Nucleophilic Carbon in Biochemistry

Barry Linkletter

Department of Chemistry, University of Prince Edward Island

Abstract

This document is intended to review some the role of carbon as a nucleophile in organic chemistry. Examples will be taken from reactions that result in the same products as biochemical pathways. It is assumed that the reader has some familiarity with organic chemistry and acid/base equilibria.[1]

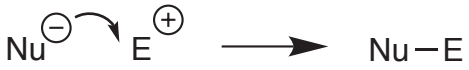
Contents

[1] This document was typeset using the LaTeX typesetting language. The LyX document processor was used to generate the LaTeX code. See "*Tutorial #26: Introducing LaTeX*" for more details. Chemical structures were created with ChemDraw 11.0 from CambridgeSoft and exported as EPS files for inclusion in this document.

1 The Basics

1.1 Electrophiles and Nucleophiles

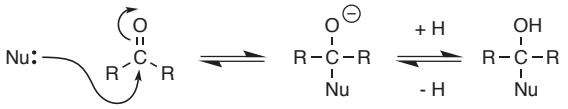
A nucleophile is an electron rich group that can donate electrons into an electrophile. An electrophile is an electron poor group that can accept electrons from a nucleophile. In a reaction that forms a covalent bond between two atoms, one atom is the nucleophile and one is the electrophile. They always come as a pair in a bond forming reaction.



1.2 Addition Reactions

A common electrophile in biochemical reactions is the carbonyl group. The carbon is electron poor due to the high electronegativity of oxygen. Electron rich nucleophiles can attach this carbon.

In an addition reaction, a nucleophile attacks the electrophilic carbon of a carbonyl group to give an alcohol product.

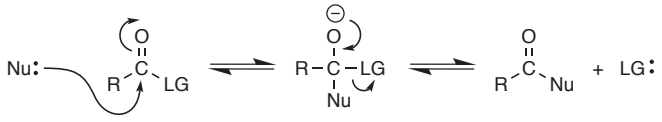


An addition reaction is a way to bond a nucleophile to a carbon atom if an alcohol product is the goal. If the nucleophile is also a carbon atom we can use an addition reaction to create a carbon-carbon bond. A common reaction of this type is the Aldol reaction.

There are many other addition reactions other than addition to a carbonyl group but we will only consider carbonyl groups in this document.

1.3 Addition/Elimination Reactions

If one of the "R" groups in the above addition reaction is a leaving group it may eliminate to give a carbonyl group. This addition of a nucleophile followed by elimination of a leaving group is another great way to form a bond between a nucleophile and a carbonyl group.



Common leaving groups are oxygen or sulfur atoms with low pK_a values (weakly to strongly acidic groups). Under exceptional circumstances a leaving group can be a very weak acid.

A common addition/elimination reaction is ester hydrolysis. If the nucleophile is a carbon and the leaving group an oxygen we would have a Claisen reaction.

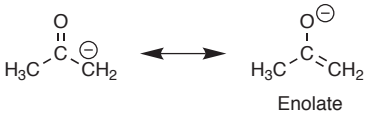
2 Nucleophilic Carbon

A carbon nucleophile in organic chemistry is an electron rich alkyl group. The electrons can donate to an electrophile to create a new carbon-carbon bond. An example of a nucleophilic carbon group would be the anion generated from deprotonating a carbon group.

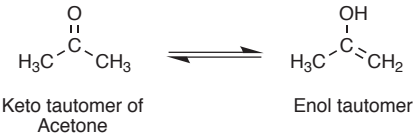
Regular alkyl groups are very, very, very difficult to deprotonate. The anion created is very, very high in energy and a very, very strong base is required. There are very few bases that are stronger than an alkyl carbon anion so these anions are usually generated using organometalic reactions and will not be discussed here.

Acidic carbon groups are carbon atoms that are next to an electron-withdrawing group that can stabilize the anion created by deprotonation. An example is a carbon group next to a carbonyl group, e.g. acetone. Anions can be generated easily by mixing acidic carbon groups with strong bases.

These anions are called enolate anions because of the enolate resonance contributor.



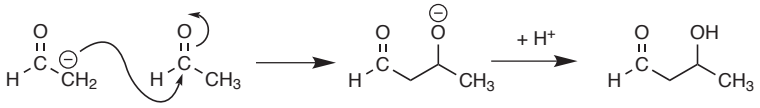
Enols are weaker nucleophiles but are more commonly used in biological systems because strong bases are not required to create them. Enols are tautomers (isomers that differ only in the arrangement of protons) of keto groups.



