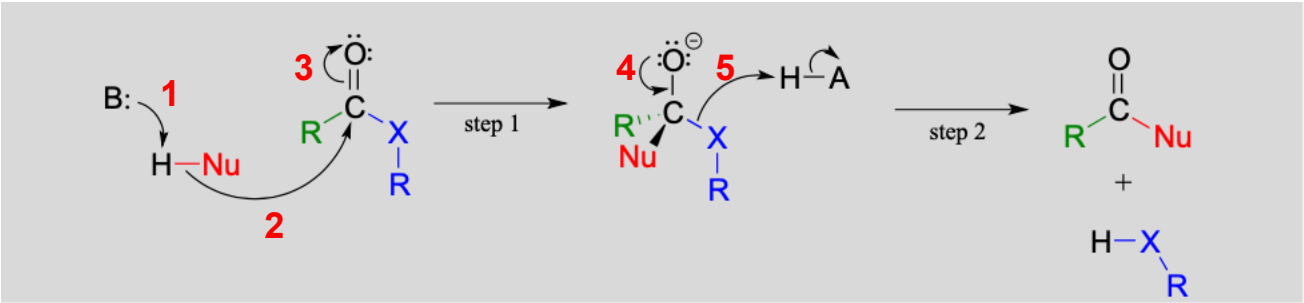
Bio-organic chemistry

Lecture 11

Nucleophilic acyl substitution mechanism



1- Base extracts proton from nucleophile – activating the nucleophile - you can think about it now as (Nu^-)

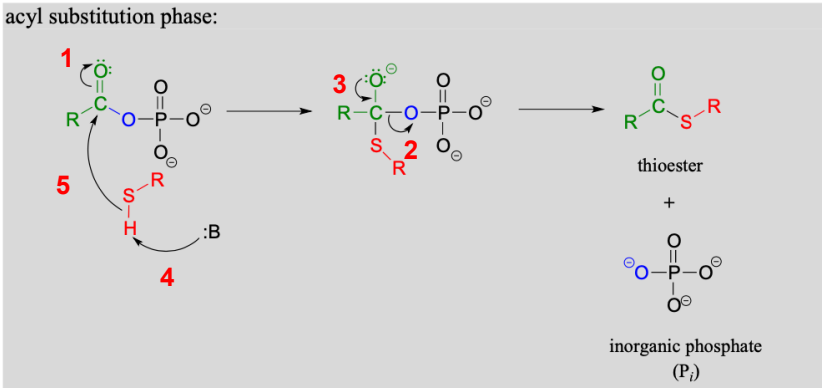
2- Attack to the electrophilic carbon

3- Delocalization of electrons to the carbonyl oxygen to fulfill the valency of the carbon

4- Delocalization of the electrons carbonyl oxygen to the acyl-X group

5 - Attack to the electron donor (acid)

Let's play a game



Pair the steps and put them in the right order

D - Attack to the electrophilic carbon

A - Delocalization of electrons to the carbonyl oxygen

C - Delocalization of the electrons from the carbonyl oxygen

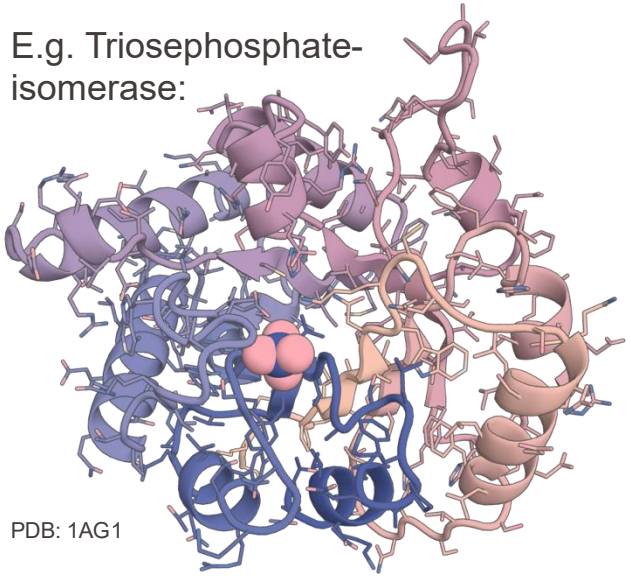
E - Extraction of proton from nucleophile

B –Bond breakage for leaving group

How can organic reactions occur in such diversity in such limited reaction conditions?

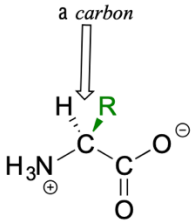
Enzymes are proteins that act as biological catalysts by accelerating chemical reactions.

E.g. Triosephosphate-isomerase:

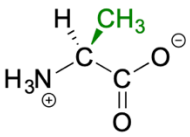


PDB: 1AG1

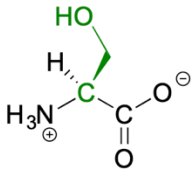
Proteins are polymers of amino acids, linked by amide groups known as peptide bonds



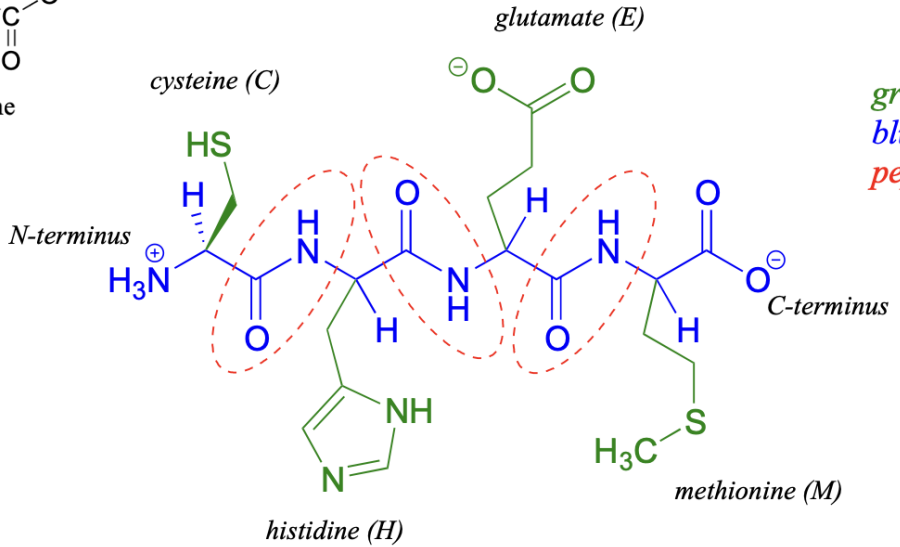
a generalized amino acid



alanine



serine



green: side chain
blue: main chain
peptide bonds circled

CHEM peptide

TWENTY-ONE PROTEINOGENIC α-AMINO ACIDS

Side chain charge at physiological pH 7.4

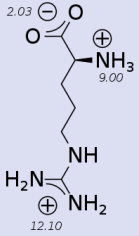
pK_a values shown italicized

⊕ Positive
⊖ Negative

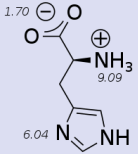
A. Amino Acids with Electrically Charged Side Chains

