



zero length cross linker

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Definition

Zero length cross linker are chemical reagents that covalently link two amino acids within a protein or between different proteins, without introducing any additional length or atoms between the linked residues



Types of zero length cross linker(ZLCL)

1-Peptide-Based

ZLCLs composed of short peptide sequences that form covalent bonds with specific protein targets.

2-Organic ZLCLs

Small organic molecules with reactive groups that can form covalent bonds with protein amino acids.

3-Metal-Based

ZLCLs containing metal ions that coordinate with specific amino acid side chains, forming stable complexes

Advantages of Zero-Length Crosslinking

Precise Localization

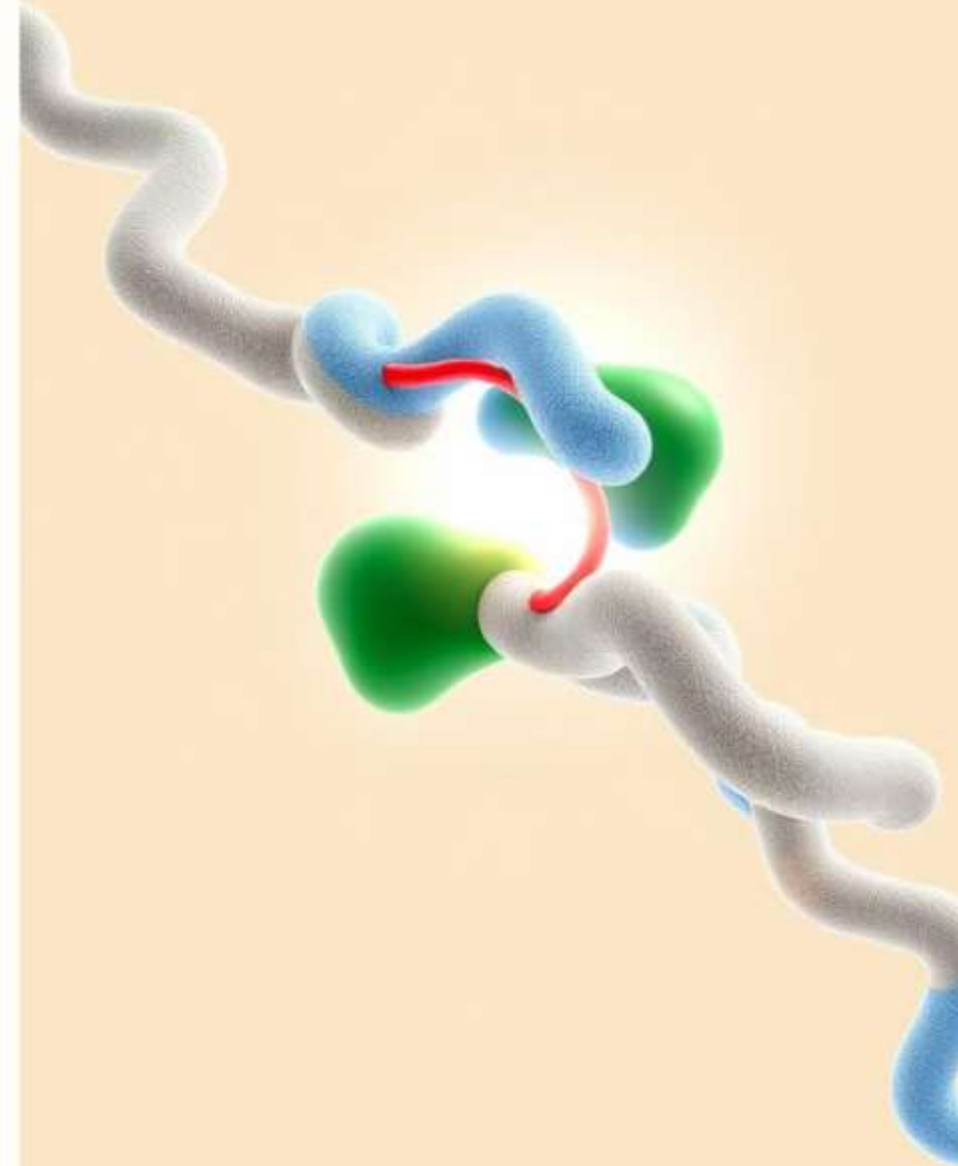
Zero-length crosslinkers pinpoint interaction sites with high accuracy.

