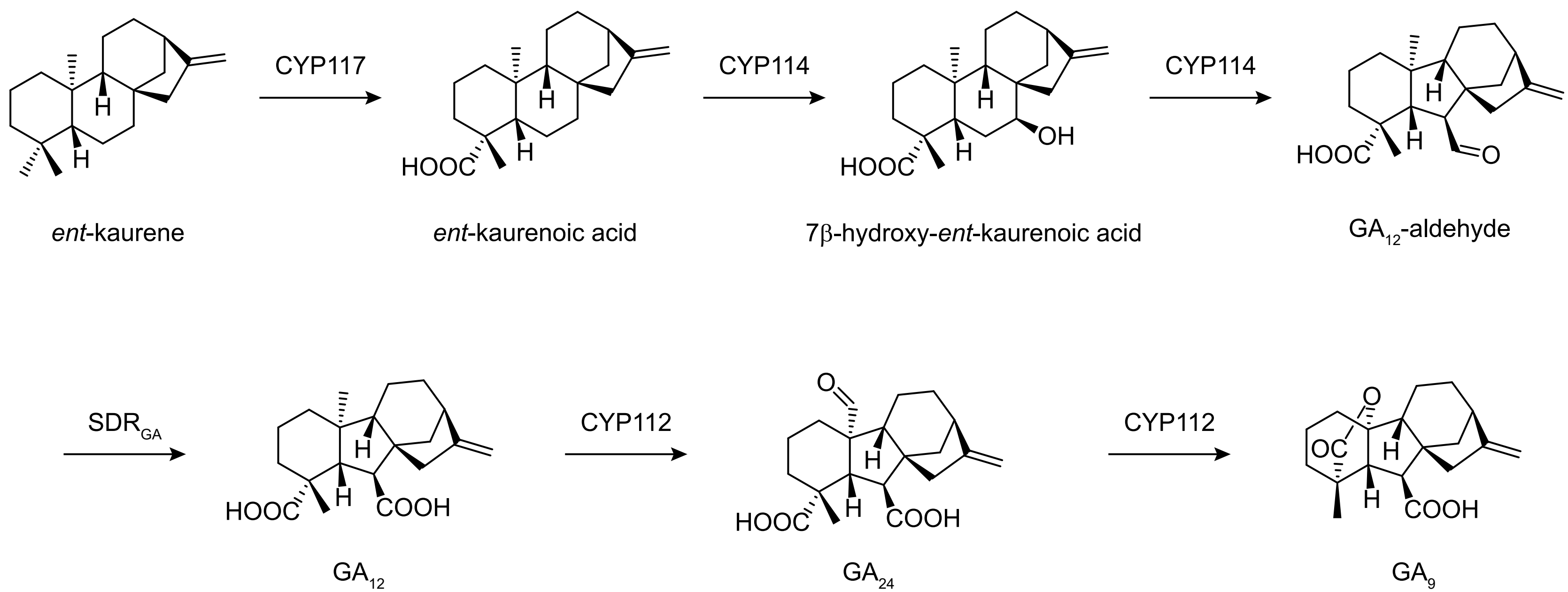
also in fungi (bifunctional enzyme) and plants (two discrete enzymes)

D. Morrone, J. Chambers, L. Lowry, G. Kim, A. Anterola, K. Bender, R. J. Peters, *FEBS Lett.* **2009**, *583*, 457.
H. Kawaide, R. Imai, T. Sassa, Y. Kamiya, *J. Biol. Chem.* **1997**, *272*, 21706.

3.2.4. Terpene Synthases

3.2.4.6. Type II diterpene synthases: gibberellin biosynthesis in *Bradyrhizobium japonicum*

downstream steps

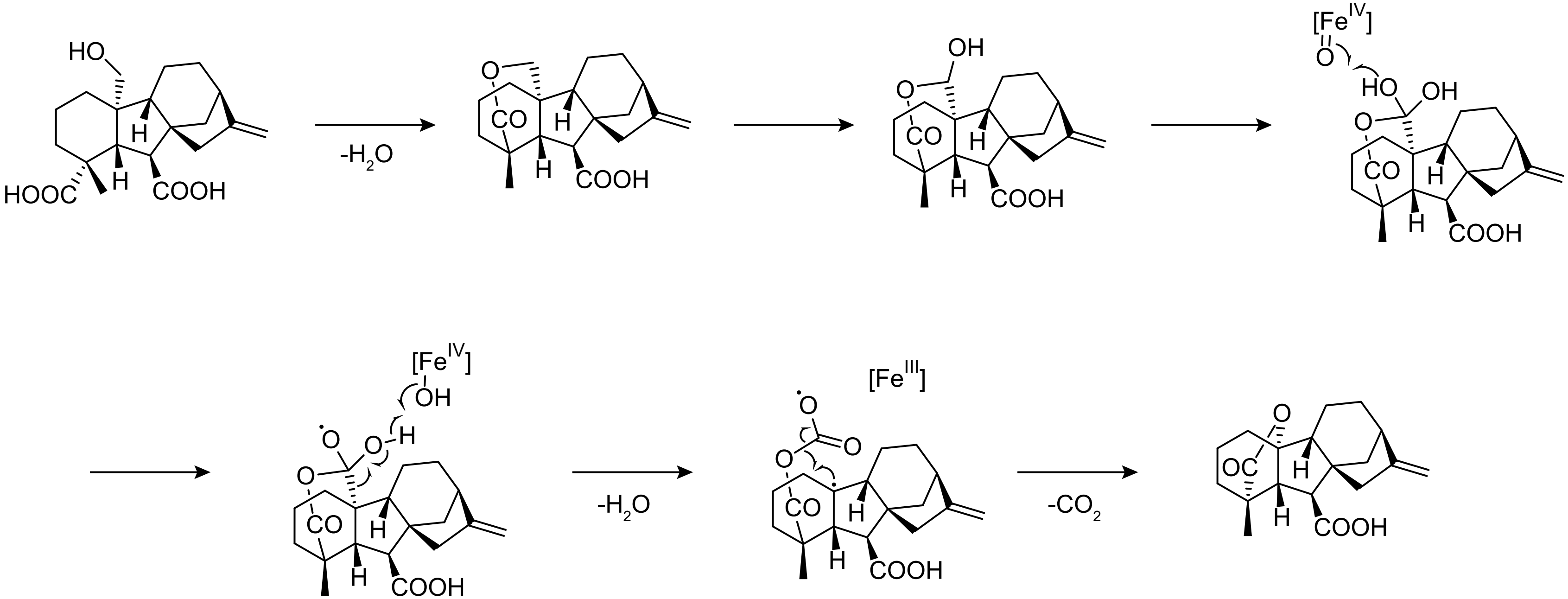


R. S. Nett, M. Montanares, A. Marcassa, X. Lu, R. Nagel, T. C. Charles, P. Hedden, M. C. Rojas, R. J. Peters, *Nat. Chem. Biol.* **2017**, *13*, 69.
R. S. Nett, J. S. Dickschat, R. J. Peters, *Org. Lett.* **2016**, *18*, 5974.

3.2.4. Terpene Synthases

3.2.4.6. Type II diterpene synthases: gibberellin biosynthesis in *Bradyrhizobium japonicum*

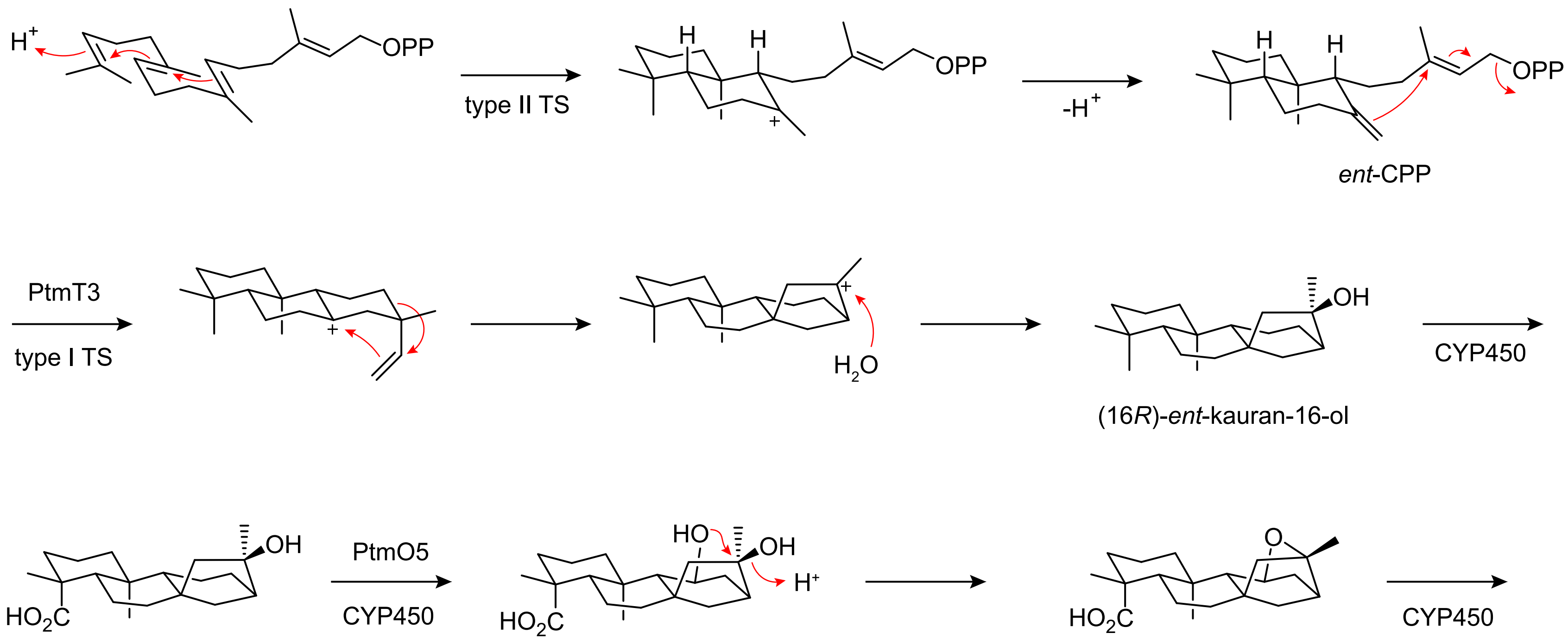
CYP112 mechanism



R. Nagel, R. J. Peters, *Angew. Chem. Int. Ed.* **2018**, *57*, 6082.

3.2.4. Terpene Synthases

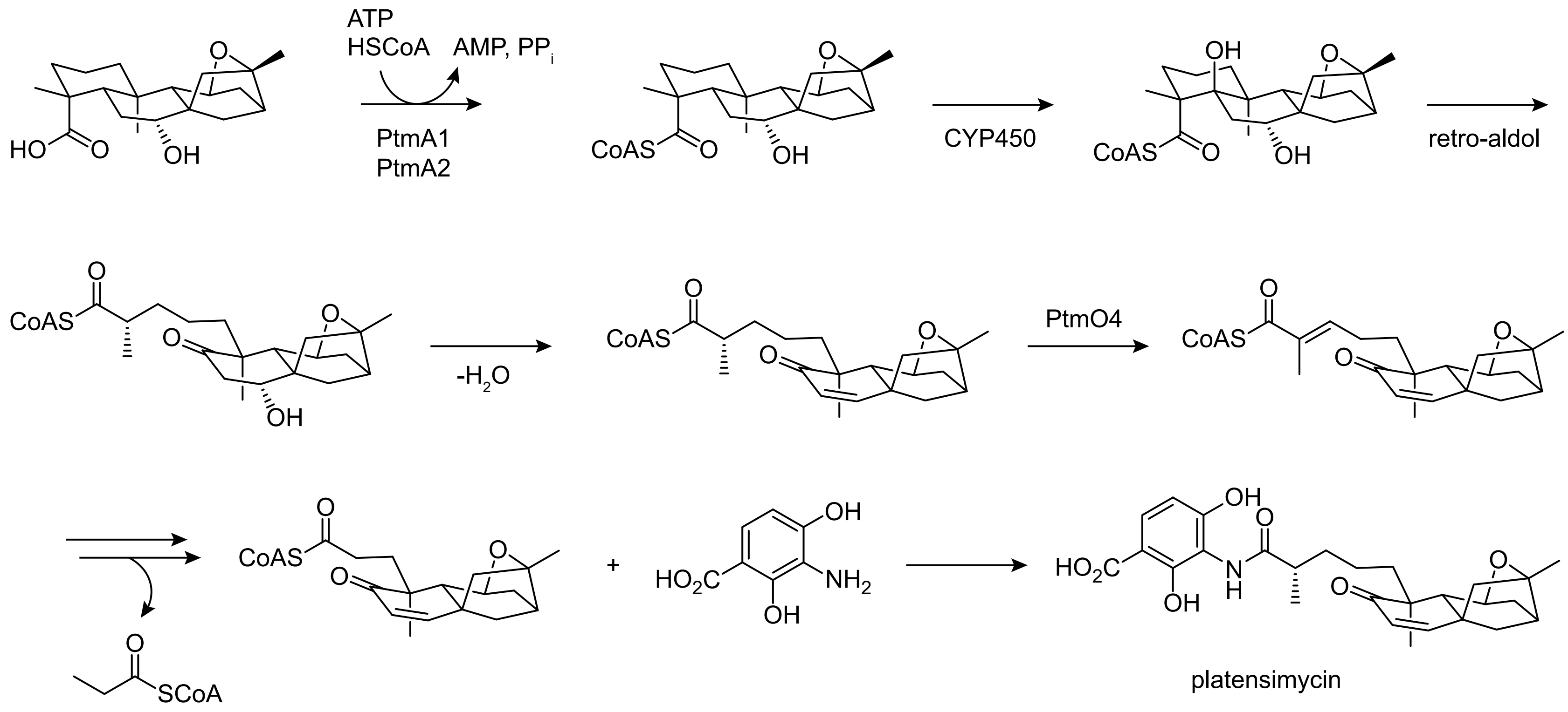
3.2.4.6. Type II diterpene synthases: platensimycin biosynthesis in *Streptomyces platensis*



M. J. Smanski, Z. Yu, J. Casper, S. Lin, R. M. Peterson, Y. Chen, E. Wendt-Pienkowski, S. R. Rajski, B. Shen, *PNAS* **2011**, *108*, 13498.
J. D. Rudolf, L.-B. Dong, K. Manoogian, B. Shen, *J. Am. Chem. Soc.* **2016**, *138*, 16711.

3.2.4. Terpene Synthases

3.2.4.6. Type II diterpene synthases: platensimycin biosynthesis in *Streptomyces platensis*



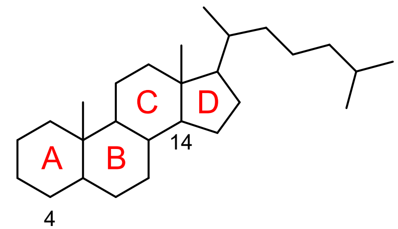
N. Wang, J. D. Rudolf, L.-B. Dong, J. Osipiuk, C. Hatzos-Skintges, M. Endres, C.-Y. Chang, ..., B. Shen, *Nat. Chem. Biol.* **2018**, *14*, 730.
J. D. Rudolf, L.-B. Dong, T. Huang, B. Shen, *Mol. Biosyst.* **2015**, *11*, 2717.