Enolization of a keto group requires that an acid protonate the oxygen of the carbonyl and a base accept the proton from the alkyl group. Most keto groups are far more stable in the keto form compared to the enol form. The enol tautomer will exist as only a small proportion of the total population. We can get around this limitation using imine chemistry, which we will discus later.

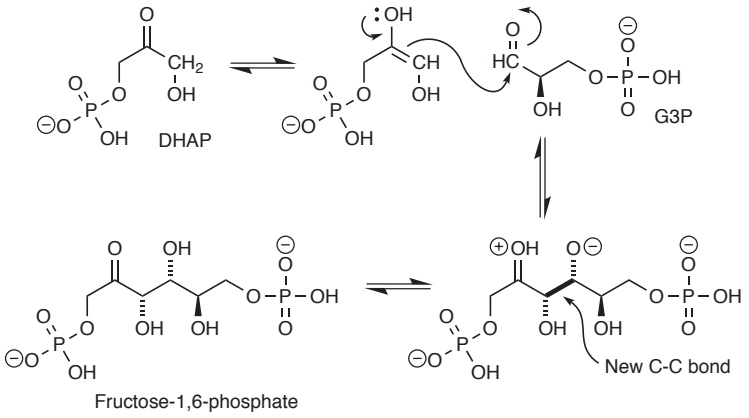
3 The Aldol Reaction

An aldol reaction is when a carbon nucleophile attacks an aldehyde group to give an alcohol product.The classic aldol reaction is the self condensation of acetaldehyde in basic water solution.



An aldol reaction that we will become familiar with is the aldolase reaction. In the gluconeogenesis pathway, the addition of the enol tautomer of dihydroxyacetone phosphate (DHAP) onto the carbonyl group of glyceraldehydes-3-phosphate (G3P) is catalyzed by the enzyme enolase. The uncatalyzed reaction could use an enol as the nucleophile. This reaction would be very slow.

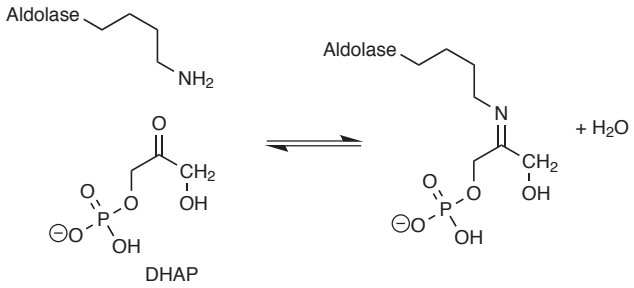
The reaction is slow because an enol is not a strong nucleophile and only a small amount of the enol tautomer will exist in equilibrium with the acetone.



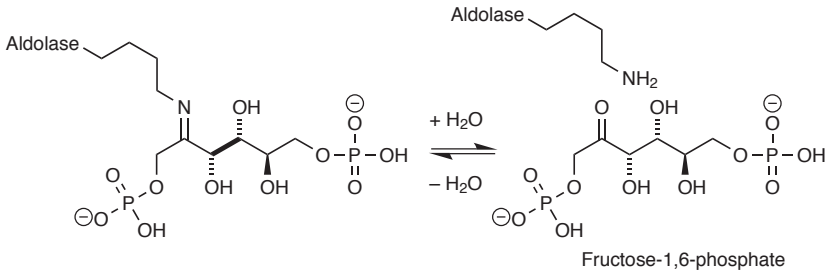
3.1 Imine catalysis

The enzyme *aldolase* greatly accelerates this reaction using covalent catalysis. A group on the enzyme becomes covalently bonded to the substrate in the course