Minimal Interference

They don't disrupt protein structure or function, yielding reliable results.

Versatile Applications

Zero-length crosslinking is suitable for a broad range of proteins and complexes.



Disadvantages of Zero-Length Crosslinking

1-Limitations

While offering advantages, zero-length crosslinking can have limitations for specific applications.

2-Accessibility

The accessibility of lysine residues in the protein can affect the efficiency of the reaction.

3-Specificity

Ensuring specificity for desired lysine residues can be challenging in complex protein mixtures.

Applications of the Zero-Length Crosslinking Technique

This technique is used to analyze protein interactions, study protein function, and develop new biomaterials, antibodies, and vaccines.



The Two-Step Zero-Length Crosslinking Procedure



Step 1: Activation

The first step involves activating one protein component using a reagent like EDC and NHS.

Step 2: Coupling

The activated protein is then reacted with the second protein component, forming a stable covalent bond between the two proteins.



The reagents can initiate the formation of three types of bonds:

An amide linkage made by the condensation of a primary amine with a carboxylic acid,

a phosphoramidate linkage made by the reaction of an organic phosphate group with a primary amine,

and a secondary or tertiary amine linkage made by the reductive amination of a primary or secondary amine with an aldehyde group.

Therefore, using these reagent systems, substances containing amines can be conjugated with other molecules containing phosphates or carboxylate.

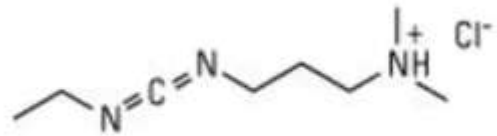
1. Carbodiimides

Carbodiimides are used to mediate the formation of amide linkages between carboxylates and amines or phosphoramidate linkages between phosphates and amines.

There are two basic types of carbodiimides: **water-soluble** and **water-insoluble**.

1. EDC (or EDAC; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl)

EDC is water-soluble, which allows for its direct addition to a reaction without prior organic solvent dissolution.



EDC
1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide • HCl
MW 191.70
Spacer Arm 0.0 Å



1. Carbodiimides

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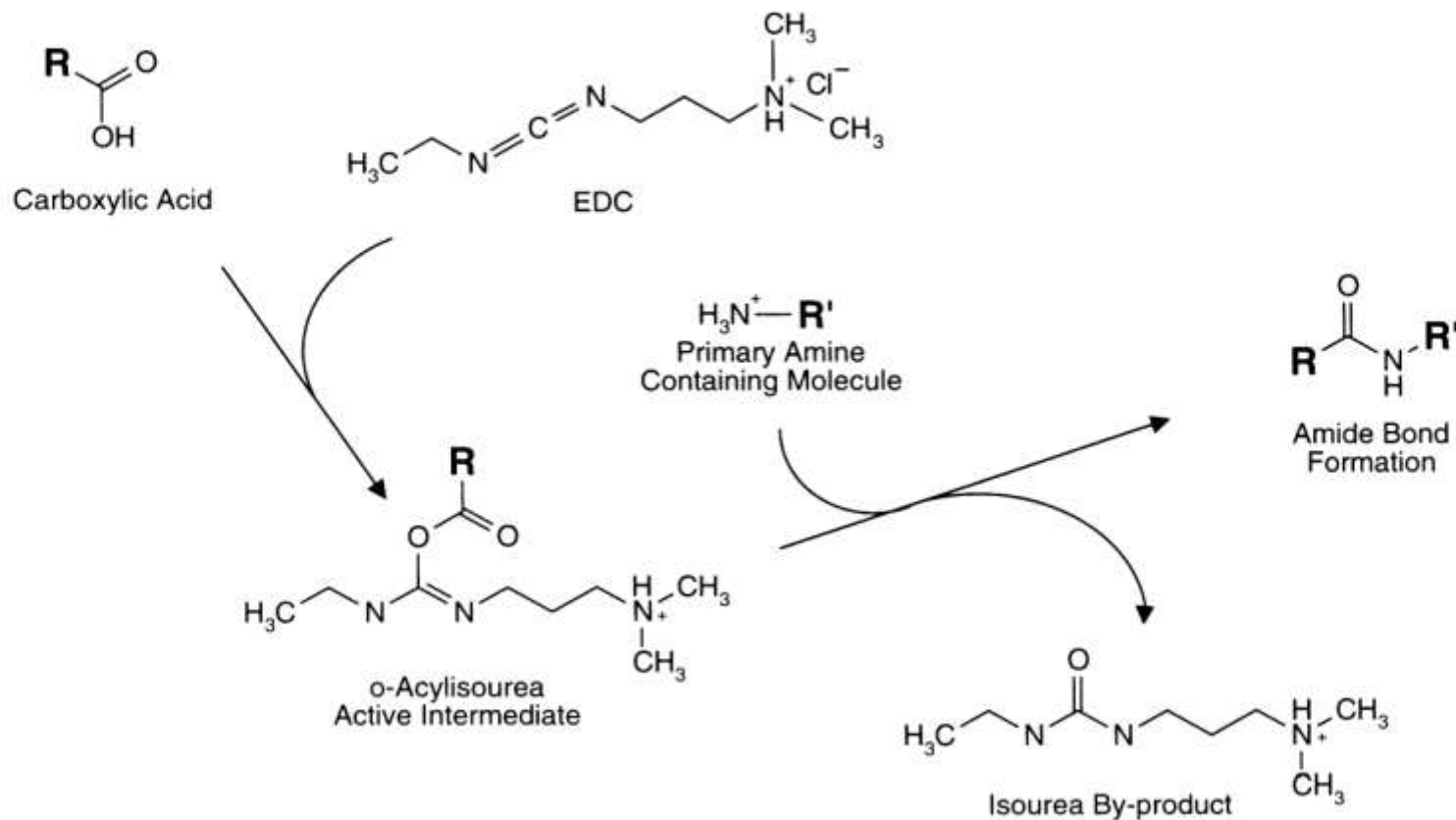
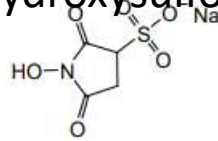


