Chemistry 5.07SC Biological Chemistry I

Fall Semester, 2013

Lecture 9 Biochemical Transformations I. Carbon-carbon bond forming and cleaving reactions in Biology (see the Lexicon).

Enzymes catalyze a limited repertoire of reactions and use the same reactions over and over again. The mechanisms of the reactions are conserved. The wealth of knowledge about organic reaction mechanism is directly applicable to biological transformations and provides an excellent foundation for thinking about the transformations that you will encounter in primary metabolism. I have handed out a Lexicon of reactions found in Biology. You will encounter examples of all of the reactions in this compendium over the course of this semester and next semester for those of you who take Chemistry 5.08. The most prevalent reactions will be introduced in the next few lectures and the rest will be introduced in the context of the metabolic pathways in which they are found.

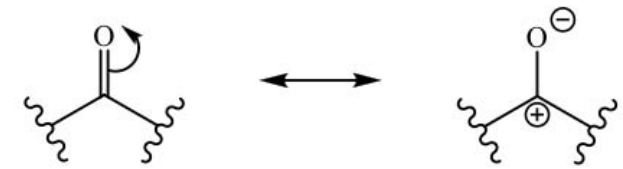
Outline:

I. Review of carbonyl chemistry
II. Aldol reaction
III. Claisen reaction
IV. Prenyl transfer reaction

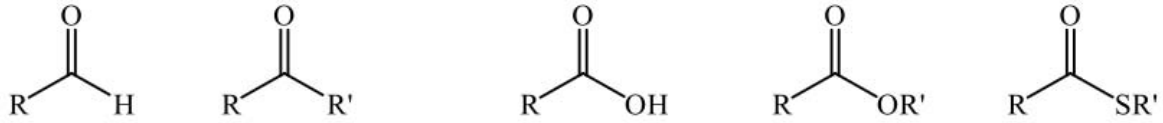
I. Review of carbonyl chemistry

A. Distinct carbonyl components of relevance in Biology

Acyl oxidation states: amides (not shown and discussed in detail in Lectures on protein structure and in Problem Set 3), acids, oxygen esters and thioesters. Nature predominantly uses thioesters in primary metabolism.

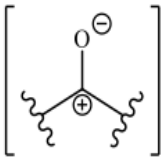


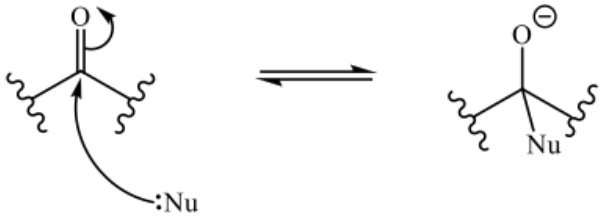
carbonyl group



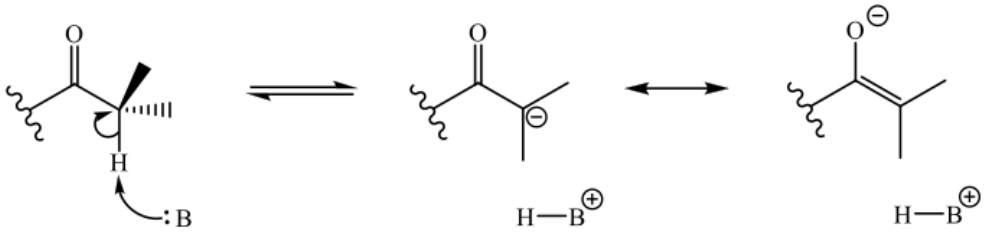
aldehydes and ketones (ex. Sugars)

B. Carbonyl compounds undergo two major reactions of biological importance:

1. Carbonyl addition reactions are prevalent where Nu is a nucleophile and the carbonyl (C=O) is the electrophile.  is an important resonance structure for the carbonyl, providing a basis for the interaction with the Nu. Carbonyl addition to simple aldehydes and ketones is reversible.

