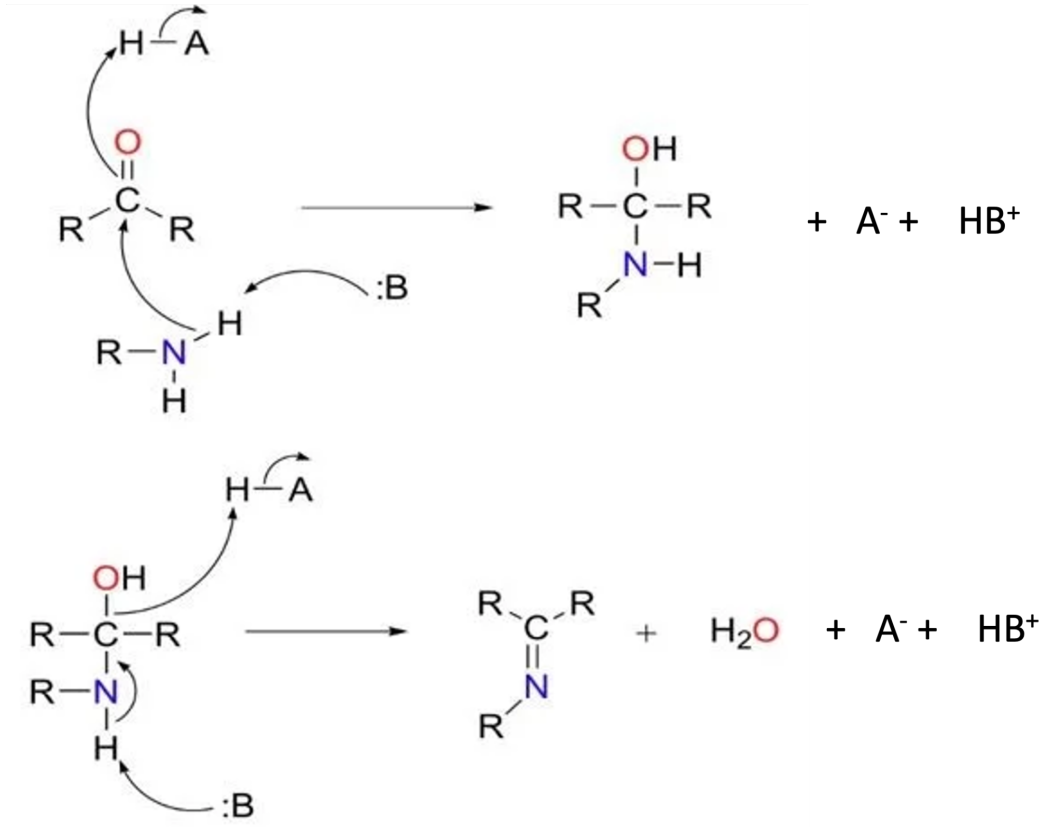
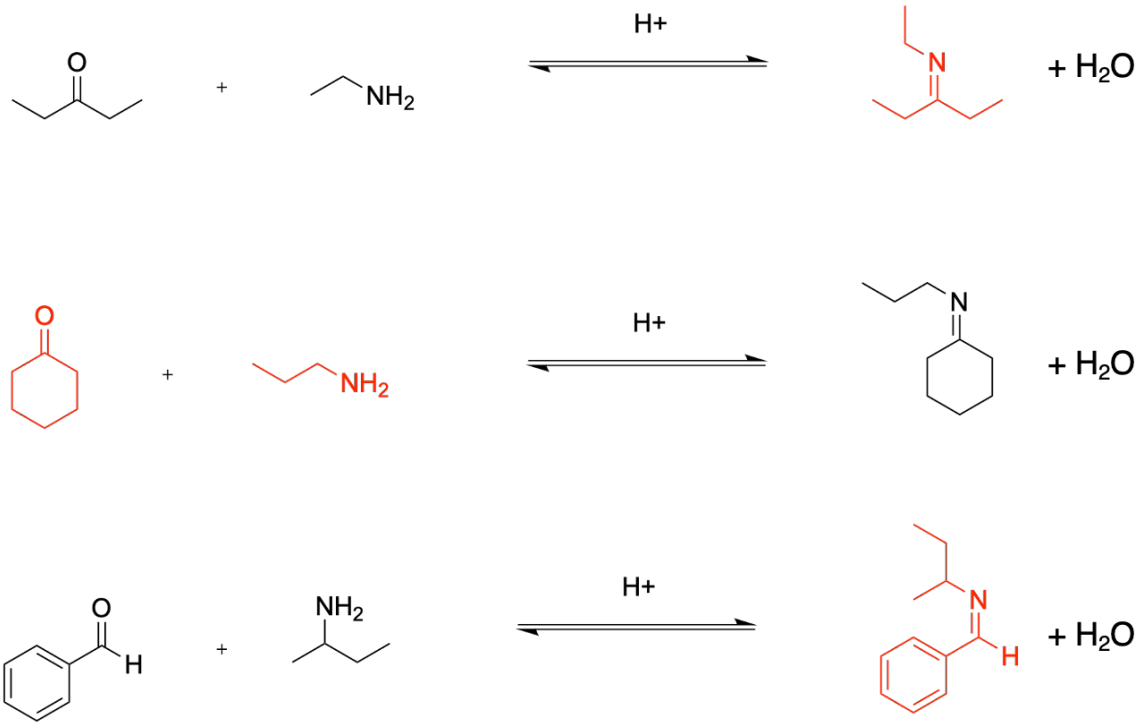
**10.1 Imine formation**