3.2.4. Terpene Synthases

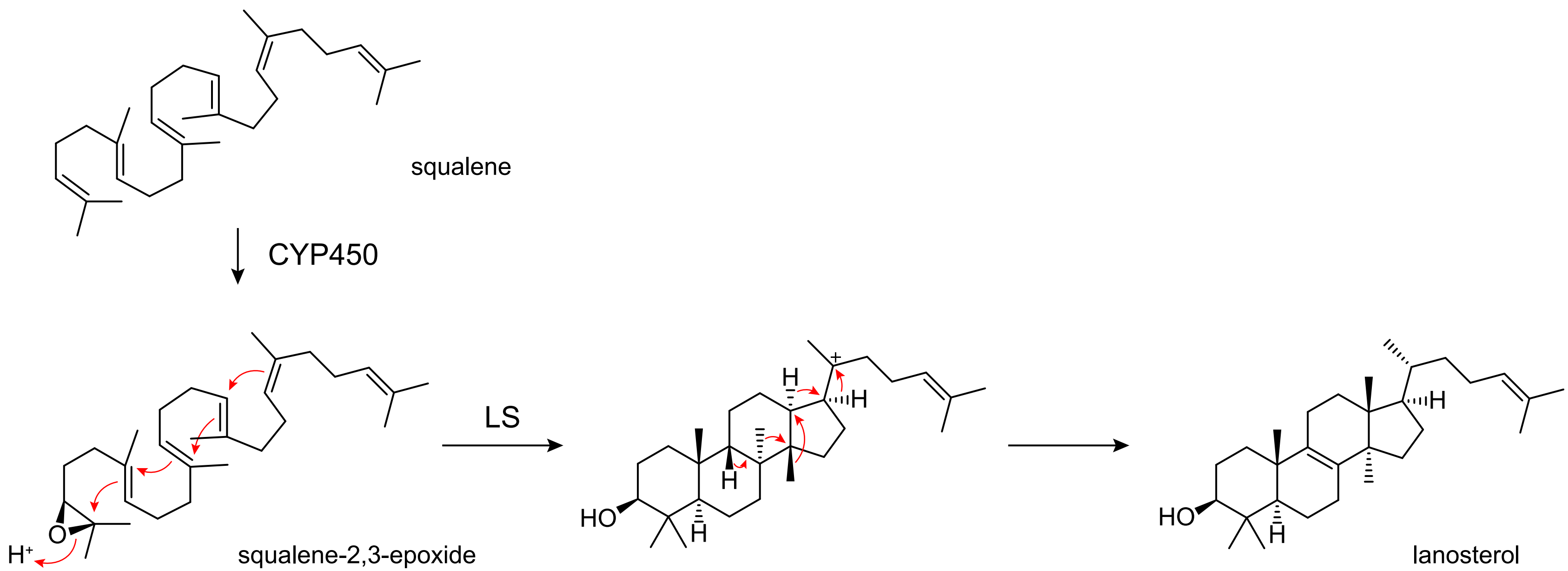
3.2.4.7. Steroids

modified triterpenes made by a type II terpene synthase

tetracyclic ring system, Me groups at C4 and C14 missing



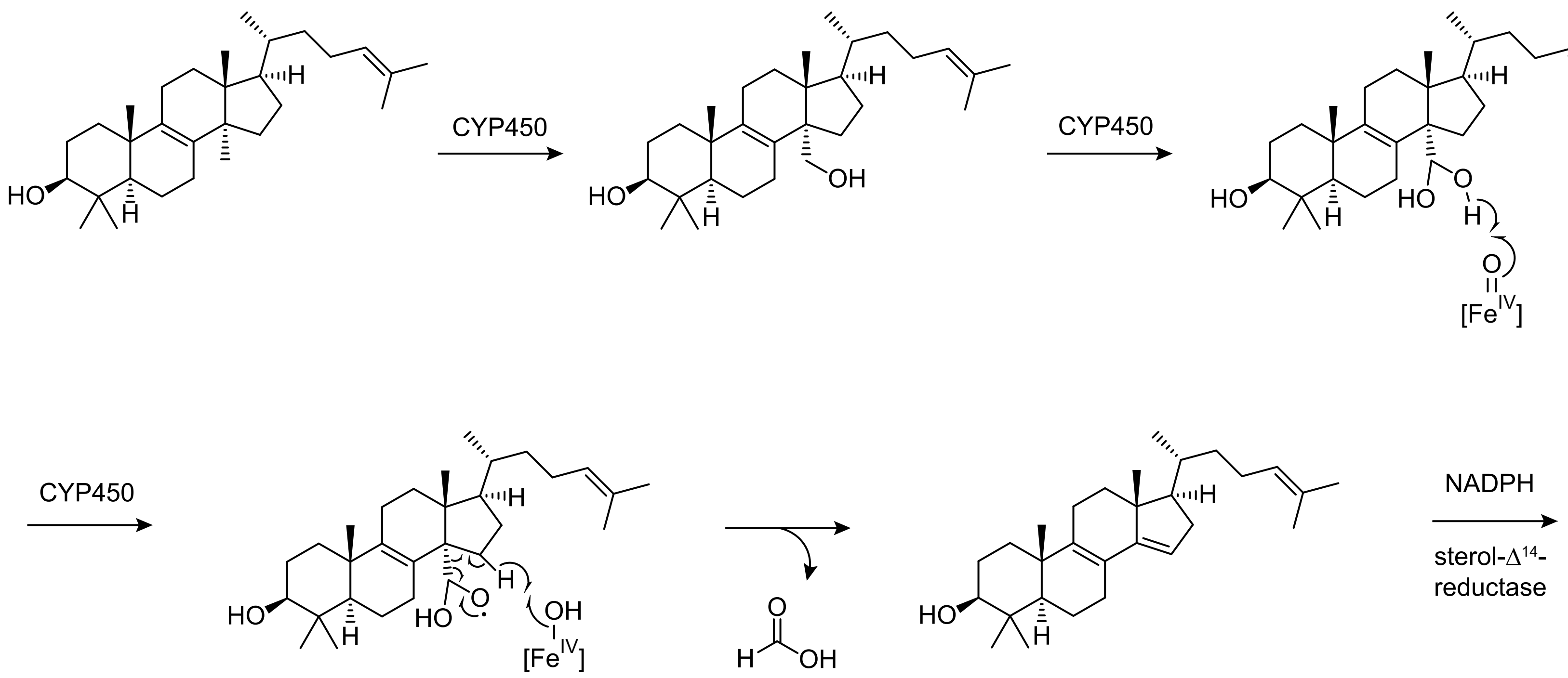
lanosterol synthase



E. E. van Tamelen, J. D. Willett, R. B. Clayton, K. E. Lord, *J. Am. Chem. Soc.* **1966**, *88*, 4752.

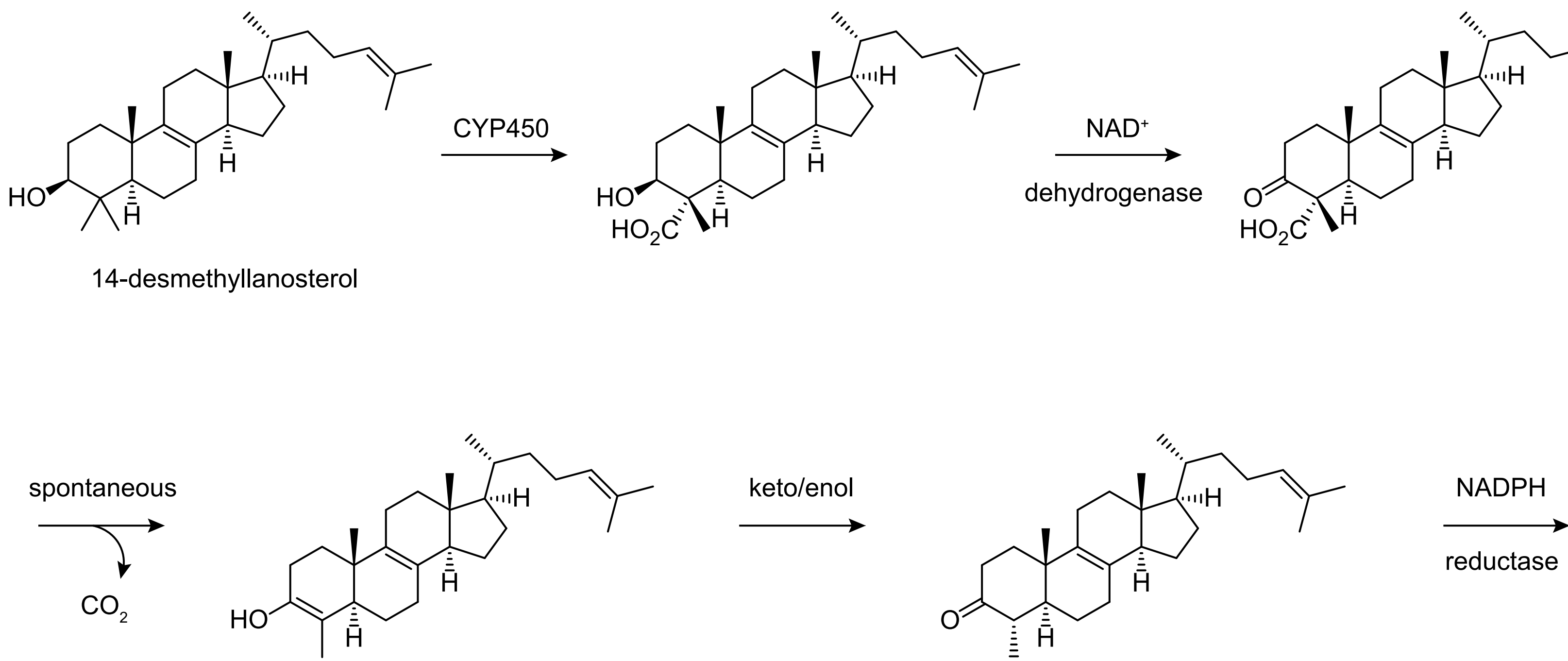
3.2.4. Terpene Synthases

3.2.4.7. Steroids: demethylation of lanosterol



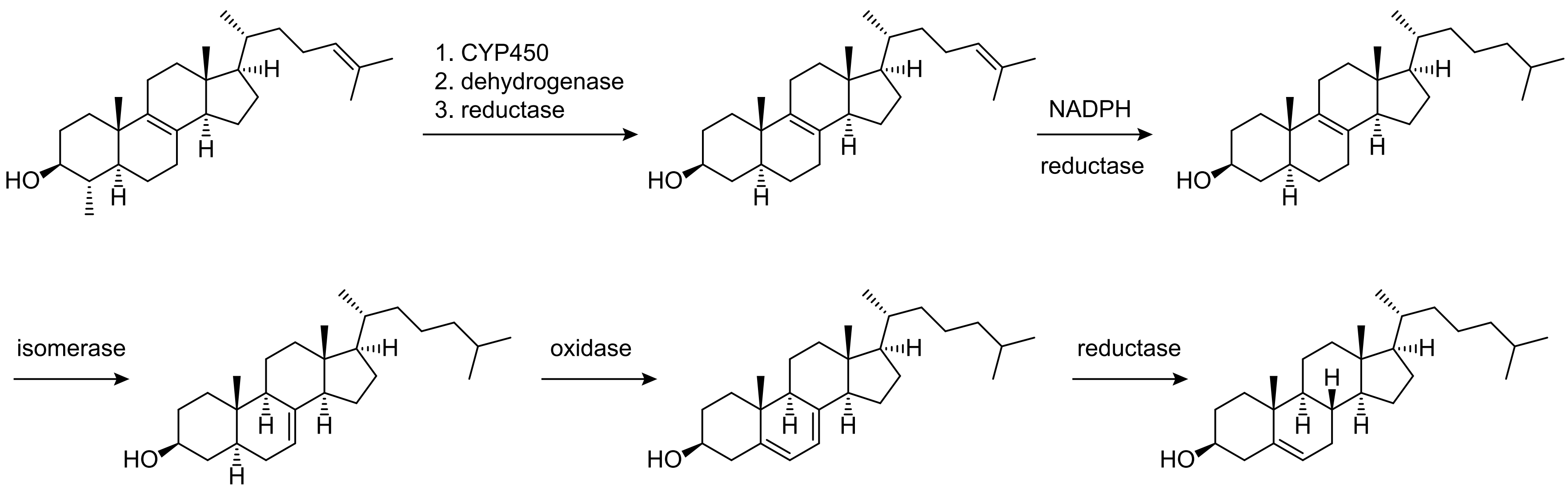
3.2.4. Terpene Synthases

3.2.4.7. Steroids: demethylation of lanosterol



3.2.4. Terpene Synthases

3.2.4.7. Steroids: demethylation of lanosterol and cholesterol biosynthesis



7-dehydrocholesterol

cholesterol

cholesterol:

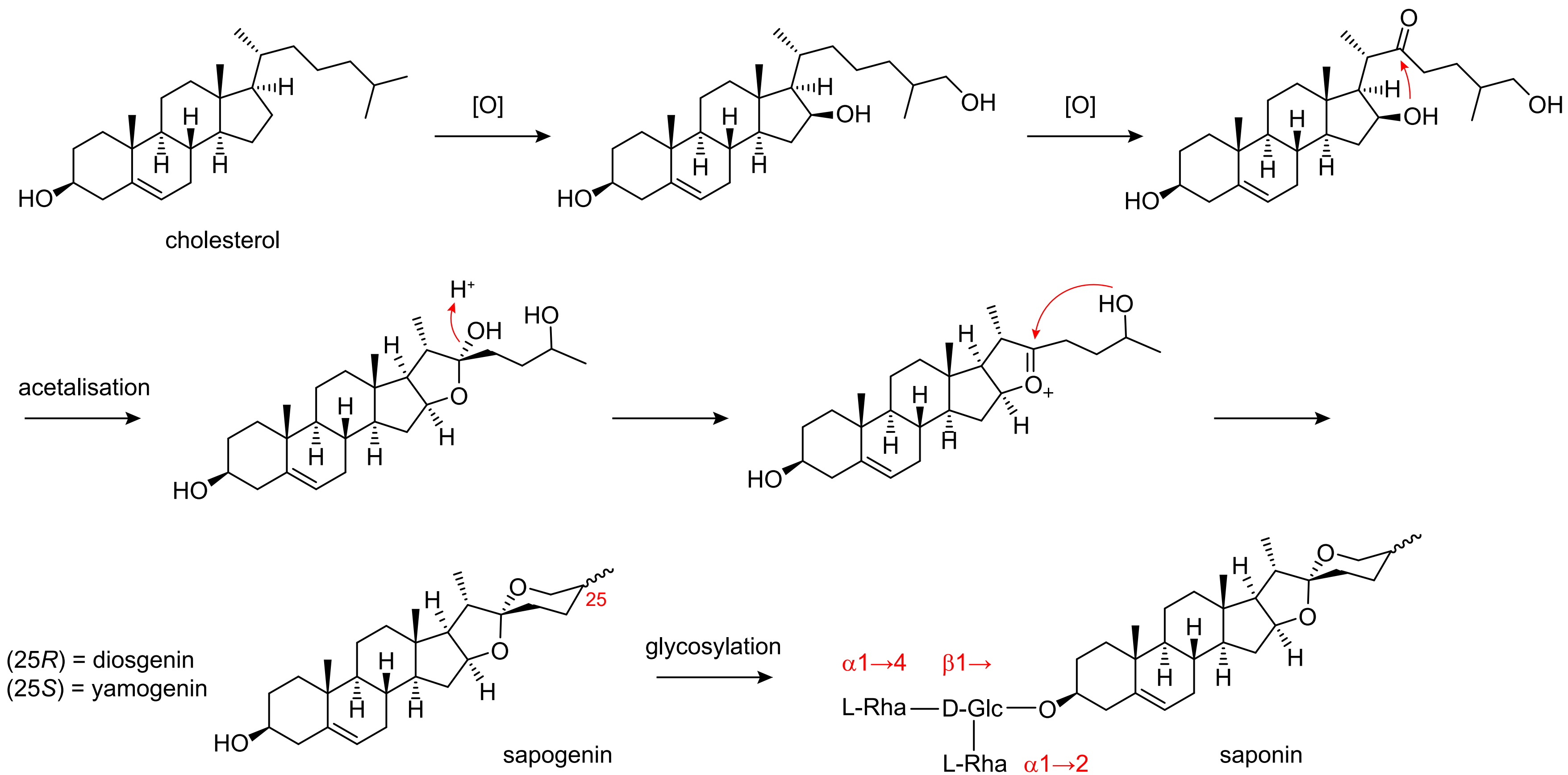
- membrane constituent
- biosynthetic precursor of steroid hormones
- gr. χολη (bile), στερεοσ (solid), main constituent of gall stones
- blood transport through binding to lipoprotein
- humans: ca. 0.2% of body weight

3. Terpenes

3.2.4. Terpene Synthases

3.2.4.7. Steroids: saponins

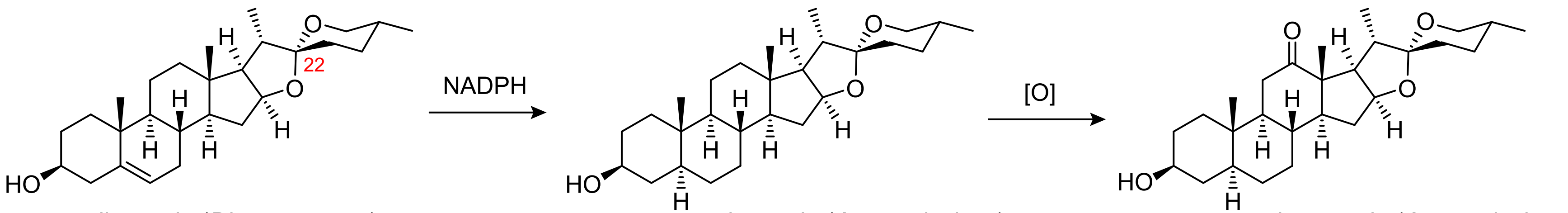
occur in many monocotyledons, contain a sugar part and a steroid part (aglycone = sapogenin)



3.2.4. Terpene Synthases

3.2.4.7. Steroids: saponins

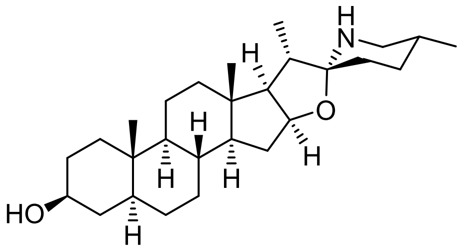
occur in many monocotyledons, contain a sugar part and a steroid part (aglycone = sapogenin)



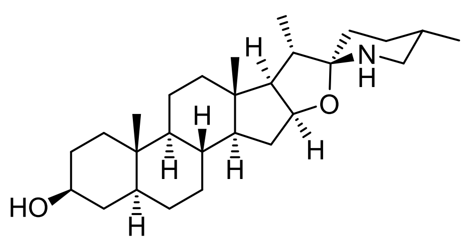
diosgenin (*Dioscorea* spp.) tigogenin (*Agave sisalana*) hecogenin (*Agave sisalana*)

always 22*R* configuration

nitrogen analogs



solasodin (*Solanum* spp.)



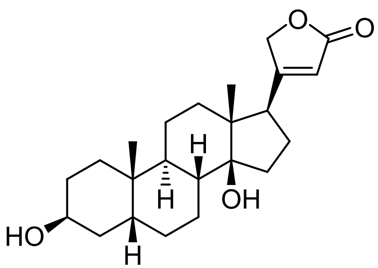
tomatidin (*Lycopersicon esculentum*)

both C22 configurations possible
toxic!

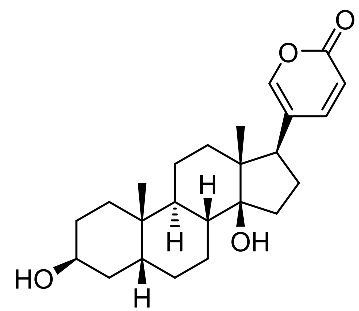
3.2.4. Terpene Synthases

3.2.4.7. Steroids: cardioactive glycosides

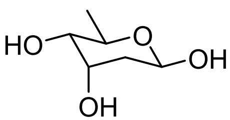
contain unsaturated lactones and an unusual sugar (D-digitoxose)



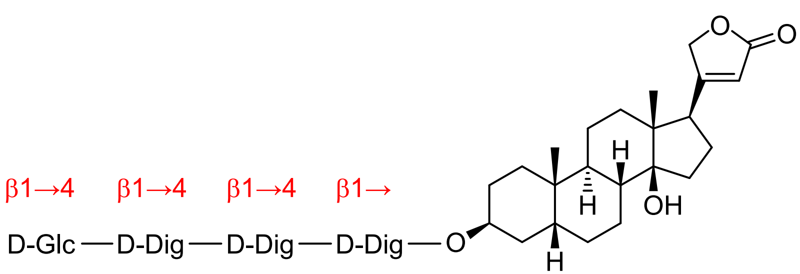
cardenolide: digitoxigenin (*Digitalis purpurea*)



bufadienolide: bufalin (*Bufo* spp.)



D-digitoxose



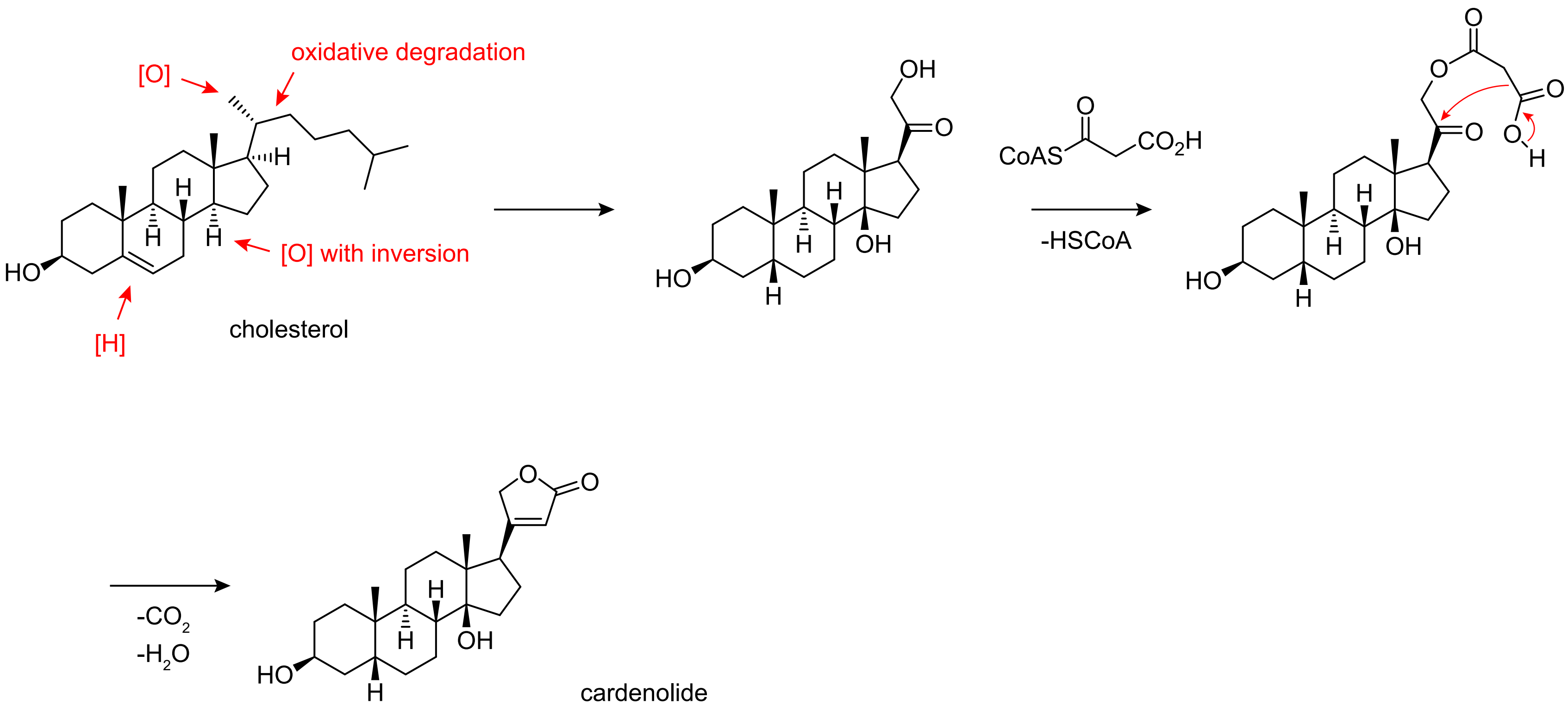
digitoxin (*Digitalis purpurea*)

strengthens heart beat
used to treat heart failure, especially for patients with poor kidney function
but narrow therapeutic window

3.2.4. Terpene Synthases

3.2.4.7. Steroids: cardioactive glycosides

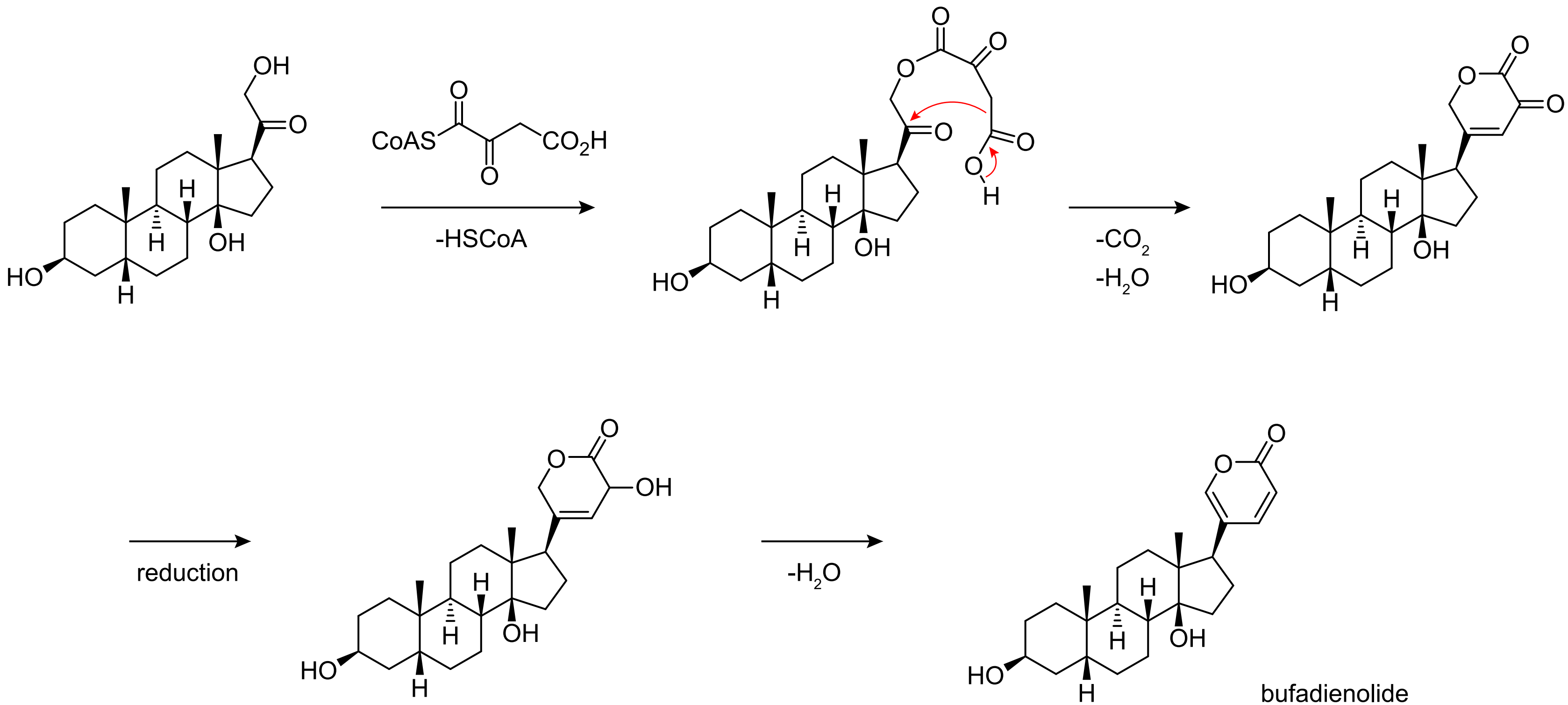
biosynthesis



3.2.4. Terpene Synthases

3.2.4.7. Steroids: cardioactive glycosides

biosynthesis

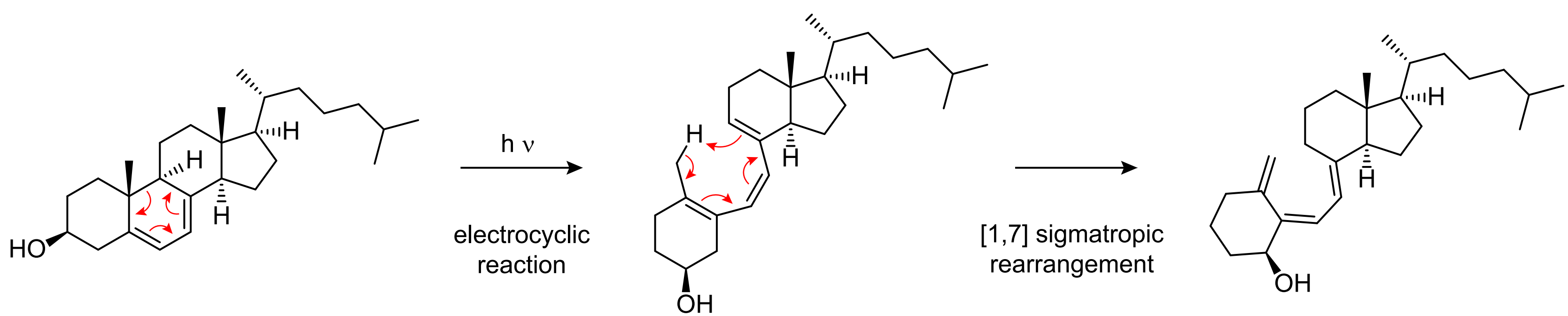


bufadienolide

3.2.4. Terpene Synthases

3.2.4.7. Steroids: vitamin D

- biosynthesis through photochemistry
- deficit leads to osteoporosis
- physiological functions in immune suppression, hormone secretion and cell differentiation