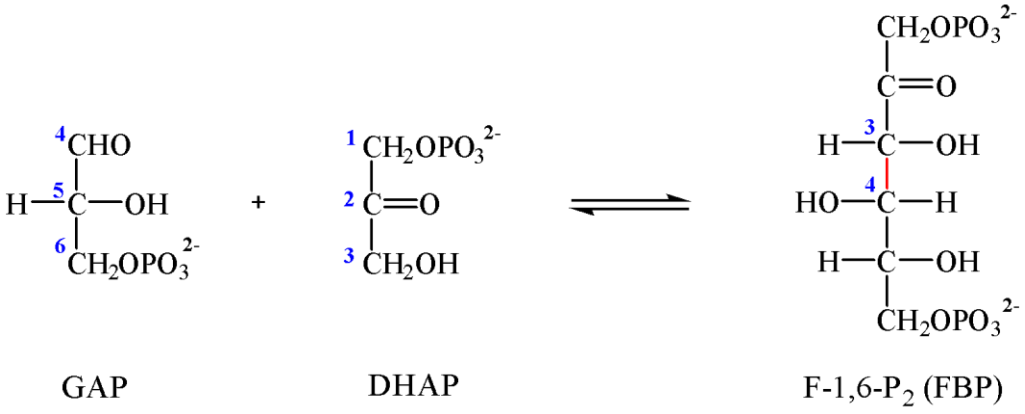


2. Carbonyls with hydrogens at the C_α position to the carbonyl can undergo enolization. The two structures on the right below show a resonance stabilized enolate anion. If the oxygen of the enolate is protonated, the resulting compound becomes an enol.



These two reactions provide the basis for many of the reactions in glycolysis / gluconeogenesis / pentose phosphate pathway.

II. One major C-C bond forming and breaking reaction that involves carbonyl chemistry is the aldol reaction. This reaction plays a major role in biosynthesis of (anabolism) and breakdown of (catabolism) carbon scaffolds. All aldol reactions are reversible and involve the formation or cleavage of a carbon-carbon bond between an aldehyde and a ketone. An example in primary metabolism that occurs in the glycolytic pathway is shown below. The enzyme is fructose-1,6-P_2 (FBP) aldolase.