Figure 3.1 EDC reacts with carboxylic acids to create an active-ester intermediate. In the presence of an amine nucleophile, an amide bond is formed with release of an isourea by-product.

2.EDC Plus Sulfo-NHS



Sulfo-NHS (N-hydroxysulfosuccinimide),



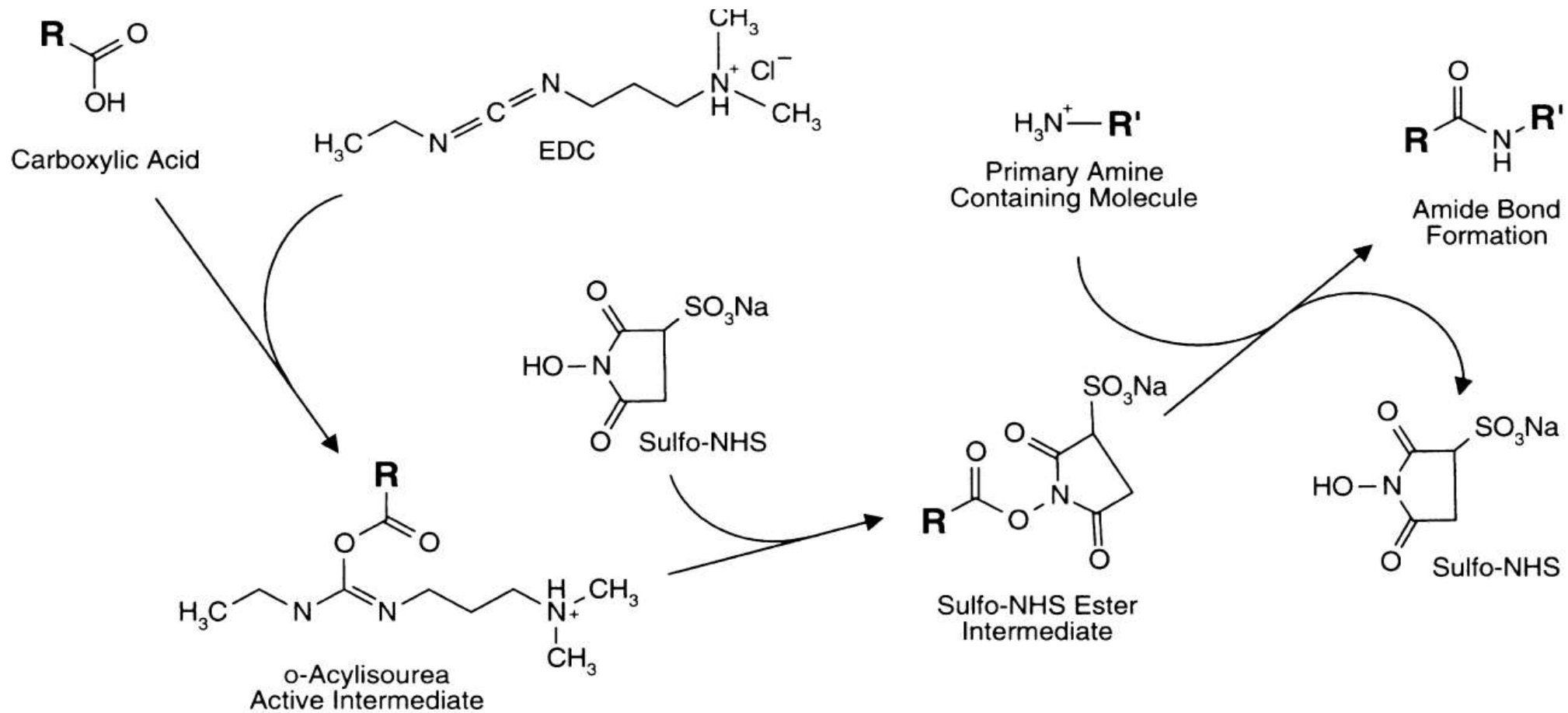
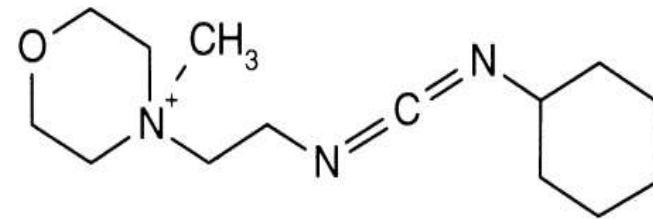