a) Draw the enzymatic mechanism of imine formation. Which step is different to the formation of acetal/ketals



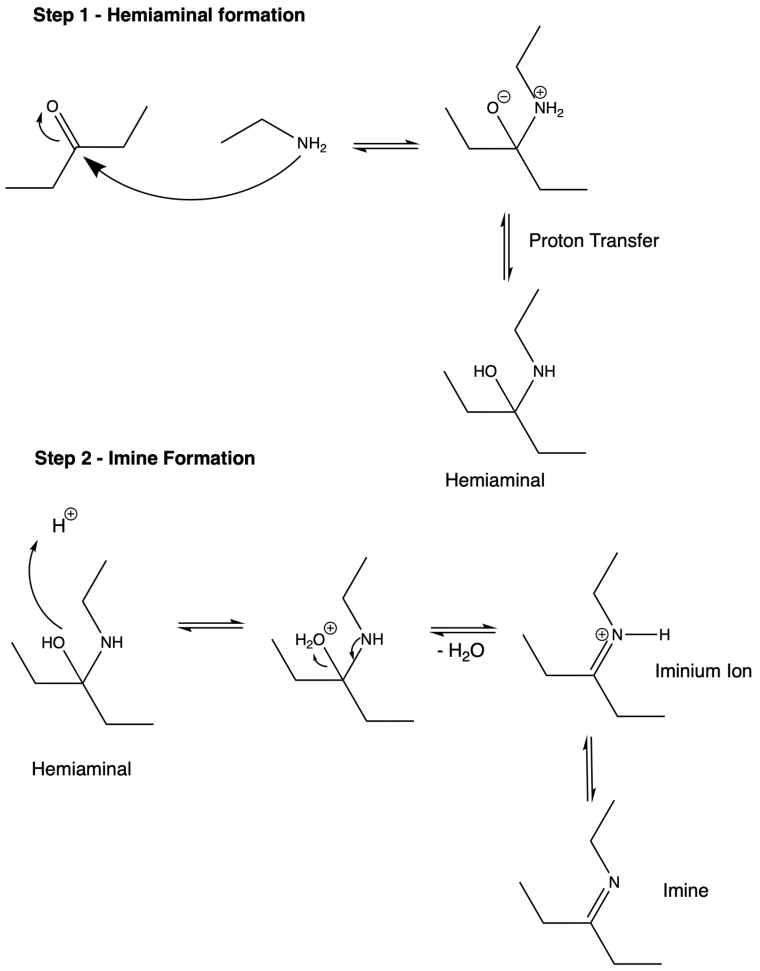

Step 1 is the same compared formation of acetal/ketals from aldehydes/ketons (formation of hemiaminal or hemiacetal/ketal respectively). However in step 2, where for acetal/ketals another addition would happen, the nitrogen lone pair electrons 'push' the oxygen off of the carbon, forming a C=N double bond (an iminium) and a displaced water molecule.

b) Fill in the blanks in the reactions below. Explain which reaction step requires acid-catalysis during imine formation.




Note: As imines have a pKa of approximately 7 at physiological pH, they can be accurately drawn as either protonated (iminium ion) or neutral (imine).