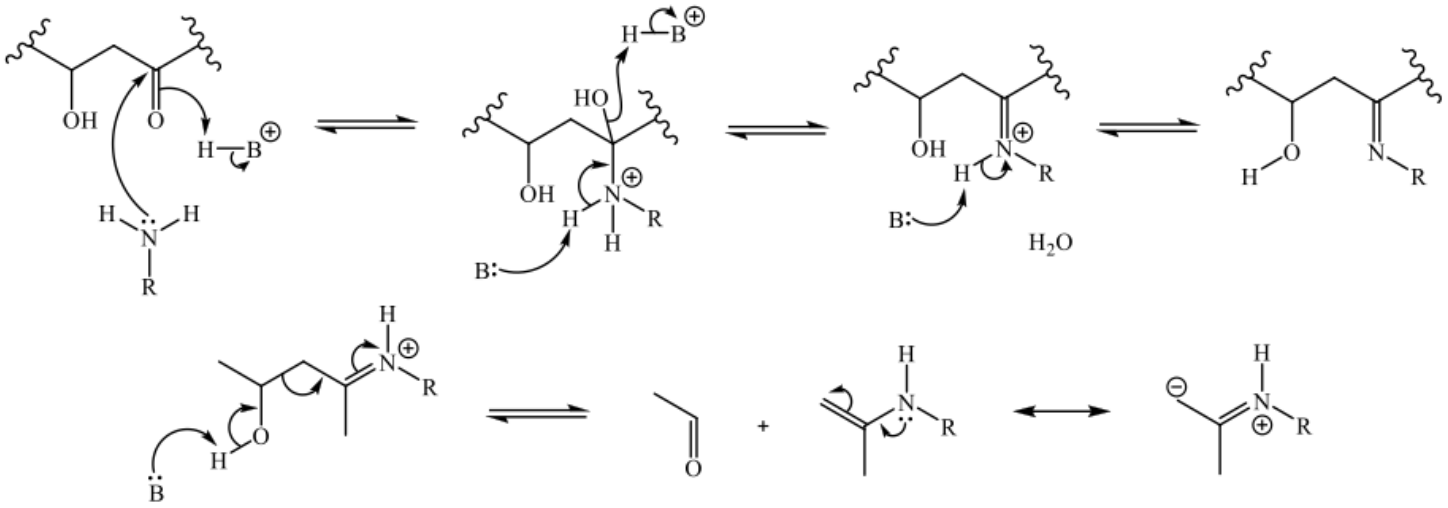


GAP DHAP F-1,6-P_2 (FBP)

A. Generalizations can be made about aldol reactions.

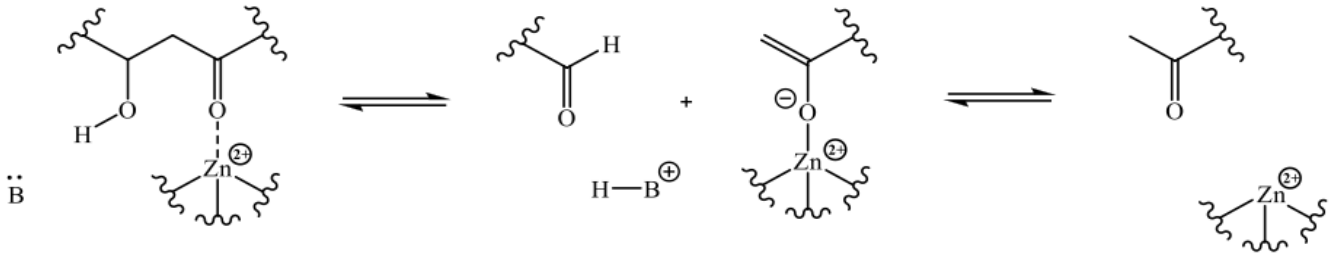
1. All aldol reactions are **reversible**.
2. Stereochemistry: all aldol reactions occur with retention of configuration.
3. Enzymes that catalyze aldol reactions use one of two mechanistic strategies to achieve the dramatic rate accelerations relative to non-enzymatic reactions. In the class I aldolases, an imine (C=N, also called a Schiffs base) is involved to activate the carbonyl. In the class II aldolases a Lewis acid cofactor (Zn^{2+}) is utilized for the same purpose.

B. The mechanism of the class I aldolase is shown below. It uses the ε NH_2 group of lysine in the active site, an example of **covalent catalysis**. The first step in the reaction involves nucleophilic attack by the lysine (NOTE K must be deprotonated----think about pKa of lysine's ε NH_2 group and how one might perturb it to so that the amine is an excellent nucleophile). It attacks the carbonyl to form a carbinolamine (an unstable tetrahedral intermediate), that then loses water in a **general acid catalyzed (GAC)** reaction to form the protonated imine. The imine N is more basic than carbonyl oxygens and can readily be protonated under physiological conditions (pK_as range from 7-9). The iminium cation is a good electron sink and lowers the



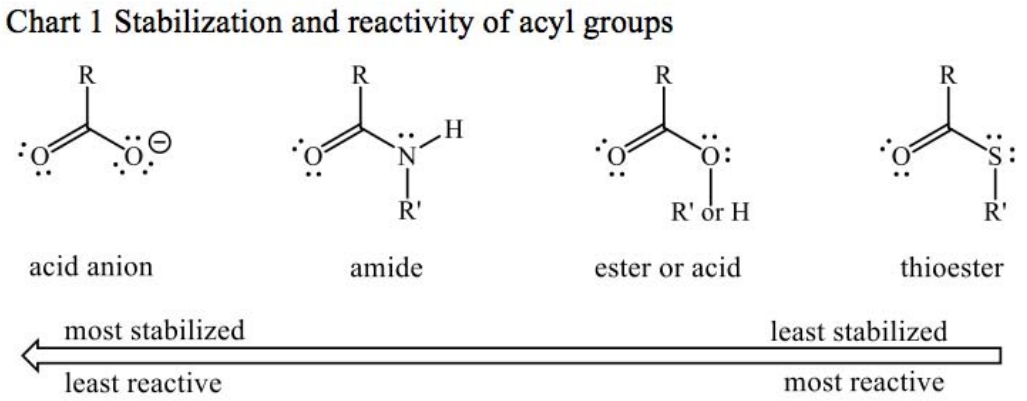
energetic barrier for C-C bond cleavage.