Figure 3.2 The efficiency of an EDC-mediated reaction may be increased through the formation of a sulfo-NHS ester intermediate. The sulfo-NHS ester is more effective at reacting with amine-containing molecules. Thus, higher yields of amide bond formation may be realized using this two-step process as opposed to using a single-step EDC reaction.

3.CMC, or 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide

is a water-soluble reagent used to form amide bonds between one molecule containing a carboxylate and a second molecule containing an amine.



CMC
1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide
MW 423.58
(as the metho-p-toluene sulfonate salt)

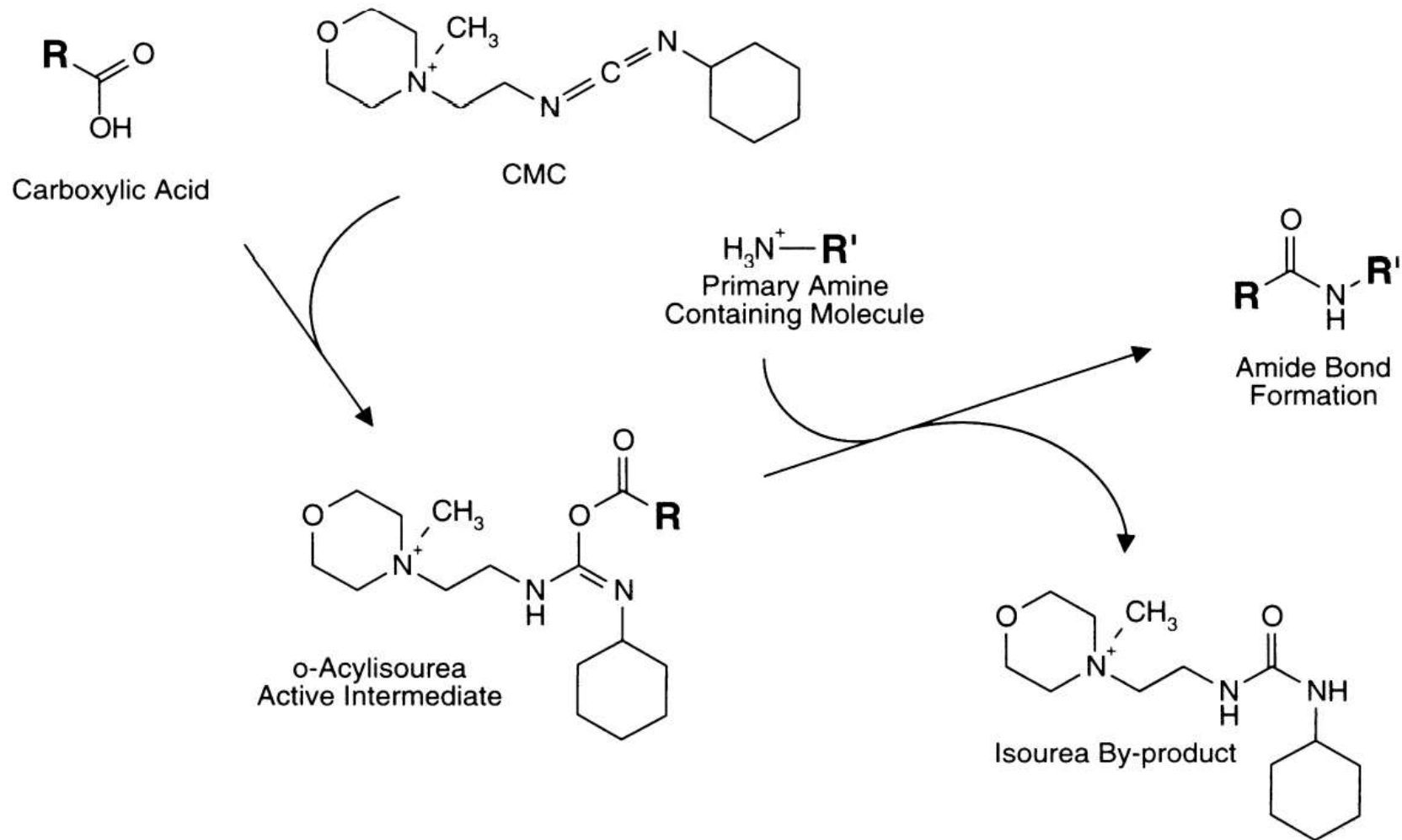


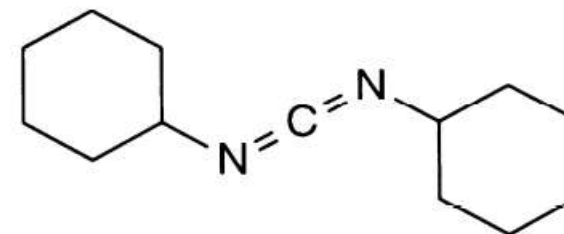
Figure 3.4 The water-soluble carbodiimide CMC reacts with carboxylates to form an active-ester intermediate. In the presence of amine-containing molecules, amide bond formation can take place with release of an isourea

4. DCC (dicyclohexyl carbodiimide)

DCC (dicyclohexyl carbodiimide) is one of the most frequently used coupling agents, especially in organic synthesis applications.

It has been used for peptide synthesis since 1955 and continues to be a popular choice for creating peptide bonds.

DCC is water-insoluble.



