Introduction to Secondary Metabolism: Natural Product Building Blocks and Common Biosynthetic Reaction Mechanisms

Lecture 2
Biofuels and Bioproducts

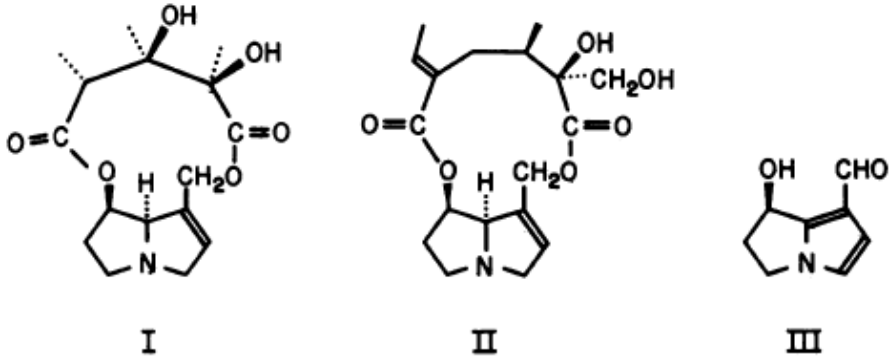
Bronx Community College - 2017
Chemistry and BioEnergy Technology for Sustainability NSF ATE 1601636

Outline

- Classes of Secondary Metabolites
- Chemical Building Blocks
- Construction Mechanisms
  - Alkylation: Nucleophilic Substitutions & Electrophilic Additions
  - Wagner-Meerwein Rearrangements
  - Aldol and Claisen Reactions

Metabolites: Primary and Secondary

- Metabolites are a vast array of organic molecules needed for the life, growth and reproduction of an organism
- *Primary metabolites* (e.g. carbohydrates, proteins, fats, nucleic acids) and their production via *metabolic pathways are conserved among diverse organisms*
- *Secondary Metabolites* are more *specialized* molecules (e.g. toxins, volatile attractants, coloring agents) and many are organism-specific
- Often referred to as natural products. Branch of organic chemistry = *natural product chemistry* or *bio-organic chemistry*

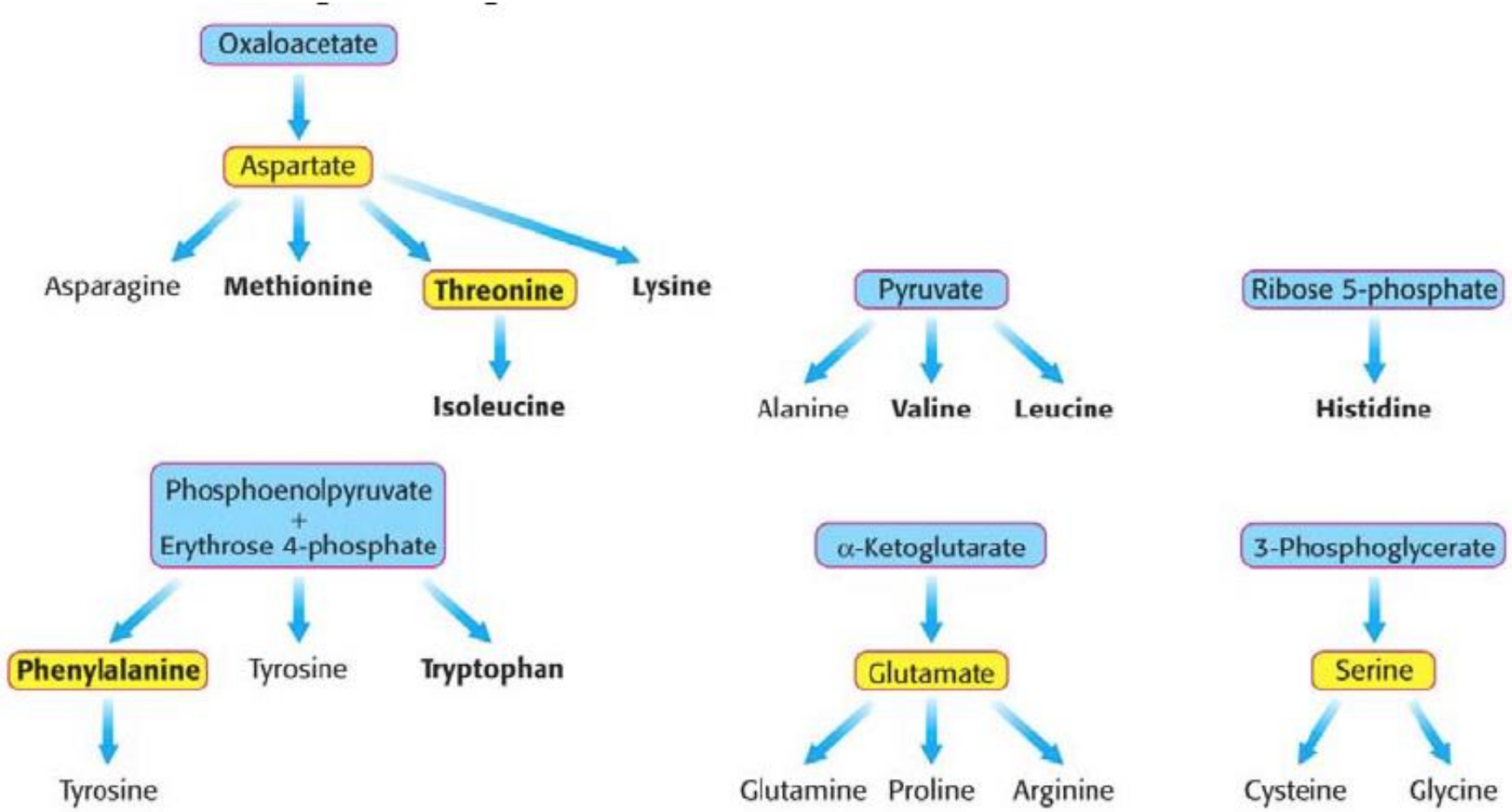


I II III

Bella moth (*Utetheisa ornatrix*) and pyrrolizidine alkaloids sequestered from food (legumes) and used to impart deterrent/toxicity to predators via the moth's eggs!

-Dussourd PNAS 85, 5992 (1988)

A side note on amino acid biosynthesis



- Humans can not bio-synthesize 9 of the 20 proteinogenic amino acids (**bold**)
- Plants and bacteria can synthesize all 20
- What does this mean for the “food vs. fuel” argument?

http://www.onegreenplanet.org/natural-health/need-protein-amino-acids-found-abundantly-in-plants/

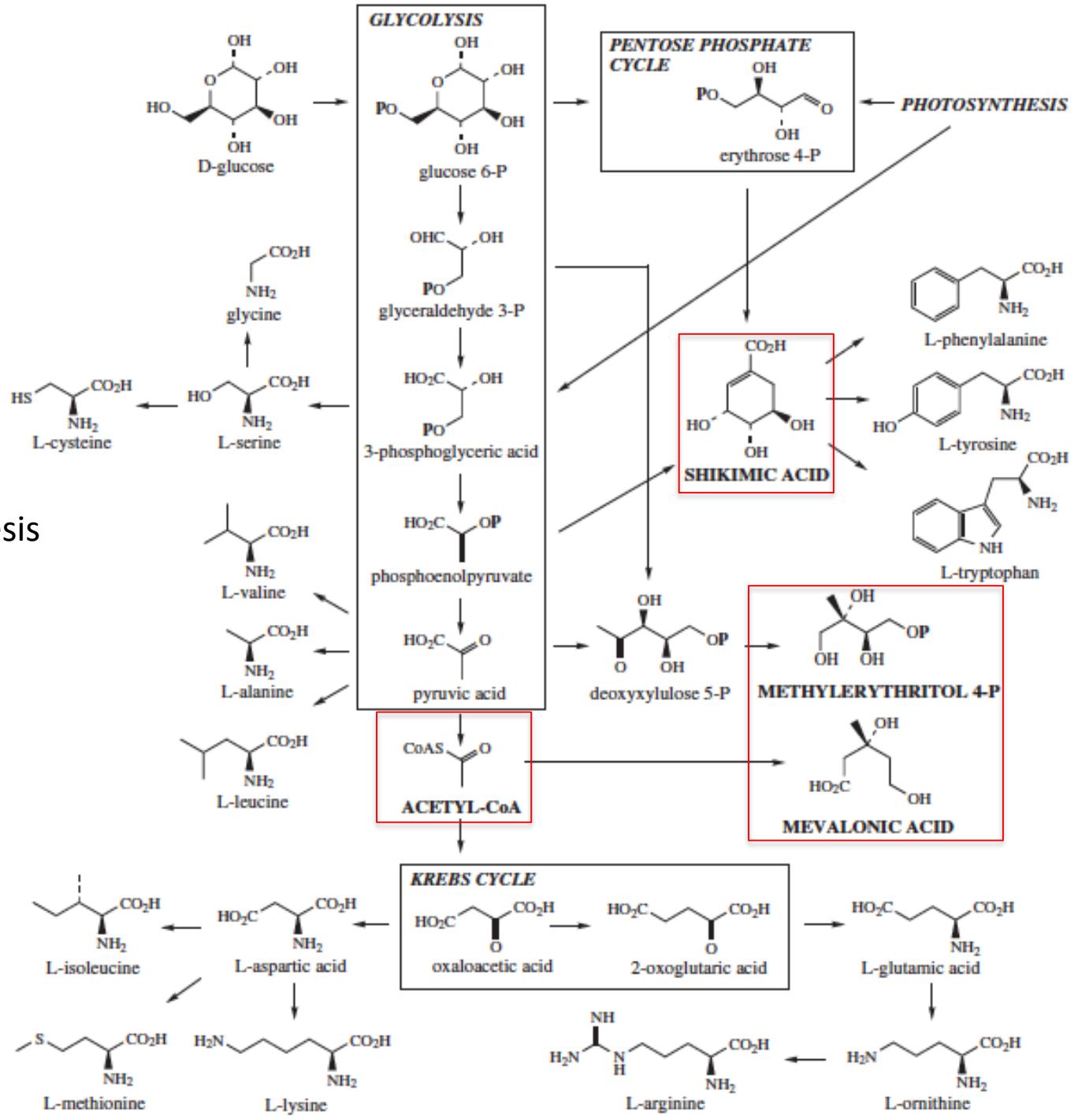
The Mile High View: Connections Between Biosynthetic Pathways