7-dehydrocholesterol

vitamin D_3

6 π-electrons
conrotatory

→ photochemical reaction

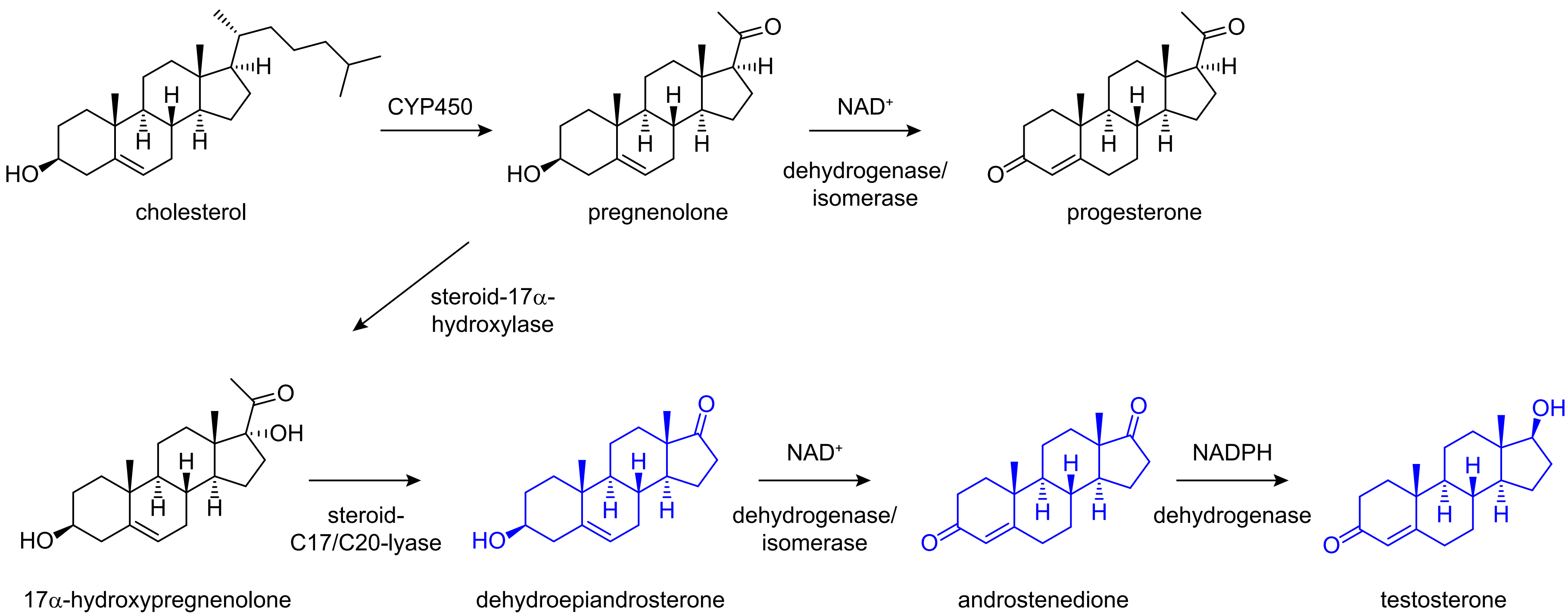
3.2.4. Terpene Synthases

3.2.4.7. Steroids: sex hormones

progestogens: C_{21} regulation of menstrual cycle and pregnancy

estrogens: C_{18} development of female sex characteristics, regulation of menstrual cycle and pregnancy

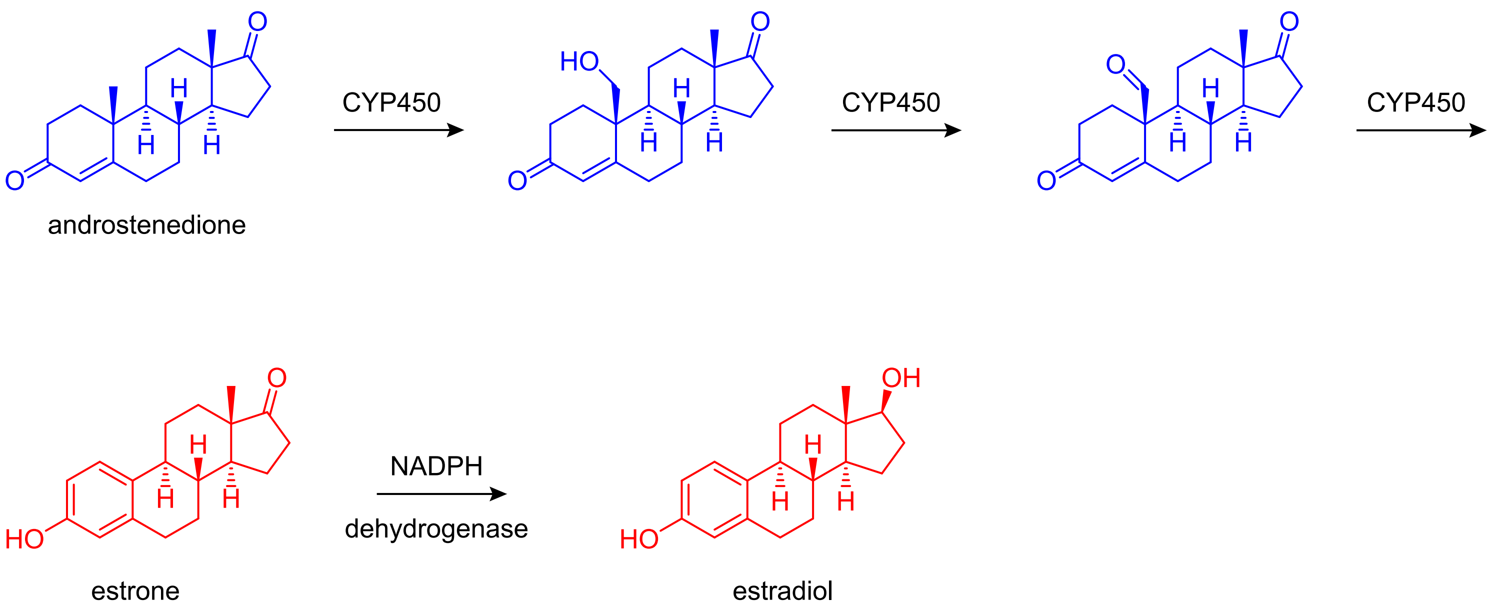
androgens: C_{19} development of male sex characteristics



3.2.4. Terpene Synthases

3.2.4.7. Steroids: sex hormones

progestogens: C_{21}	regulation of menstrual cycle and pregnancy
estrogens: C_{18}	development of female sex characteristics, regulation of menstrual cycle and pregnancy
androgens: C_{19}	development of male sex characteristics

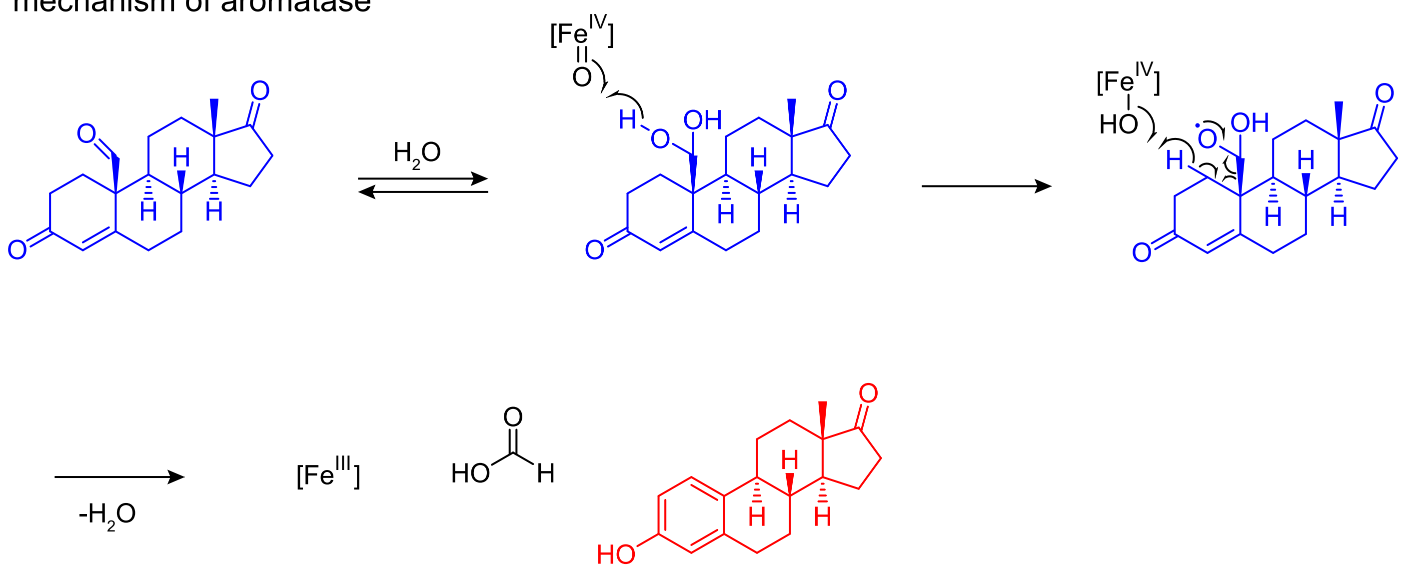


3.2.4. Terpene Synthases

3.2.4.7. Steroids: sex hormones

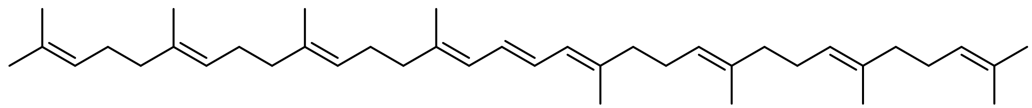
progestogens: C_{21}	regulation of menstrual cycle and pregnancy
estrogens: C_{18}	development of female sex characteristics, regulation of menstrual cycle and pregnancy
androgens: C_{19}	development of male sex characteristics

mechanism of aromatase



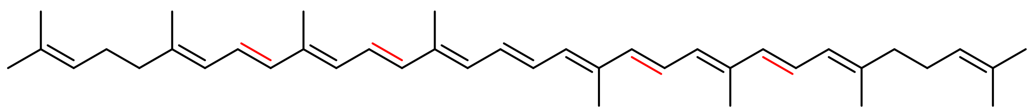
3.2.4. Terpene Synthases

3.2.4.8. Tetraterpenes

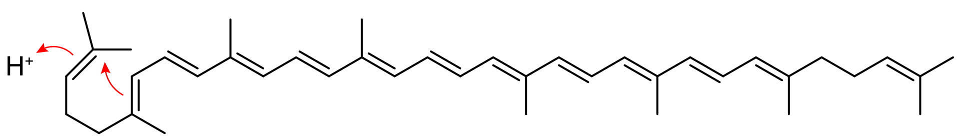


phytoene

↓ phytoene desaturase

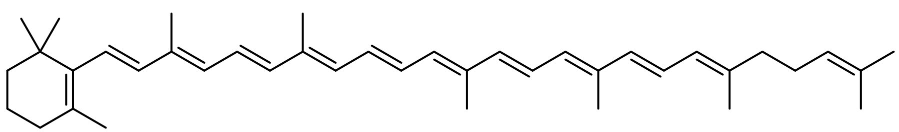


lycopene



lycopene

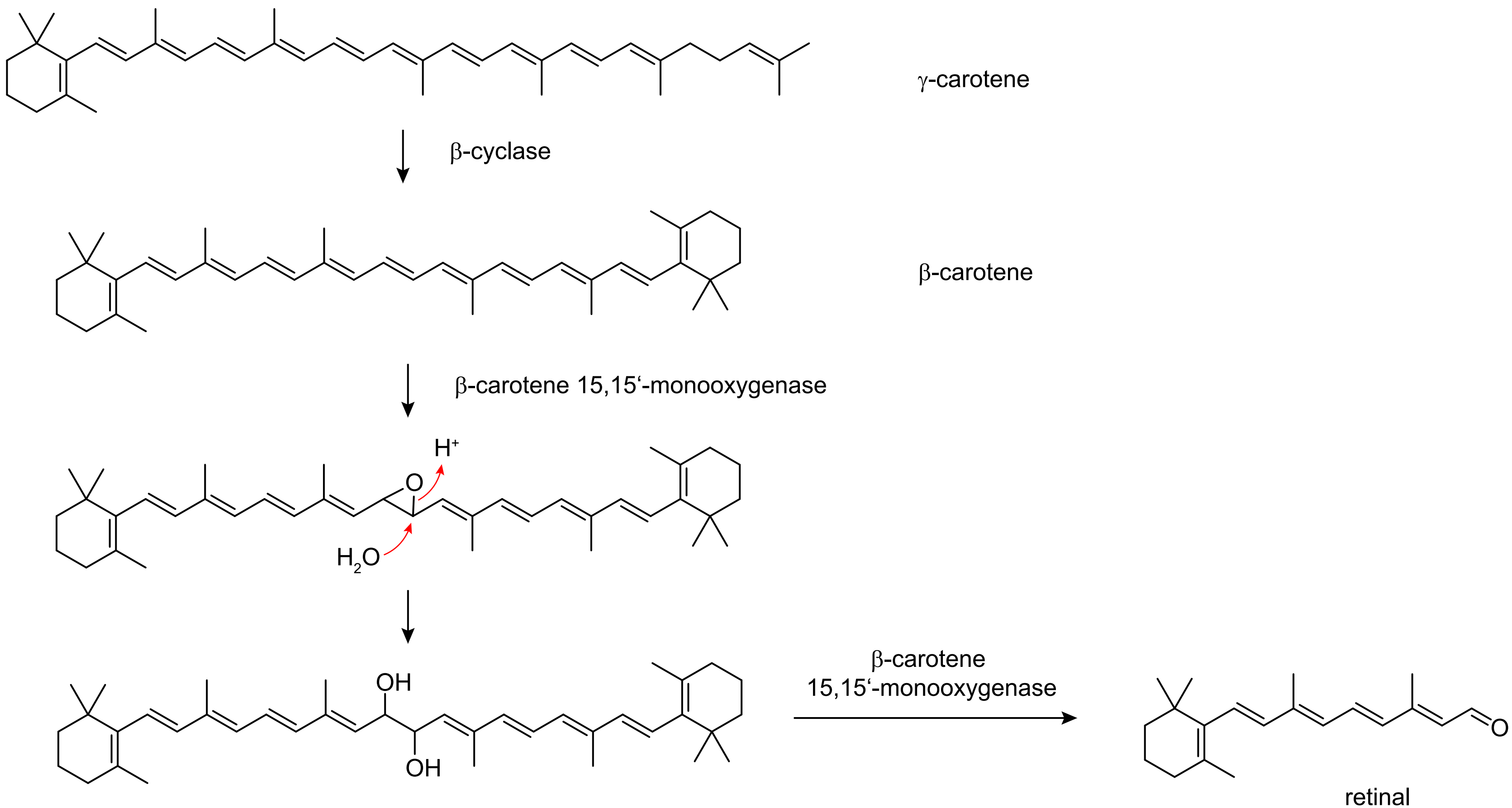
↓ β-cyclase



γ-carotene

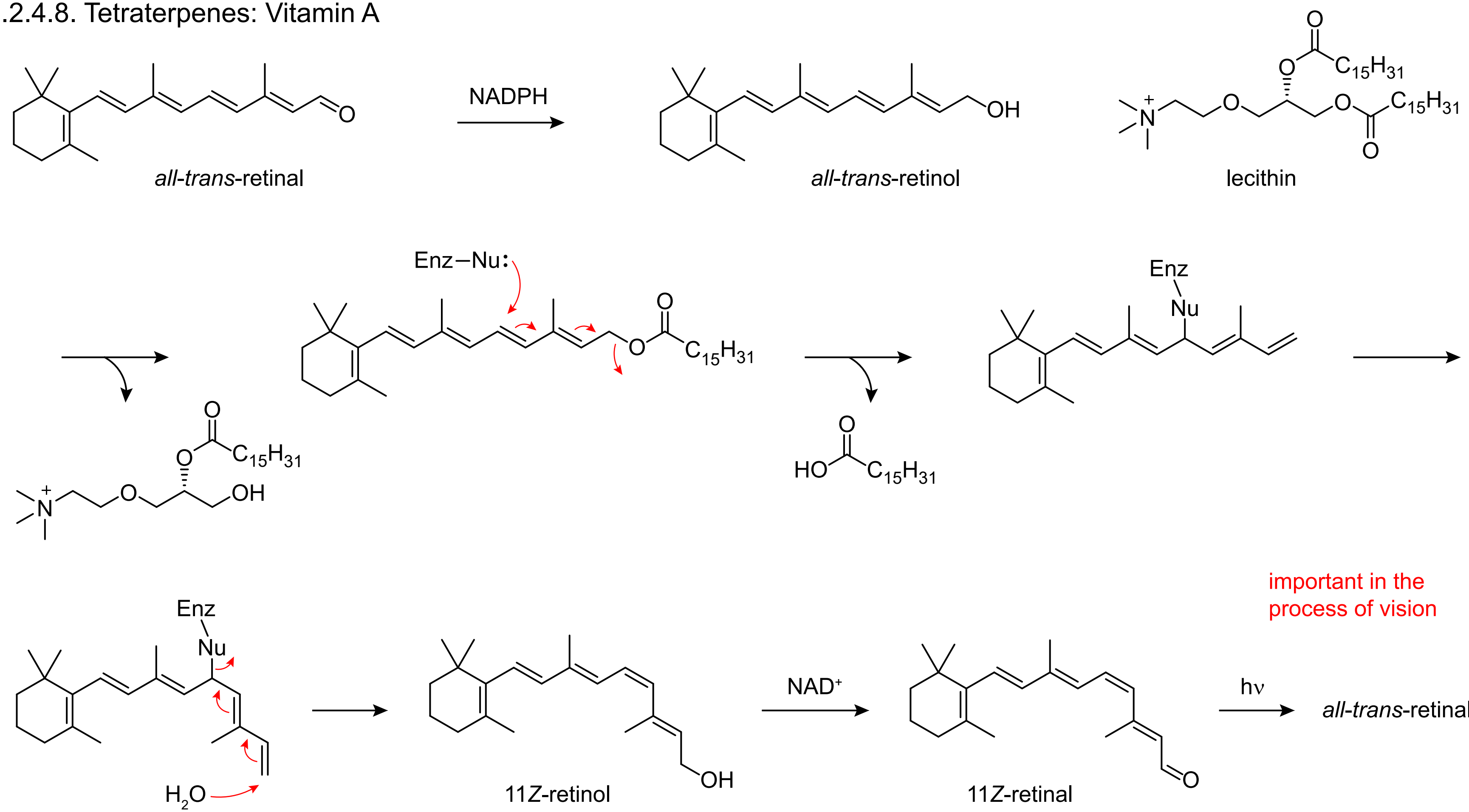
3.2.4. Terpene Synthases

3.2.4.8. Tetraterpenes



3.2.4. Terpene Synthases

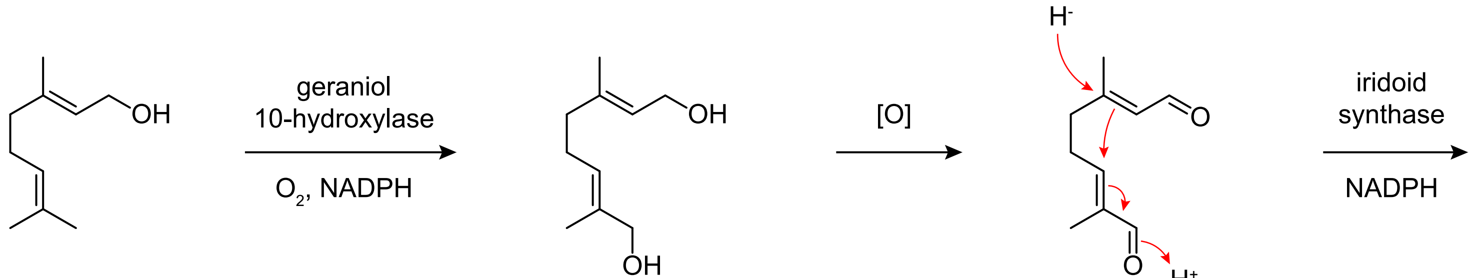
3.2.4.8. Tetraterpenes: Vitamin A



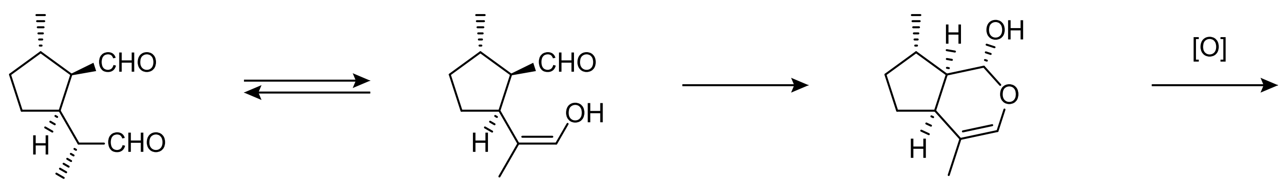
3.2.4. Terpene Synthases

3.2.4.9. Iridoid biosynthesis

Iridoids = special form of monoterpenes



geraniol



iridodial (keto form)