Positive

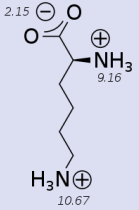
Arginine Arg R



Histidine His H

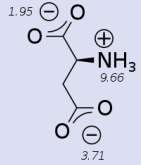


Lysine Lys K

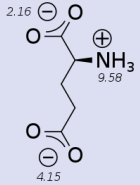


Negative

Aspartic Acid Asp D

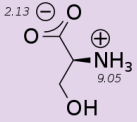


Glutamic Acid Glu E

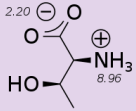


B. Amino Acids with Polar Uncharged Side Chains

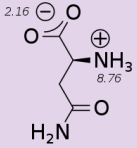
Serine Ser S



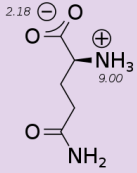
Threonine Thr T



Asparagine Asn N

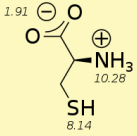


Glutamine Gln Q

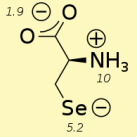


C. Special Cases

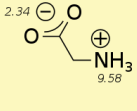
Cysteine Cys C



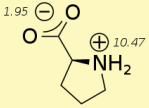
Selenocysteine Sec U



Glycine Gly G

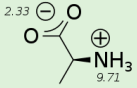


Proline Pro P

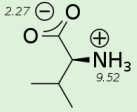


D. Amino Acids with Hydrophobic Side Chains

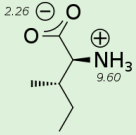
Alanine Ala A



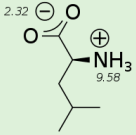
Valine Val V



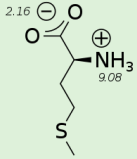
Isoleucine Ile I



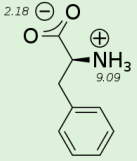
Leucine Leu L



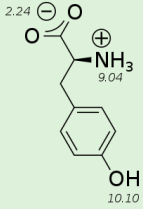
Methionine Met M



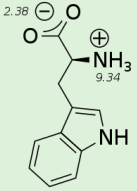
Phenylalanine Phe F



Tyrosine Tyr Y



Tryptophan Trp W



Diversity of peptides / proteins – a number's game

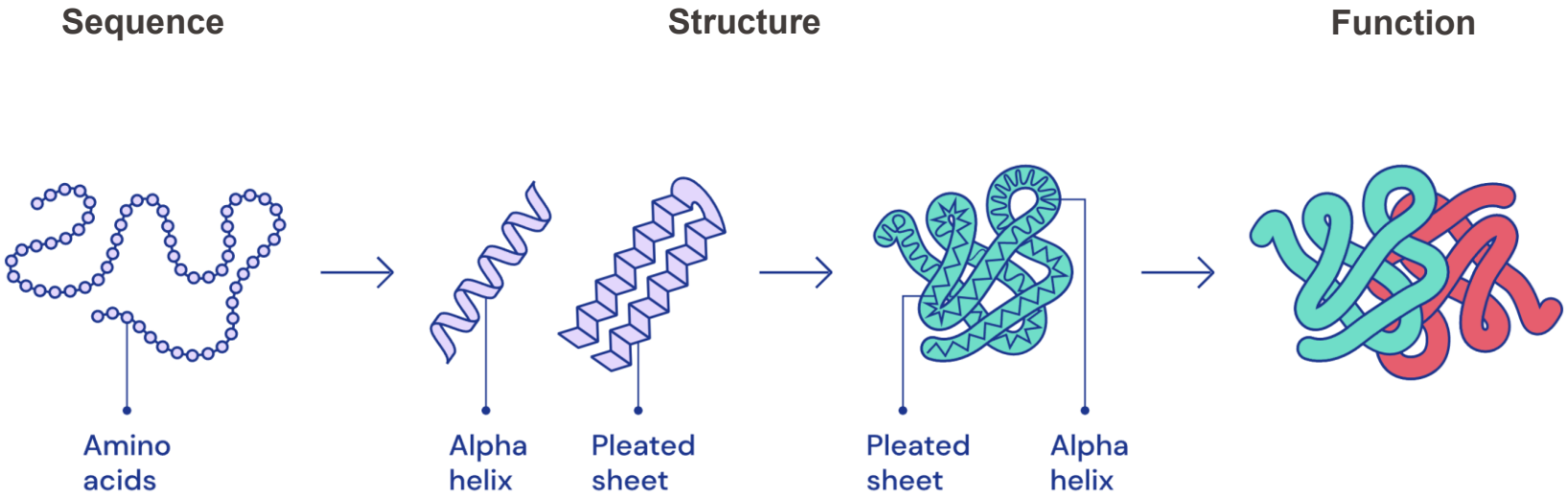
Random combinations of 20 amino acids

3-mer:	$20^3 = 8000$ different peptides
5-mer:	$20^5 = 3.2 \times 10^6$ different peptides
10-mer:	$20^{10} = 1.0 \times 10^{13}$ different peptides
50-mer:	$20^{50} = 1.1 \times 10^{65}$ different peptides

Weight of 1.1×10^{65} single 50-mer peptide molecules:
1.3×10^{39} kg

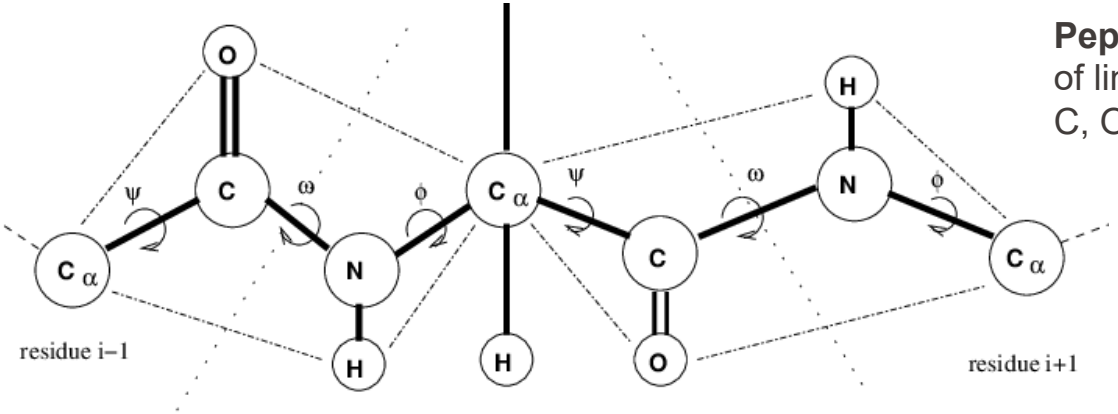
Weight of the earth: 5.9×10^{24} kg

Anfinsen-Dogma: The Sequence Determines the Protein Structure and Function

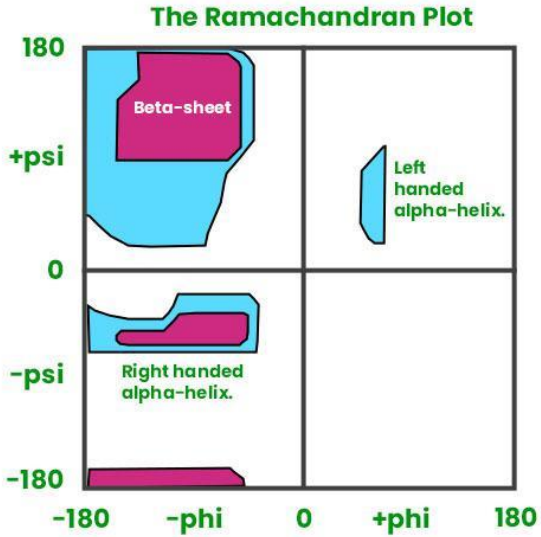


1. Image taken from: https://www.deepmind.com/blog/alphafold-using-ai-for-scientific-discovery-2020

The Protein Backbone

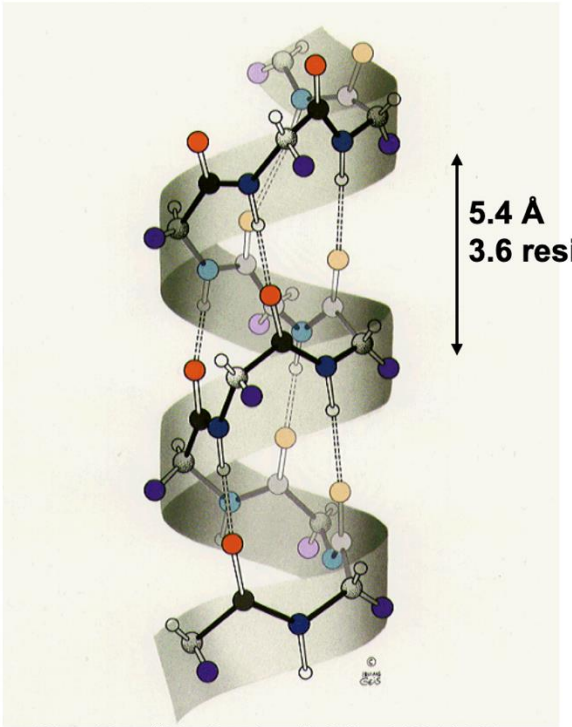


Peptide Bonds Are Planar: In a pair of linked amino acids, six atoms (C_α, C, O, N, H, and C_α) lie in a plane.



2° Structures: α-Helix

Right handed α-helix (Φ = -57°, Ψ = -47°)



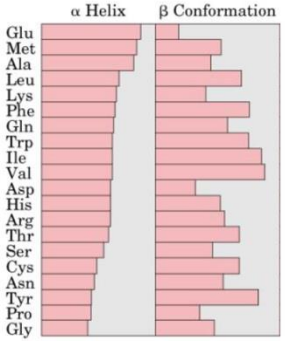
From: Voet, Voet "Biochemistry", Wiley VCH 1990

H-bonds

- between C=O of AA^n and N-H of AA^{n+4}
- parallel to helix axis
- N••••O distance: ~2.8 Å
- angle N•••H•••O: ~9°

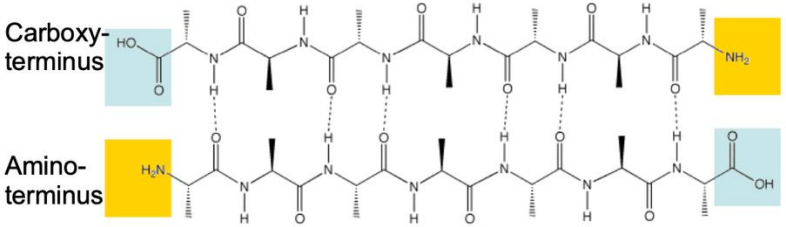
A. Helen, L. Pauling

Amino acid propensity to form α-helices and β-sheets

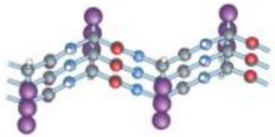


Parallel and Antiparallel β-sheets

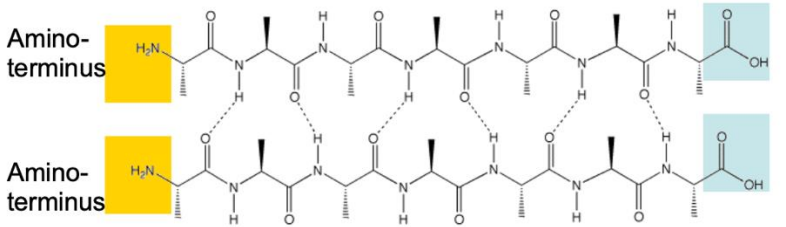
Anti-parallel (Φ = -139°, Ψ = 135°)