The class II enzymes use another commonly used strategy by chemists: a metal ion functioning as a Lewis acid. In this case the carbonyl polarization is facilitated by coordination to Zn^{2+} and activates C-C bond cleavage.



Aldolase reactions will be encountered in glycolysis/gluconeogenesis and the pentose phosphate pathway.

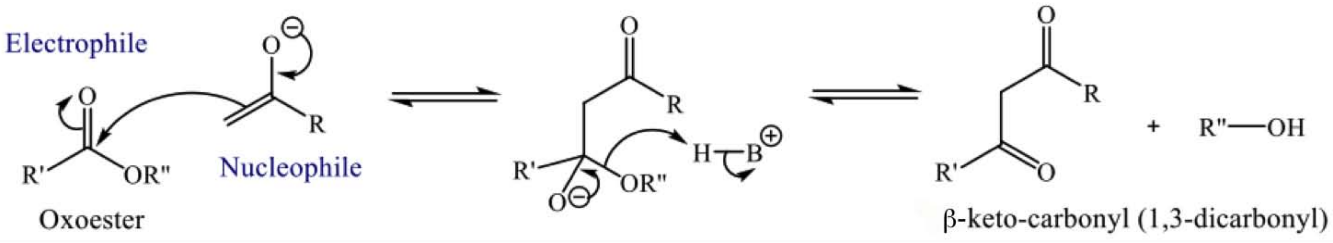
Example: fructose-1,6-P_2 aldolase. You will also see this type of reaction in Problem Set 4.



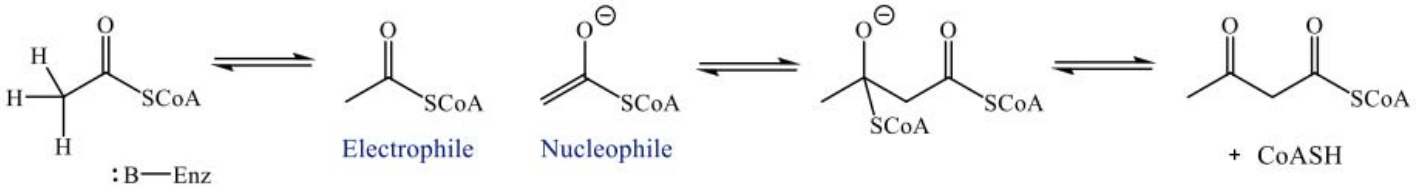
III. The second type of carbon-carbon bond forming reaction is the Claisen reaction. This type of reaction usually occurs in Biology between two thioesters or a ketone and a thioester.