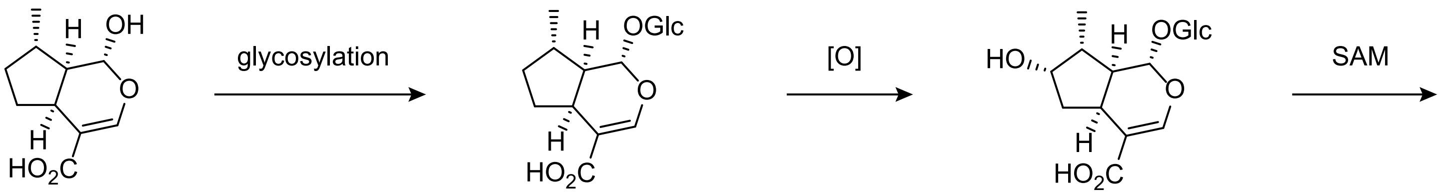
iridodial (enol form)

iridodial (hemiacetale)

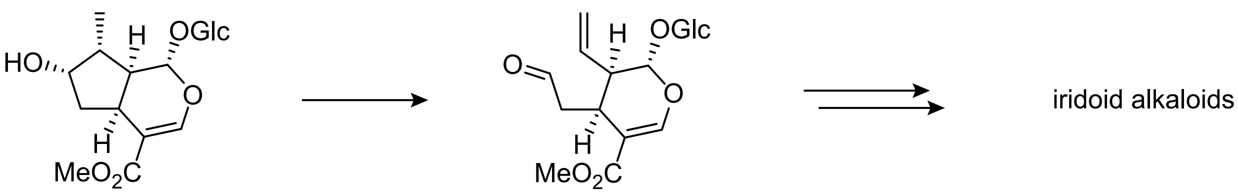
3.2.4. Terpene Synthases

3.2.4.9. Iridoid biosynthesis

Iridoids = special form of monoterpenes



loganic acid



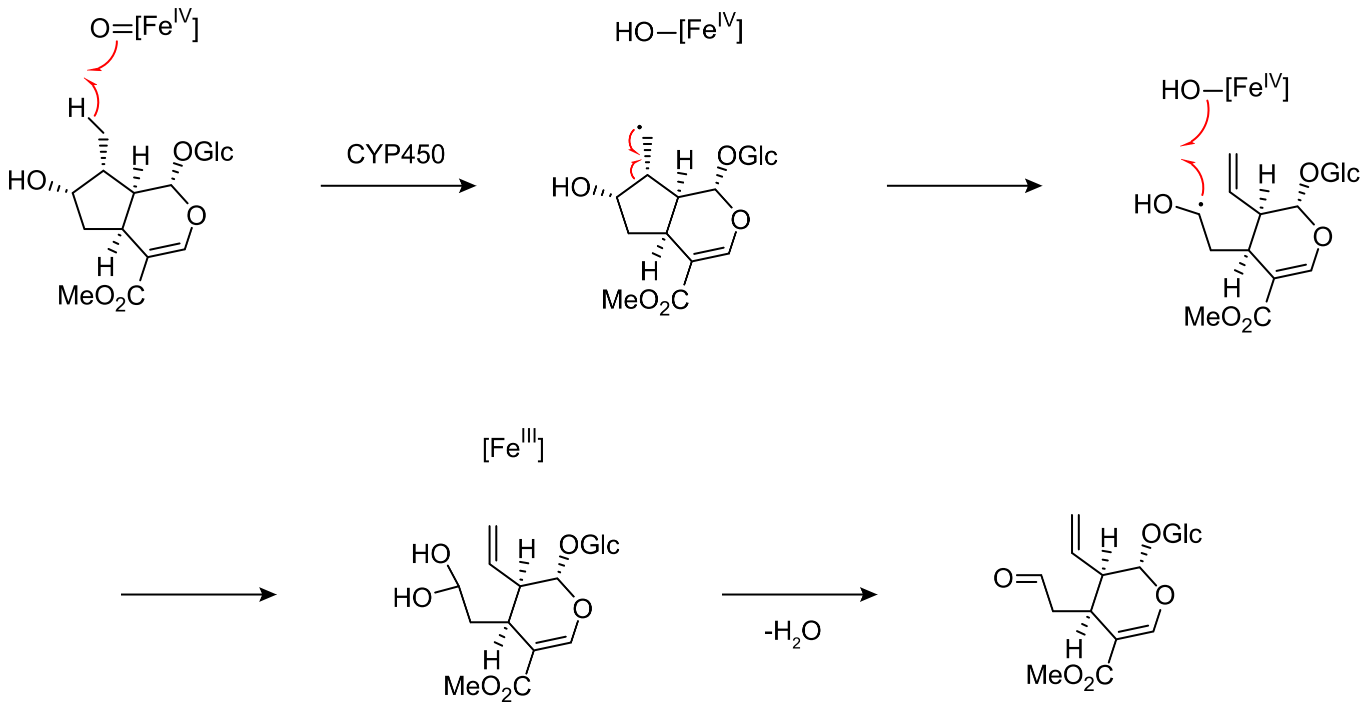
loganin

secologanin

3.2.4. Terpene Synthases

3.2.4.9. Iridoid biosynthesis

mechanism of ring opening reaction



4. Alkaloids

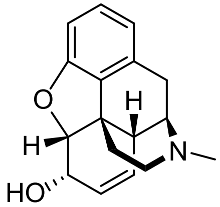
4.1. Definition, occurrence, biological activity

ca. 30,000 known compounds, nitrogen containing

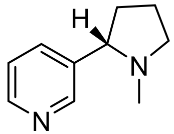
heterogeneous class of natural products, this complicates a satisfying definition

mostly from plants, but also known from bacteria, fungi and animals

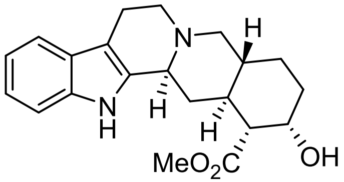
arabic: al-qalya = plant ashes



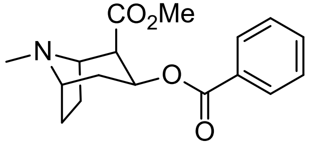
morphine
(*Papaver somniferum*)
strong pain killer



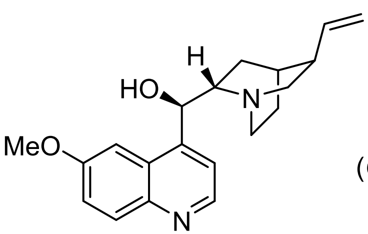
nicotine
(*Nicotiana tabacum*)
intoxicant



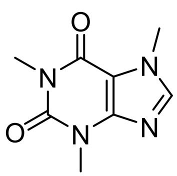
yohimbine
(*Pausinystalia yohimbe*)
aphrodisiac



cocaine
(*Erythroxylum coca*)
intoxicant and pain killer

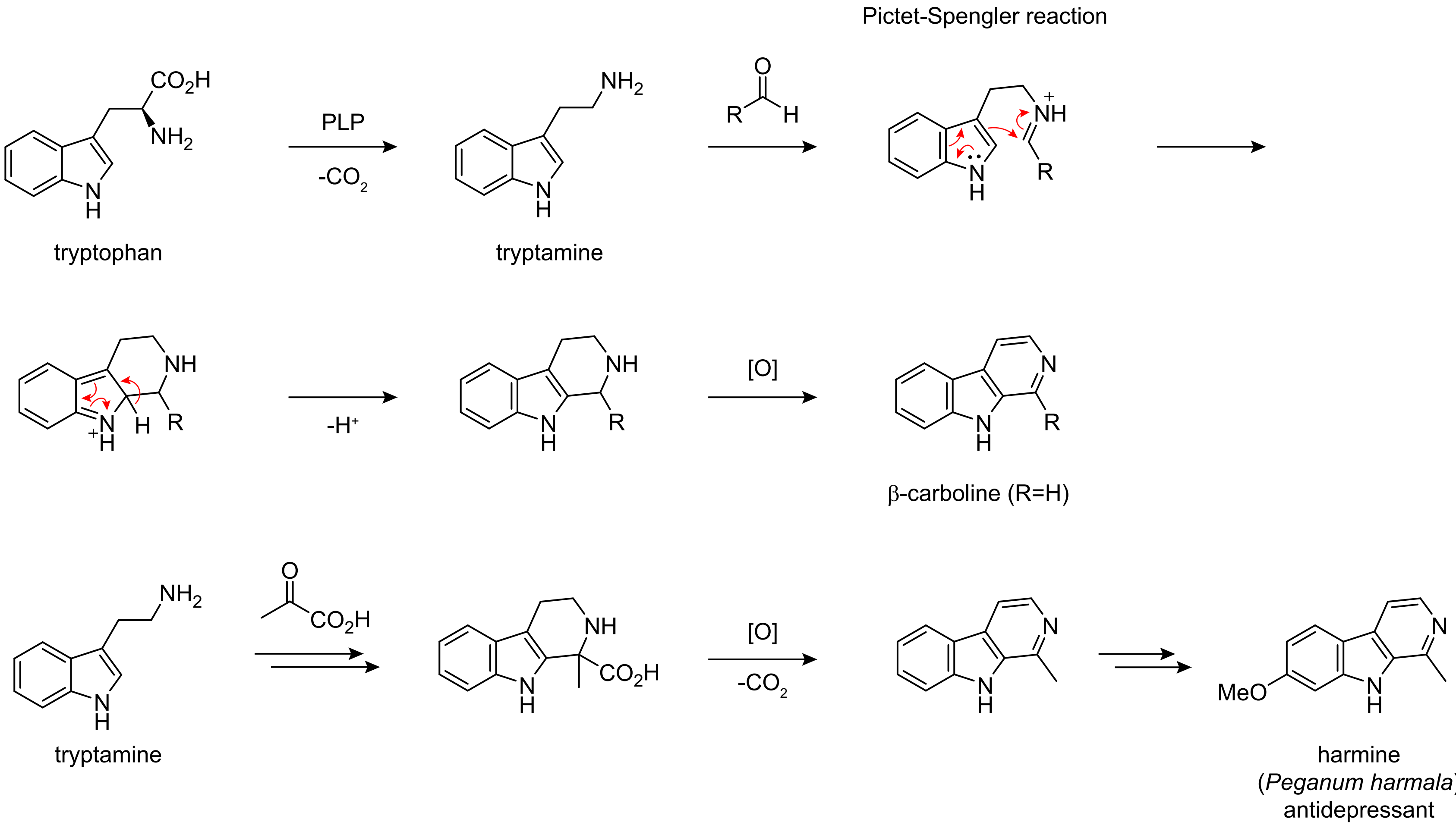


quinine
(*Chinchona officinalis*)
anti-malaria drug



caffeine
(*Coffea arabica*)
stimulans

4.2. Indole alkaloids



4.2. Indole alkaloids

4.2.1. Strictosidine and terpene indole alkaloids

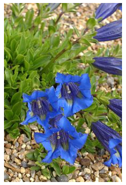
> 3,000 known compounds from 8 plant families

- Apocynaceae (dogbane family, Apocynum = dog-away)

- Loganiaceae (family of highly poisonous plants)

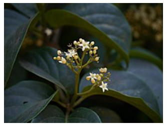
- Rubiaceae (bedstraw or coffee family)

- Gentianaceae (named after the Illyrian king Gentius)



- Gelsemiaceae

- Icacinaceae



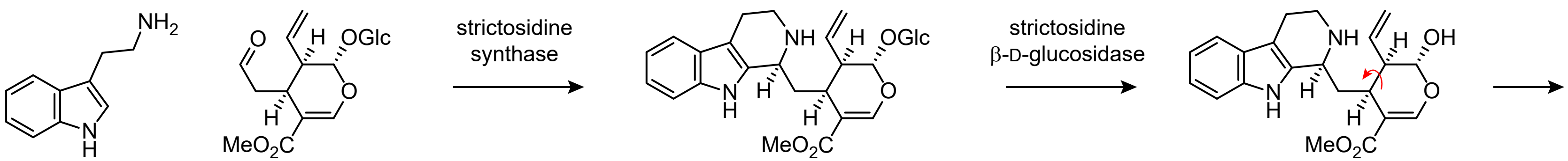
- Cornaceae (dogwood family)

- Alangiaceae

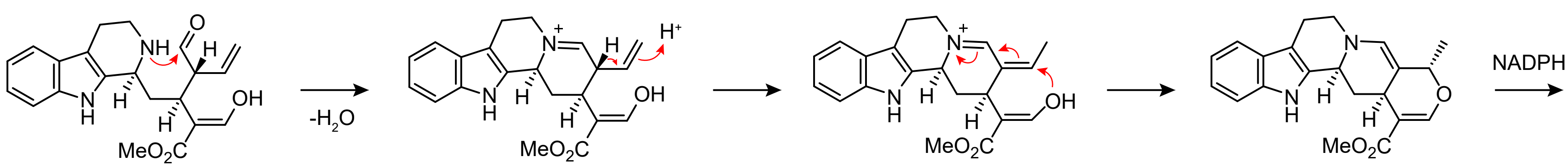
4. Alkaloids

4.2. Indole alkaloids

4.2.1. Strictosidine and terpene indole alkaloids



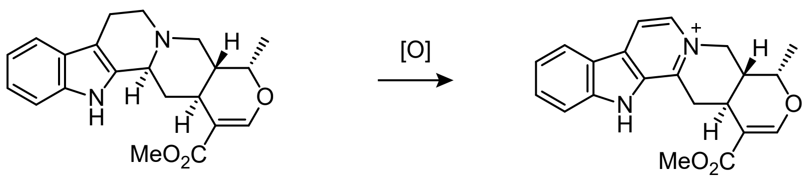
tryptamine | secologanin | strictosidine (*Catharanthus roseus*, Apocynaceae)



dehydrogeissoschizine (enol form) | cathenamine

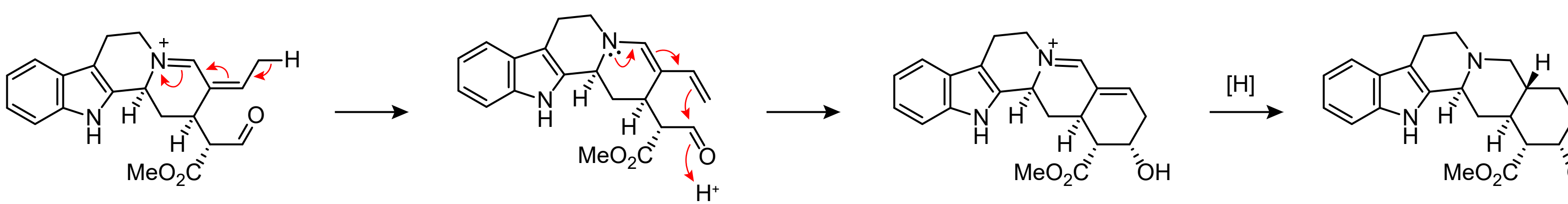
4.2. Indole alkaloids

4.2.1. Strictosidine and terpene indole alkaloids



ajmalicine
(*Rauwolfia serpentia*, Apocynaceae)

serpentine
(*Rauwolfia serpentia*)

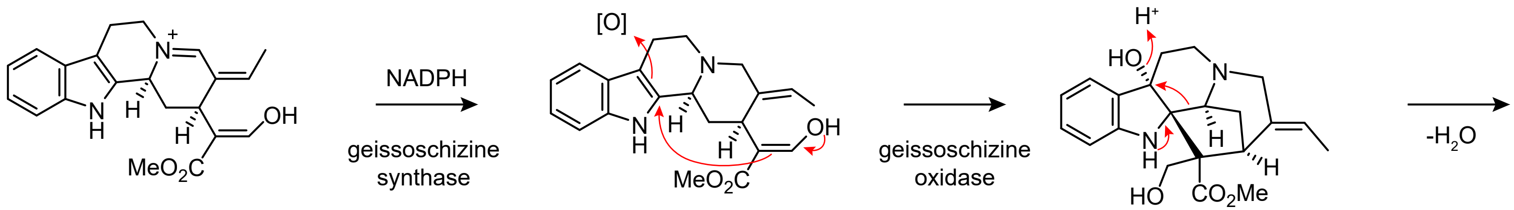


dehydrogeissoschizine
(keto form)

yohimbine
(*Pausinystalia yohimbe*, Rubiaceae)

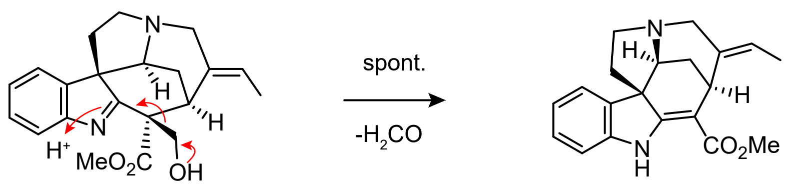
4.2. Indole alkaloids

4.2.1. Strictosidine and terpene indole alkaloids



dehydrogeissoschizine (enol form)

geissoschizine (*Catharanthus roseus*, Apocynaceae)

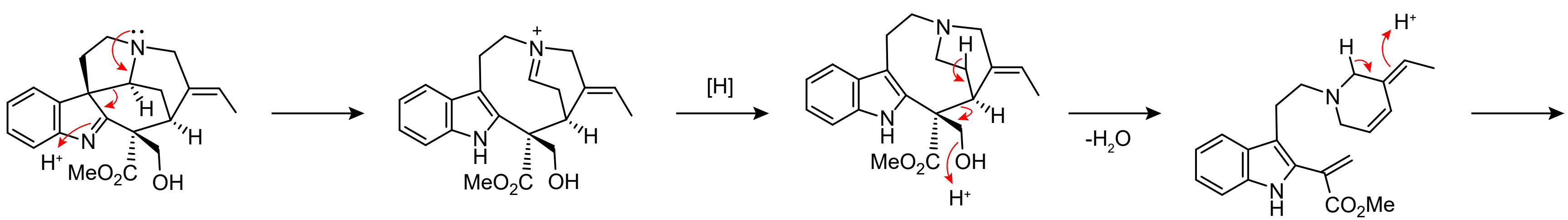


preakuammicine (*Catharanthus roseus*, Apocynaceae)

akuammicine (*Picralima nitida*, Apocynaceae)

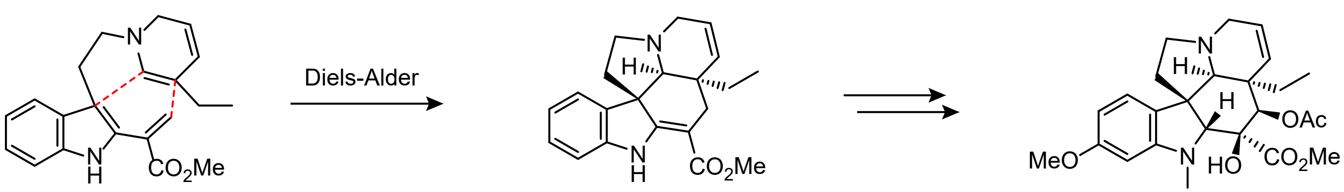
4.2. Indole alkaloids

4.2.1. Strictosidine and terpene indole alkaloids



preakuammicine

stemmadenine



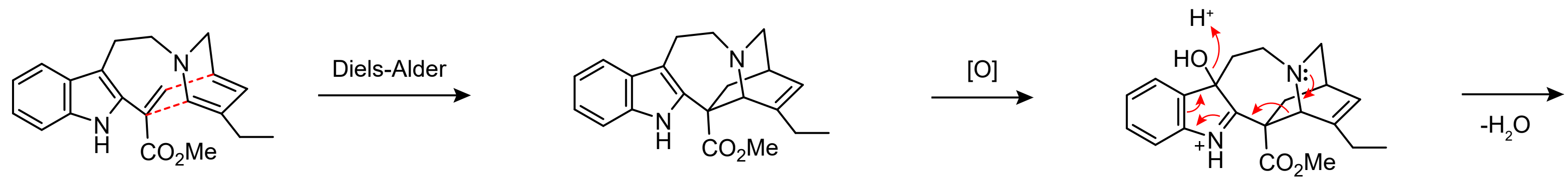
dehydrosecodine

tabersonine
(*Amsonia tabernaemontana*, Apocynaceae)

vindoline
(*Catharanthus roseus*, Apocynaceae)