Take the first reaction as an example for imine formation:



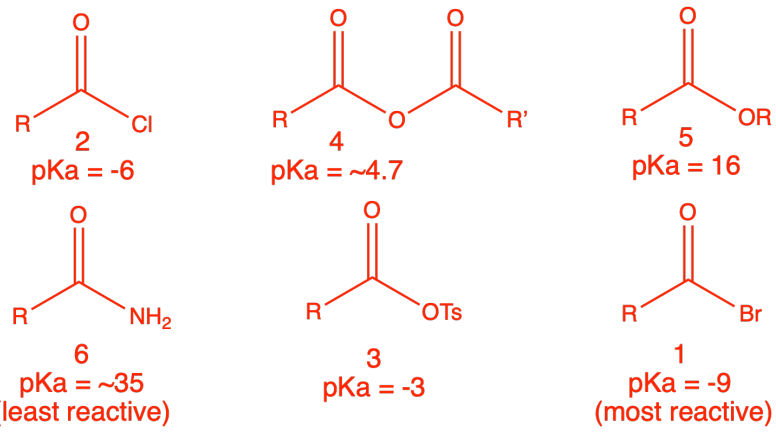

Only the second reaction step requires acid catalyses. In the first step, acid would protonate the amine and remove it from the equilibrium and hence slow this step down.
**Detailed**: Imine formation involves the addition of carbonyl compounds to a primary amine. It begins with the generation of carbinolamine through a series of steps involving an initial nucleophilic attack and then several proton transfer reactions. The second part includes the elimination of water, as a leaving group, to give the imine. Imines are formed under mildly acidic conditions. A pH of 4.5 is ideal for the reaction.

If the pH is low or the solution is too acidic, the reaction slows down in the first step—this is when the lone pair on the nitrogen atom of the amine attacks the carbonyl carbon of the substrate. A low pH indicates a high concentration of the protonated form of amine. The protonated amine cannot function as a nucleophile, slowing down this step's rate.

If the pH is high or the solution is too basic, the fourth step of the reaction mechanism is affected. This is when the hydroxyl group of the carbinolamine is protonated to generate the leaving group, water. Highly basic conditions inhibit the process of water elimination by making the protonated amine, the proton donor in this step, less available for the reaction.

**10.2 Nucleophilic acyl substitutions: Reactivity**

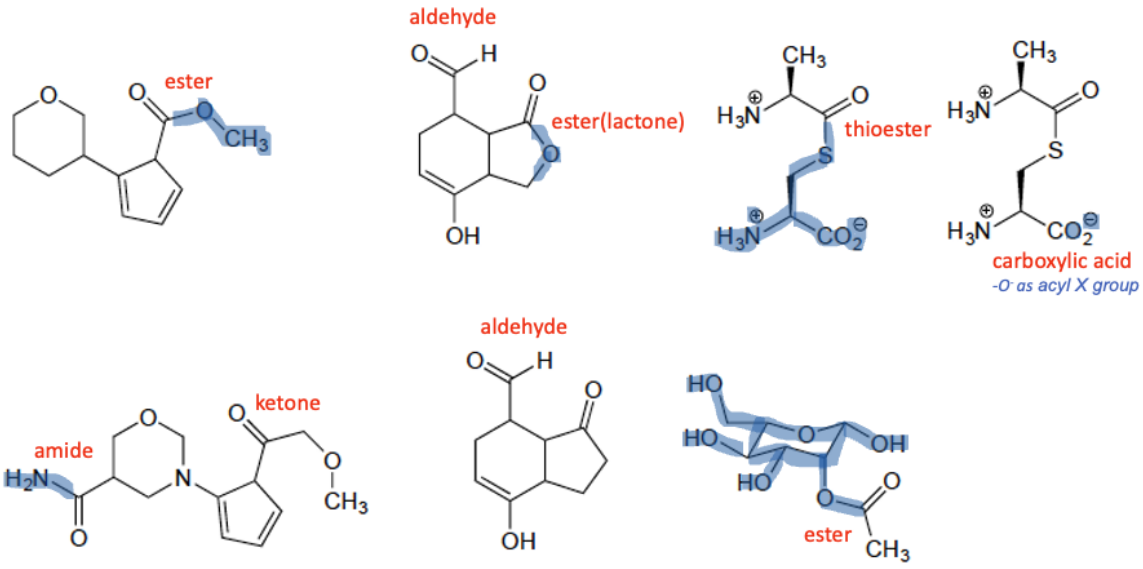
Rank the following carboxylic acid derivatives by their reactivity with the nucleophile water and hypothesize about the required reaction conditions.