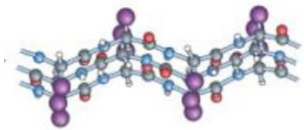


Side views

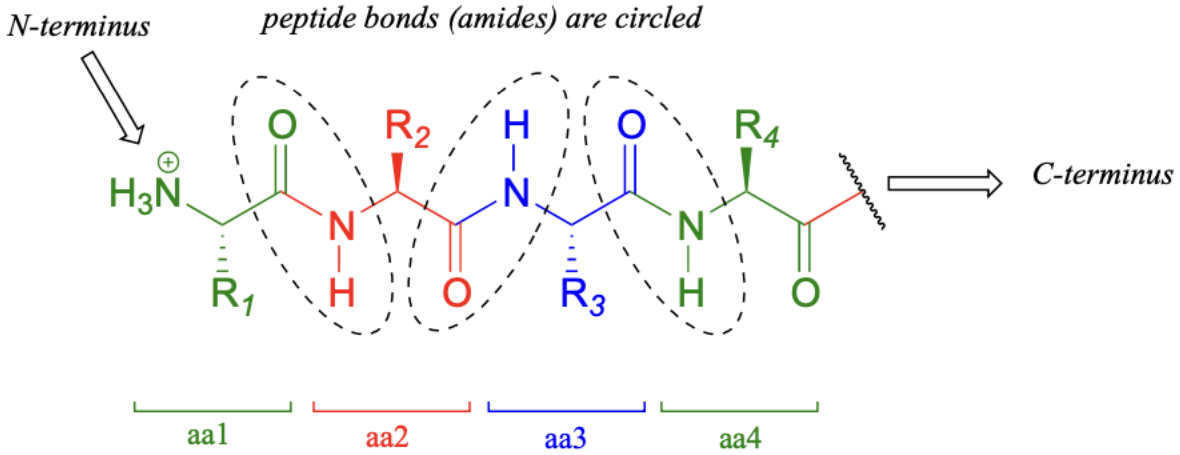


Parallel (Φ = -119°, Ψ = 113°)





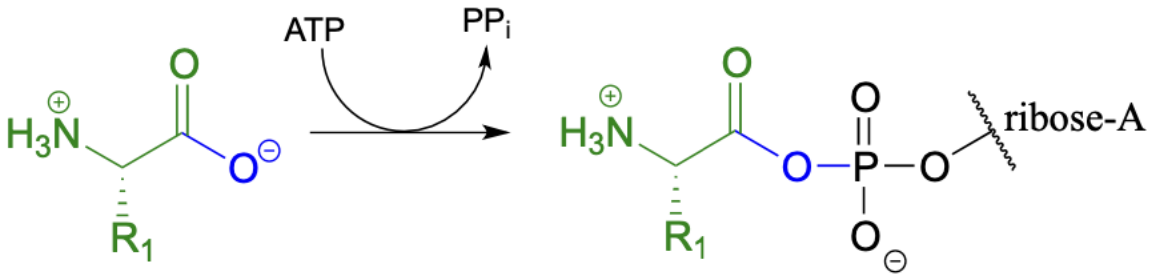
Protein Biosynthesis



(Bio-)Chemical Challenges:

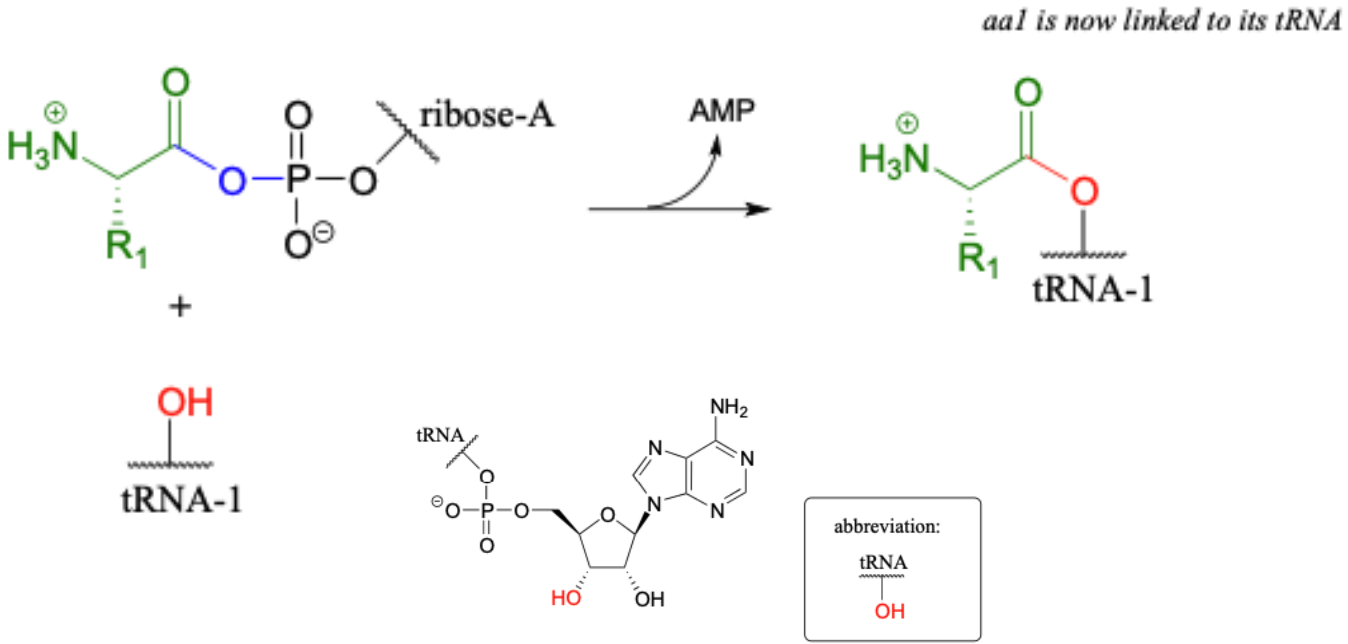
- Activation?
- Sequence?
- Selectivity?

The carboxylate group of aa-1 is first transformed to an acyl-AMP intermediate

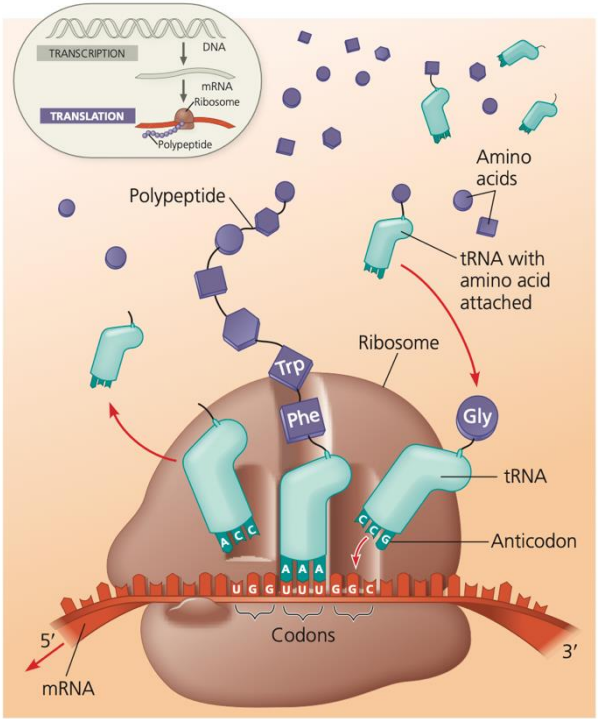


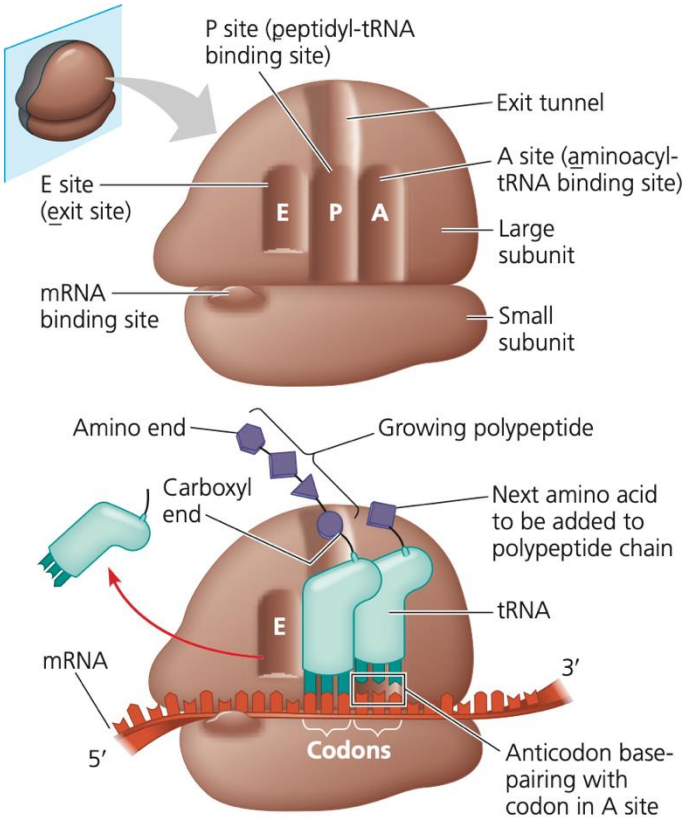
activated acyl-AMP intermediate

The amino acid is transferred to a special kind of RNA polymer called transfer RNA (tRNA)



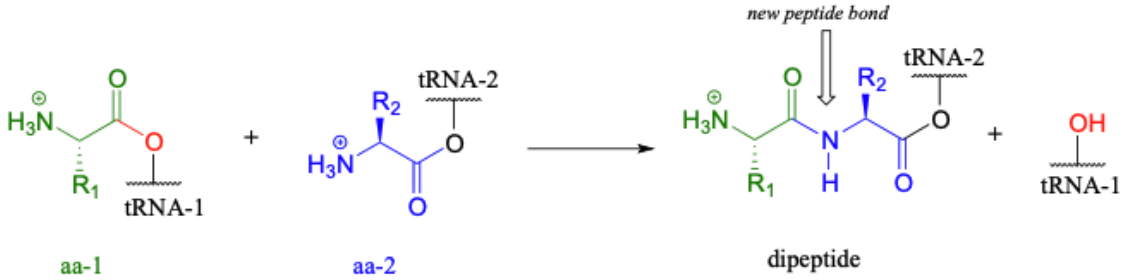
Selectivity of Protein Biosynthesis is Based on "Codons"