Photosynthesis
- Erythrose 4-P
  - Shikimate

Glycolysis
- Cys, Ser, Gly
- Val, Ala, Leu
- Acetyl Co-A
  - Lipid biosynthesis
  - Mavalonate
- Shikimate
  - Phe
  - Tyr
  - Try

Krebs Cycle
- Asp, IsoLeu
- Lys, Met
- Glu, Orn, Arg

Can you look at a structure and discern its bio-synthetic origin?



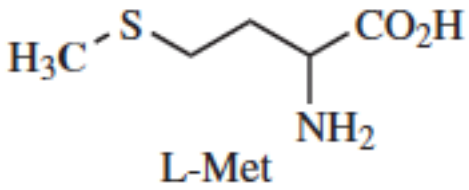
Major Pathways and Classes of Secondary Metabolites

- **Acetyl Co-A Pathway**: e.g. fatty acids, polyketides, phenols, prostaglandins, macrolide antibiotics
- **Shikimic Acid Pathway**: e.g. phenols, cinnamic acid derivatives, lignin, lignans, alkaloids (phenylalanine, tyrosine, tryptophan)
- **Mevalonic acid Pathway** (and methylerythritol pathway): e.g. terpenes and steroids

Many natural products are derived from a combination of pathways ("mixed biosynthesis") and can also include sugar motifs (glycosides). The 'aglycone' is the non-sugar portion

Building Blocks of Secondary Metabolites: C1 and C2

Krebs →



L-Met

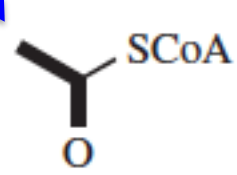
⟹ —X—CH$_3$ (X = O, N, S, C)

C_1

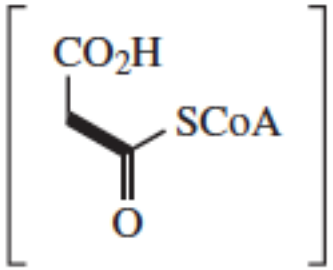
Often used for heteroatom methylations

Reactivity of L-Met is increased by converting it to SAM (slide 13)

Glycolysis →



acetyl-CoA



malonyl-CoA

(more reactive, why?)

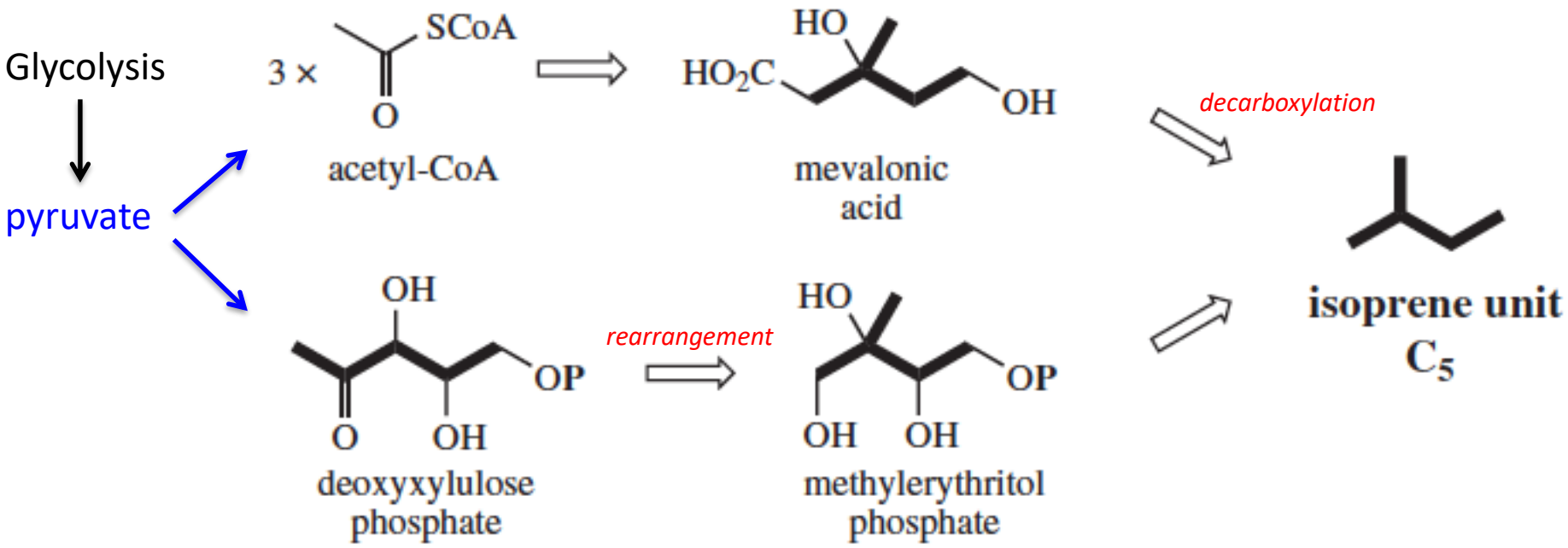
⟹ C=C

C_2

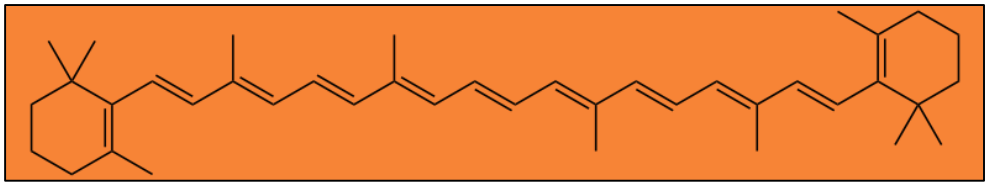
Often used for extending alkyl chains (e.g. fatty acid) or building aromatic systems

Reactivity of Acetyl Co-A is increased by converting it to malonyl-CoA (slide 28), and crucial for fatty acid and polyketide biosynthesis (Lecture 4)

Building Blocks of Secondary Metabolites: C5 (isoprene)