In general, carboxylic acid derivatives are more reactive when they carry better leaving groups. In general, weaker bases are better leaving groups. Hence, the lower the pKa of the conjugate acid of the leaving group, the better the leaving group and the more reactive the carboxylic acid derivative (see above for the ordering and pKa values of the conjugate acids). The conditions necessary for successful reaction vary drastically and illustrate the differences in reactivity. Acyl chlorides react violently with water, while amides need refluxing with 10% NaOH or concentrated HCl in a sealed tube at 100 °C overnight.

**10.3 Nucleophilic acyl substitutions: Carboxylic acid derivatives**

a) Name all aldehydes, ketones and carboxylic acid derivative groups in the molecules below and indicate which part is the “acyl X group” (the acyl X group corresponds to the leaving group in an acyl substitution).

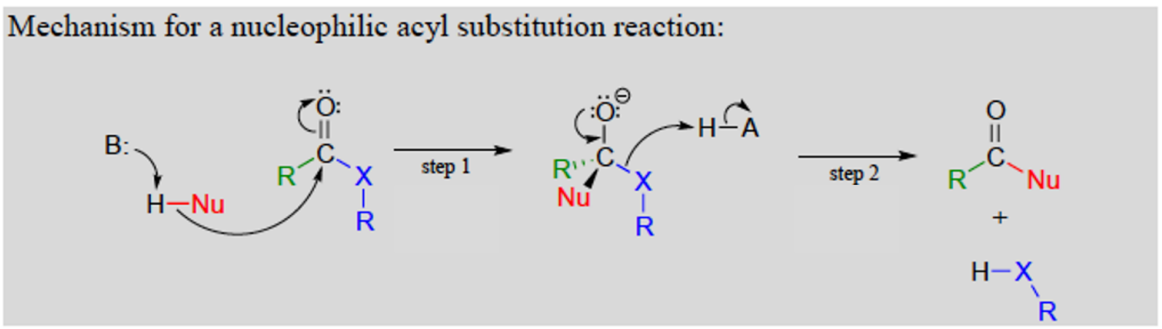



All acyl X groups are marked in blue.

For reference see Organic Chemistry With a Biological Emphasis Tim Soderberg Chapter 11, p 96: Carboxylic acid derivatives can be distinguished from aldehydes and ketones by the presence of a group containing an electronegative heteroatom - usually oxygen, nitrogen, or sulfur – bonded directly to the carbonyl carbon. You can think of a carboxylic acid derivative as having two sides. One side is the acyl group, which is the carbonyl plus the attached alkyl (R) group. On the other side is the heteroatom-linked group: in this text, we will sometimes refer to this component as the 'acyl X' group (this, however, is not a standard term in organic chemistry, mostly it is just referred to as a leaving group).

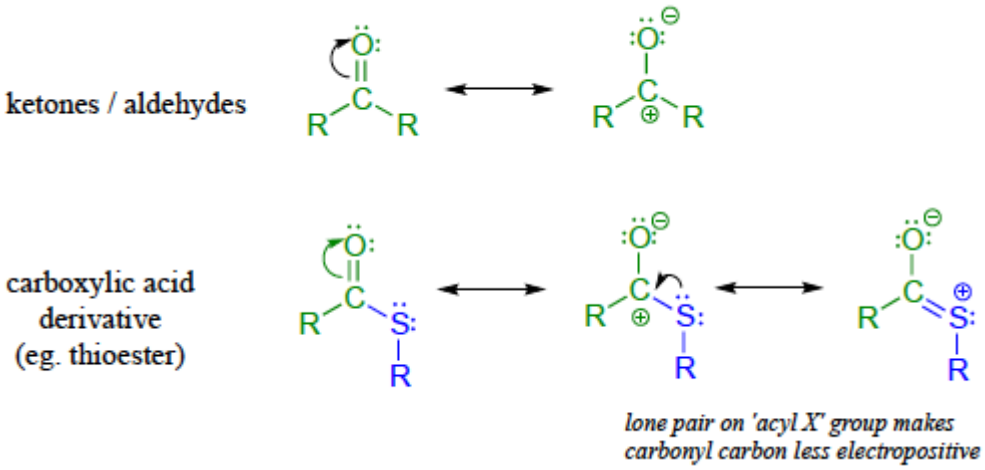
**10.4 Nucleophilic acyl substitutions: Carboxylic acid derivatives**

a) Draw the 2 steps of a nucleophilic substitution to a carbonyl. Use arrows to indicate the movement of electrons.



b) The first mechanistic step is the same as in nucleophilic additions to carbonyls. Explain why this step is faster for aldehydes and ketones compared to carboxylic acid derivatives.