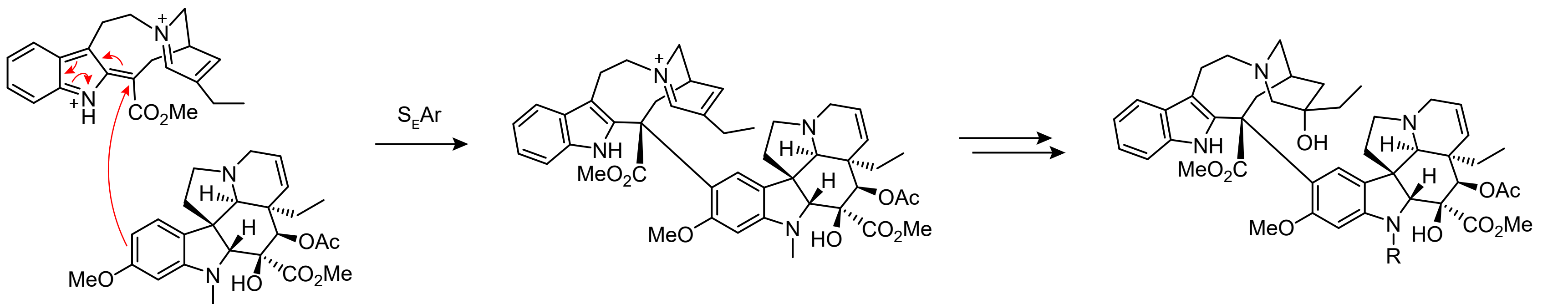
4.2. Indole alkaloids

4.2.1. Strictosidine and terpene indole alkaloids



dehydrosecodine

catharanthine
(*Catharanthus roseus*, Apocynaceae)



vinblastine (R=Me)
vinchristine (R=CHO)
(*Catharanthus roseus*, Apocynaceae)

used in chemotherapy
tubulin binders that inhibit tubulin polymerisation

4.2. Indole alkaloids

4.2.2. Ergoline alkaloids

from fungi (*Claviceps*, *Aspergillus*, *Rhizopus*, *Penicillium*)

growth on crops, leading to contamination with ergot

in history often causing food poisonings

→ ergotism: spasms, diarrhea, mania, psychosis, headaches, nausea, vomiting, weak peripheral pulses, loss of peripheral sensation, edema

during the middle ages reason for witchcraft accusations

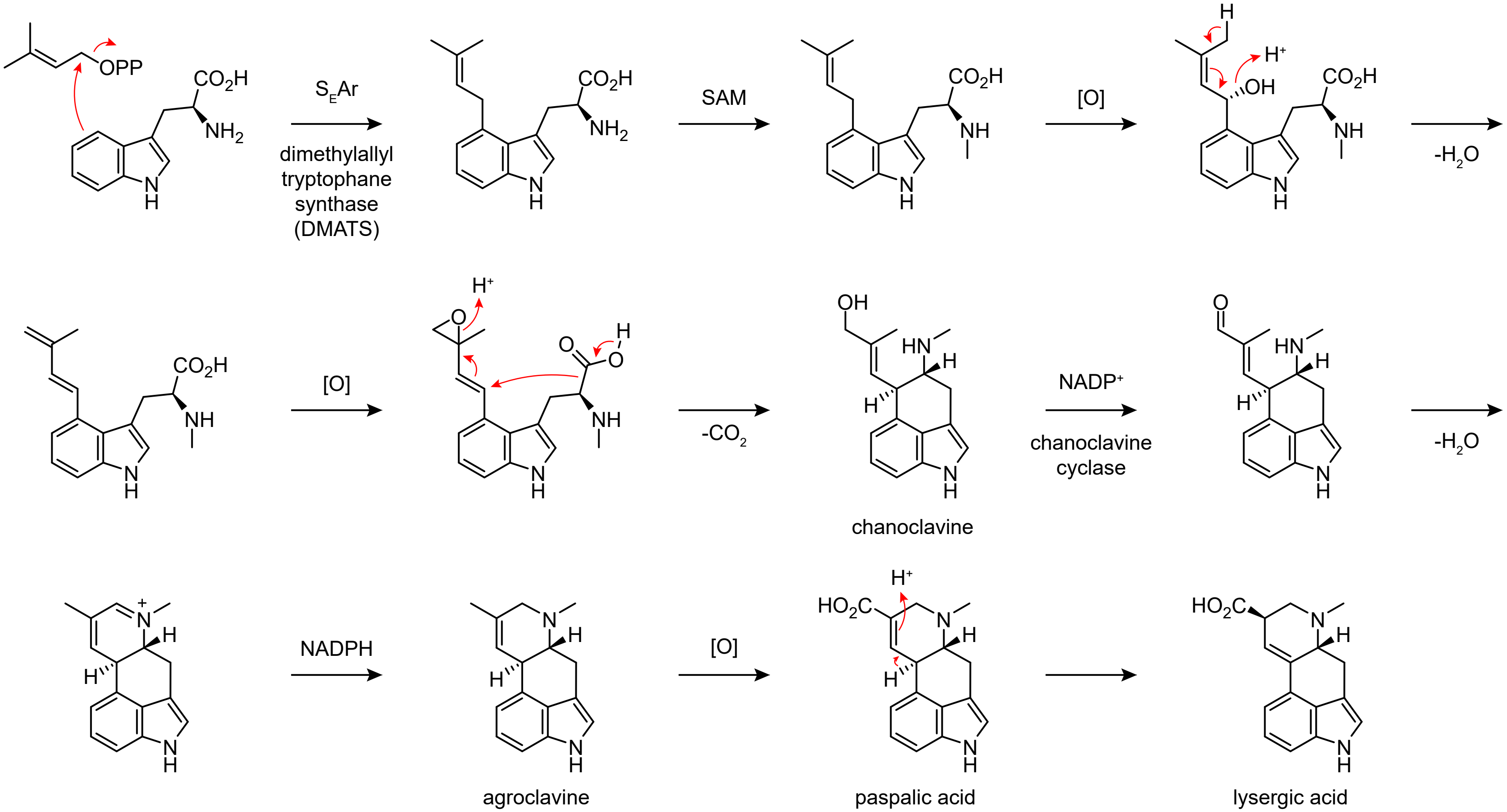
ergot in barley

Temptation of St Anthony (Matthias Grünwald)

showing a patient suffering advanced ergotism

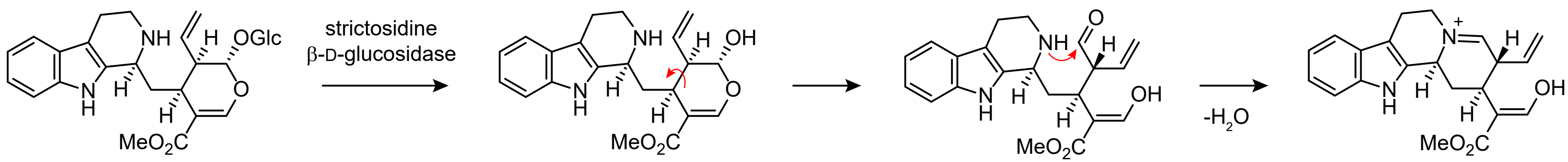
4.2. Indole alkaloids

4.2.2. Ergoline alkaloids

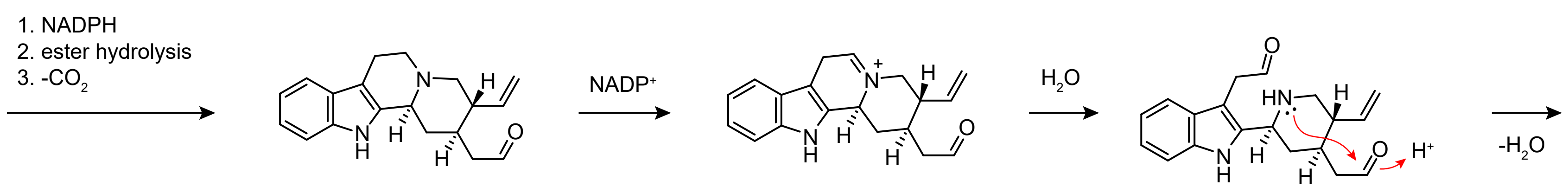


4.3. Quinoline alkaloids

from Chinchona (Rubiaceae)



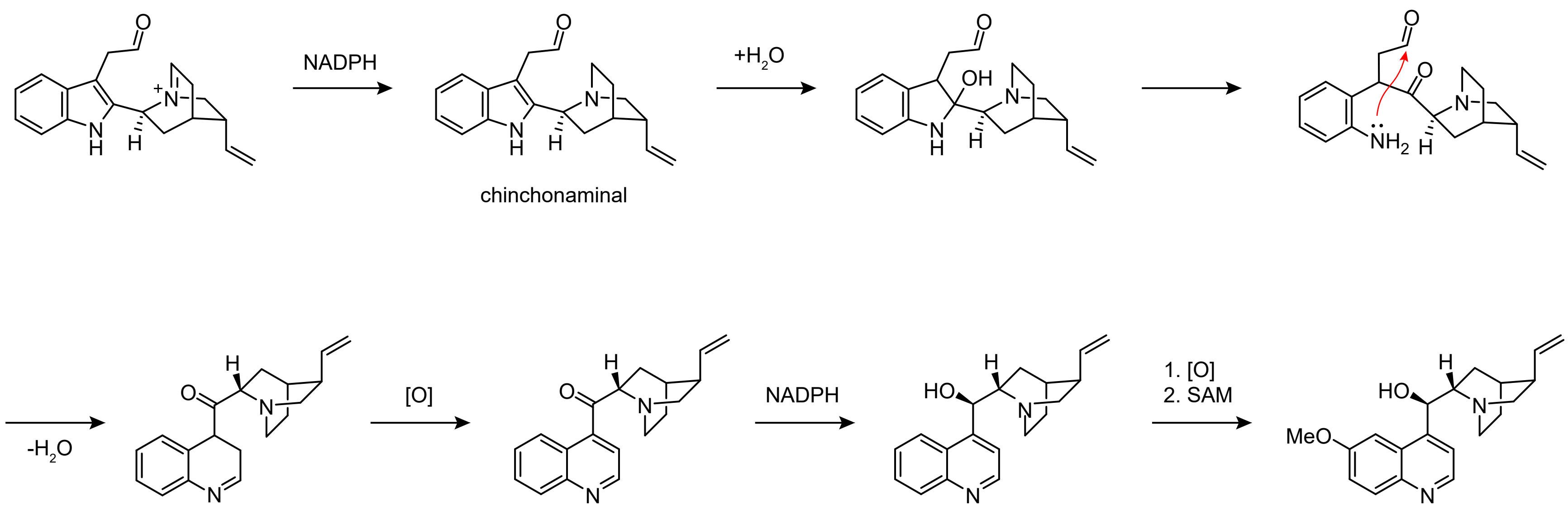
strictosidine



corynantheal

4.3. Quinoline alkaloids

from Chinchona (Rubiaceae)



