In this section, we will look at two more common biochemical reactions that proceed through enolate intermediates. In a typical **conjugate addition**, a nucleophile and a proton are 'added' to the two carbons of an alkene which is conjugated to a carbonyl (i.e. in the \(\alpha-\beta\) position). An \(\beta\)-elimination step, the reverse process occurs:

\[
\text{Nu-H} + \text{R} \xrightarrow{\text{conjugate addition}} \text{R} \xrightarrow{\beta\text{-elimination}} \text{Nu-O}
\]

In [chapter 9](#) we learned about nucleophilic carbonyl addition reactions, including the formation of hemiacetals, hemiketals, and imines. In all of these reactions, a nucleophile directly attacks a carbonyl carbon.

If, however, the electrophilic carbonyl is \(\beta\)-unsaturated - if, in other words, it contains a double bond conjugated to the carbonyl - a different reaction pathway is possible. A resonance structure can be drawn in which the \(\beta\)-carbon has a positive charge, meaning that the \(\beta\)-carbon also has the potential to be an electrophilic target.

If a nucleophile attacks at the \(\beta\)-carbon, an enol or enolate intermediate results (step 1 below). In many cases this intermediate collapses and the \(\alpha\)-carbon is protonated (step 2). This type of reaction is known as a **conjugate addition**.

Mechanism of a conjugate addition reaction
The reverse of a conjugate addition is a \(\text{\(\beta\)}\)-elimination, and is referred to mechanistically the abbreviation \(\text{E1cb}\).

**Mechanism of an E1cB elimination**

![Mechanism Diagram](image)

The \(\text{E}\) stands for 'elimination'; the numeral 1 refers to the fact that, like the \(\text{S_N1}\) mechanism, it is a stepwise reaction with first order kinetics. "\(\text{cB}\)" designation refers to the intermediate, which is the conjugate base of the starting compound. In step 1, an \(\text{\(\alpha\)}\)-carbon is deprotonated to produce an enolate, just like in aldol and Claisen reactions we have already seen. In step 2, the excess electron density on the enolate expels a leaving group at the \(\text{\(\beta\)}\) position (designated 'X' in the figure above). Notice that the \(\text{\(\alpha\)}\) and \(\text{\(\beta\)}\) carbons change from \(\text{sp^3}\) to \(\text{sp^2}\) hybridization with the formation of a conjugated double bond.

(In chapter 14 we will learn about alternate mechanisms for alkene addition and \(\text{\(\beta\)}\)-elimination reactions in which there is not an adjacent carbonyl (or imine) group, and in which the key intermediate species is a resonance-stabilized carbocation.)

**Step II of fatty acid degradation is a conjugate addition of water, or hydration.**

![Fatty Acid Degradation Diagram](image)

Note the specific stereochemical outcome: in the active site, the nucleophilic water is bound behind the plane of the conjugated system (as drawn in the figure above), and the result is \(\text{\(S\)}\) configuration in the \(\text{\(\beta\)}\)-hydroxy thioester product.

In step III of the fatty acid synthesis cycle we saw an \(\text{E1cb}\) \(\text{\(\beta\)}\)-elimination of water (dehydration):

![Fatty Acid Synthesis Cycle Diagram](image)

Notice that the stereochemistry at the \(\text{\(\beta\)}\)-carbon of the starting alcohol is \(\text{R}\), whereas the hydration pathway (step II) reaction in the fatty acid degradation cycle pathway results in the \(\text{\(S\)}\) stereoisomer. These two reactions are not the reverse of one another!

Here are two more examples of \(\text{\(\beta\)}\)-elimination reactions, with phosphate and ammonium respectively, as leaving groups. The first, 3-dehydroquinate synthase (EC 4.2.3.4) is part of the biosynthesis of aromatic amino acids, the
second, aspartate ammonia lyase (EC 4.3.1.1) is part of amino acid catabolism.

Exercise $\PageIndex{1}$

In the glycolysis pathway, the enzyme 'enolase' (EC 4.2.1.11) catalyzes the dehydration of 2-phosphoglycerate. Predict the product of this enzymatic step.

Exercise $\PageIndex{2}$

N-ethylmaleimide (NEM) is an irreversible inhibitor of many enzymes that contain active site cysteine residues. Inactivation occurs through conjugate addition of cysteine to NEM: show the structure of the labeled residue. (Michael addition)

Exercise $\PageIndex{3}$
Argininosuccinate lyase (4.3.2.1), an enzyme in the metabolic pathway that serves to eliminate nitrogen from your body in the form of urea in urine, catalyzes this $\beta$-elimination step:

Propose a complete mechanism.

**Hint**

Don't be intimidated by the size or complexity of the substrate - review the $\beta$-elimination mechanism, then identify the leaving group and breaking bond, the $\alpha$-carbon which loses a proton, the carbonyl that serves to stabilize the negatively-charged (enolate) intermediate, and the double bond that forms as a result of the elimination. You may want to designate an appropriate 'R' group to reduce the amount of drawing.

**Contributors**