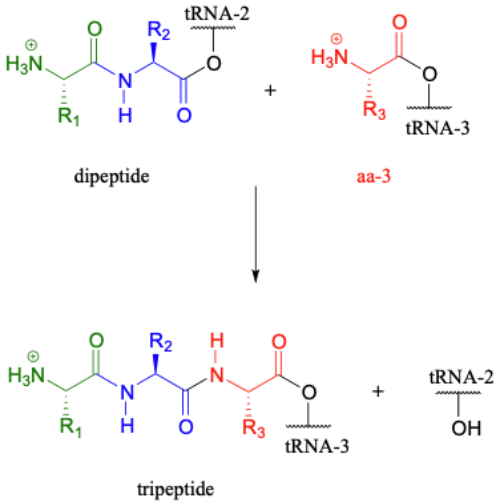




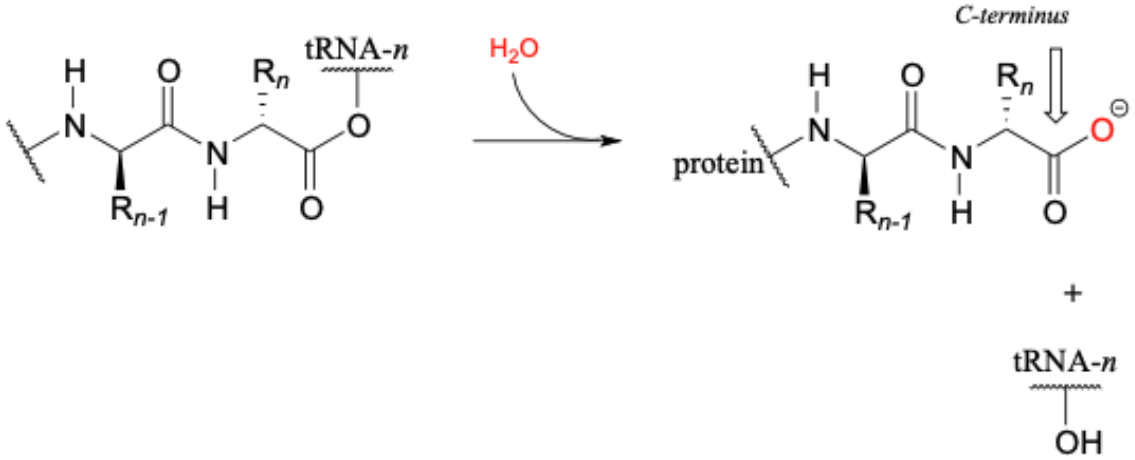
Reece, J. B., Urry, L. A., & Cain, M. L. (2017). Campbell biology. Pearson.

Elongation: Ester to amide conversion by an enzymatic component of the ribosome called peptidyl transferase



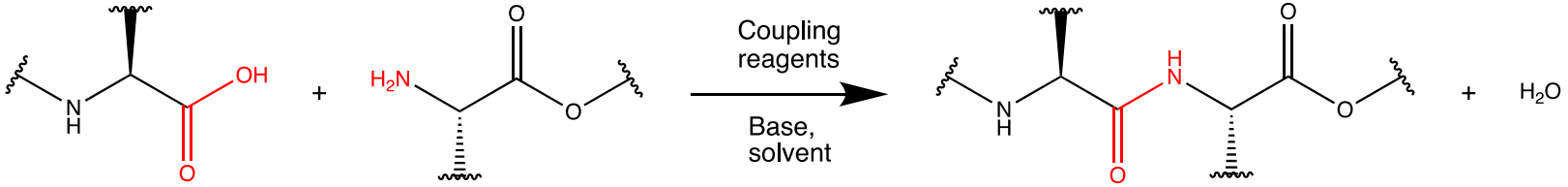


Ester hydrolysis by release factors (RFs)



In-vitro Protein Production

Activation?
Sequence?
Selectivity?
Questions the Chemist
also asks *in vitro*

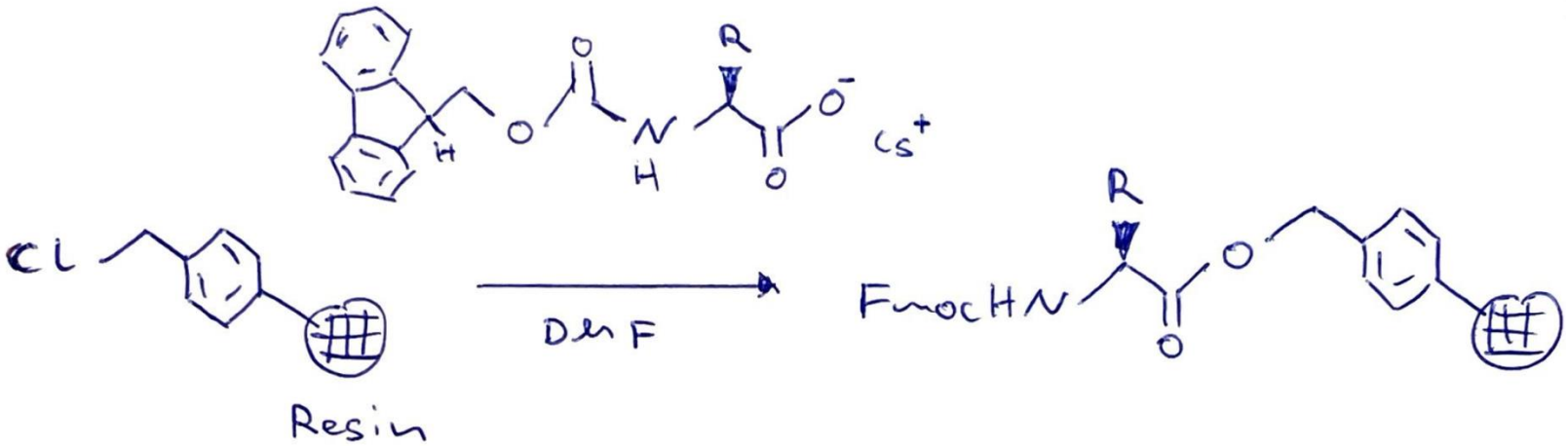


Carboxylic Acid Amine Amide

Solid Phase Peptide Synthesis (SPPS)

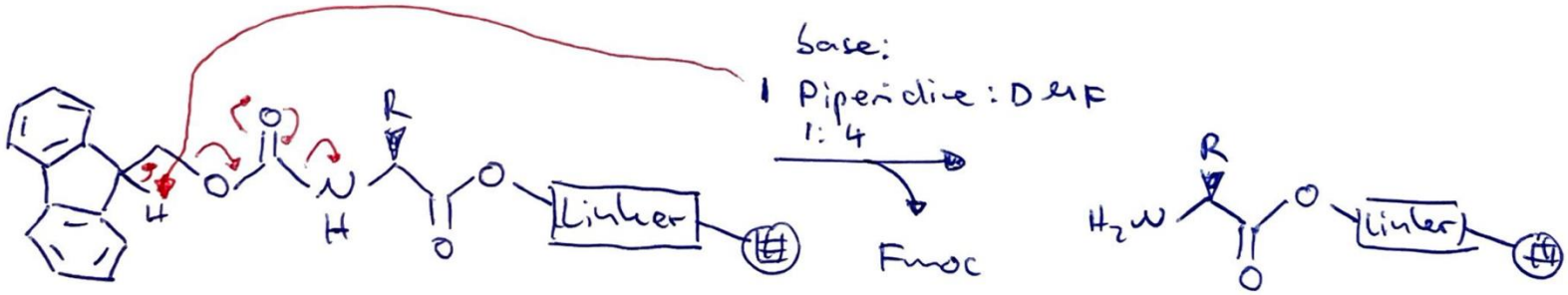
1. Coupling to Resin

Fmoc = Protecting Group to avoid undesired side reaction



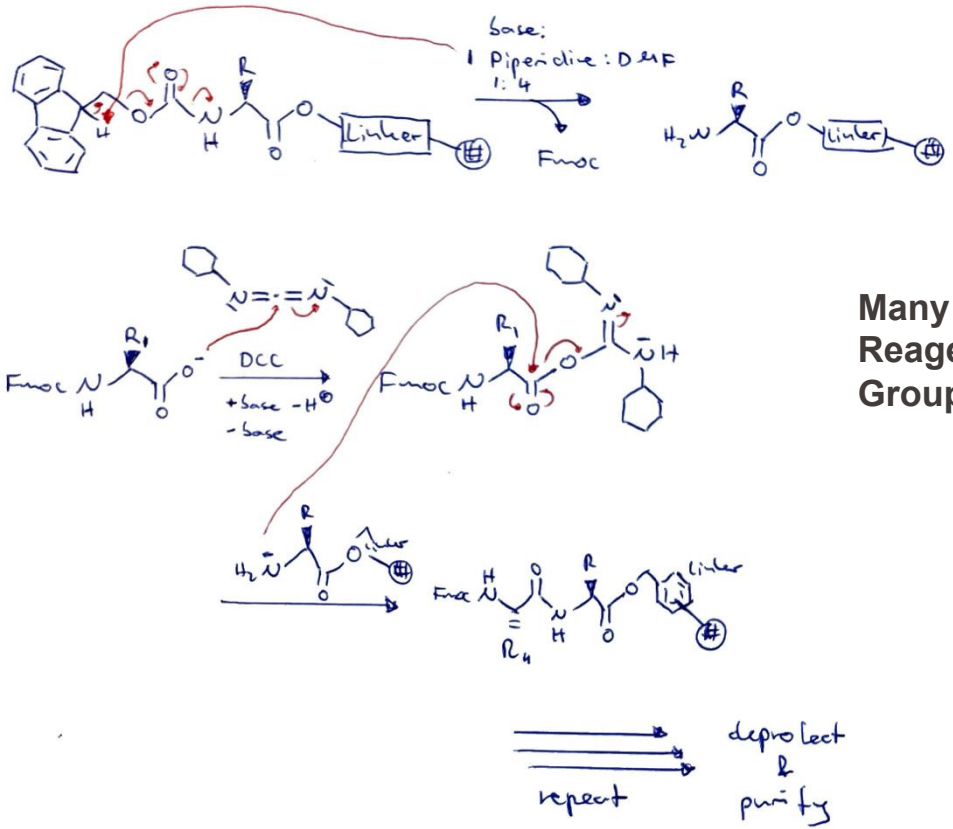
Solid Phase Peptide Synthesis (SPPS)