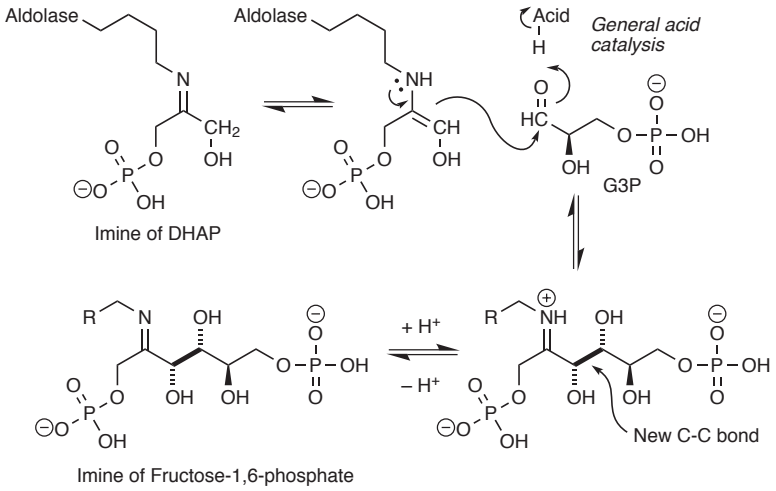
of the reaction. As a result, a different mechanism is available that is faster than the enol mechanism. This new route to product involves the formation of an imine.



In *aldolase*, a lysine residue condenses with the DHAP to form an imine. The nitrogen atom is not as electronegative as oxygen, so the eneamine (the imine version of an enol) tautomer will be more electron-rich and a better nucleophile. The reaction can then proceed in a manner similar to the enol mechanism to give the imine of fructose-1,6-bisphosphate. An acidic group in the enzyme assists with general acid catalysis.



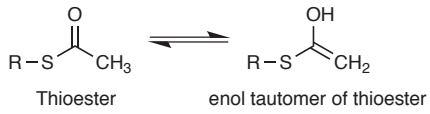
The imine is as easily hydrolyzed as it is formed and the fructose product is released from the enzyme.



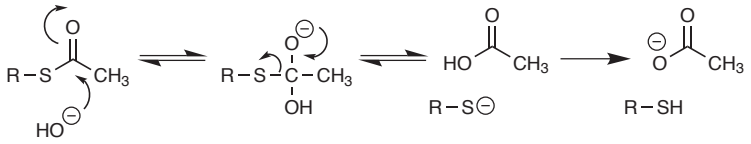
The mechanism for the analogous reaction in the glycolysis pathway is the reverse of the steps outlined above. The enzyme *aldolase* catalyzes the reaction that results in the equilibrium between fructose-1,6-bisphosphate and DHAP & G3P.

3.2 Thioester catalysis

Thioesters are similar to esters but the larger orbitals of a sulfur atom do not interact with carbon orbitals as well as the orbitals of an oxygen atom. So the electrons are not delocalized into the sulfur to the extant that they are in an ester. As a result, the enols of thioesters are more nucleophilic than those of esters.



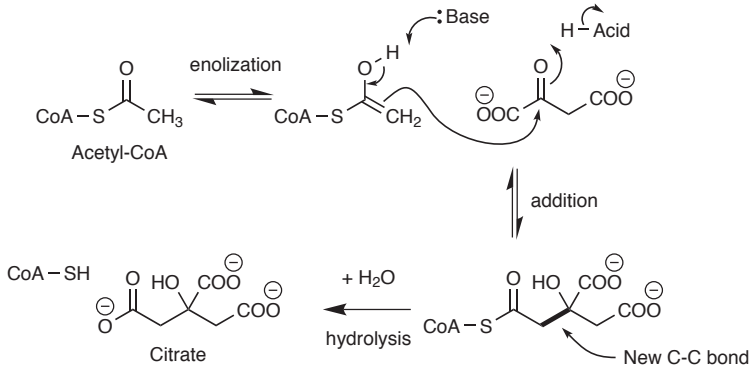
Thioesters are also easily hydrolyzed to give a thiol and a carboxylic acid.



As you can see below, reactions using activated thioesters as nucleophiles will give a carboxylic acid as a final product. The synthesis of thioesters is not

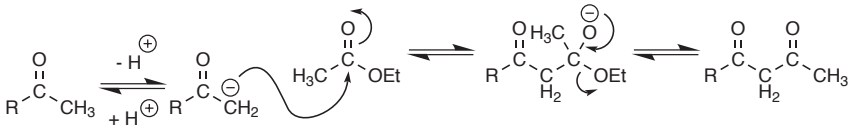
covered in this document. Read about pyruvate oxidation for an example of this synthesis (acetyl-CoA synthesis).

In the enzyme *citrate synthase,* we see an example a thioester catalyzed nucleophilic addition. The thioester is acetyl-CoA and the electrophile is the keto group of oxaloacetate. The aldol addition product is an alcohol and the thioester hydrolysis results in a carboxylic acid. Additionally, the addition is catalyzed by general acid/base catalysis. You will see this type of chemistry used by the enzyme *citrate synthase* and many others.