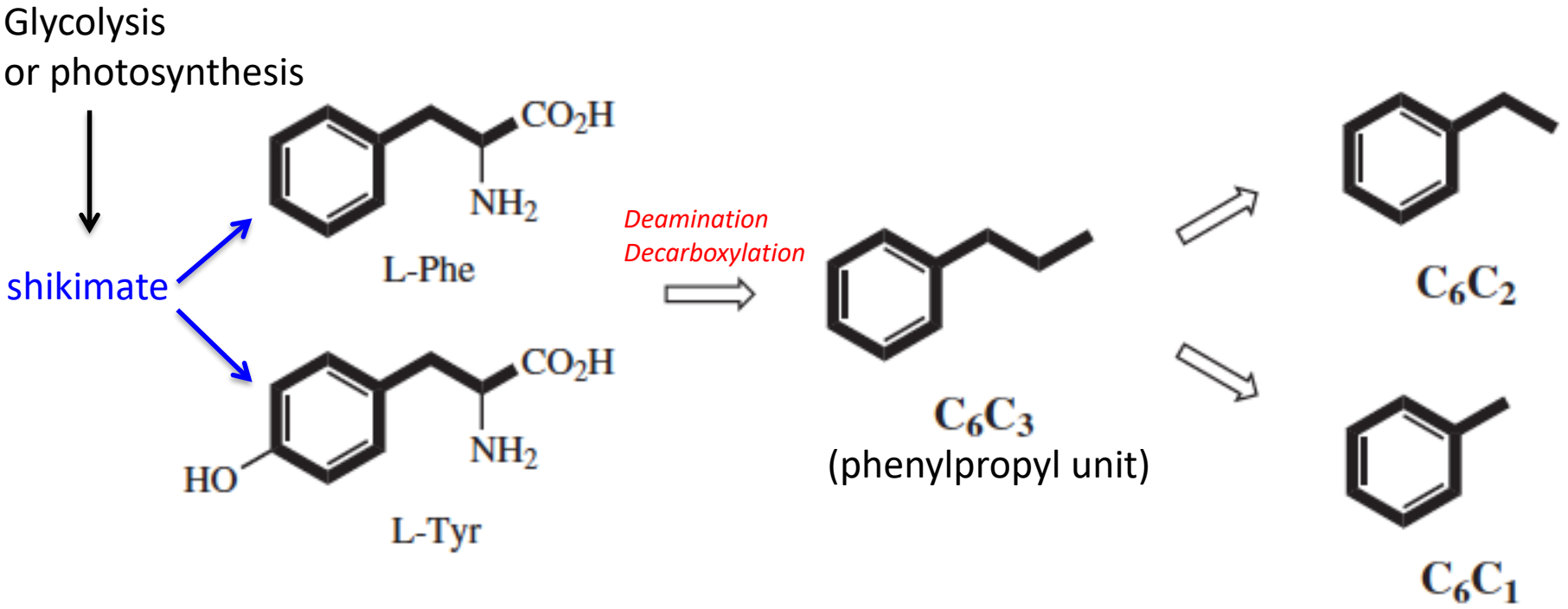


- Isoprene units are combined to make terpenes and steroids
  - C10 = monoterpene
  - C15 = sesquiterpene
  - C20 = diterpene
  - C25 = sesterterpene
  - C30 = triterpenene…



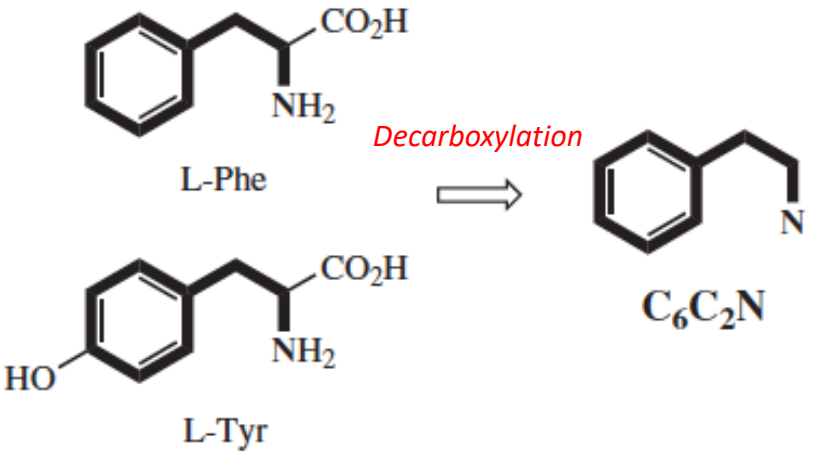
β-carotene ($C_{40}H_{56}$), a tetraterpene

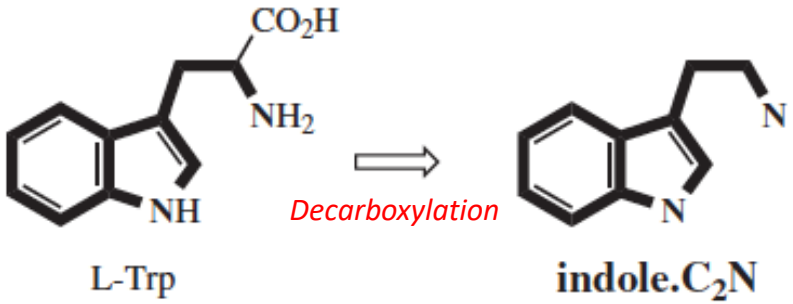
Building Blocks of Secondary Metabolites: C6 (propyl, ethyl, methyl)



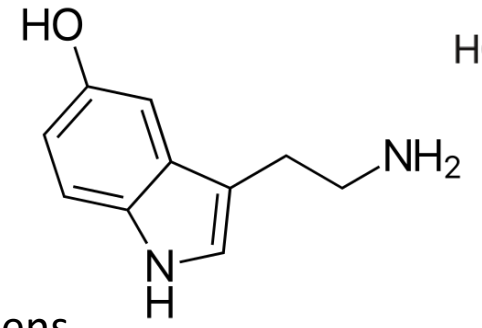
- Among other structures, phenylpropyl units are used to make lignin, a component of secondary plant cell walls and major obstacle/opportunity for 2nd generation (lignocellulosic) biofuels

Building Blocks of Secondary Metabolites: C6 ethylamine & indole

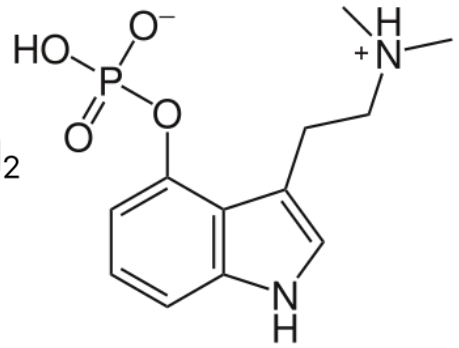




- Indole is the core component of many alkaloids, including serotonin and some hallucinogens

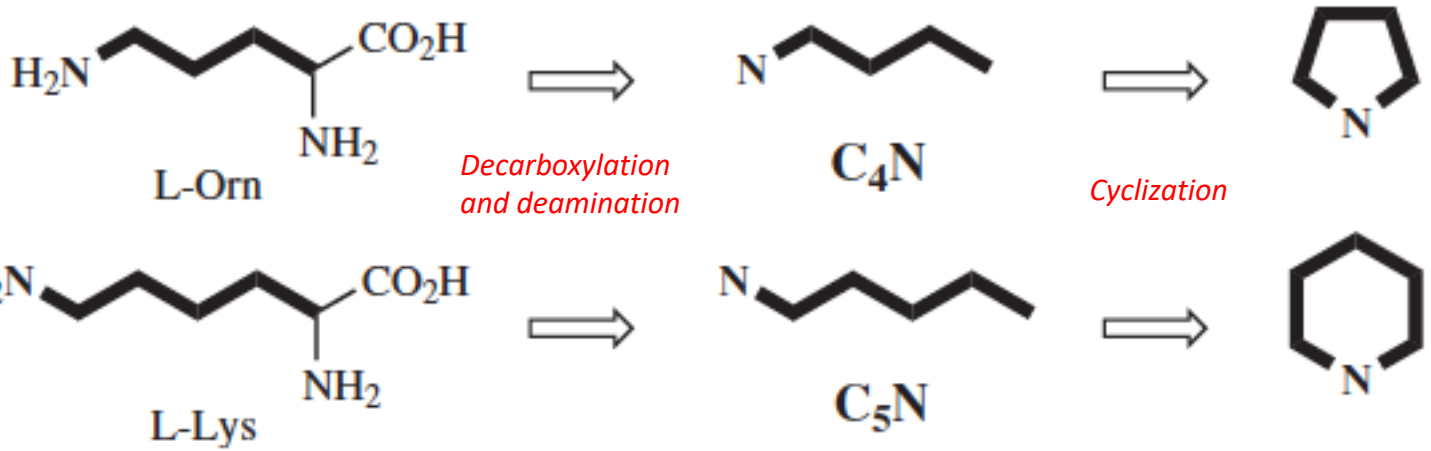


serotonin

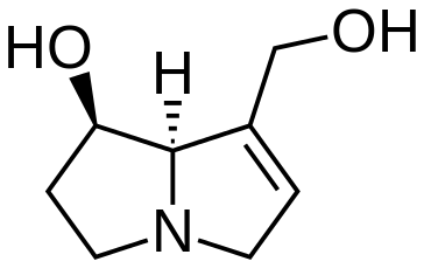


psilocybin

Building Blocks of Secondary Metabolites: Pyrrolidines and Piperidines



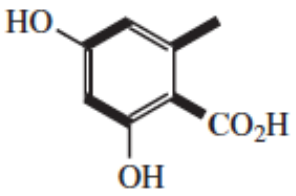
- Pyrrol based alkaloids are responsible for many plant defense compounds (aka 'natural biocides')
- Piperidine is principal constituent of conium alkaloids (e.g. poison Hemlock)



Retronecine

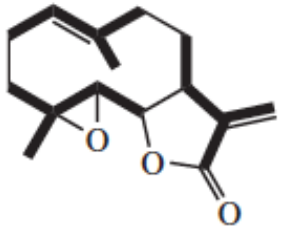
Scenecio nemorensis

Now can you look at a structure and discern its biosynthetic origin?