A. This type of reaction involves acyl transfer.

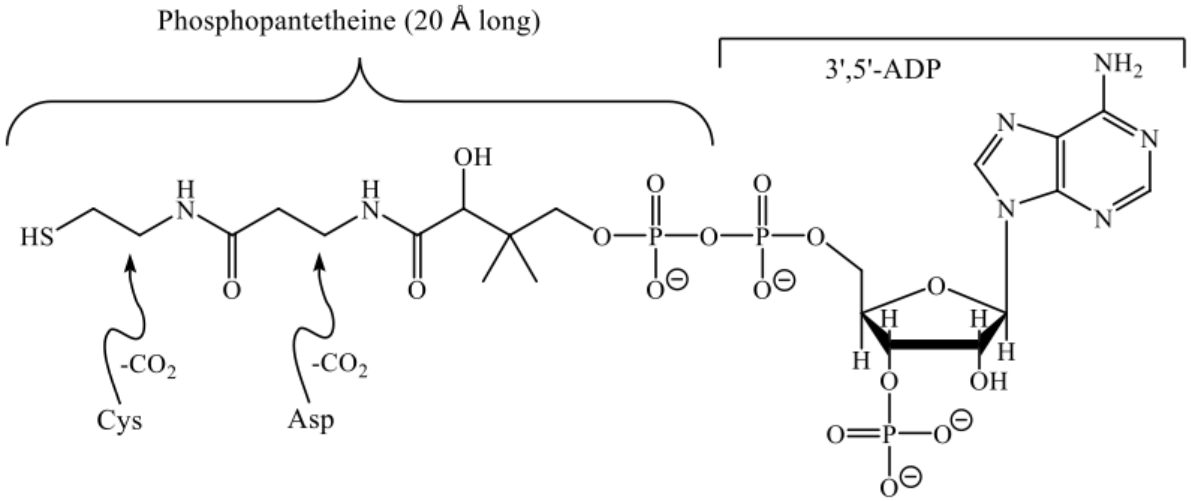
The acyl series of functional groups are ranked from left to right in order of decreasing resonance stabilization and hence increasing reactivity. [Think about the amide resonance structures and their importance in the allowed hierarchy of protein structures]. Shown below is the reaction of a ketone and an oxygen ester (a typical organic Claisen reaction). In the Claisen reaction, in contrast with the aldol reaction, the tetrahedral addition product is not stable and loss of a thiolate (alcohol below) occurs.



An example of a biological Claisen reaction is shown below using acetylCoA as the substrate (see below the structure of coenzyme A (CoA)). AcetylCoA is one of the metabolites that plays a central role in primary metabolism. From a chemical (reactivity) perspective this entire coenzymeA moiety could be replaced with a SCH_3 group. Thus the complexity of this molecule must be related to specificity.



where the structure of CoASH is given below



B. Generalizations about the mechanism of Claisen reactions in biology:

1. All reactions go through a carbanion intermediate.
2. Stereochemistry: all reactions go with inversion of configuration.

Why has nature chosen to use thioesters over oxygen esters? AcetylCoA plays two roles in the reactions shown above. The thioester is activated for nucleophilic attack and the C_α C-H bond is cleaved to generate an enolate. The difference in chemical reactivity for both of these reactions, when compared to an oxygen ester, is apparent when one considers that "S" of the thioester is much more carbon-like than "O". Thus in the oxygen ester, the non-bonded electrons associated with "O" can delocalize with the π electrons of the carbonyl of the oxygen ester. This delocalization is energetically unfavorable with the "S" of the thiol ester. The pKa of the H attached to C_α of the thioester is estimated to be 18, closer to that of acetone (a ketone), while the pKa of the H attached to C_α of the oxygen ester is closer to 22. The differences in these pKas can also be rationalized by resonance structures.