4 Claisen Reactions

In a Claisen reaction a nucleophile attacks a carbonyl group and the alcoxide intermediate eliminates a leaving group to give a new carbonyl compound with the nucleophile replacing the leaving group. In a Claisen reaction, the nucleophile is a carbon group and the leaving group is an oxygen group.

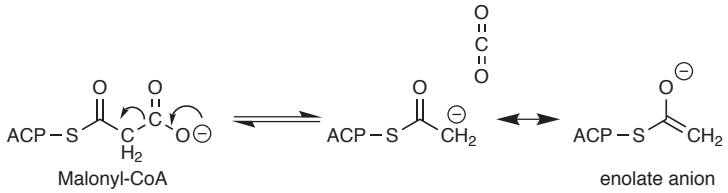


We see addition/elimination reactions with carbon nucleophiles in many cases in biochemistry. A classic case is the formation of carbon-carbon bonds in fatty acid biosynthesis.

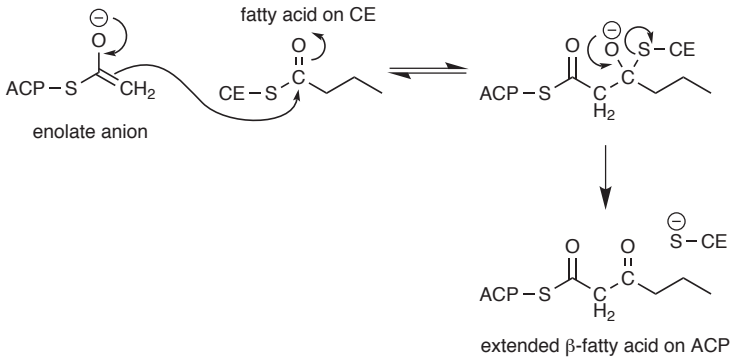
4.1 Claisen-like reactions in fatty acid synthesis

Another way to create nucleophilic carbon is to have a carboxylate group on the carbon atom next to a keto group. This is called an α-carboxylate because the carboxylate is on the α-carbon (the carbon next to a carbonyl group). The

carboxylate group is easily eliminated as carbon dioxide and leaves behind a nucleophilic enolate anion.



We see this type of reaction is the Acyl-malonyl condensing enzyme component of fatty acid synthase. In this reaction the α-carboxylate on a malonyl-CoA is eliminated to give a nucleophilic carbon. The resulting anion is stabilized by resonance with its enolate form. The sulfur atom does not delocalize the charge because of poor orbital overlap and so enolates of thioesters are better nucleophiles than enolates of esters.



Removal of carbon dioxide by respiration will ensure that the reaction is unidirectional. Now we have a nucleophile that can attack an electrophile. In this case, the electrophile is a carbonyl group with a leaving group. The leaving group is the sulfur group of the cysteine residue of the condensing enzyme domain in *FA synthase* (see your biochemistry textbook). In Claisen reactions the leaving group is oxygen but in this case the sulfur atom of the cysteine residue of the Acyl-malonyl condensing enzyme (CE) is the leaving group.

The product has a new carbon-carbon bond between the two carbon unit derived from malonyl-ACP and the growing fatty acyl group. The product is a β-keto acyl group on the ACP component of FA synthase. A reduction reaction, an elimination reaction and another reduction reaction will result in a fatty acyl group ready to be transferred to CE and then extended again. See your textbook for more on FA synthesis.

5 Summary

We have seen that a carbon nucleophile can add to a carbon electrophile to create a new carbon-carbon bond. This type of reaction is essential if living things are to synthesize complicated carbon compounds from simple building blocks. A common method used to join two carbon building blocks is the aldol reaction. We have seen how this reaction can be accomplished in living systems using imine groups of thioesters to generate nucleophilic carbon atoms and using carbonyl groups as electrophiles.

6 Further Reading

I suggest that you read the following sections of your textbook from organic chemistry and the textbook for this course.

Organic chemistry textbook.

- Nucleophilic carbon
- Aldol reaction
- Claisen reaction

Biochemistry Textbook.

- Aldol reaction and the cleavage of fructose-1-phosphate by *aldolase*
- Aldol reaction and *transketolase*
- Aldol reaction and *citrate synthase*
- Addition/elimination reactions and fatty acid synthesis