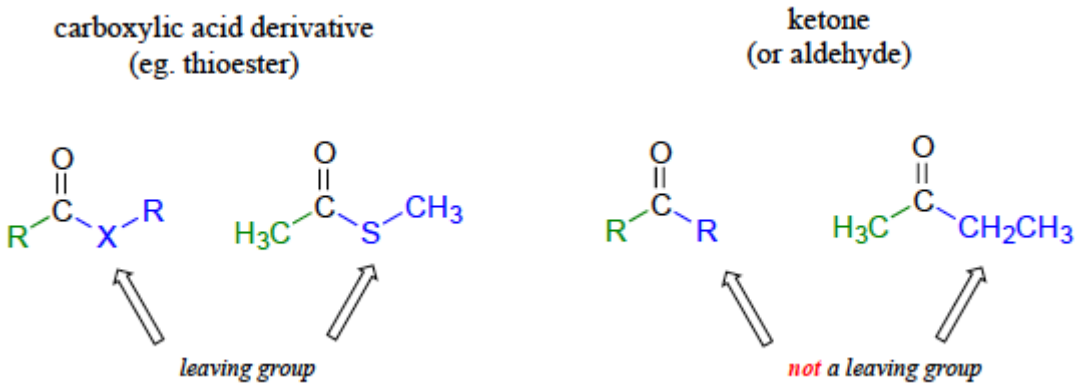
The rate of the first mechanistic step is mainly affected by how well the partial positive charge on the carbonyl carbon is stabilized. Stabilization reduces the electrophilic character of the carbonyl carbon by reducing its partial positive charge which reduces the rate of this step. The Y group heteroatom's ability to stabilize a carbonyl by donating electrons through resonance makes most carboxylic acid derivatives less reactive compared to aldehydes or ketones.




(see Organic Chemistry With a Biological Emphasis Tim Soderberg Chapter 11, p 101).

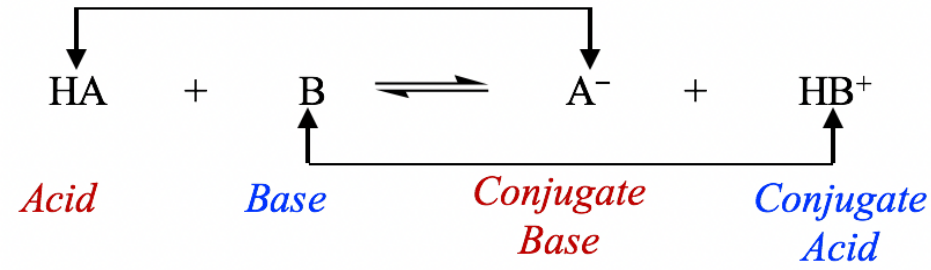
c) Explain why carboxylic acid derivatives undergo nucleophilic acyl substitutions and aldehyde and ketones do not.

The carbonyl carbon of aldehydes and ketones do not contain suitable leaving groups (R- or H- are too unstable). Aldehydes and ketones tend to undergo **nucleophilic addition** to form a tetrahedral alkoxide intermediate. Once formed, the negative charge of the alkoxide intermediate cannot be transferred to a suitable leaving group, instead protonation converts it into an alcohol (see Organic Chemistry With a Biological Emphasis Tim Soderberg Chapter 11, p 99).