DCC
N,N'-Dicyclohexyl
carbodiimide
MW 206.32

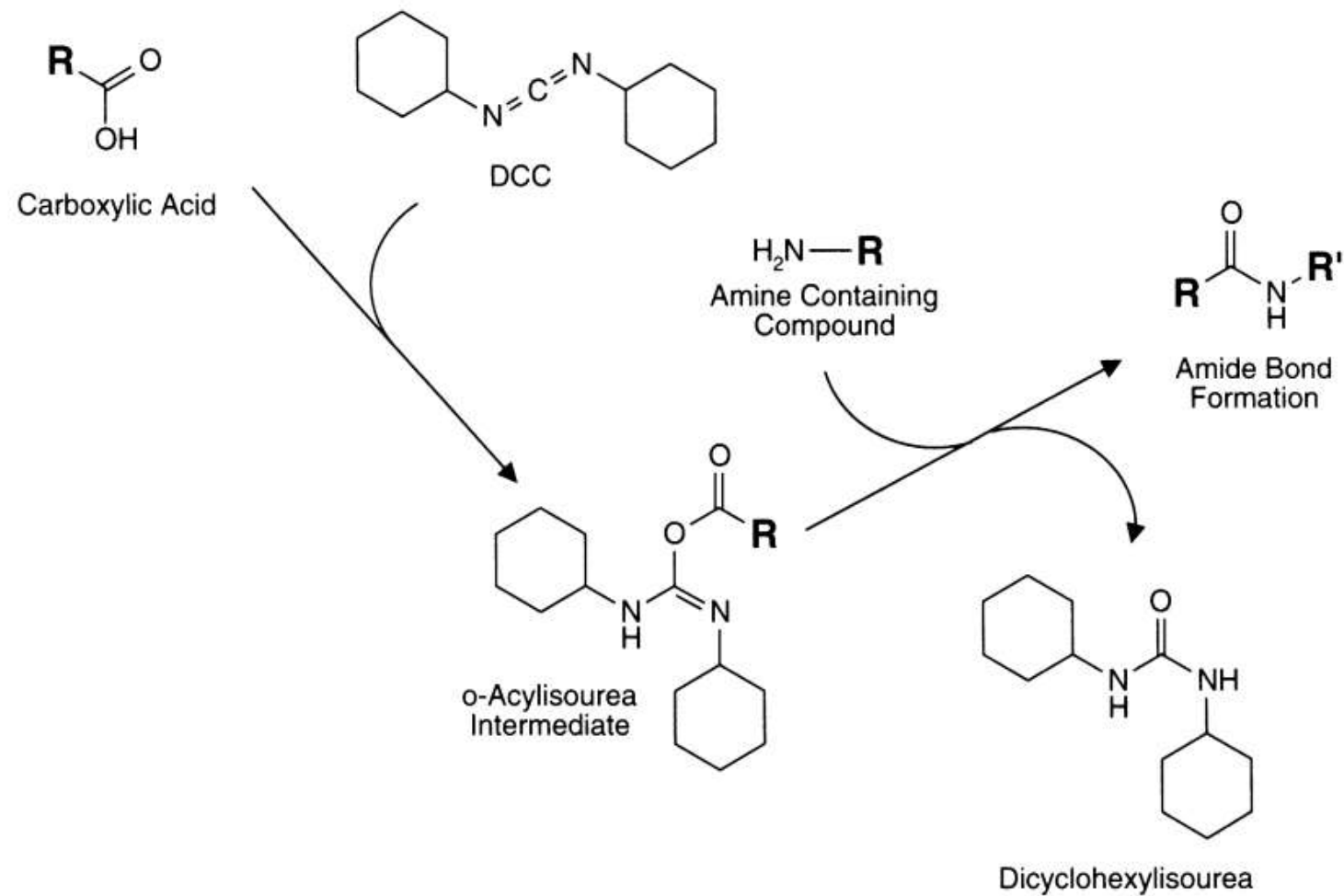
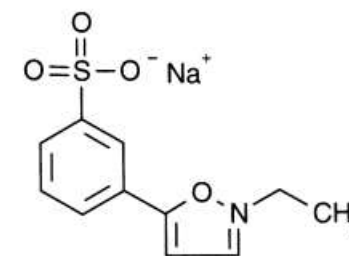


Figure 3.5 The organic-soluble carbodiimide DCC is often used to create amide bonds, especially between water-insoluble compounds.

2. Woodward's Reagent K

Woodward's reagent K is N-ethyl-3-phenylisoxazolium-3-sulfonate, a zero-length crosslinking agent able to cause the condensation of carboxylates and amines to form amide bonds.

The reaction mechanism involved in **activating** a carboxylate includes the conversion of the reagent under alkaline conditions to a reactive **ketoketenimine**. This intermediate then reacts with a carboxylate to create an **enol ester**. The enol ester is highly susceptible to **nucleophilic** attack.