Protecting Group Removal



Solid Phase Peptide Synthesis (SPPS)

Amino acid activation via coupling reagents

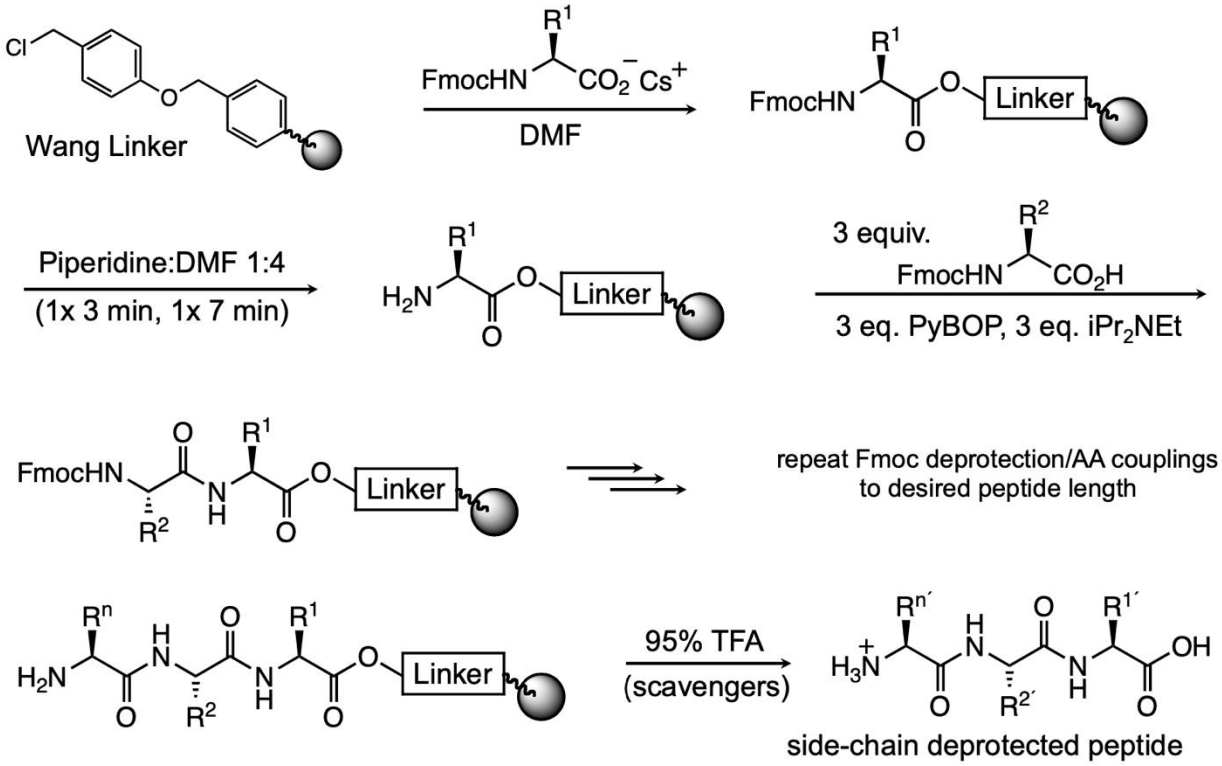


Many linkers & Coupling Reagents & Protecting Groups possible!

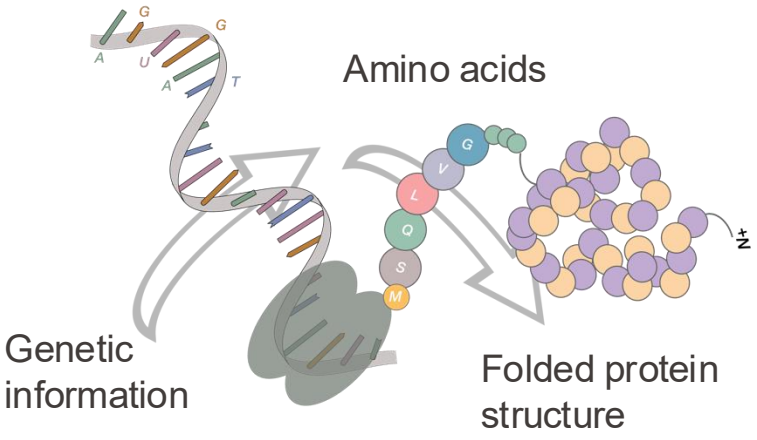
Solid Phase Peptide Synthesis (SPPS)

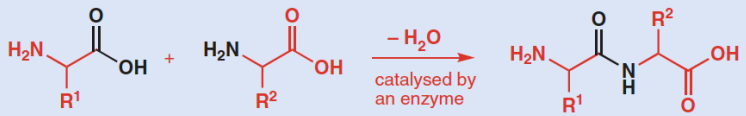
Summary

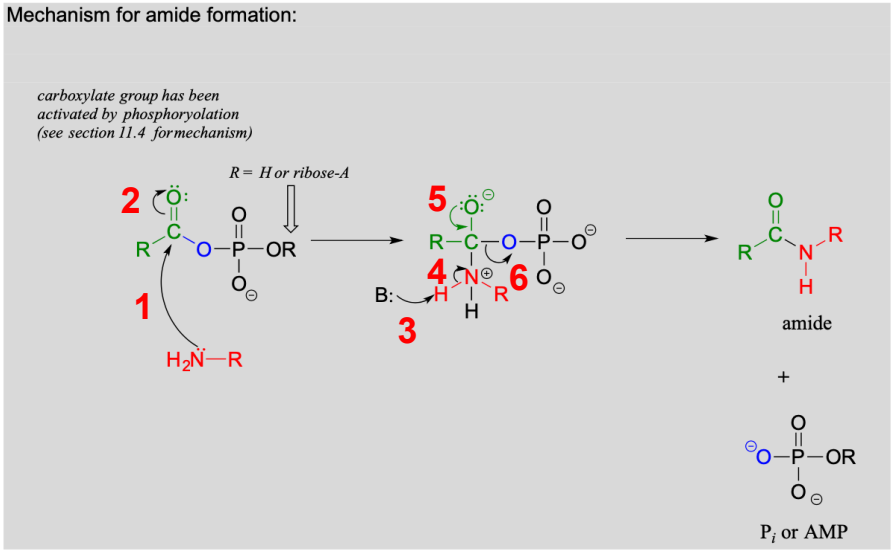
Synthesis in C-term -> N-term direction



Amide formation from carboxylic acids







1 - Amine attacks the electrophilic carbonyl carbon

2 – Delocalization of electrons

3 – Base attack to the nitrogen proton

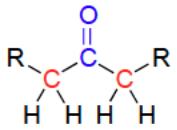
4 – Electrons become a nonbonded pair in N

5 – Delocalization of electrons to make a double bond

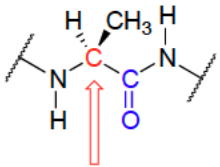
6 – Bond breakage to release leaving group

Let’s do one of these together without the phosphate leaving group

Reactions at the α- carbon of Carbonyls, Carboxylic acids and derivatives



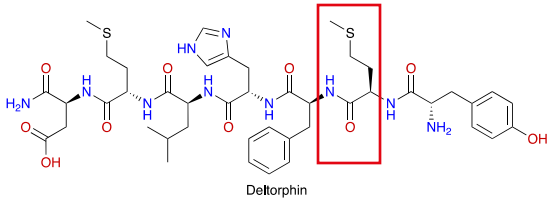
α-carbons on a ketone



the α-carbon of an alanine amino acid residue in a protein

How the chemistry of the α- carbon is related to biology

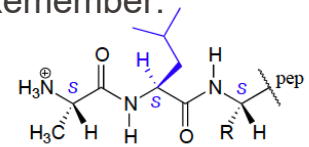
Note: Venoms usually contain a pool of different peptides and active compounds.



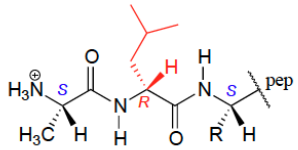
IMFFEMQACWSHSGVCRDKSE
RNCKPMAWTYCENRNQKCCEY
(DLP2)

The peptides are initially synthesized using all *L*-amino acids, and then the amino acid at position #2 undergoes a 'post-translational modification' to the *D*-amino acid

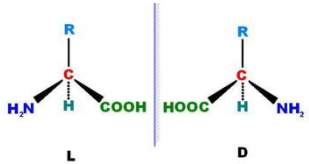
Remember:



peptide with L-Leu at position 2

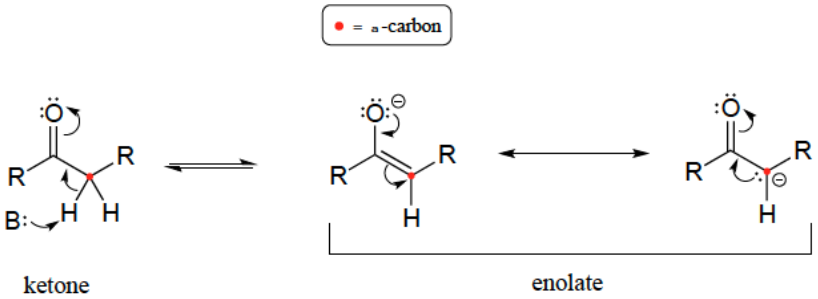


peptide with D-Leu at position 2



How does this exchange work?