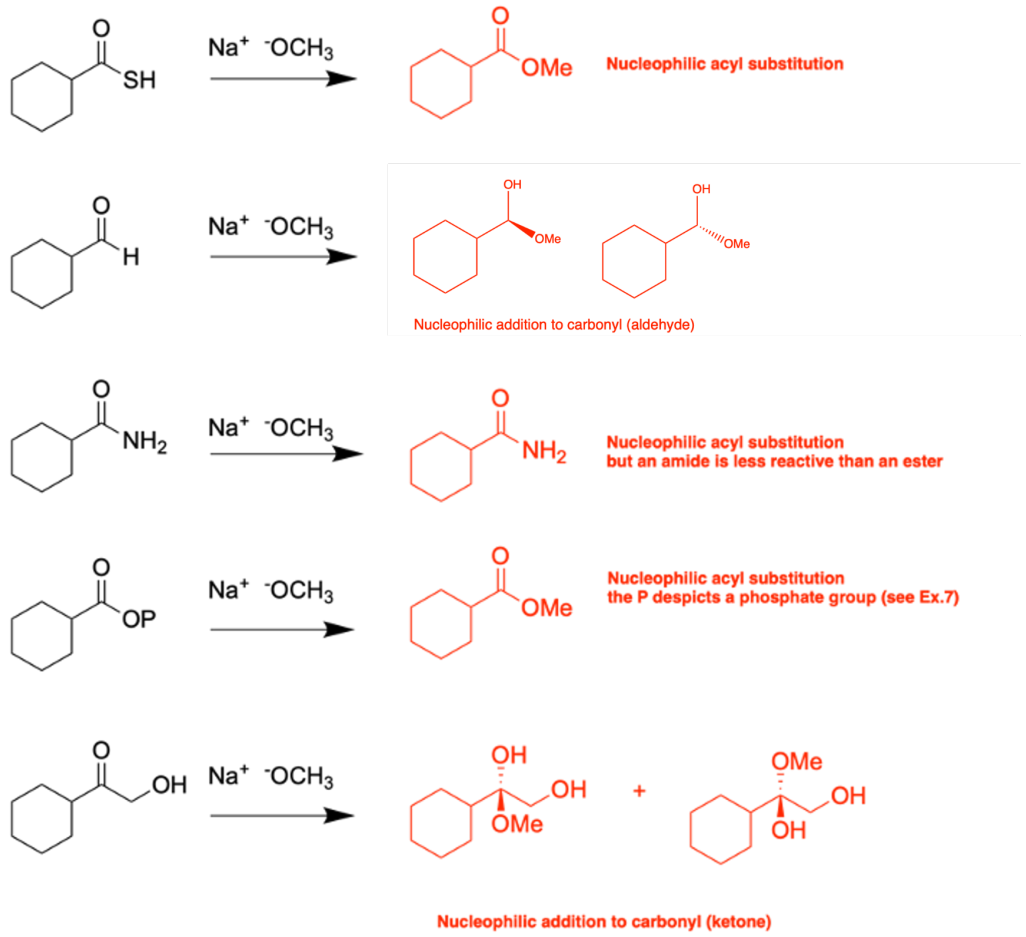



d) Repetition question/Important to understand concept: How does the strength of a leaving group (Y) correspond to the stability of the negative charge, basicity and the pKa of its corresponding conjugate acid (HY)?

The better the negative charge is stabilized on the leaving group, the weaker it is as a base (A-), the lower is the pKa of its corresponding conjugate acid (HA).

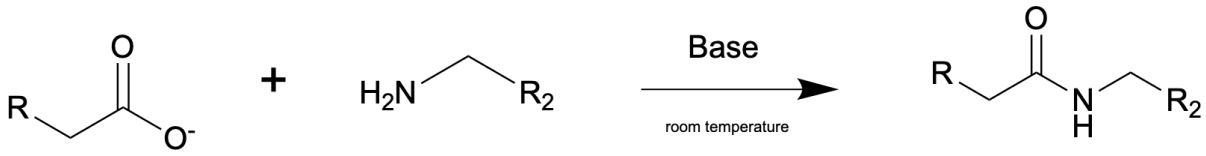


**10.5 Draw the products of following reactions**




**10.6 Activation of Carboxylic acid derivatives**

a) Why does the following reaction not happen spontaneously?

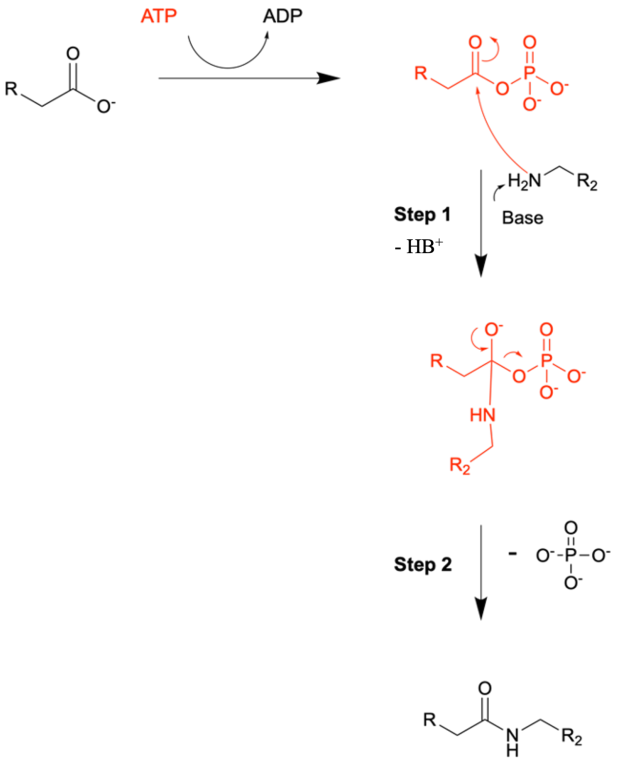



This reaction won't happen spontaneously as the carboxylate is a highly unreactive carboxylic acid derivative (O-, is no leaving group, as the additional negative charge would be very badly stabilized). Formation of an amide bond would therefore require energy (uphill reaction).

b) How, then, does a living system accomplish a reaction such as the one shown above? To what other, highly reactive carboxylic acid derivative are carboxylates mostly converted to in biological systems in order to become activated?