Woodward's Reagent K
N-Ethyl-5-phenylisoxazolium-3'-
sulfonate, sodium salt
MW 176

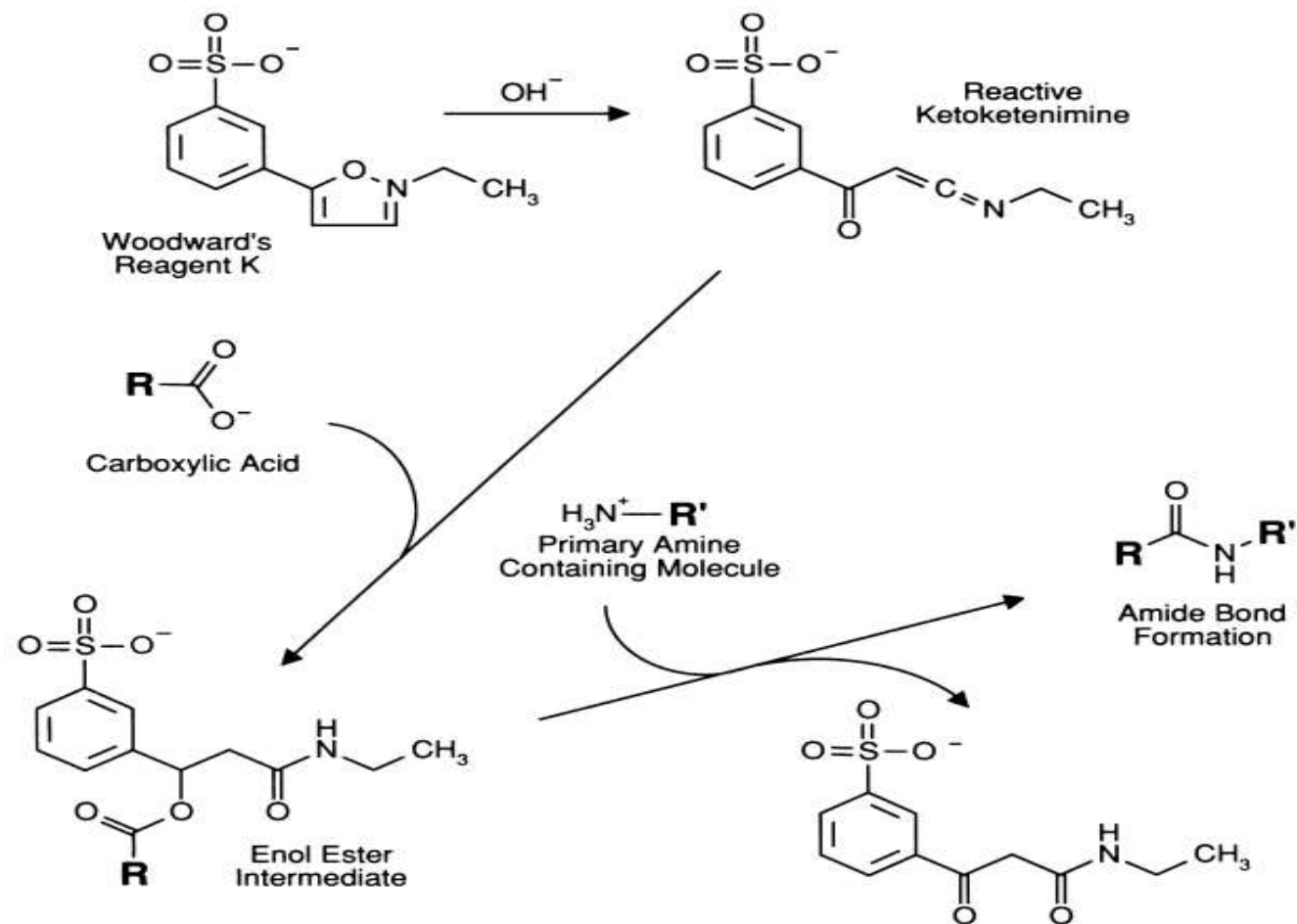


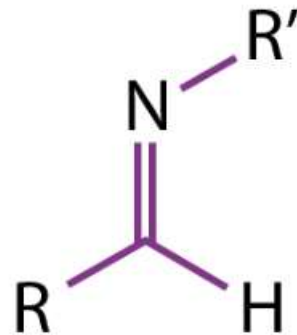
Figure 3.11 Woodward's reagent K undergoes a rearrangement in alkaline solution to form a reactive ketoketenimine. This active species can react with a carboxylate group to create another active group, an enol ester derivative. In the presence of amine nucleophiles, amide bond formation takes place.