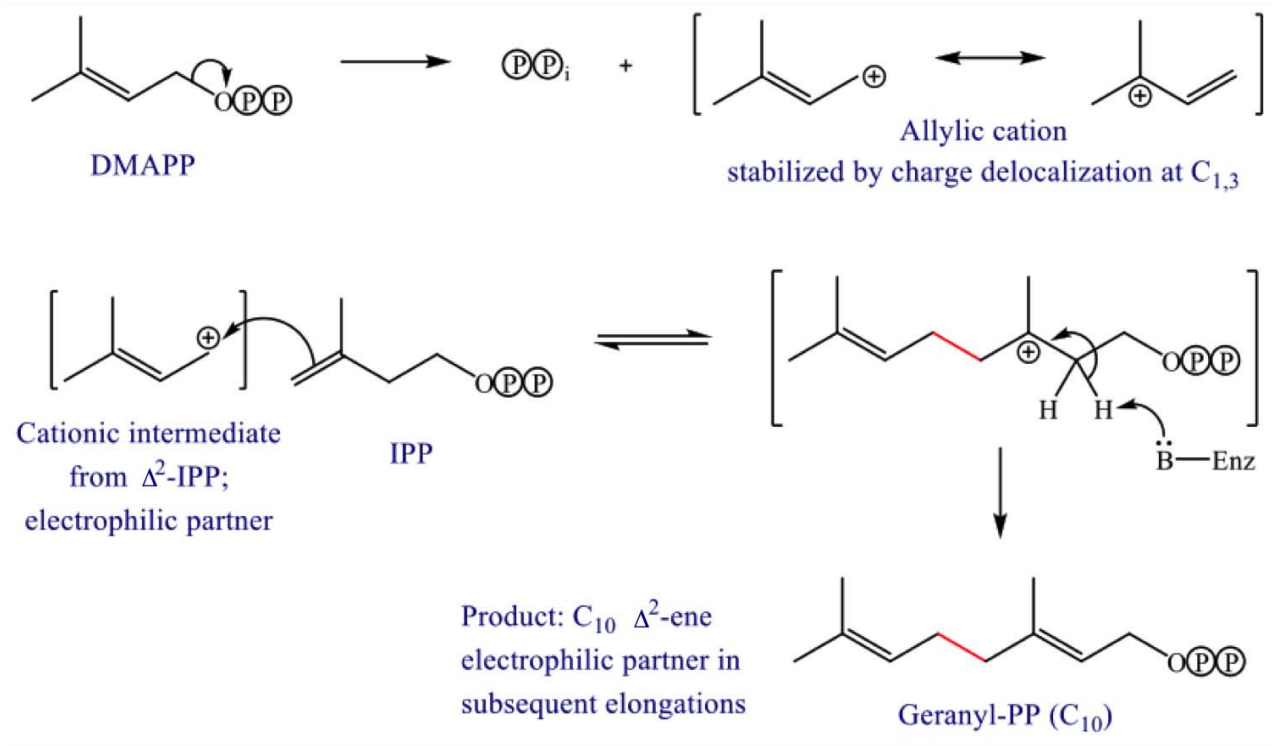
Finally the K_{eq} for hydrolysis of acetylCoA to acetate and CoA is 10^5, reflecting thermodynamic activation. The thioester is kinetically stable and thermodynamically labile, as you will see with ATP in the next lecture. The reactions with acetylCoA play a central role in fatty acid biosynthesis and degradation.

IV. You will cover Prenyl Transfer reactions in Chemistry 5.08 and are NOT RESPONSIBLE for this section in Chem. 5.07. It is here as an introduction to prenyl transfer.

Carbon-carbon bond forming reactions can also occur by prenyl transfer reactions, that is via transfer of a 5 carbon unit (C-5) all at once. This type of reaction is used in cholesterol and sterol biosynthesis (see lanosterol cyclase in Lecture 6) and these five carbon units represent the building blocks for an estimated 30,000 natural products (terpenes). The two key players in these types of reactions are isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). All of the reactions require Mg^{2+} that binds to the pyrophosphate moiety to neutralize the charge, converting the pyrophosphate moiety into a good leaving group (inorganic pyrophosphate, $Mg^{2+}\bullet PP_i$).

A. Generalizations about mechanism:

1. These reactions involve carbocation intermediates and are one of the few examples of an SN1 reaction used in Biology.
2. In SN1 reactions in solution the intermediate carbocation, if it has three different substituents gives a racemate on reaction with a nucleophile. The Nu can attack from the top face or bottom face of the planar cation. In enzymatic systems, the reaction can proceed with either inversion or retention of stereochemistry. The steric constraints in the chiral active site of the enzyme, dictate the stereochemistry observed.
3. In general, the PP moiety of IPP or DMAPP is complexed with Mg^{2+}



The new ten carbon species (C-10) is the precursor to Vitamin D, Vitamin E, Vitamin K, steroids, cholesterol and rubber. These fat-soluble vitamins are discussed in Chem. 5.08.

SUMMARY: The three general ways to form C-C bonds are aldol and claisen reactions that utilize carbanion intermediates and prenyl transfer reactions (C5 transfers) that involve carbocation intermediates.

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