Enolate formation is an important property of carbonyl groups



Remember the electron withdrawing effect of the carbonyl!

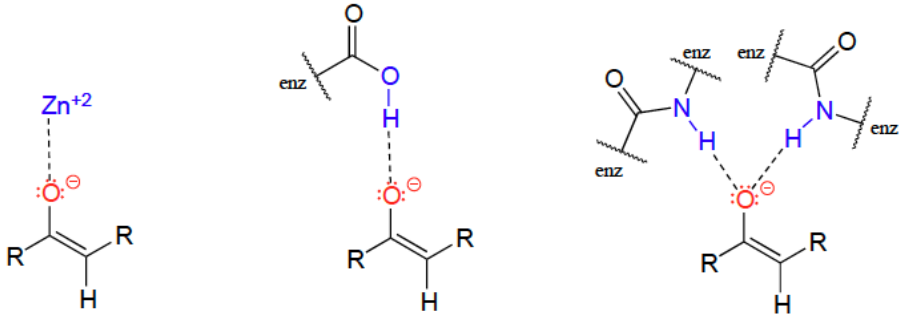
Acidity of α-protons:

- pKa range of approximately 18-20 in aqueous solutions.
- Resonance stabilization of the enolate conjugate base spreads the negative charge over both the α-carbon and the carbonyl oxygen.

Enolate Ion Structure:

- α-carbon is sp2-hybridized with trigonal planar geometry, akin to the carbonyl carbon and oxygen atoms.
- Resonance delocalization of negative charge involves π-bonding in conjugated systems.

Different structural properties of enzymes can stabilize enolates



Enzymatic Influence on pKa:

- Enzymes modify the effective pKa of functional groups in their active sites.
- Stabilization of the negative charge on the oxygen atom of the enolate is crucial for reactions involving α-proton abstraction.

Stabilization Mechanisms:

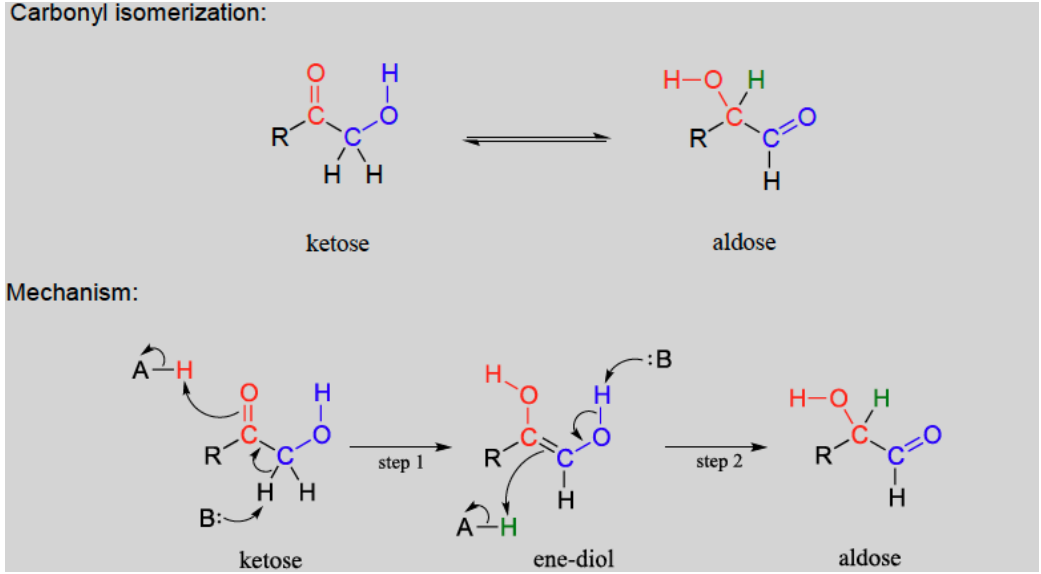
- Metal cations (e.g., Zn^{+2}) form stabilizing ion-ion interactions in the active site.
- Proton-donating groups near the oxygen atom assist in stabilization.
- Active site architecture can provide hydrogen bond donor groups for stabilization purposes.

Isomerization at the α- carbon

Ketose and Aldose Conversion:

Ketose: Sugar molecules containing ketone functional groups.

Aldose: Sugar molecules containing aldehyde functional groups.



1. Enol Formation

The ketose species undergoes a conversion to its enol tautomer, specifically an 'ene-diol' intermediate.

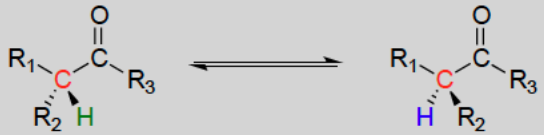
2. Tautomerization to Aldose

In the subsequent step, another tautomerization occurs, leading to the formation of the aldose.
(structural isomers)

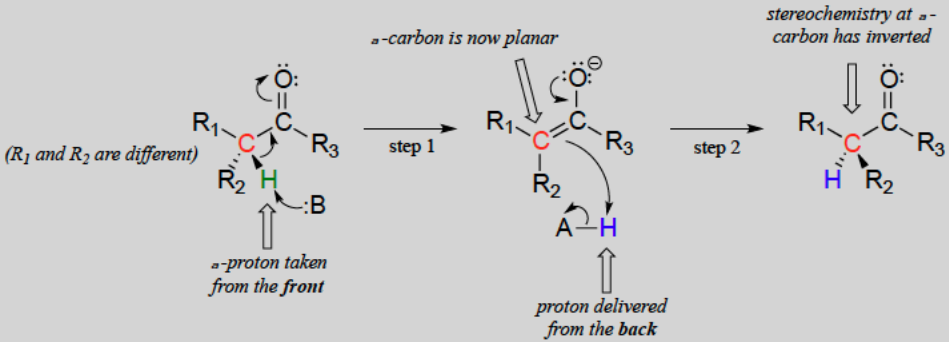
Stereoisomerization at the α- carbon

Involvement of enolates as intermediates allows for the **alteration of stereochemical configurations** at the α-carbon, contributing to the formation of **enantiomers in racemization**

Racemization/epimerization:



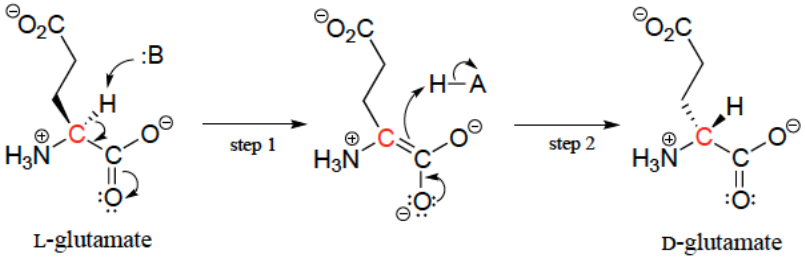
Mechanism:



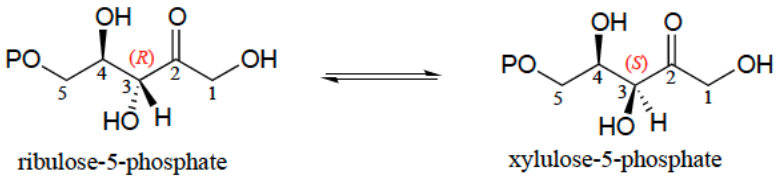
Racemization:

Involves the conversion between enantiomers, resulting in a racemic mixture.

Stereoisomerization at the α- carbon



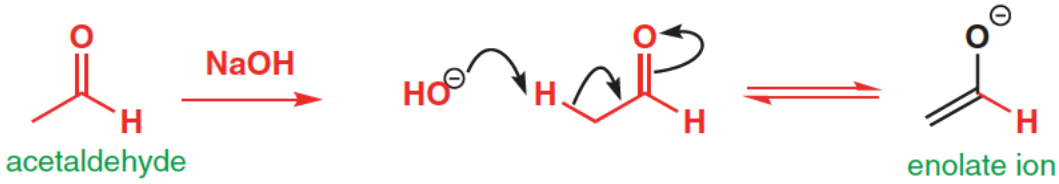
Cell walls of bacteria are constructed in part of peptides containing D-glutamate, converted from L-glutamate by the enzyme **glutamate racemase**.