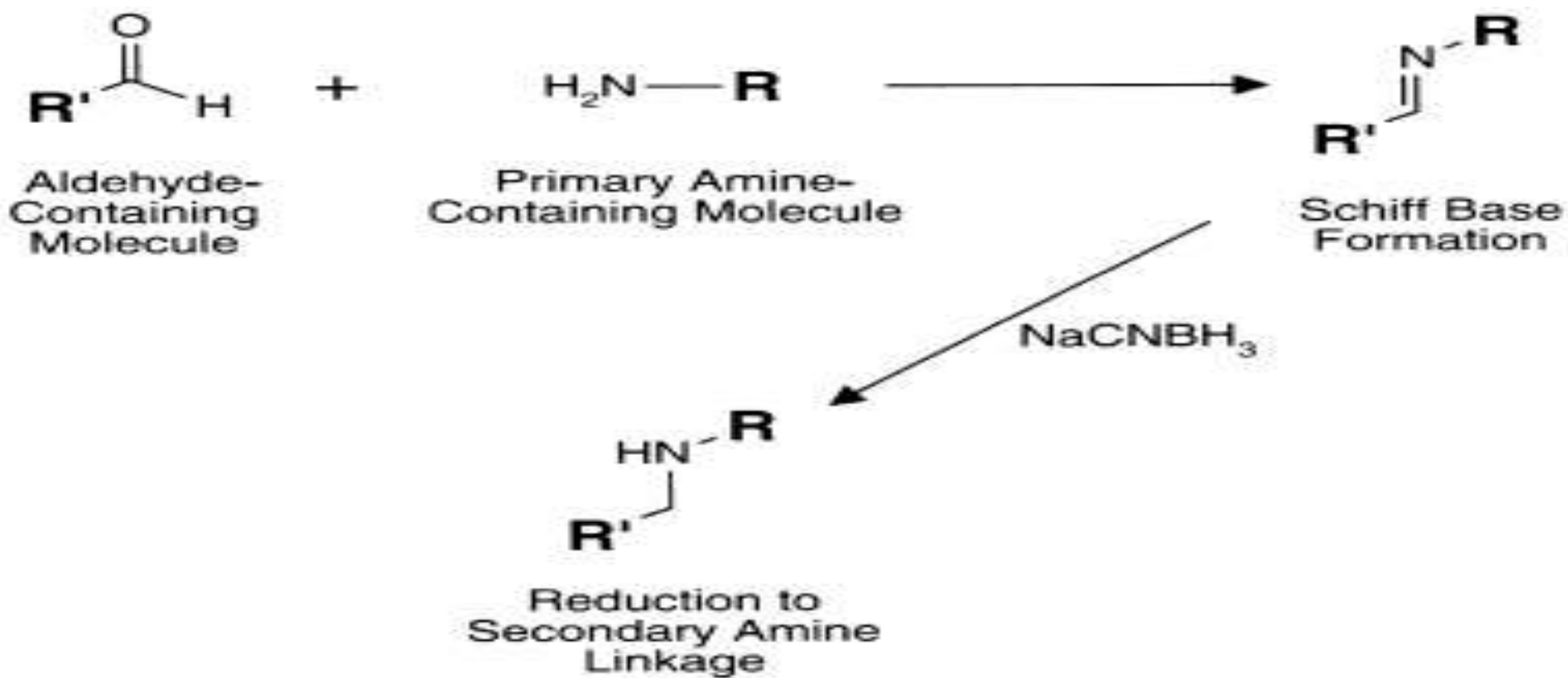
3-SCHIFF BASE FORMATION AND REDUCTIVE AMINATION

Aldehydes and ketones can react with primary and secondary amines or tertiary amine linkages to form Schiff bases. Carbonyl groups such as in aldehydes and ketones can react with amine nucleophiles to form reversible Schiff base intermediates. In the presence of a suitable reductant, such as sodium cyanoborohydride, the Schiff base is stabilized to a secondary amine bond. or tertiary amine linkages

A Schiff base

“A Schiff base is a compound with the general structure $R_2C=NR'$ and is considered as a subclass of imines, (imines are the compounds consisting of carbon-nitrogen double bond) being either secondary aldehydes or secondary ketimines depending on their structure.” Schiff's bases are aldehyde- or ketone compounds where the carbonyl group is replaced by imine group





Reference


Book Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine

Article Chemistry of Crosslinking 2019

G Biosciences The Protein Man's Blog 2016

Bioconjugate Techniques (Third edition) 2013

Article PMC pubmed central Probing structures of large protein complexes using zero-length cross-linking 2016



Thank you

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