chinchonaminal

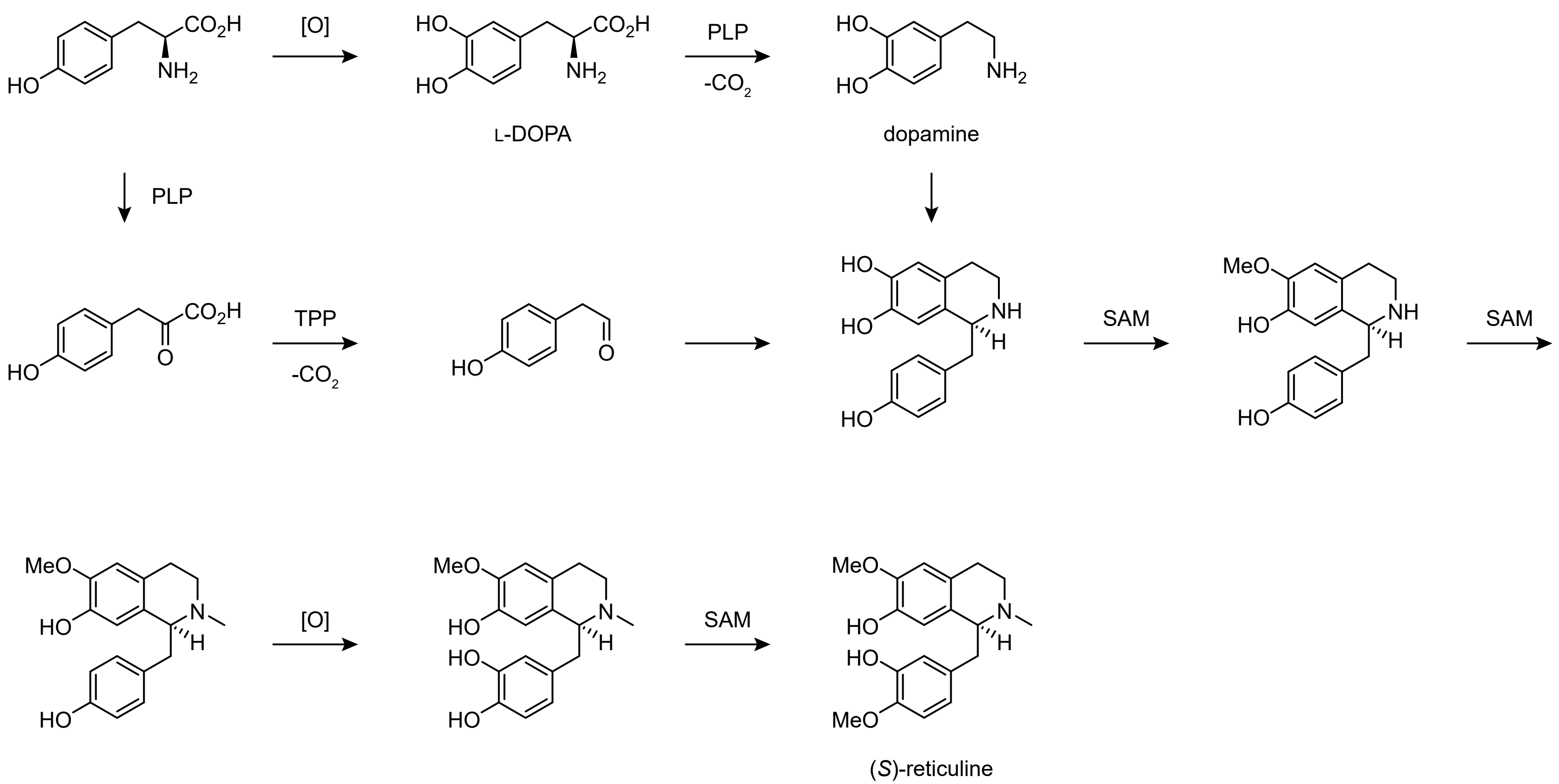
chinchonidinone

chinchonidine

quinine

4.4. Isoquinoline alkaloids

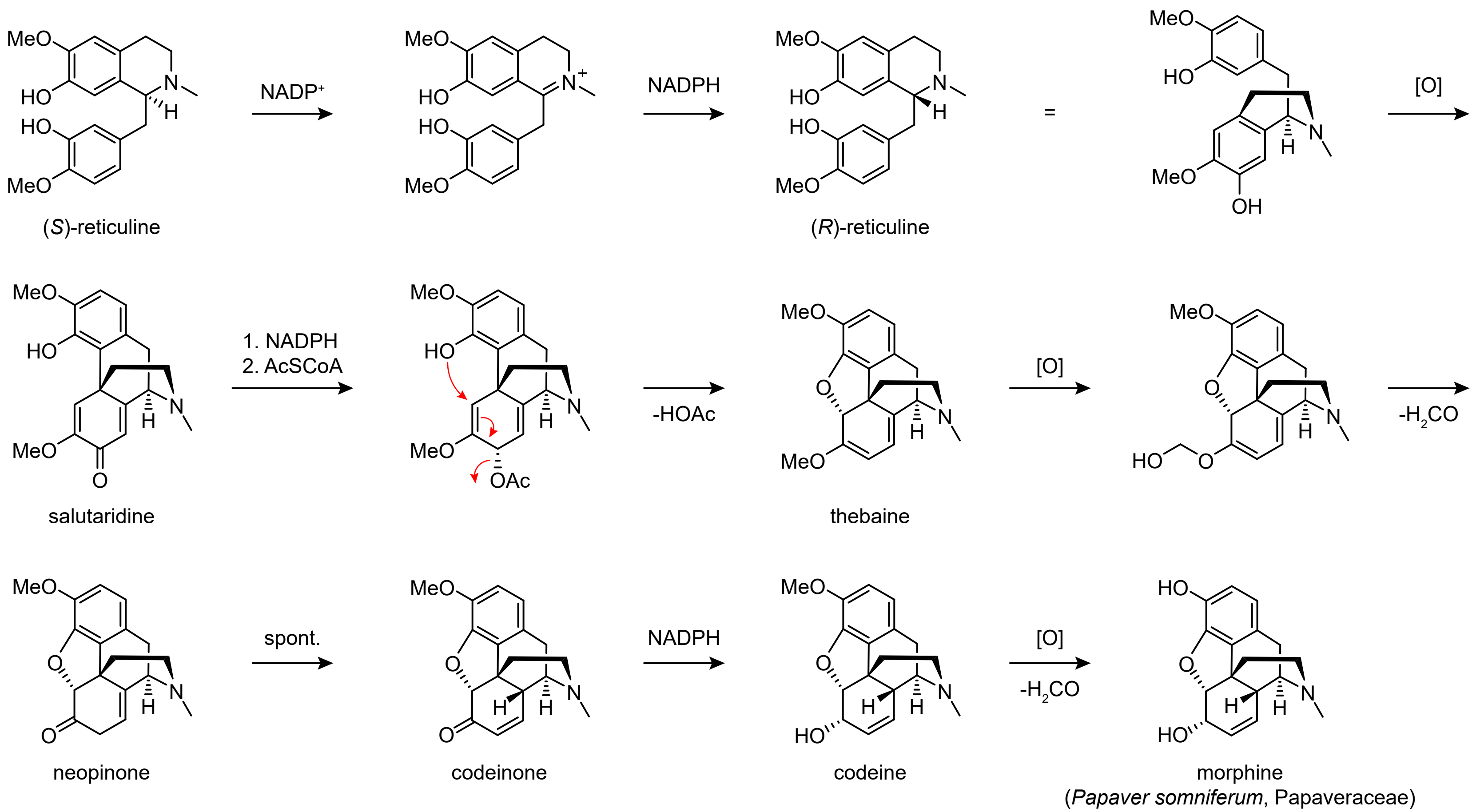
Reticuline, codeine, morphine



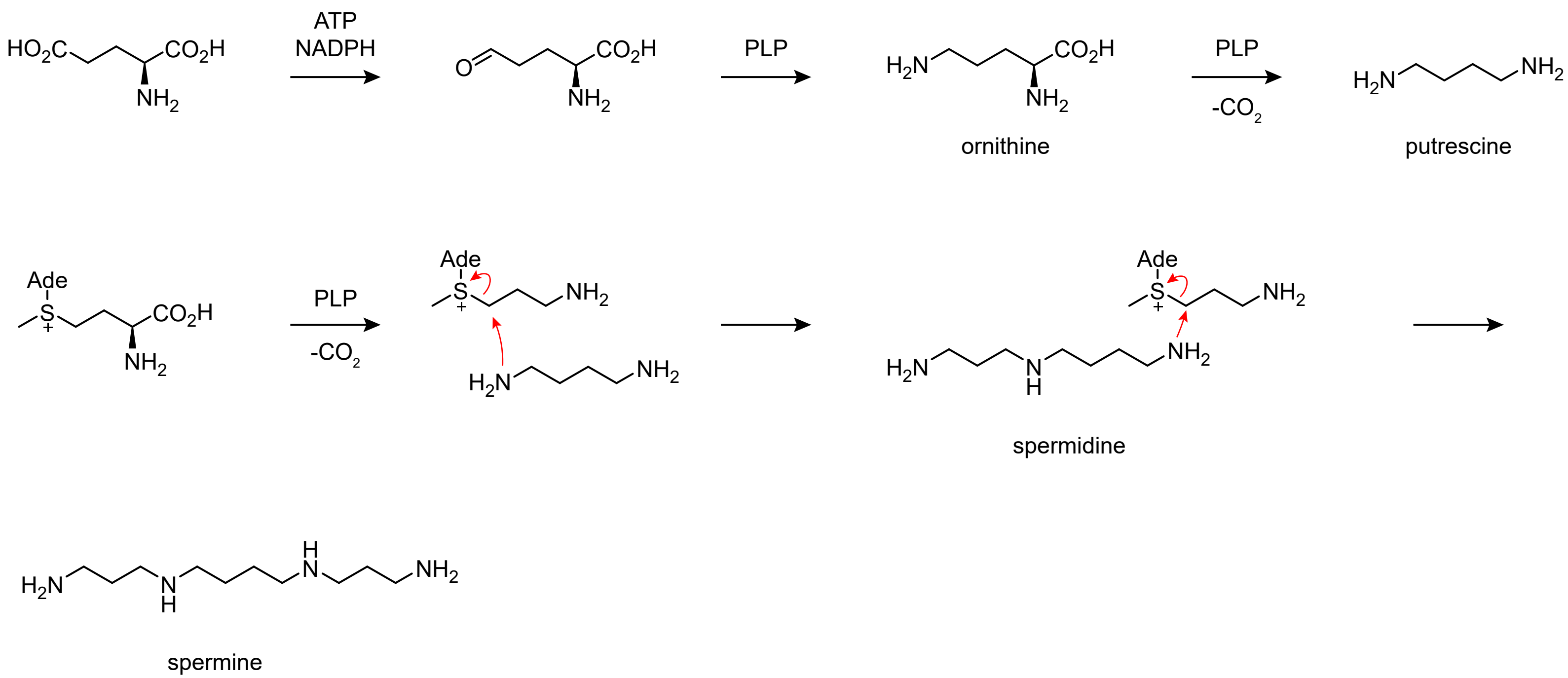
4. Alkaloids

4.4. Isoquinoline alkaloids

Reticuline, codeine, morphine

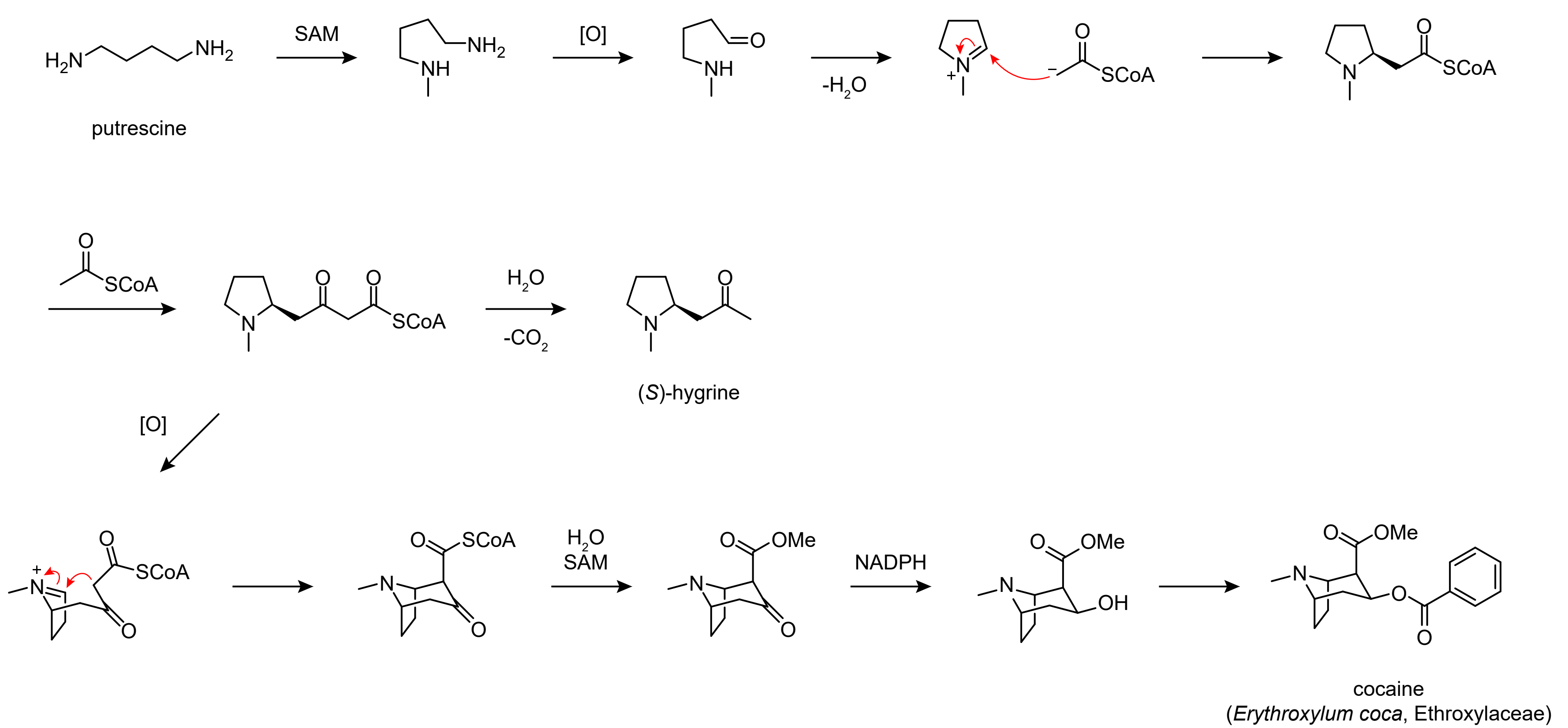


4.5. Polyamines, pyrrolidine and tropane alkaloids

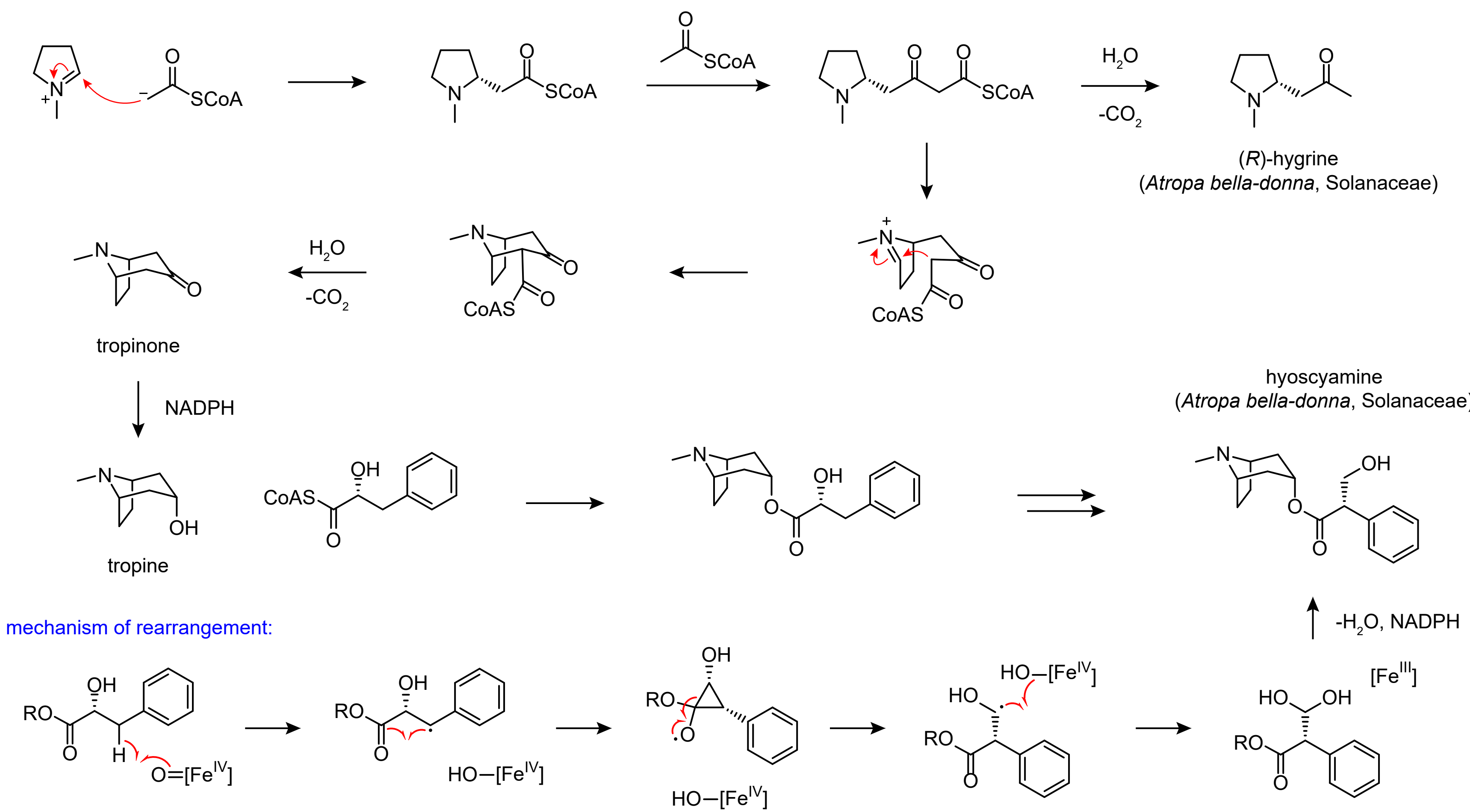


- spermidine and spermine occur in nearly all species
- regulators of cell growth, development and death
- polyamine biosynthesis is an important target in chemotherapy

4.5. Polyamines, pyrrolidine and tropane alkaloids



4.5. Polyamines, pyrrolidine and tropane alkaloids

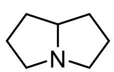


4. Alkaloids

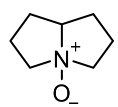
4.6. Pyrrolizidine alkaloids

ca. 1,000 known compounds from > 6,000 plant species (particularly Asteraceae)

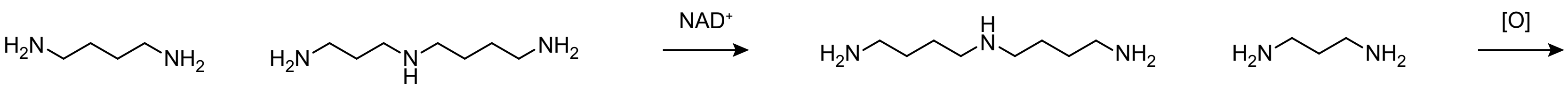
defense against herbivores, catabolites are hepatotoxic



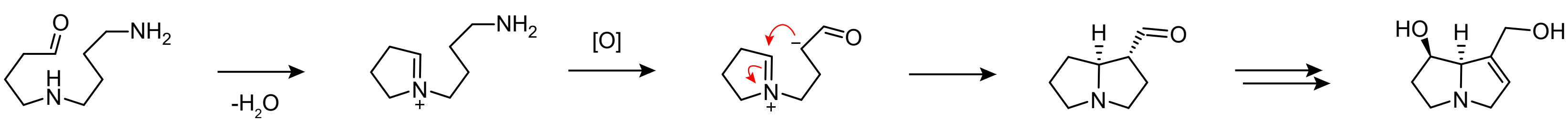
pyrrolizidine



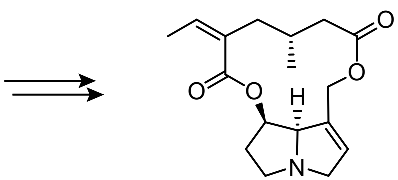
pyrrolizidine N-oxide



putrescine spermidine homospermidine 1,3-diaminopropane



retronecine

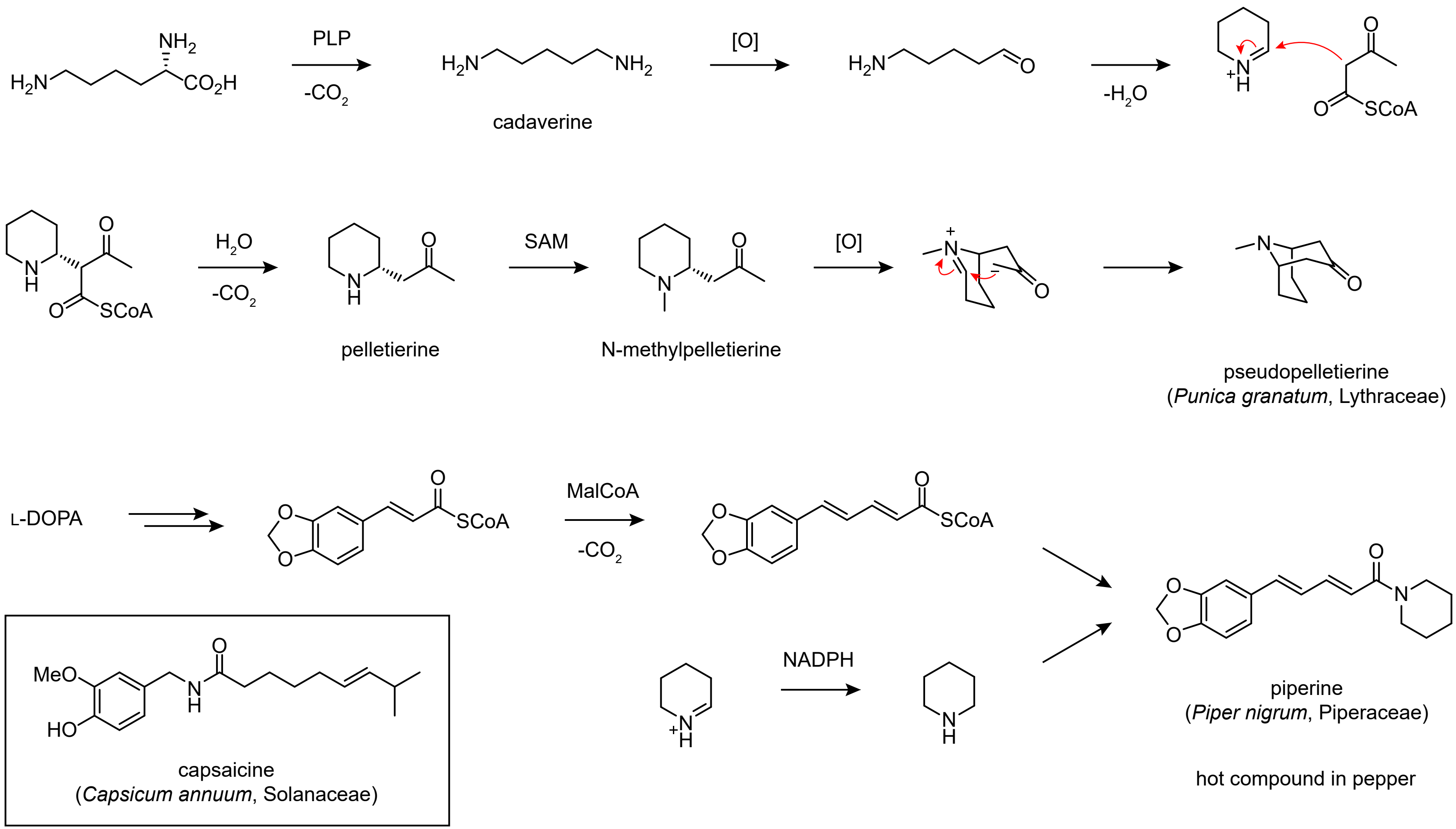


senecionine
(*Senecio vulgaris*, Asteraceae)

- sequestration by *Arctia villica* (Lepidoptera)
- male sex pheromone, „wedding present“
- used to protect eggs against predators

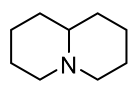
4. Alkaloids

4.7. Piperidine alkaloids

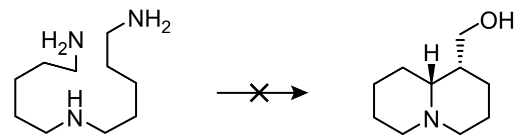


4.8. Quinolizidine alkaloids

Lupinine, Sparteine (from Leguminosae)

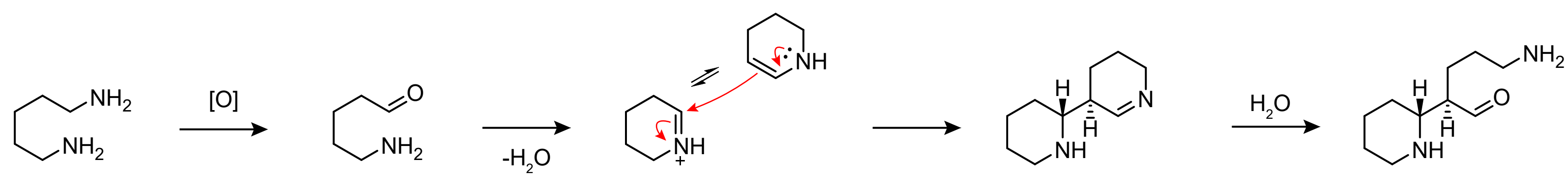


quinolizidine

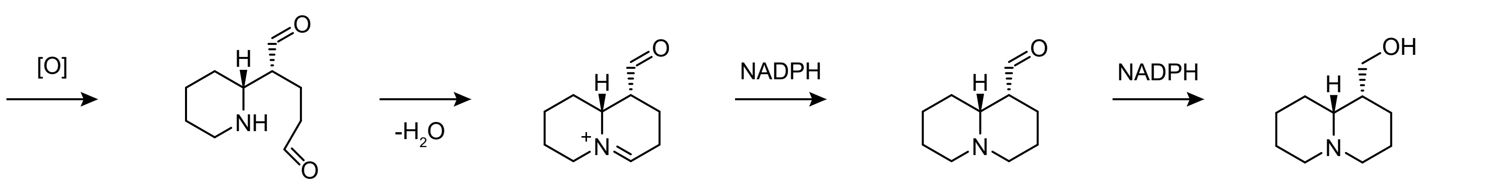


lupinine

compare to pyrrolizidine alkaloids!



cadaverine

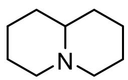


lupinine
(*Lupinus luteus*, Leguminosae)

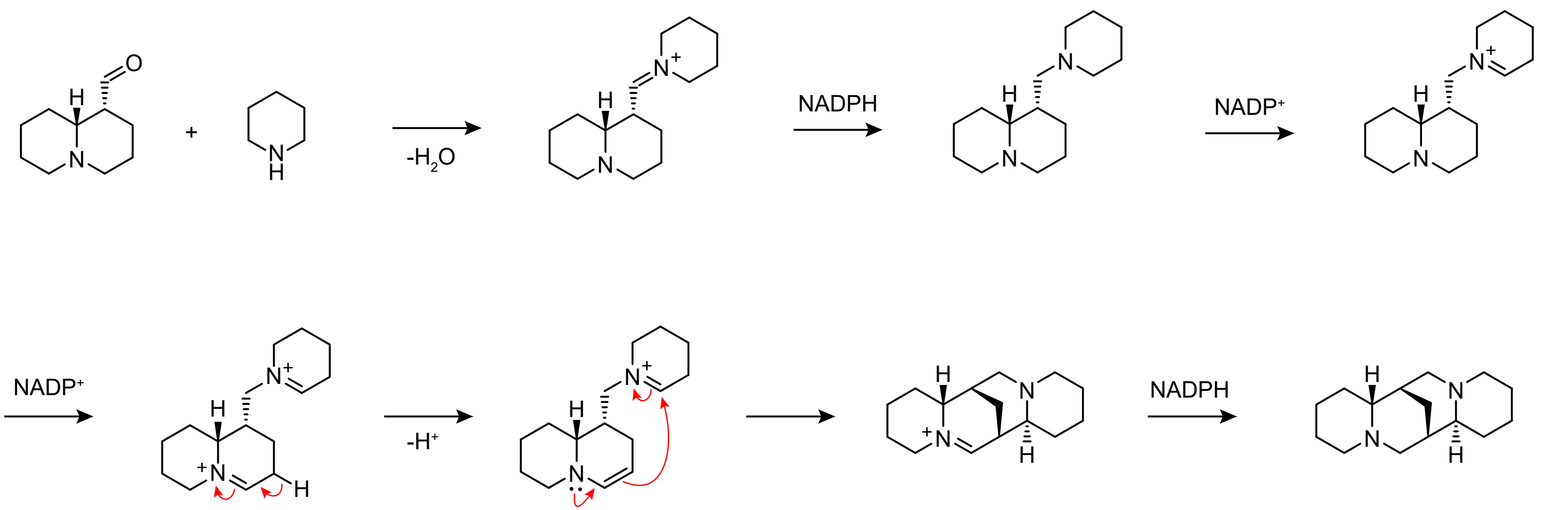
4. Alkaloids

4.8. Quinolizidine alkaloids

Lupinine, Sparteine (from Leguminosae)



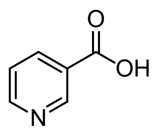
quinolizidine



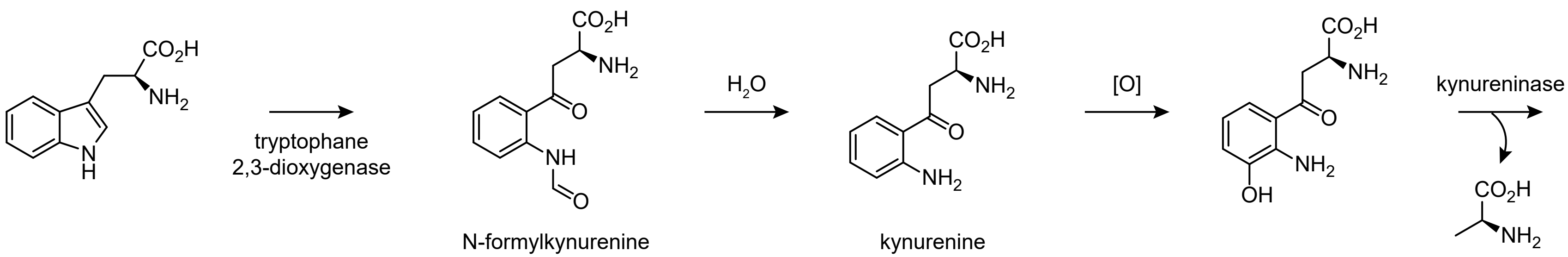
sparteine
(*Cytisus scoparius*, Leguminosae)

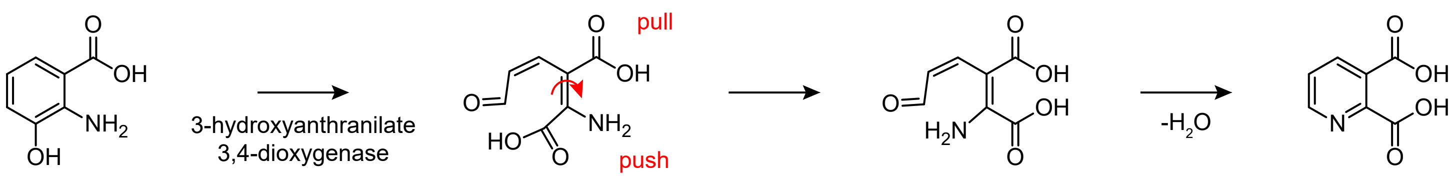
4.9. Pyridine alkaloids

Nicotine (from Solanaceae)