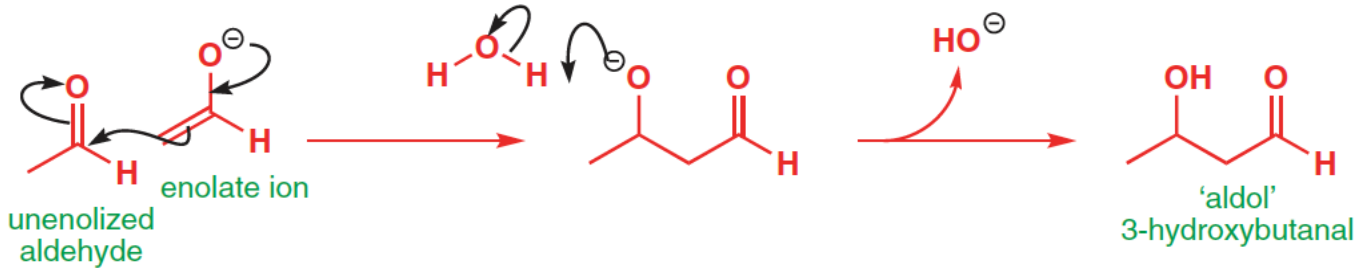
The epimerization of ribulose-5-phosphate and xylulose-5-phosphate catalyzed by the **epimerase** is a pivotal step in the pentose phosphate pathway

Aldol reactions

-One of the most important mechanisms in metabolism
-Creation of carbon-carbon bonds

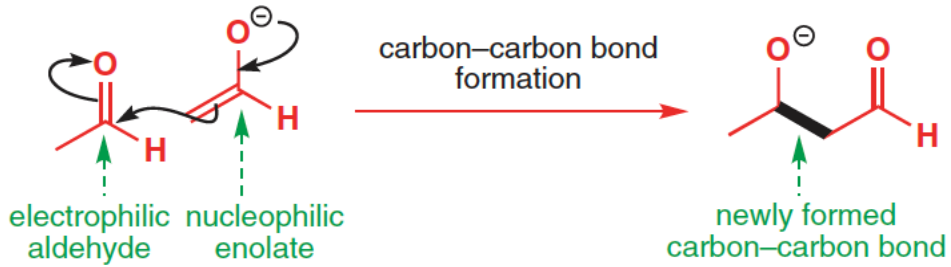


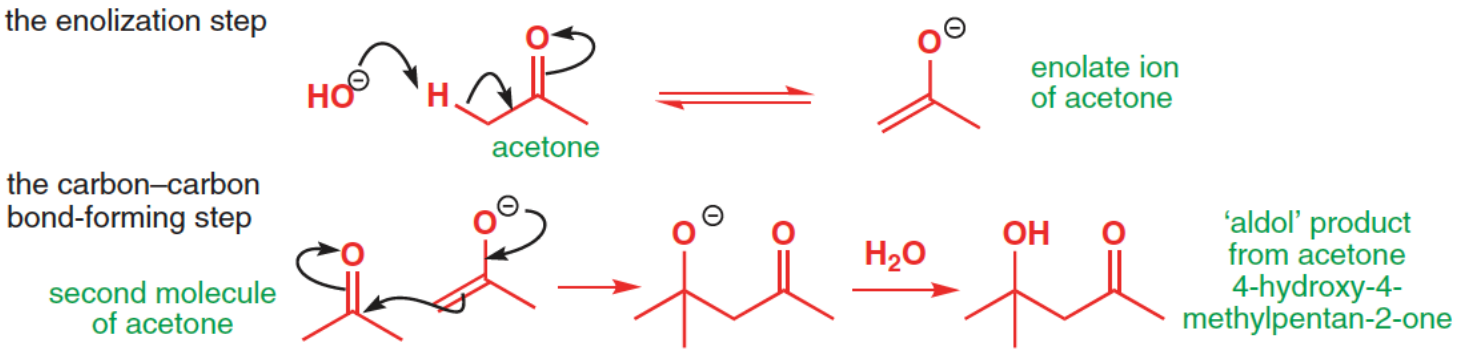
- Basic conditions (NaOH) generates enolate ions – not to completion
- Formed enolate ions act as nucleophiles, attacking unenolized aldehyde molecules
- Water, produced in the process, protonates the alkoxide ion.



The product is an aldehyde with a hydroxy (ol) group, and it has the name aldol

Aldol reactions





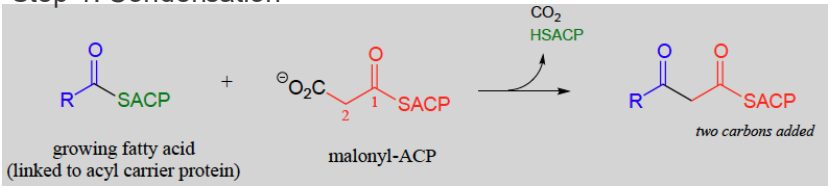
Ketones, like acetone, undergo enolization akin to acetaldehyde's aldol sequence. Acetone forms an enolate ion, leading to a hydroxy-ketone product via carbon-carbon bond formation.

Let's do one of these together

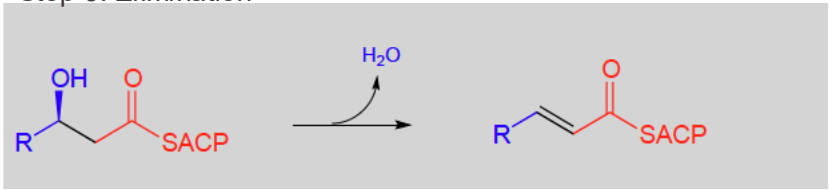
Application: Fatty Acid Synthesis

Two carbons are added at a time to a growing fatty acid chain, and in the degradative direction, two carbons are removed at a time. In each case, there is a four-step reaction cycle that gets repeated over and over.

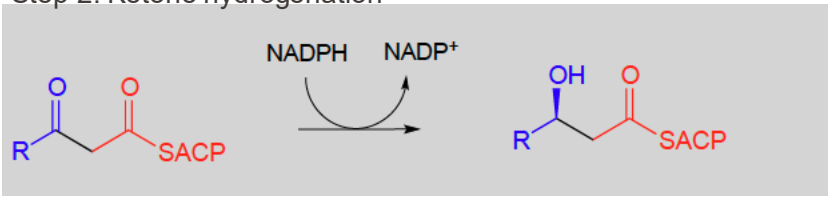
Step 1: Condensation



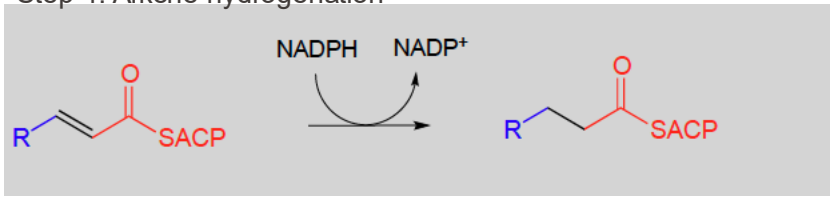
Step 3: Elimination



Step 2: Ketone hydrogenation

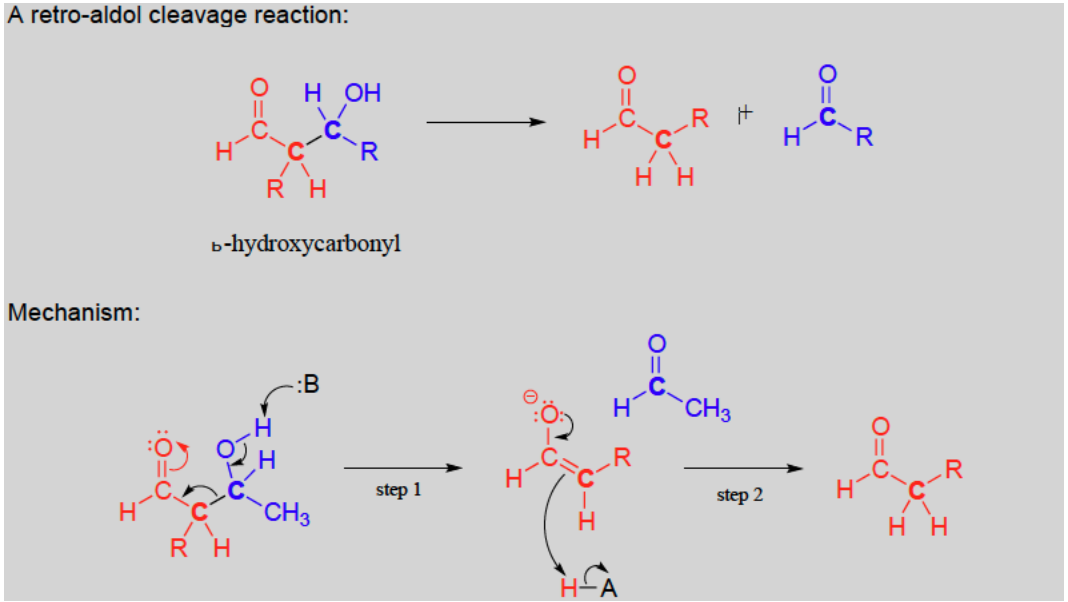


Step 4: Alkene hydrogenation



Breaking C-C bonds: Retro-Aldol Reactions

- Aldol reactions can go both ways are reversible due to closely balanced energy levels between reactants and products. Under specific metabolic conditions, aldolases can catalyze retro-aldol reactions, breaking carbon-carbon bonds in a reversal of aldol reactions.



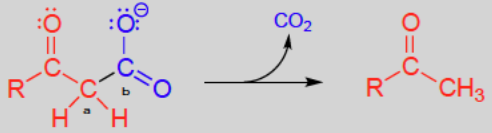
Retro-aldol cleavage steps:
Step 1: Deprotonation of the β-hydroxy group, forming a carbonyl and transitioning the enolate carbon into a leaving group.
Step 2: Reprotonation of the enolate, regenerating the initial aldehyde.