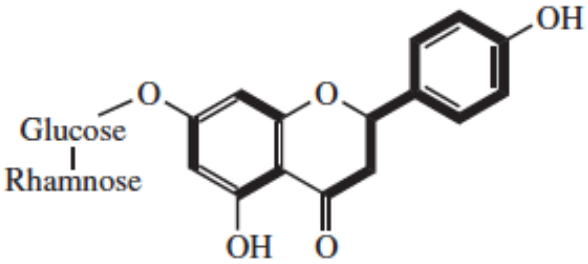
orsellinic acid

$4 \times C_2$



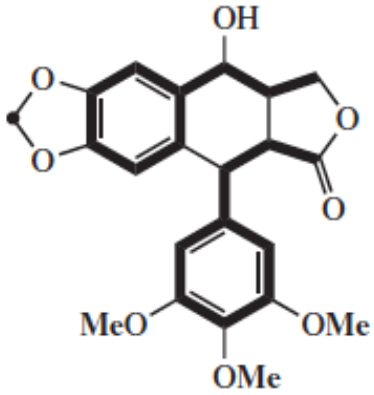
parthenolide

$3 \times C_5$



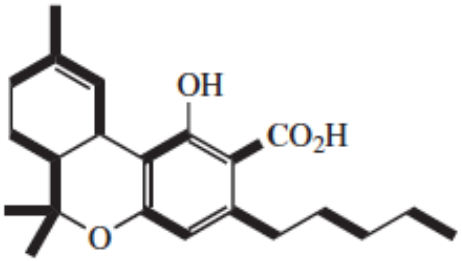
naringin

$C_6C_3 + 3 \times C_2 + \text{sugars}$



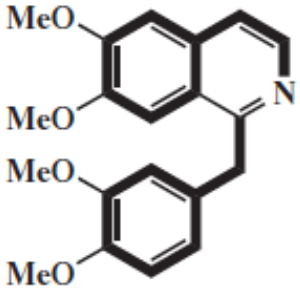
podophyllotoxin

$2 \times C_6C_3 + 4 \times C_1$



tetrahydrocannabinolic acid

$6 \times C_2 + 2 \times C_5$

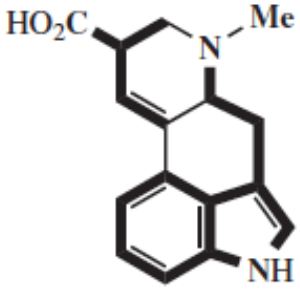


papaverine

$C_6C_2N + (C_6C_2) + 4 \times C_1$

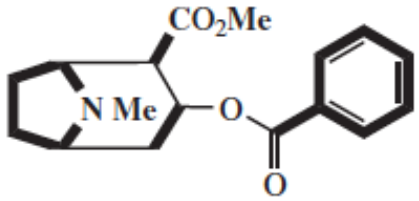
$\Uparrow$

C_6C_3



lysergic acid

indole.$C_2N + C_5 + C_1$



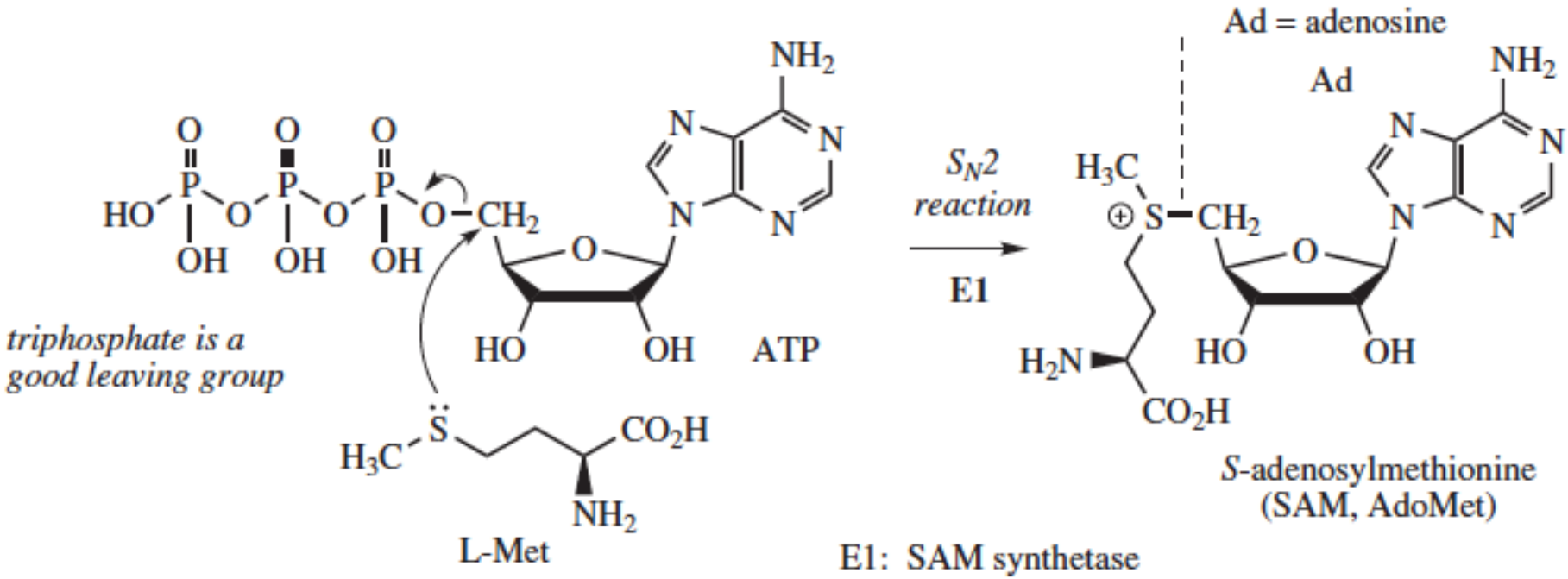
cocaine

$C_4N + 2 \times C_2 + (C_6C_1) + 2 \times C_1$

$\Uparrow$

C_6C_3

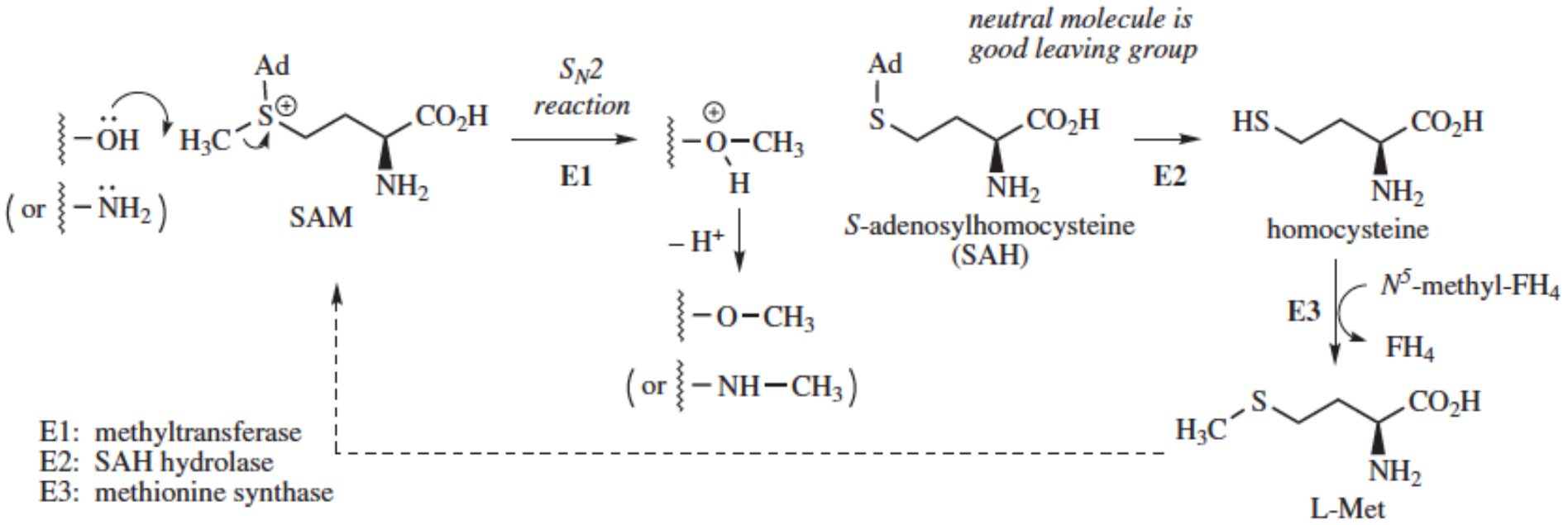
Alkylation Reactions: Nucleophilic Substitutions (SN2 and SN1)



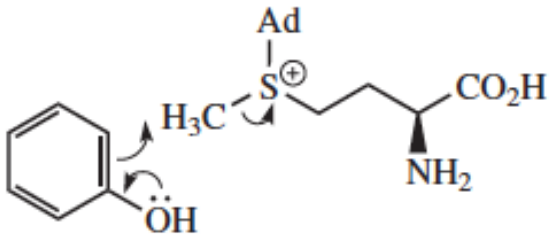
Step 1: Formation of SAM

- Why is triphosphate a good leaving group?
- Why is SAM preferred over L-Met as an alkylating agent?