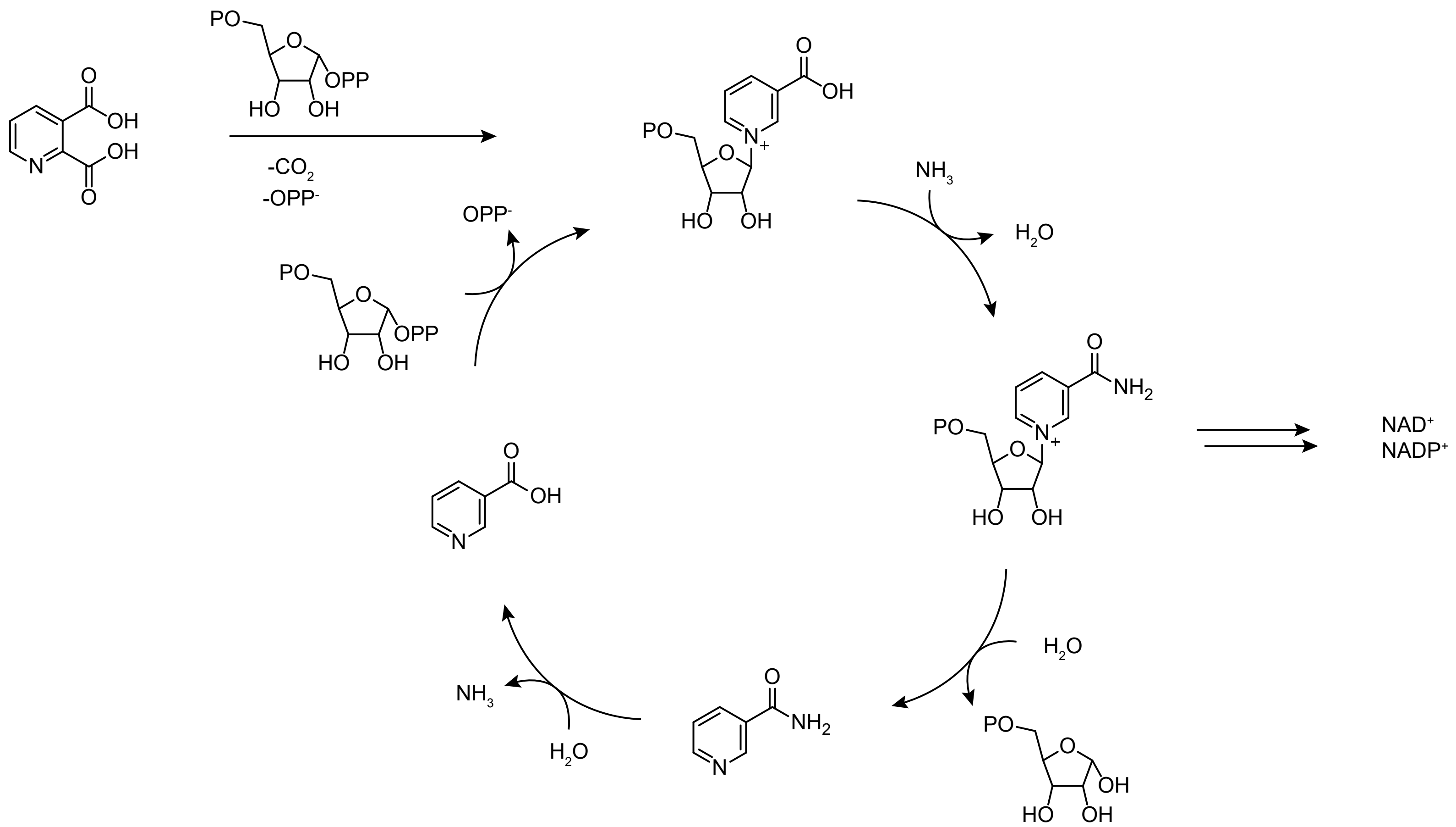
nicotinic acid





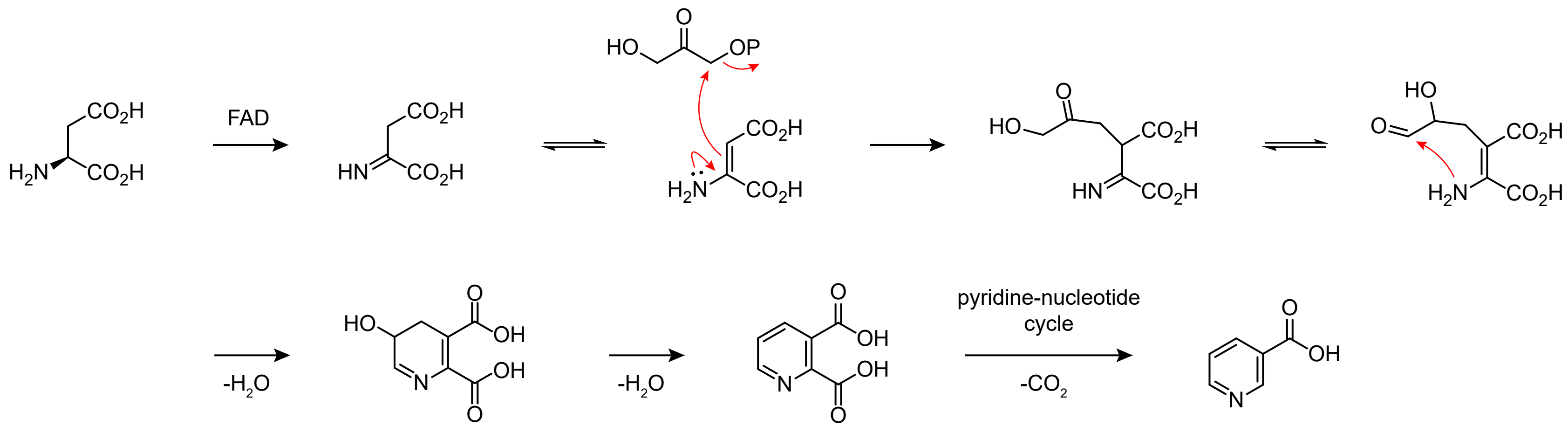
4.9. Pyridine alkaloids

Pyridine-Nucleotide Cycle

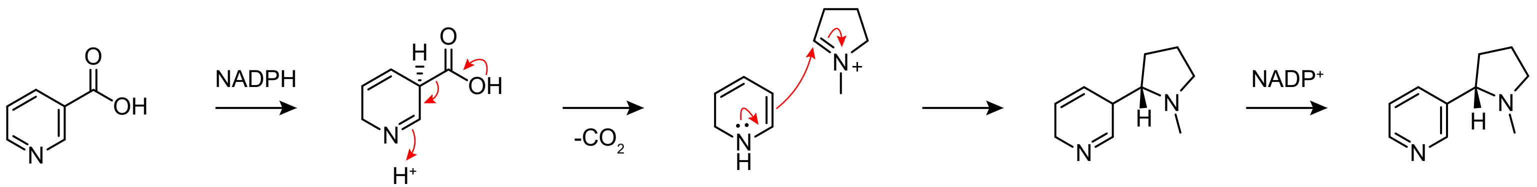


4.9. Pyridine alkaloids

alternative pathway from aspartate and dihydroxyacetone phosphate



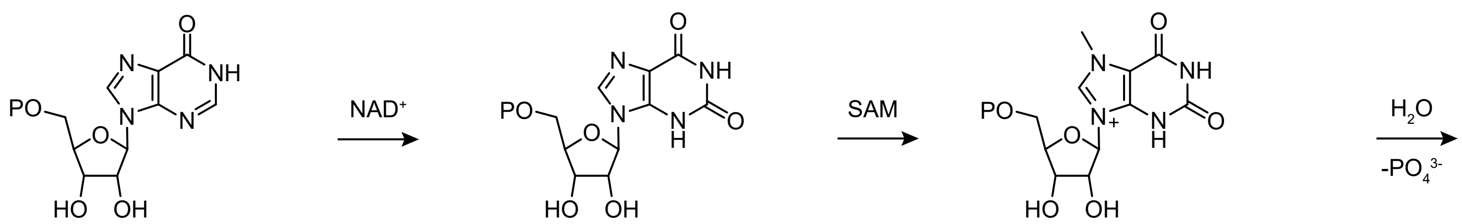
nicotine biosynthesis



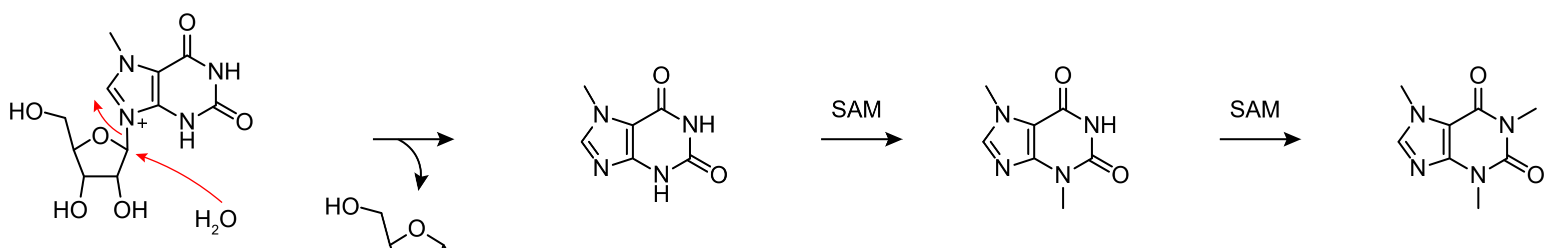
nicotine
(*Nicotiana tabacum*, Solanaceae)

4.10. Purine alkaloids

theobromine, caffeine



inosine monophosphate (IMP) — xanthosine monophosphate (XMP) — 7-methyl-XMP

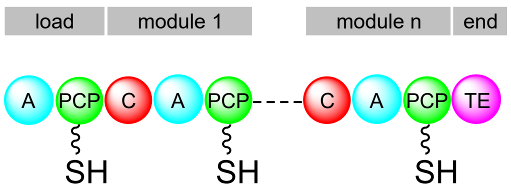


7-methylxanthosine — 7-methylxanthine — theobromine (*Theobroma cacao*, Malvaceae) — caffeine (*Coffea arabica*, Rubiaceae)

5. Non-ribosomal peptides

5.1. Enzymology: Non-ribosomal peptide synthases (NRPS)

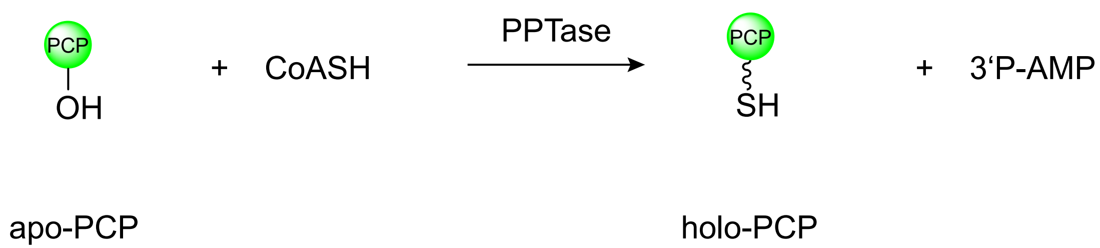
modular (as for modular type I PKS)



A	adenylation domain	(PKS: AT)
PCP	peptidyl carrier protein	(PKS: ACP)
C	condensation domain	(PKS: KS)
TE	thioesterase	(PKS: TE)

PCP

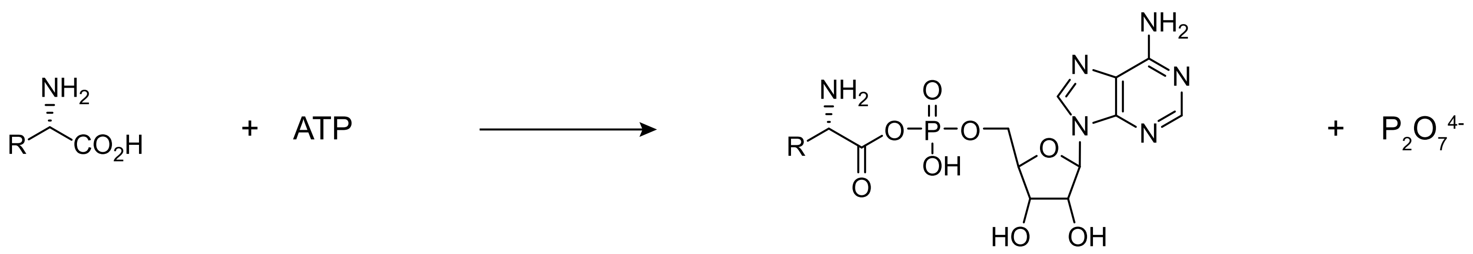
highly conserved serine, requires activation by PPTase



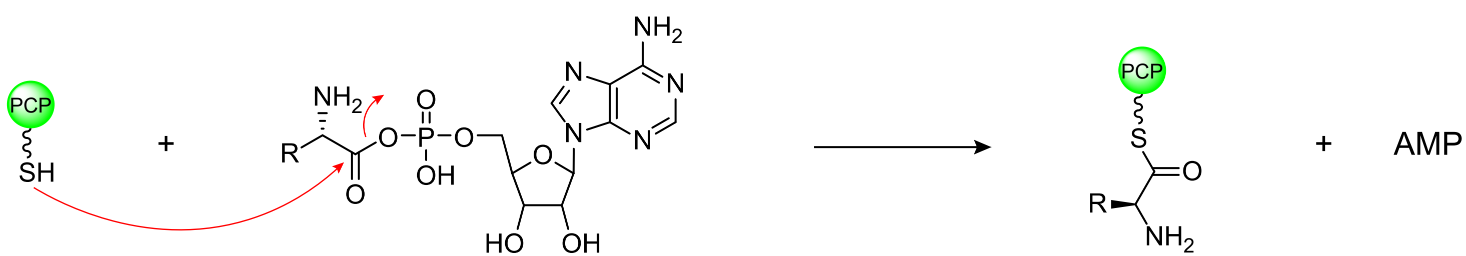
5.1. Enzymology: Non-ribosomal peptide synthases (NRPS)

A domains

substrate activation



upload to PCP

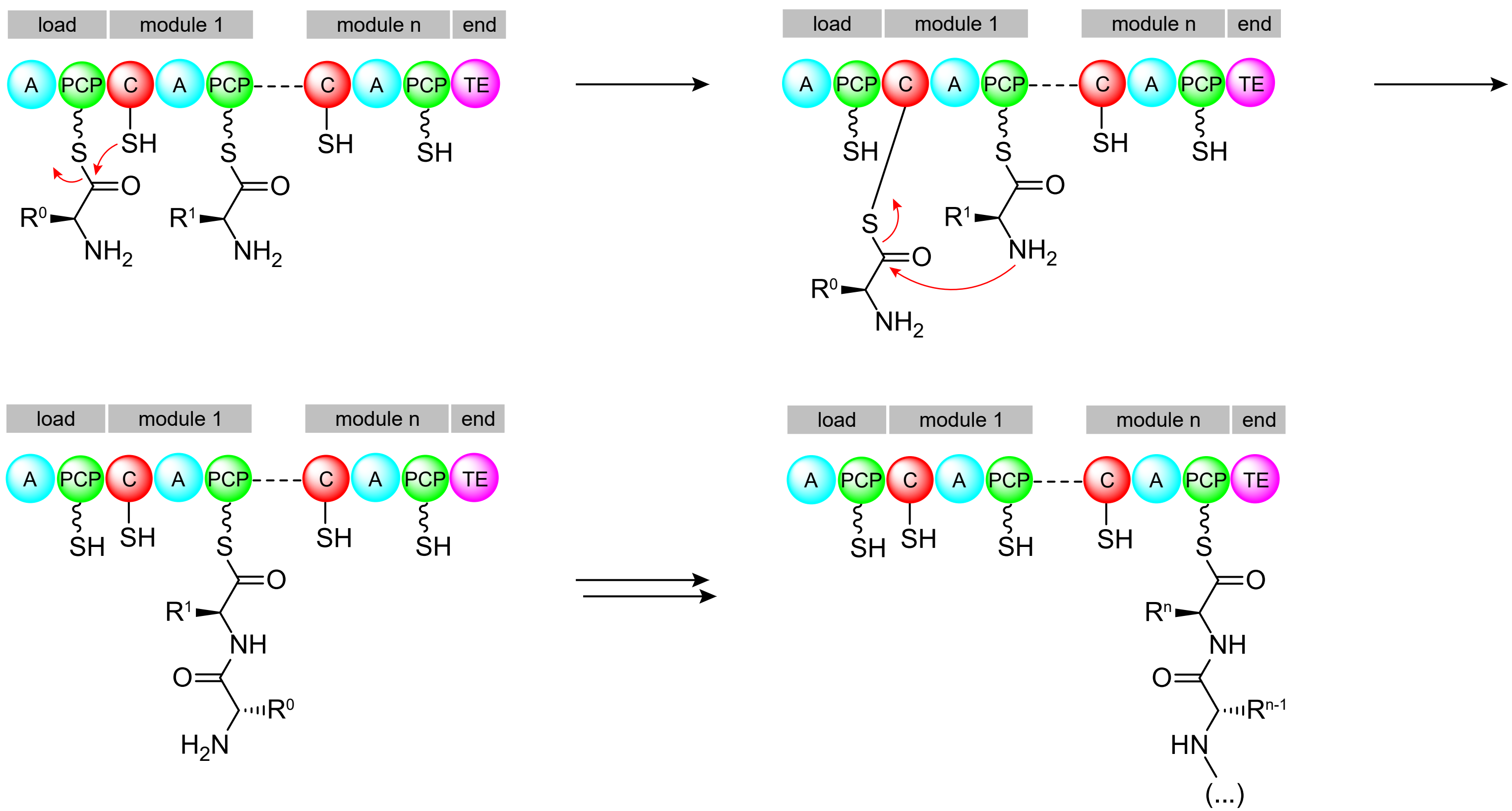


5. Non-ribosomal peptides

5.1. Enzymology: Non-ribosomal peptide synthases (NRPS)

C domains

condensation of amino acid units (peptide bond formation), highly conserved cysteine

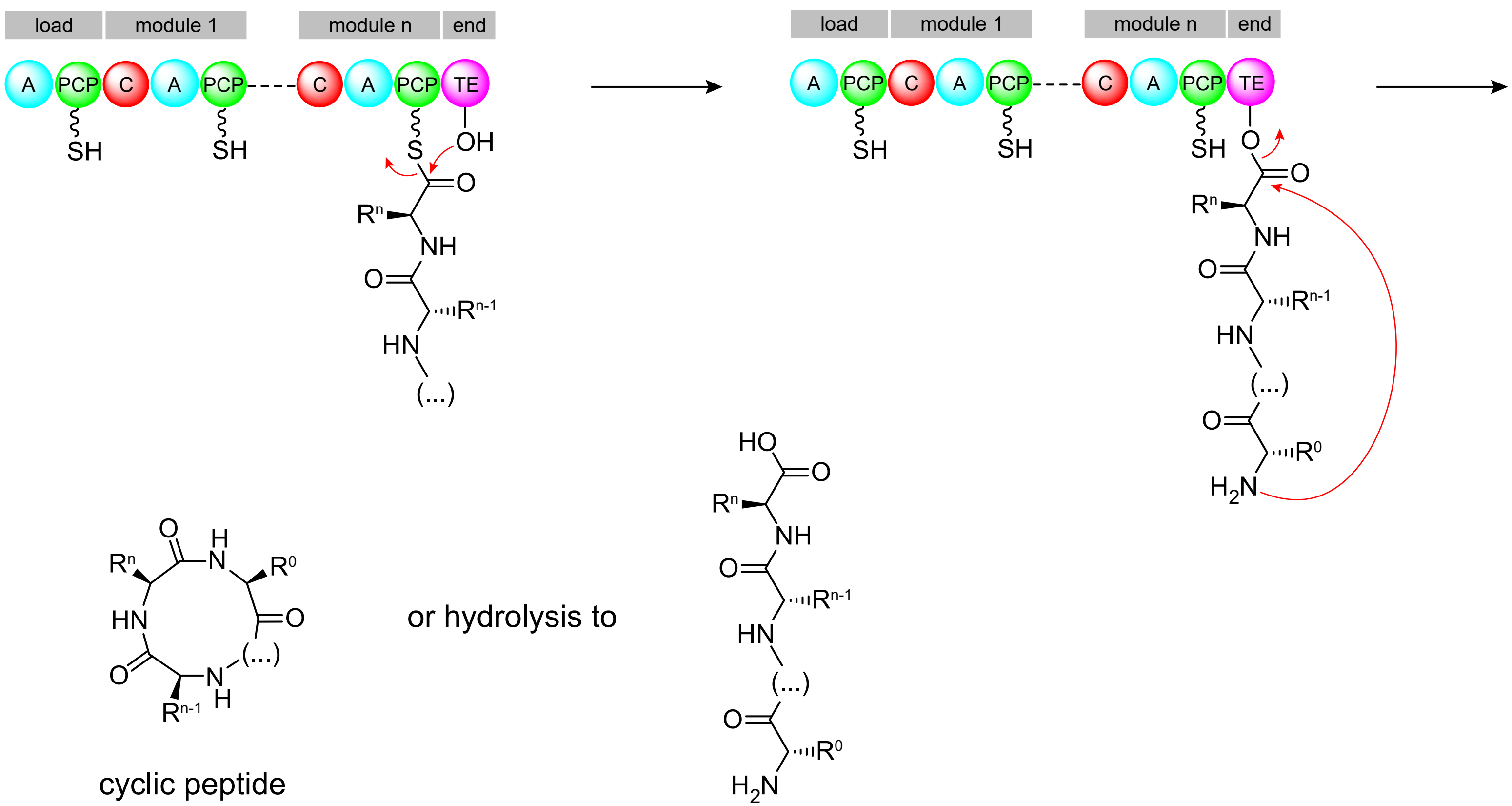


5. Non-ribosomal peptides

5.1. Enzymology: Non-ribosomal peptide synthases (NRPS)

TE domains

product release, highly conserved serine



cyclic peptide

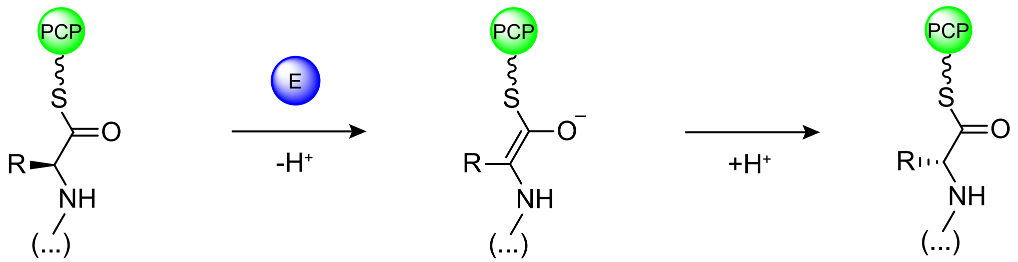
or hydrolysis to

5. Non-ribosomal peptides

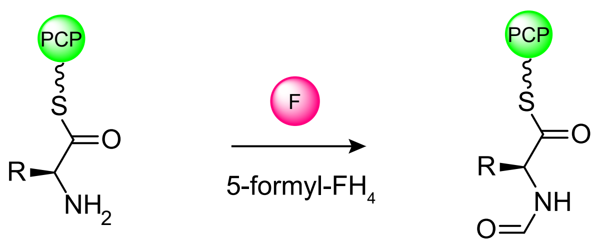
5.1. Enzymology: Non-ribosomal peptide synthases (NRPS)

Optional domains

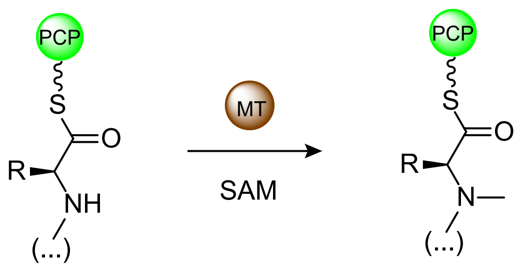
E (epimerisation domains)



F (formylation domains, only loading module)



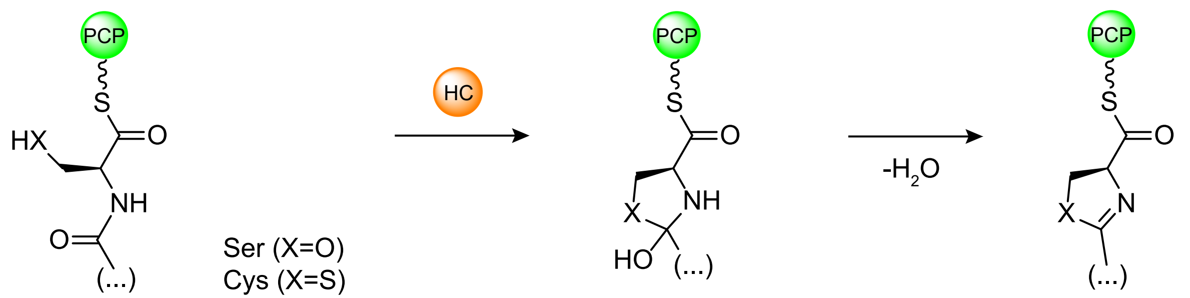
MT (methyltransferase domains, usually N-methylation)



5.1. Enzymology: Non-ribosomal peptide synthases (NRPS)

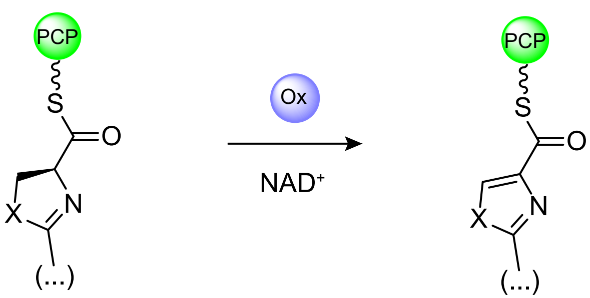
Optional domains

HC (heterocyclisation domains)



dihydrooxazole (X=O)
dihydrothiazole (X=S)

Ox (oxidation domains)



oxazole (X=O)
thiazole (X=S)