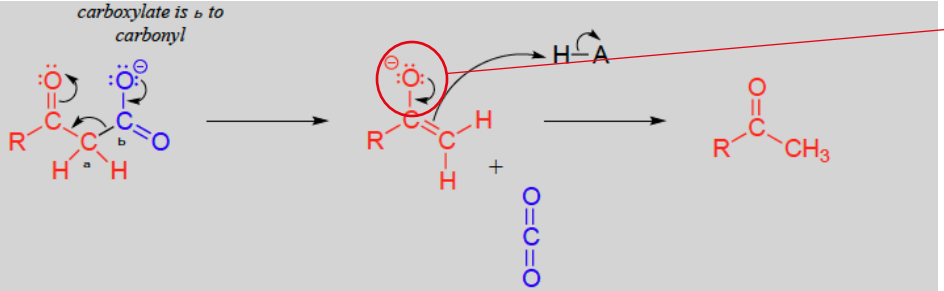
Decarboxylation Reactions

The gain or loss of a single carbon atom, often in the form of carbon dioxide (CO_2), is integral in numerous carbon-carbon bond-forming and bond-breaking processes within biological chemistry

$$6CO_{2(g)} + 6H_2O_{(l)} + \text{energy} \rightarrow C_6H_{12}O_{6(aq)} + 6O_{2(g)}$$

Decarboxylation of a β-carboxy ketone or aldehyde:



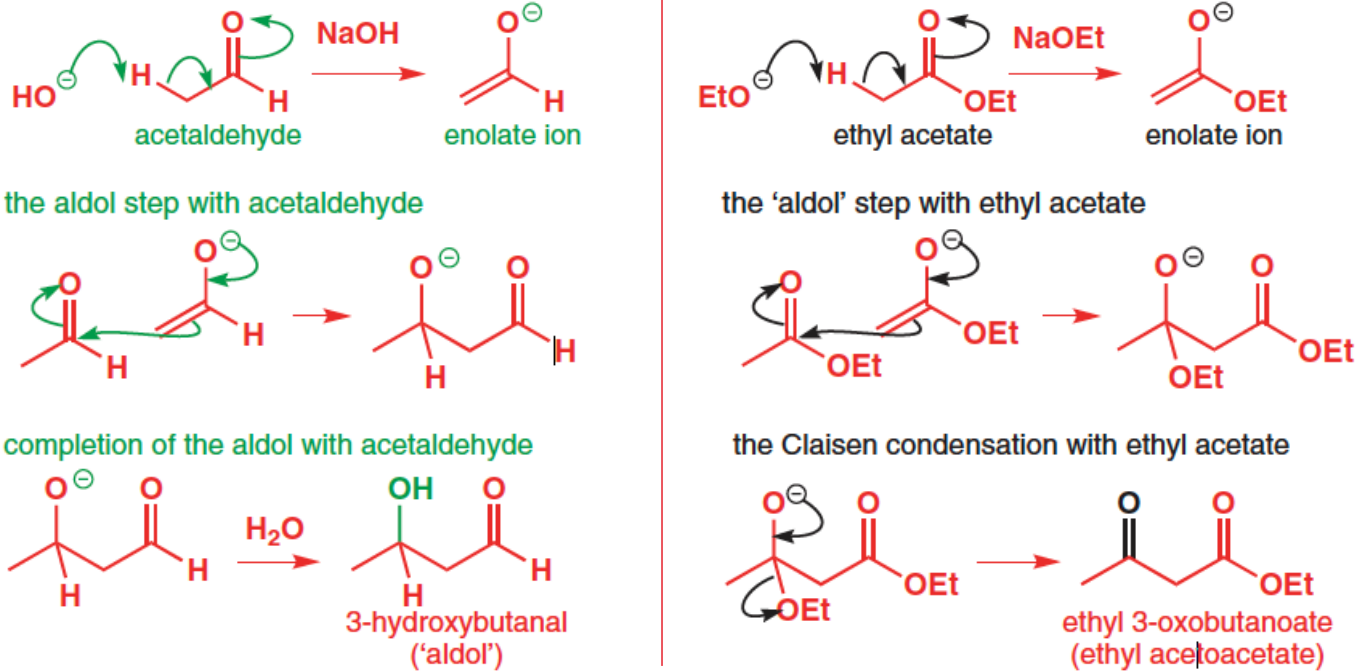


Electrons need to be stabilized!

➔ Usually by resonance with an oxygen or nitrogen

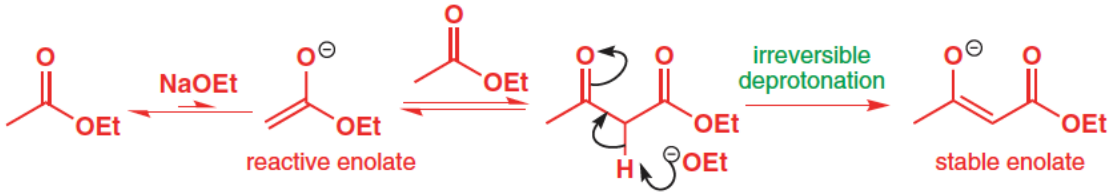
Similar to a retro-aldol reaction, the breaking of a carbon-carbon bond necessitates the stabilization of the resulting electrons

The Claisen Condensation

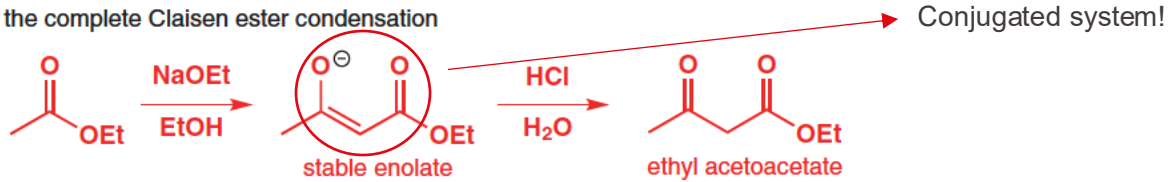


*A Claisen condensation reaction is simply a nucleophilic acyl substitution reaction with an **enolate** carbon nucleophile*

The Claisen Condensation



The base partially forms the enolate from the ester, leading to a limited equilibrium concentration that reacts with the ester electrophile. While appearing catalytic at first, the subsequent step progresses by consuming ethoxide through irreversible deprotonation, driving the reaction toward product formation



-> The product formation involved acylating the enolate carbon of an ester.

Let's do one of these together

What to know

-Understand the formation of the enolate species

-Isomerization effects at the alpha-carbon

-Aldol reaction and its mechanism

-Retro-aldol reaction and its mechanism

-Decarboxylation reaction and its mechanism

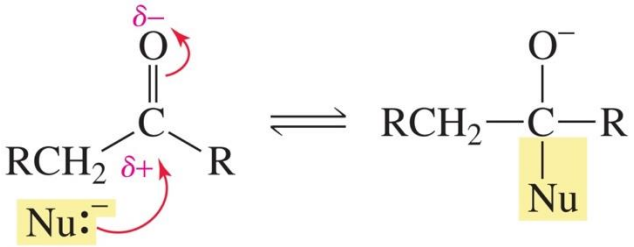
-Claisen condensation

Many other aspects are covered in chapter 12+13 for alpha-carbon reactions – for exam purposes focus only on the topics covered this lecture and the exercises

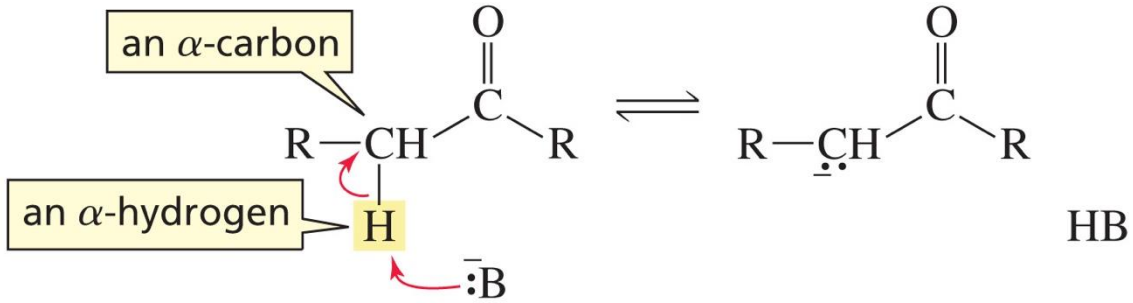
Questions ?

Many Carbonyl Compounds Have Two Sites of Reactivity

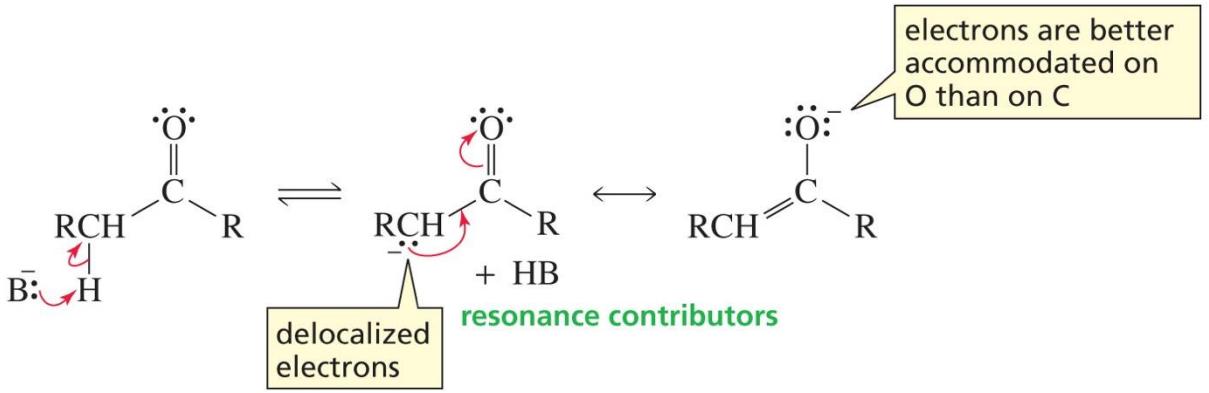
a nucleophile can add to the carbonyl carbon



a base can remove a proton from an a-carbon
(an α-carbon is a carbon that is adjacent to a carbonyl carbon)



The Electrons Left Behind When a Proton is Removed from an α-Carbon are Delocalized onto an Oxygen



delocalization increases stability

(the more stable the base, the stronger its conjugate acid)

oxygen is better able to accommodate the electrons than carbon