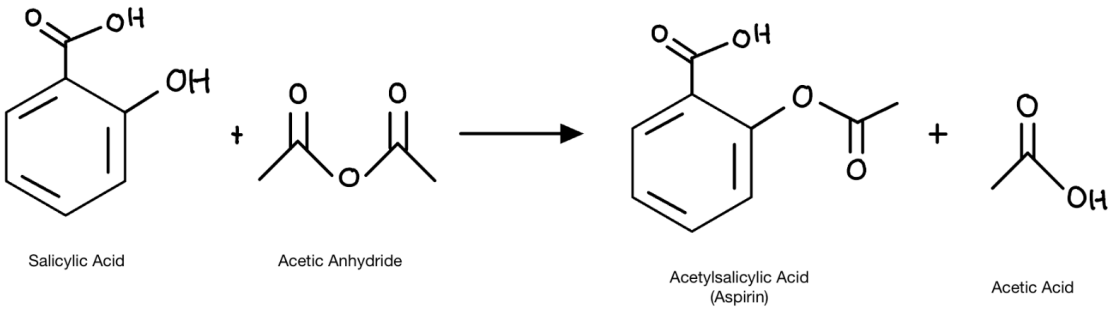
In biochemistry, all thermodynamically unfavorable “uphill” reactions are linked to an energy-releasing, 'downhill' reaction. In many cases this linked reaction that pays for the uphill reaction is hydrolysis of ATP, where enzymes activate the carboxylate by converting it to an **acyl phosphate,** while ATP gets converted to ADP.

c) Fill in the blank in following reaction scheme



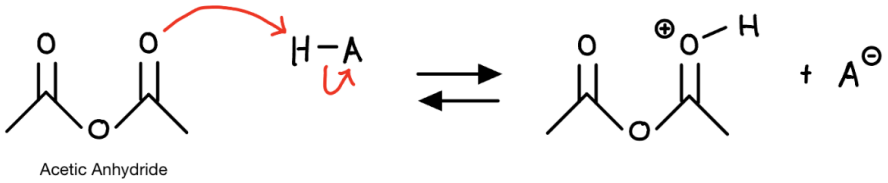
**10.7 Aspirin Synthesis**

The commercial preparation of Aspirin involves the following reaction of a benzene derivative with an anhydride (under acidic cat.). Draw a complete mechanism for this reaction:

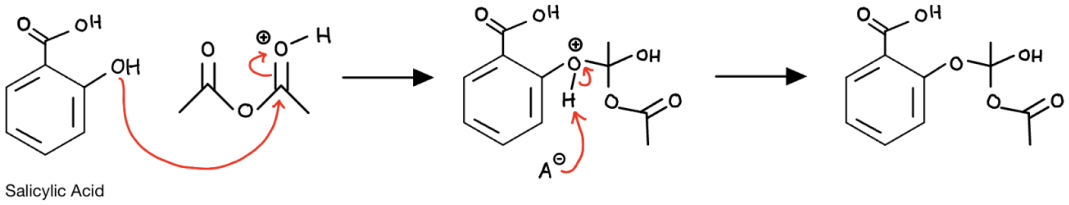



Mechanism:

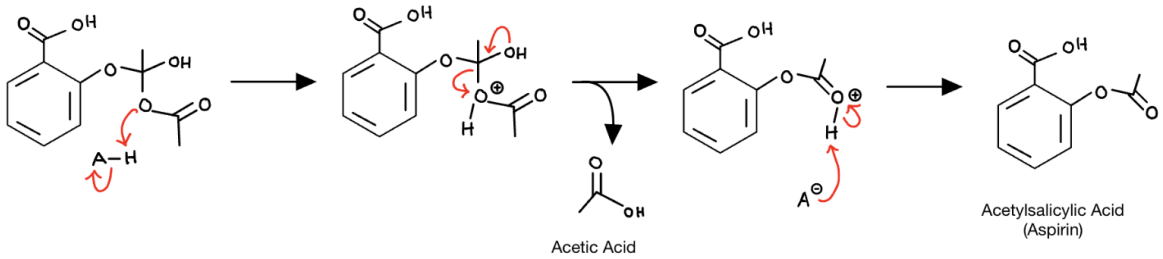
**1. Activation of Acetic Anhydride**: Acetic anhydride is activated in the presence of an acid catalyst, protonating the carbonyl oxygen and making it more electrophilic.