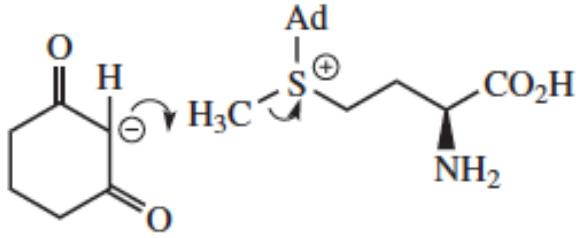
O- , *N*-, and C-alkylation using SAM via SN2 Mechanism (and regeneration of Met)



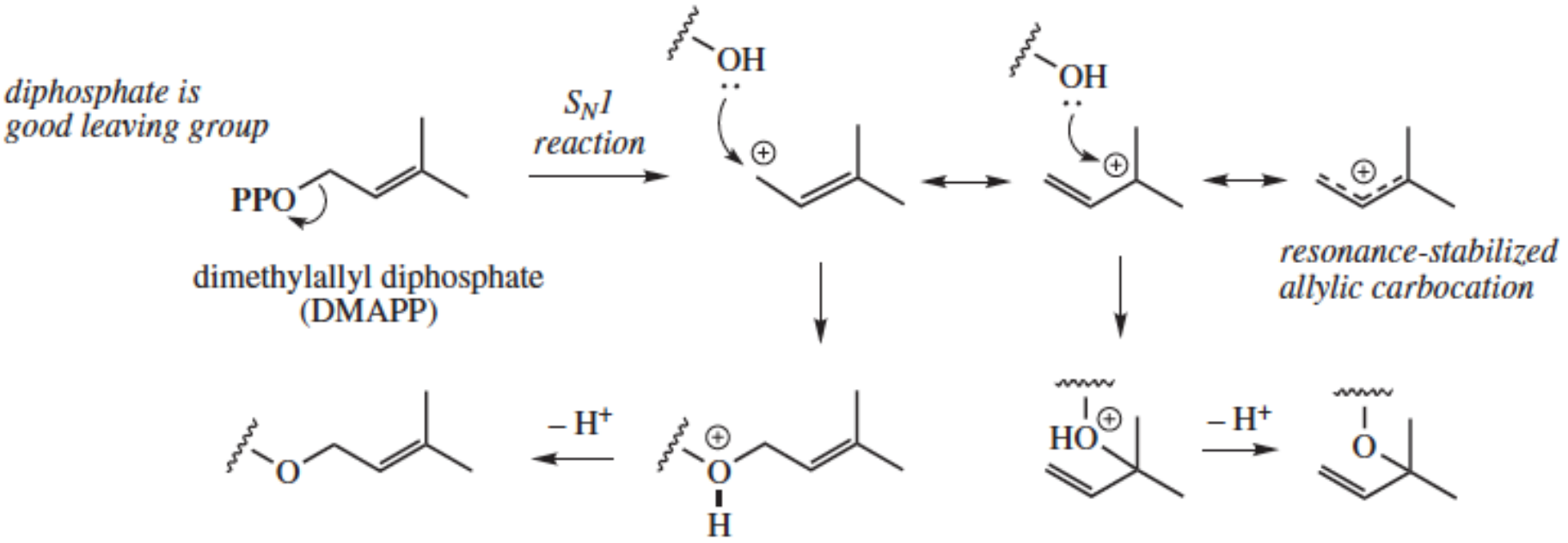
ortho (and para) positions are activated by OH



carbonyl groups increase acidity and allow formation of enolate anion



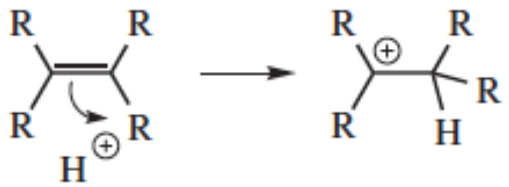
Alkylation using dimethylallyl diphosphate (DMAPP) via SN1 Mechanism



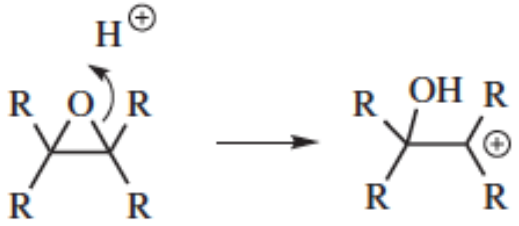
What is evidence for the SN1 mechanism?

Alkylation Reactions: Carbocation Formation & Electrophilic Additions (inter- and intra-molecular)

loss of leaving group

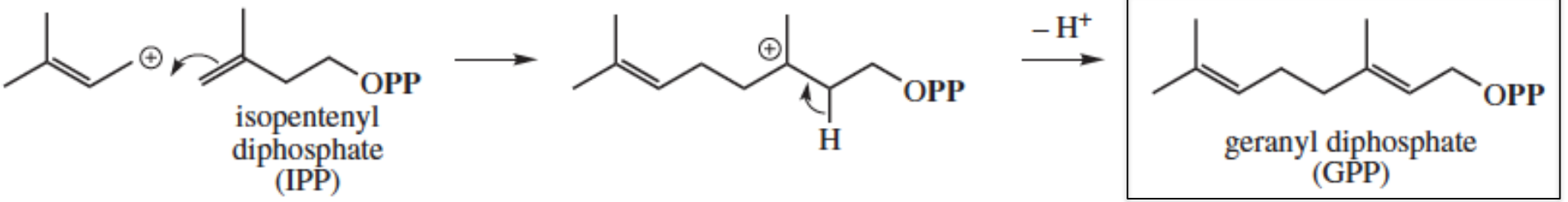


protonation of alkene



protonation and ring opening of epoxide

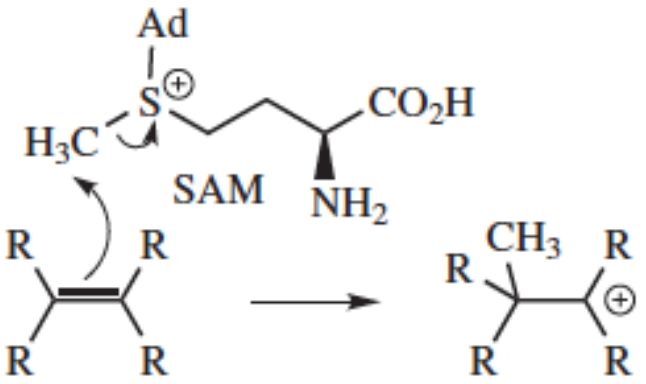
electrophilic addition of cation onto alkene: intermolecular addition



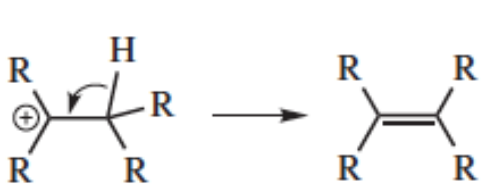
intramolecular addition: cyclization

Alkene Methylation via SAM

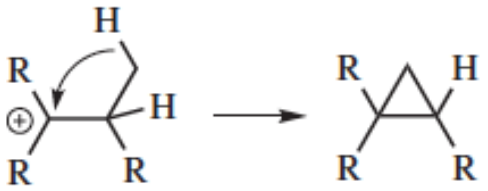
- Also an electrophilic addition reaction



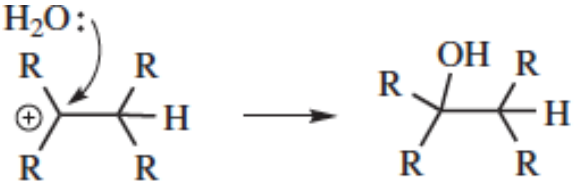
- Carbocation formed can be "quenched" several ways



loss of proton



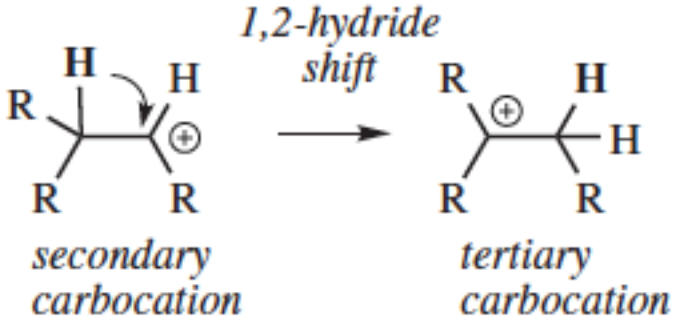
cyclization / loss of proton

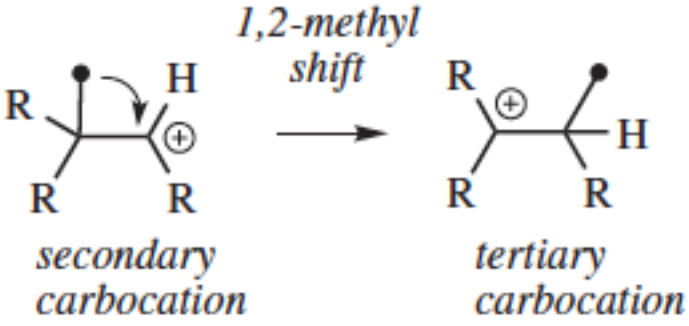


quenching with nucleophile (water)

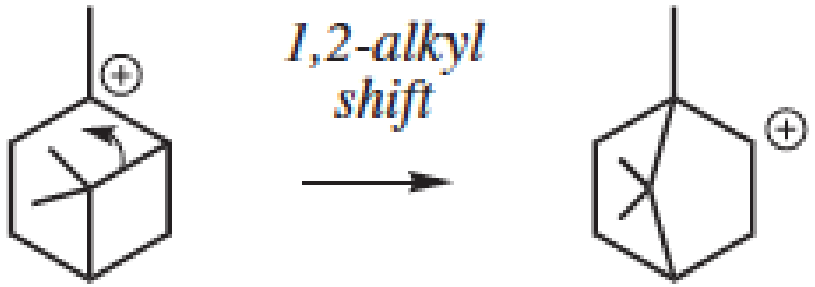
Wagner-Meerwein Rearrangements

- Favor most stable carbocation (3>2>1)
- Favor reduced ring strain (larger rings more stable)
- Enzyme mediated and therefore extraordinary (e.g. “cascade” rearrangements) chemistry is possible





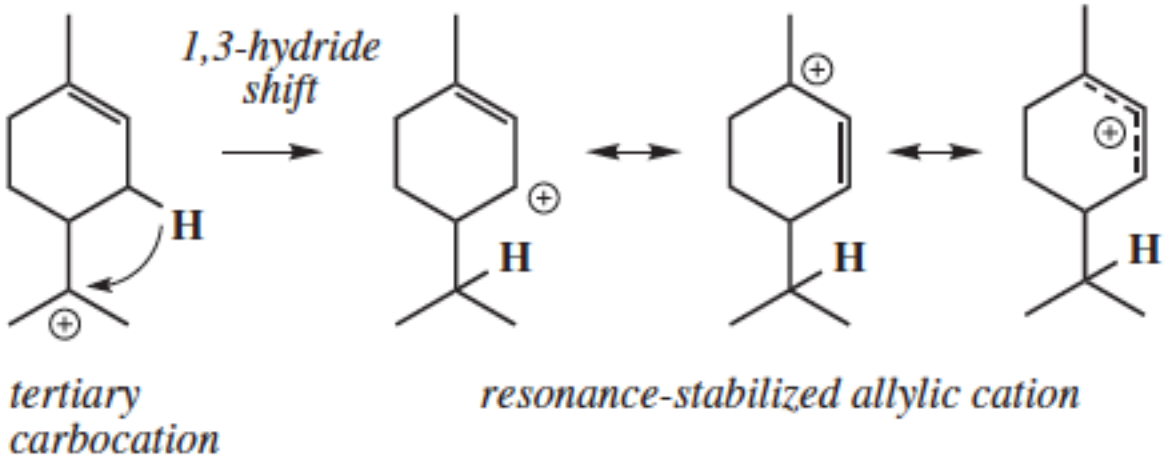
Reducing Ring Strain via 1,2-alkyl rearrangement of a cyclic monoterpene

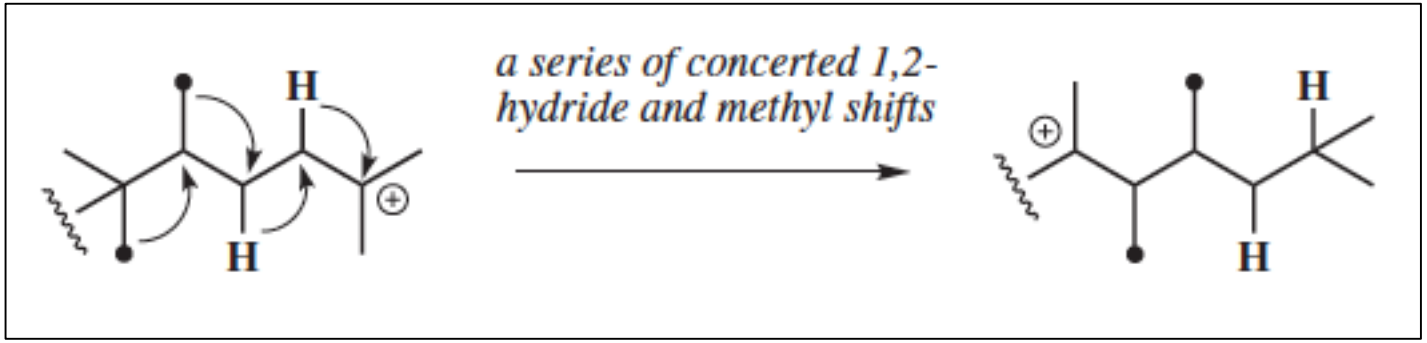


tertiary carbocation, but strained 4-membered ring

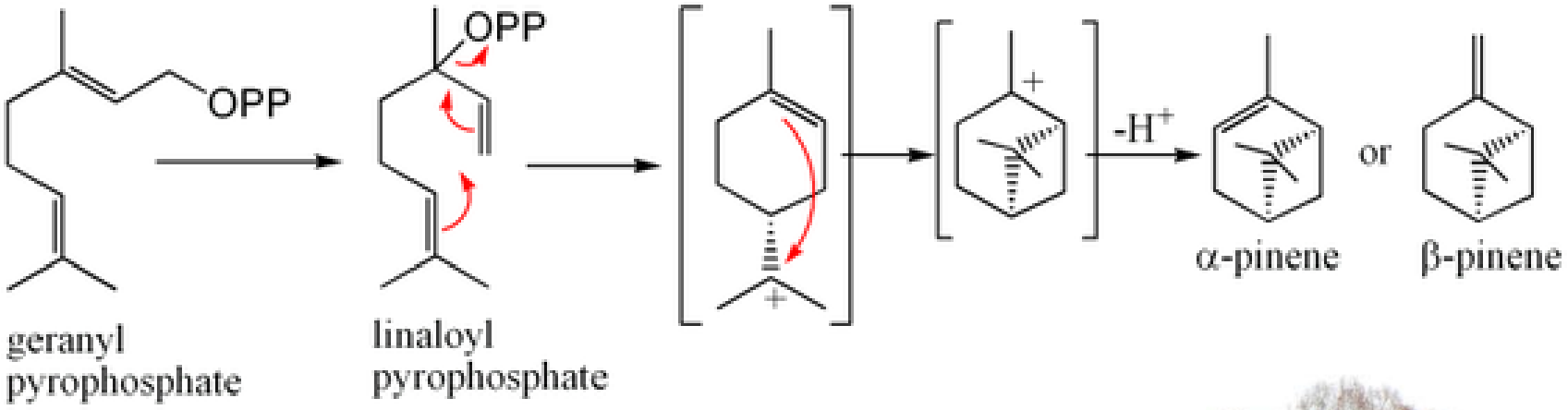
secondary carbocation, but reduced ring strain in 5-membered ring

The (occasional) 1,3-hydride shift and a concerted reaction mechanism



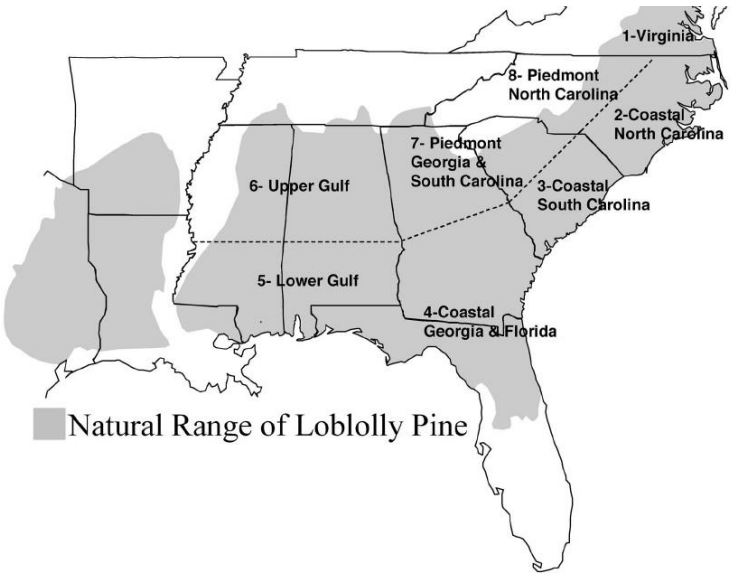


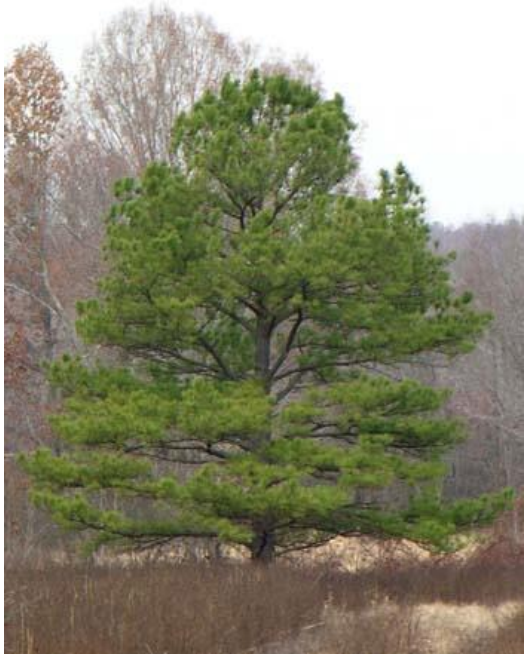
Formation of +/- pinenes: (and the smell of Christmas Trees)



Is there a market for pinene oil?

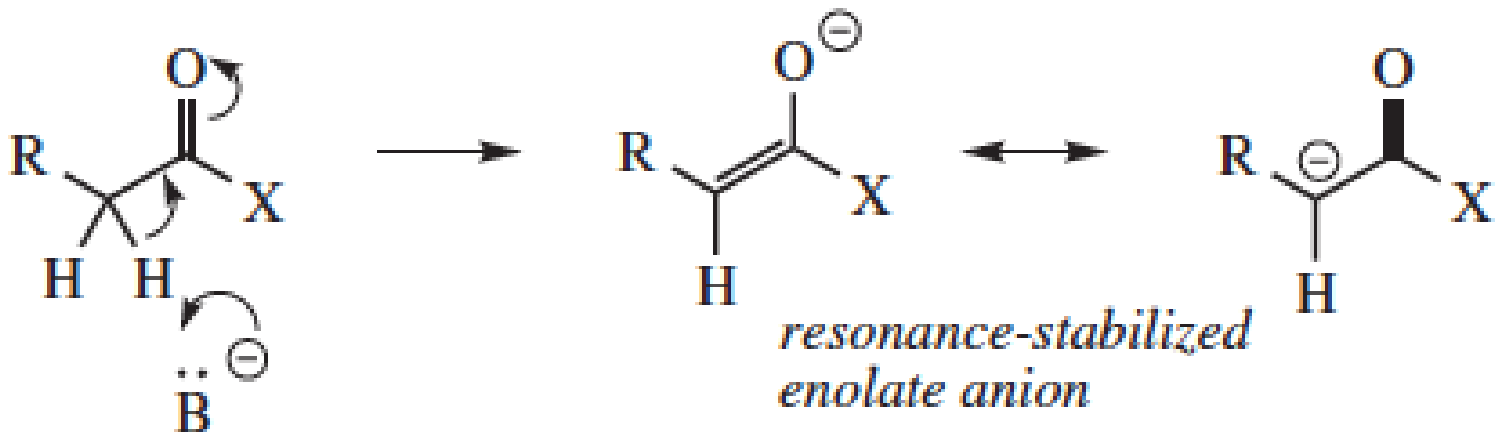
How could it be isolated prior to biofuel production from Loblolly pine (*Pinus taeda*)?



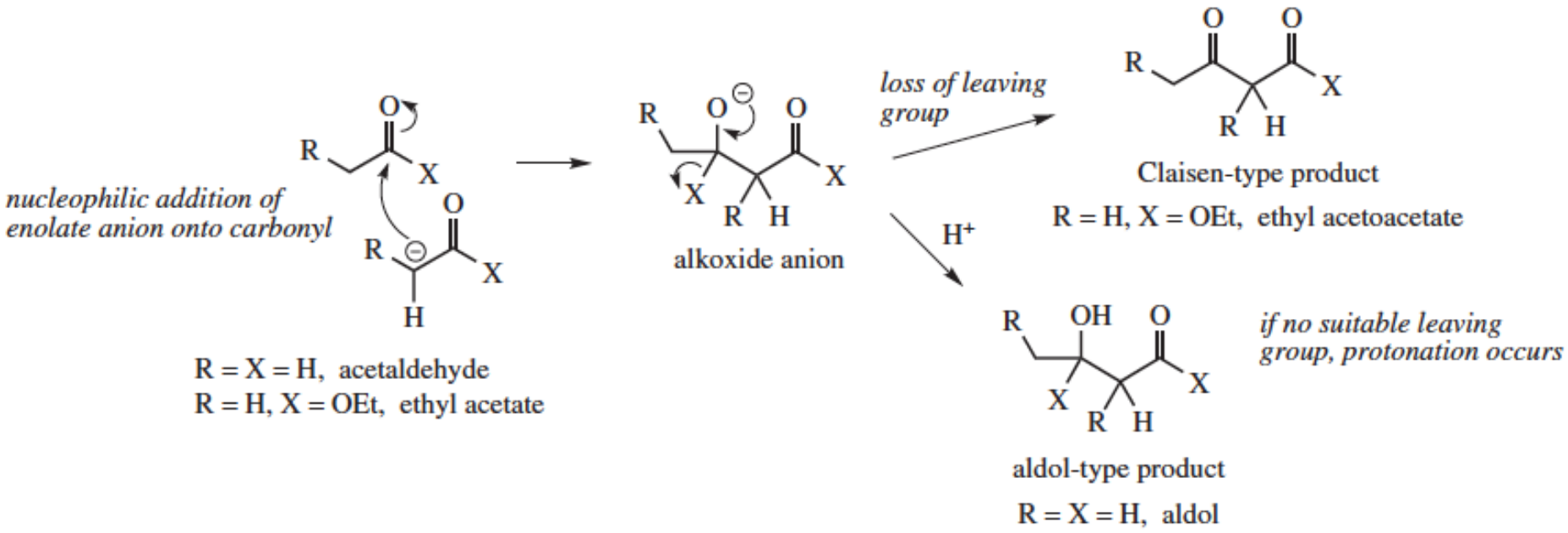


Aldol and Claisen reactions occur by first generating an enolate

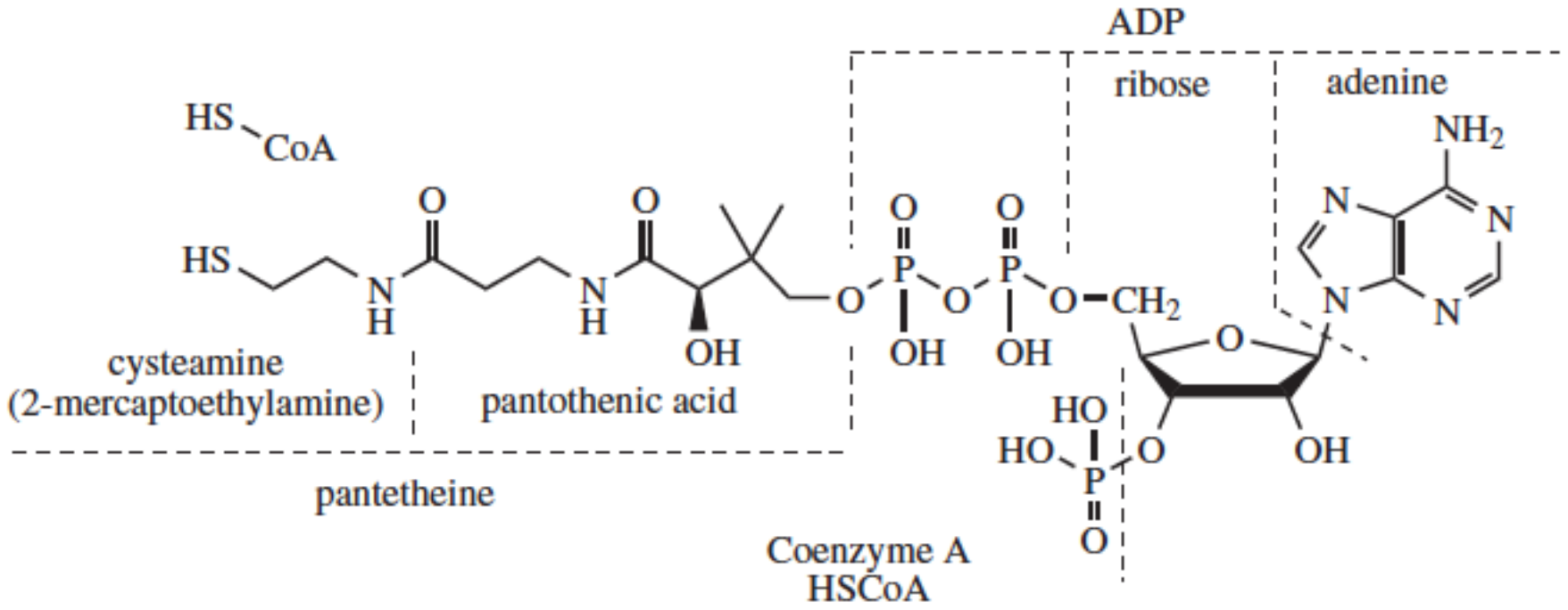
- C-C bond formation reactions (frequently between two C_2 acetate groups)
- Base catalyzed (Enzyme mediated)
- *Formation of enolate followed by nucleophilic attack by enolate into carbonyl*
- Aldol vs. Claisen Products depend on LG



Aldol vs. Claisen Product depends on Loss of LG vs. Protonation

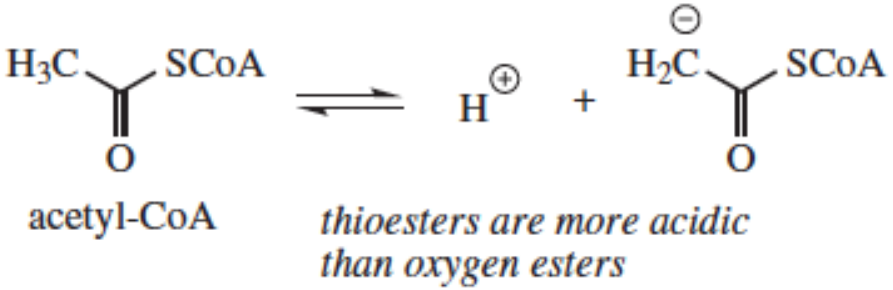


Aldol/Claisen Reactions often involve Acetyl CoA as a Leaving Group



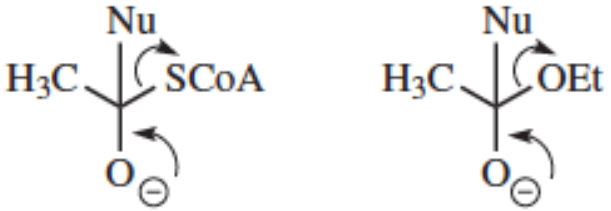
Thioesters form better Enolates as compared to Oxygen Esters

- Sulfur has a larger electron orbital cloud than oxygen and can more easily delocalize electrons when a proton is removed
- Therefore, thioesters are more acidic that oxygen esters, and better for generating ***enolate*** anions
- RS- is a better LG than RO- (Favors Claisen Product)



acetyl-CoA

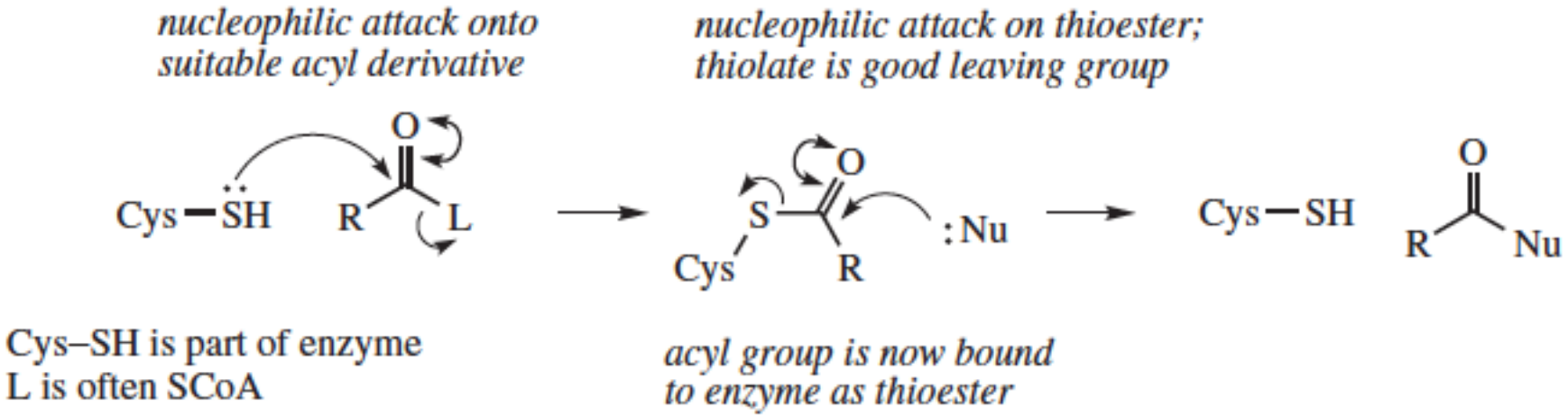
thioesters are more acidic than oxygen esters



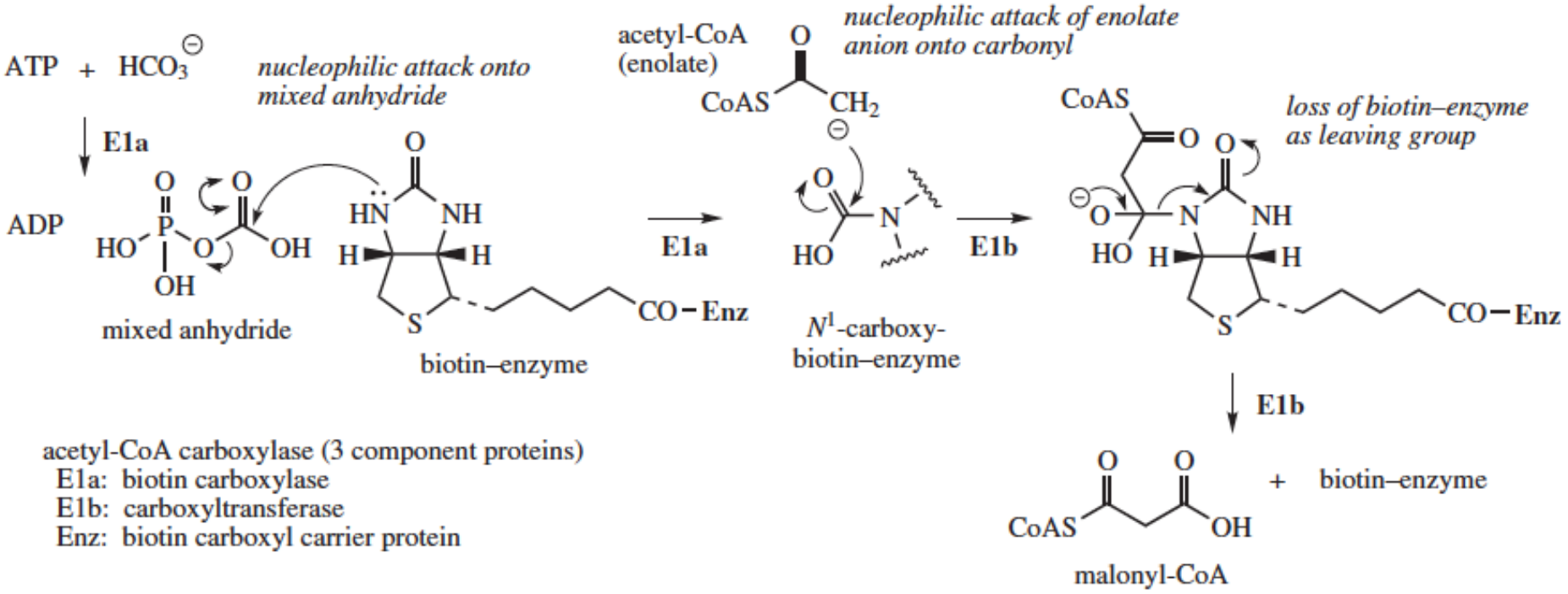
RS^- *is a better leaving group than* RO^-

Thioesters also allow Enzyme Binding, Molecular Modification and Release

- Thioester linkages allow for substrates to covelently bond to enzymes for modification and subsequent release of product



Synthesis of a Superior Claisen Substrate: Malonyl CoA



- ATP and CO_2 form mixed anhydride
- Biotin is carboxylated while enzyme bound and undergoes a Claisen reaction
- Enzyme-bound Biotin is a good leaving group
- Malonyl Co-A (a β-carboxythioester) has even more acidic α protons than acetyl CoA and is therefore a better substrate for Claisen rxns.