5. Non-ribosomal peptides

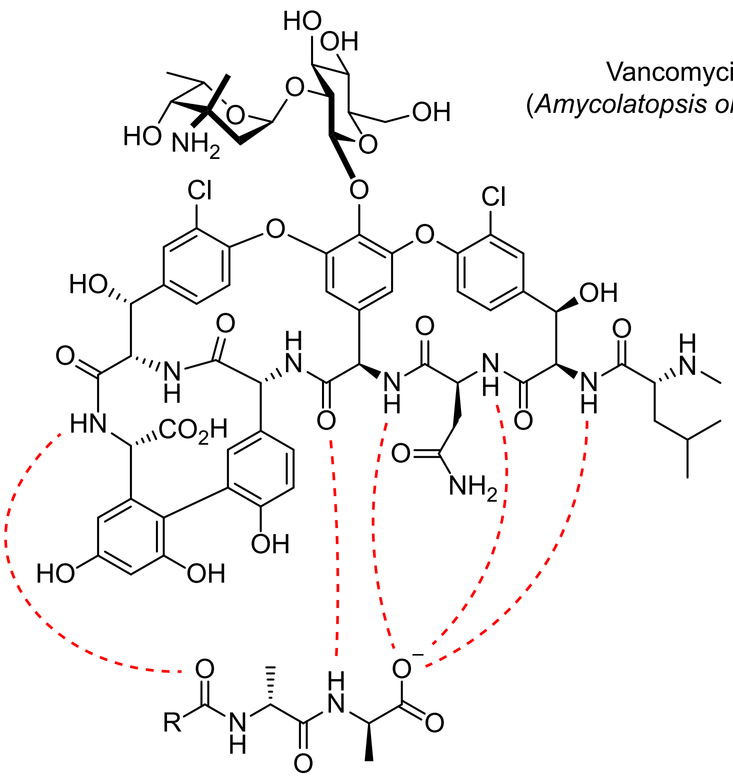
5.2. Vancomycin biosynthesis

Vancomycin blocks peptidoglycan biosynthesis

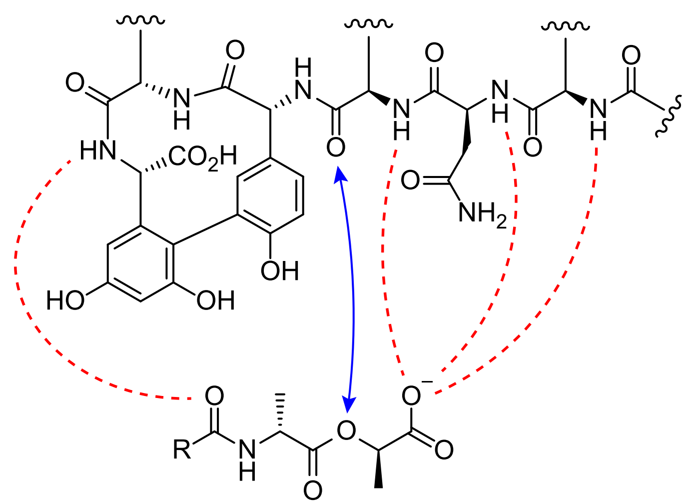
Peptidoglycan is a constituent of the bacterial cell wall

Resistance mechanism: change to D-Ala-D-Lac

Vancomycin
(*Amycolatopsis orientalis*)



D-Ala-D-Ala motif in peptidoglycan

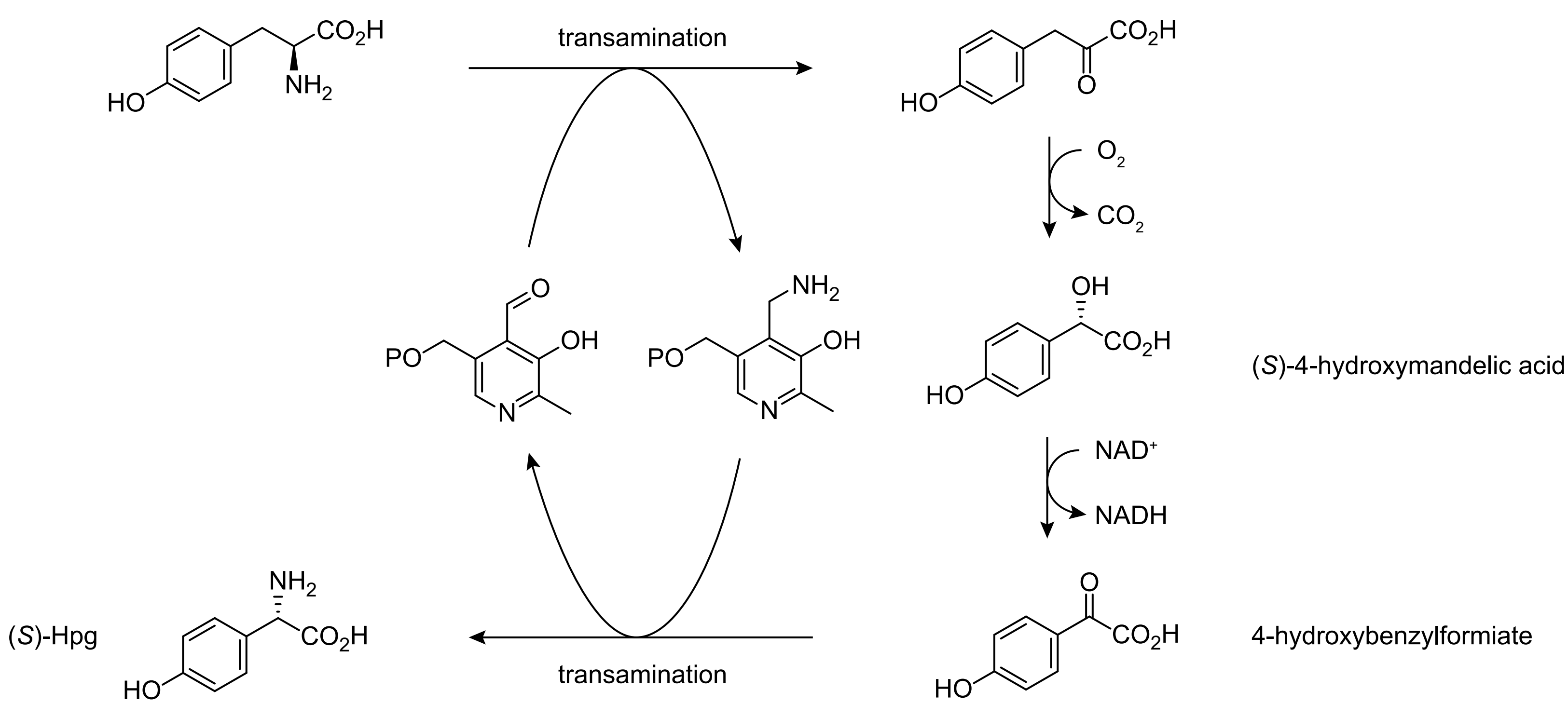


D-Ala-D-Lac motif in modified peptidoglycan

5.2. Vancomycin biosynthesis

Biosynthesis of monomers

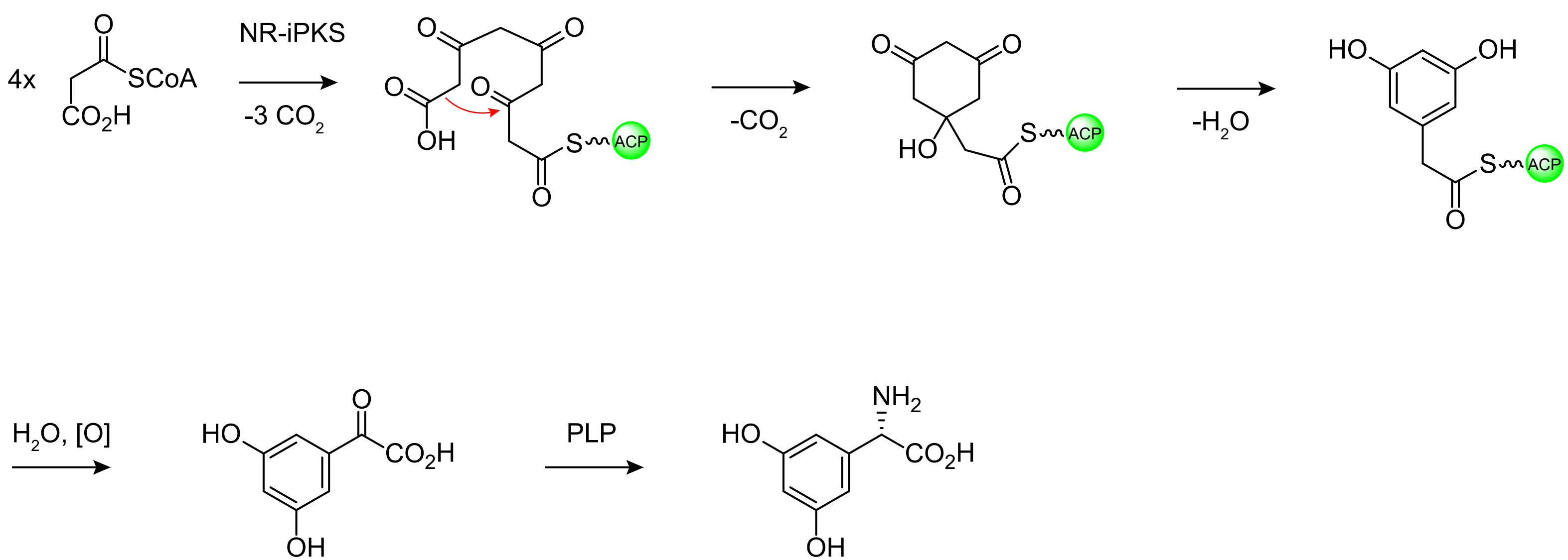
a) (*S*)-4-Hydroxyphenylglycine (Hpg)



5.2. Vancomycin biosynthesis

Biosynthesis of monomers

b) (*S*)-3,5-Dihydroxyphenylglycine (Dpg)

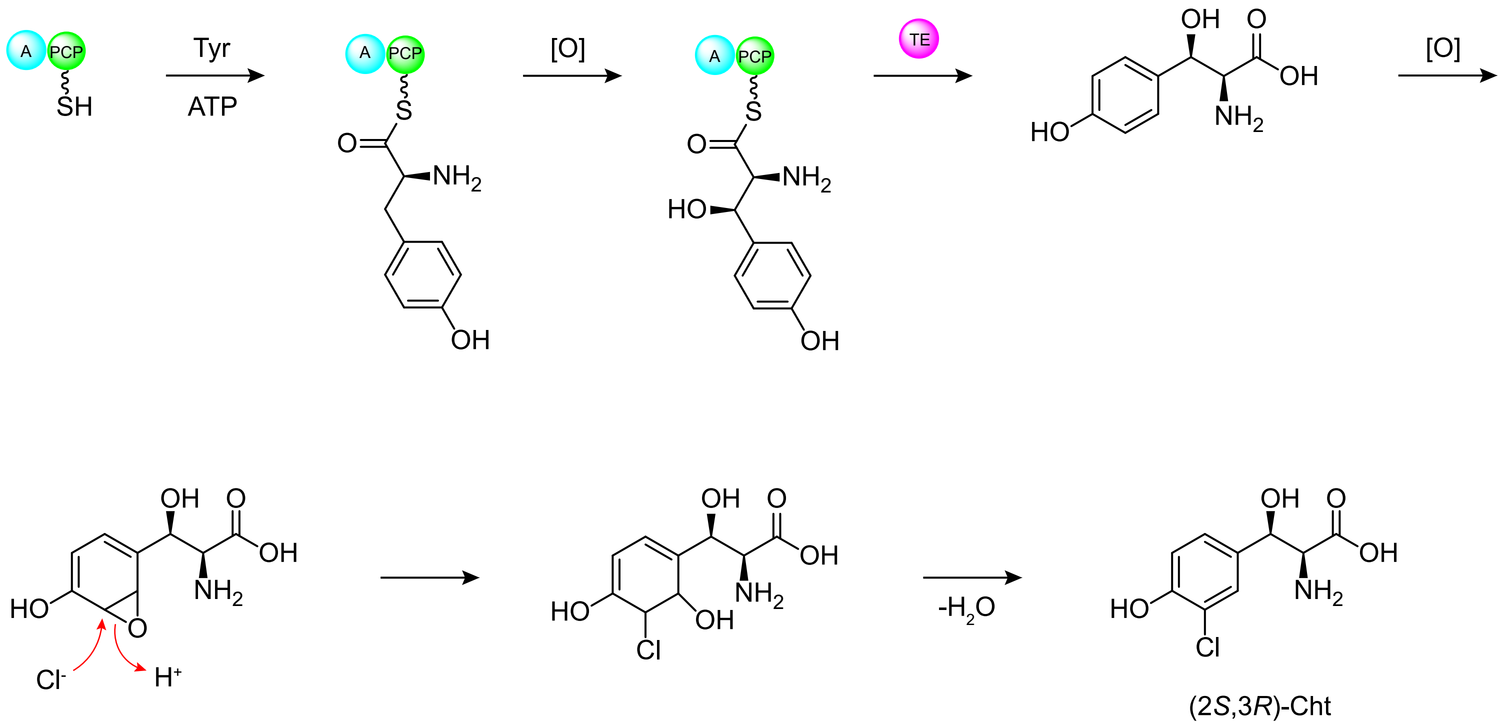


(*S*)-Dpg

5.2. Vancomycin biosynthesis

Biosynthesis of monomers

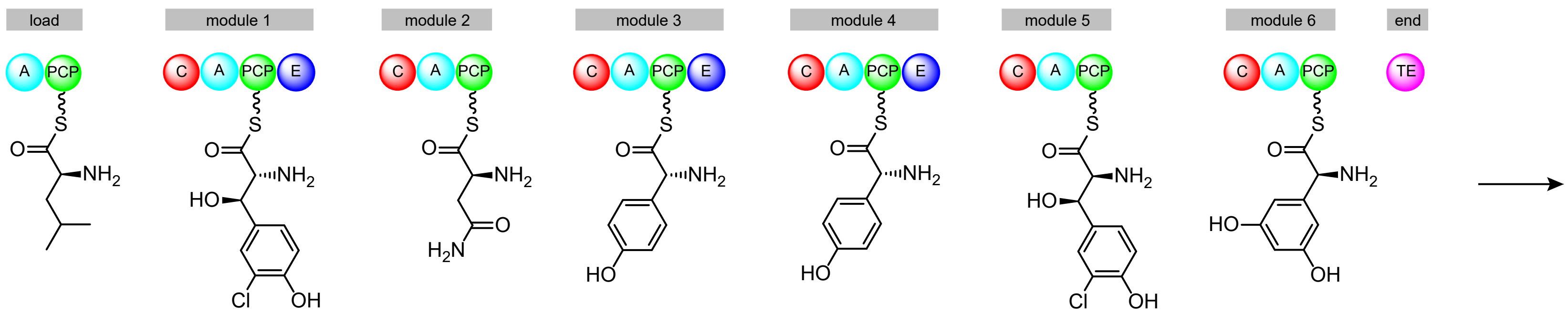
b) (2*S*,3*R*)-3-Chloro-β-hydroxytyrosine (Cht)

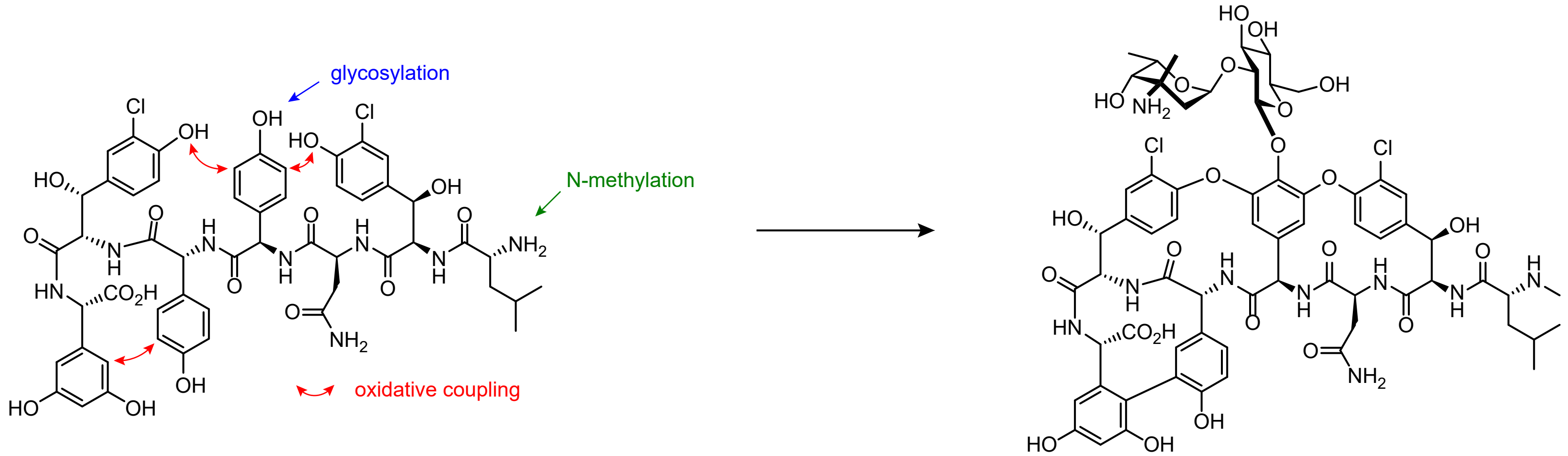


5. Non-ribosomal peptides

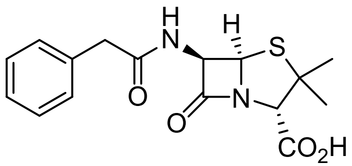
5.2. Vancomycin biosynthesis

NRPS



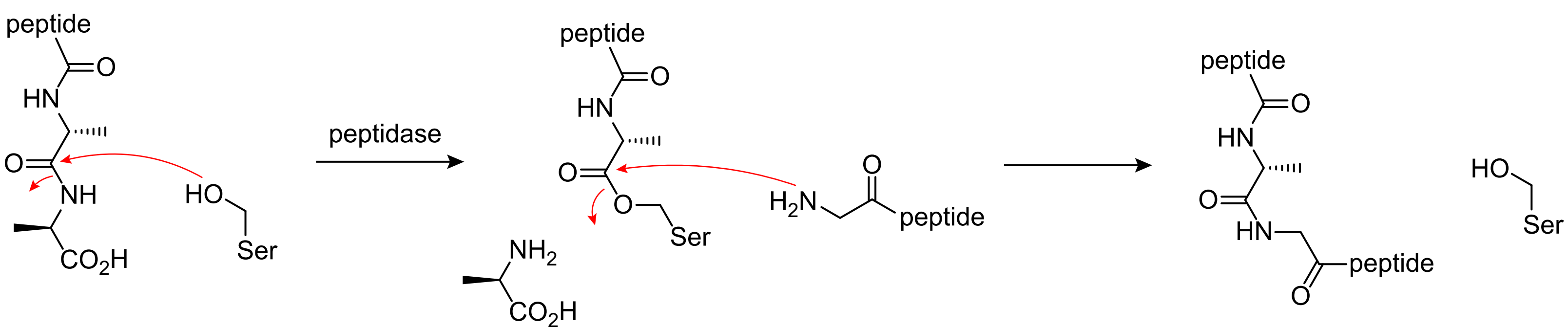


5.3. Penicillin biosynthesis

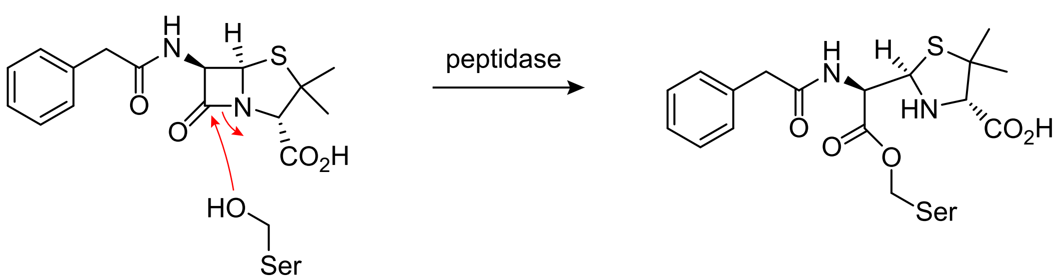


penicillin G
(*Penicillium chrysogenum*)

mode of action: inhibition of peptidoglycan crosslinking



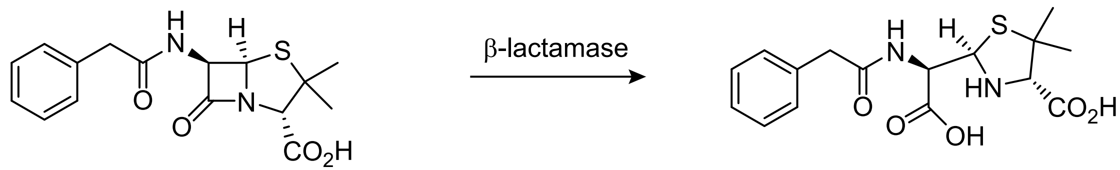
with penicillin:



blocks peptidase

5.3. Penicillin biosynthesis

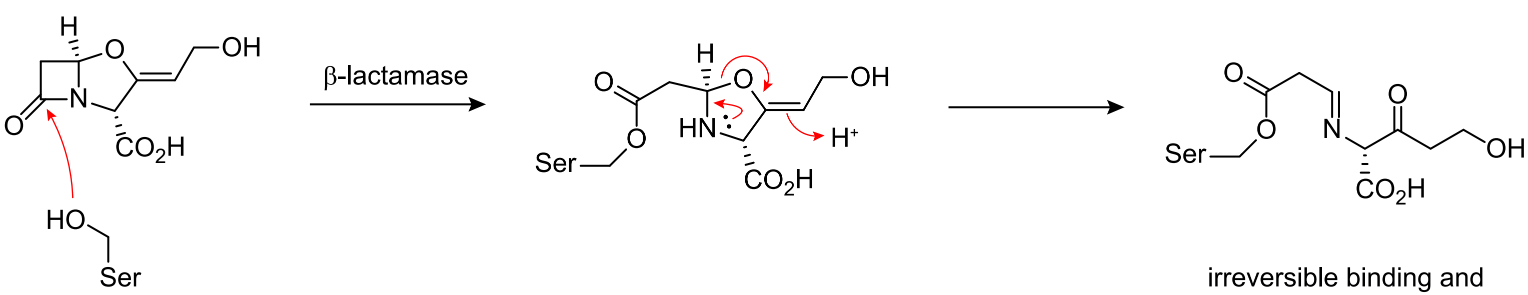
resistance mechanisms



or modified peptidase that does not react with penicillin

clavulanic acid: blocking of β-lactamase to overcome resistance mechanism

clavulanic acid
(*Streptomyces clavuligerus*)



irreversible binding and
blocking of β-lactamase

5. Non-ribosomal peptides

5.3. Penicillin biosynthesis