**2. Nucleophilic attack by Salicylic Acid**: the hydroxyl group on the benzene of salicylic acid acts as a nucleophile and attacks the electrophilic carbon of the acetic anhydride. This forms a tetrahedral intermediate



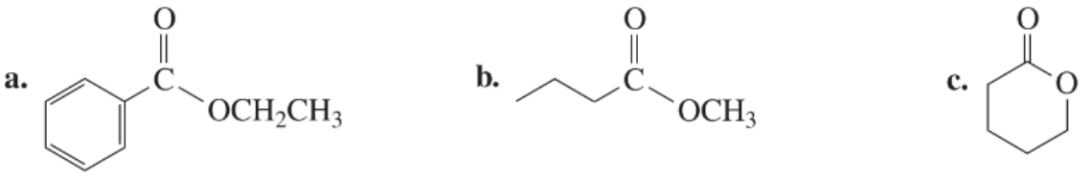

**3. Formation of Acetylsalicylic acid**: the tetrahedral intermediate collapses expelling acetic acid as a leaving group and forming aspirin.



**10.8 Ester Hydrolysis**

You have learned about ester hydrolysis under basic conditons. Try to reason from your previous experience of how acids react with carbonyl groups how hydrolysis works under acidic conditions.

a) What products are formed from an acid-catalyzed hydrolysis of the following esters.




Hydrolysis products:

a.

Benzoic acid ($C_6H_5COOH$)

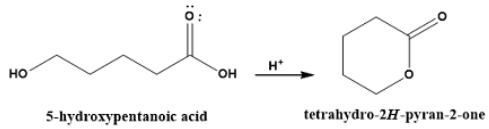
Ethanol ($CH_3CH_2OH$)

b.

Butanoic acid ($CH_3CH_2CH_2COOH$)

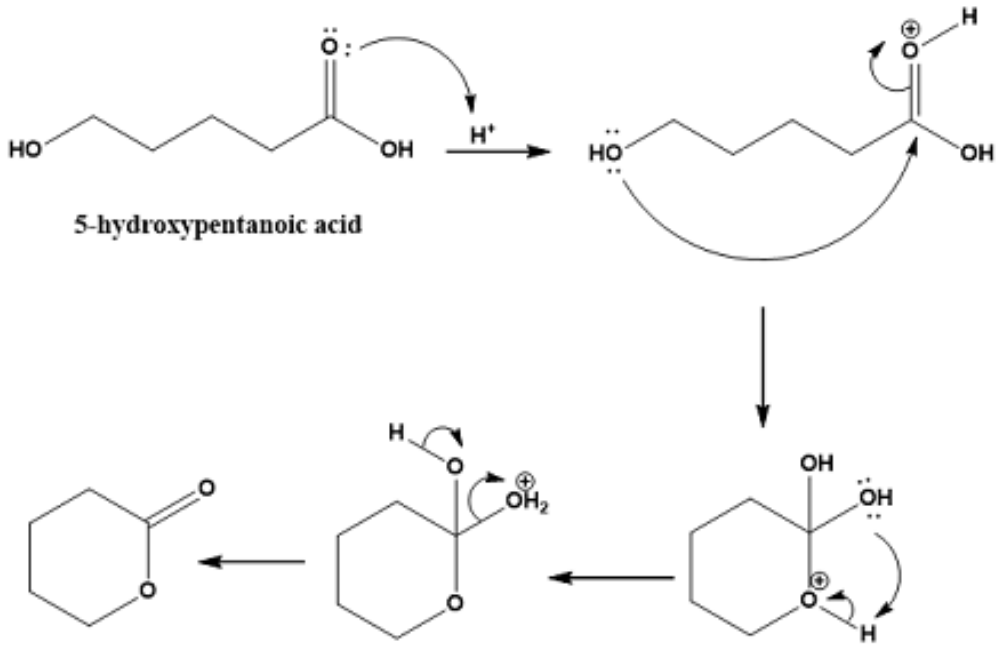
Methanol ($CH_3OH$)

c.




a) Show the mechanism of the ester-hydrolysis under acidic conditions for c.

Ester hydrolysis just reverse of ester formation:



Mechanism: