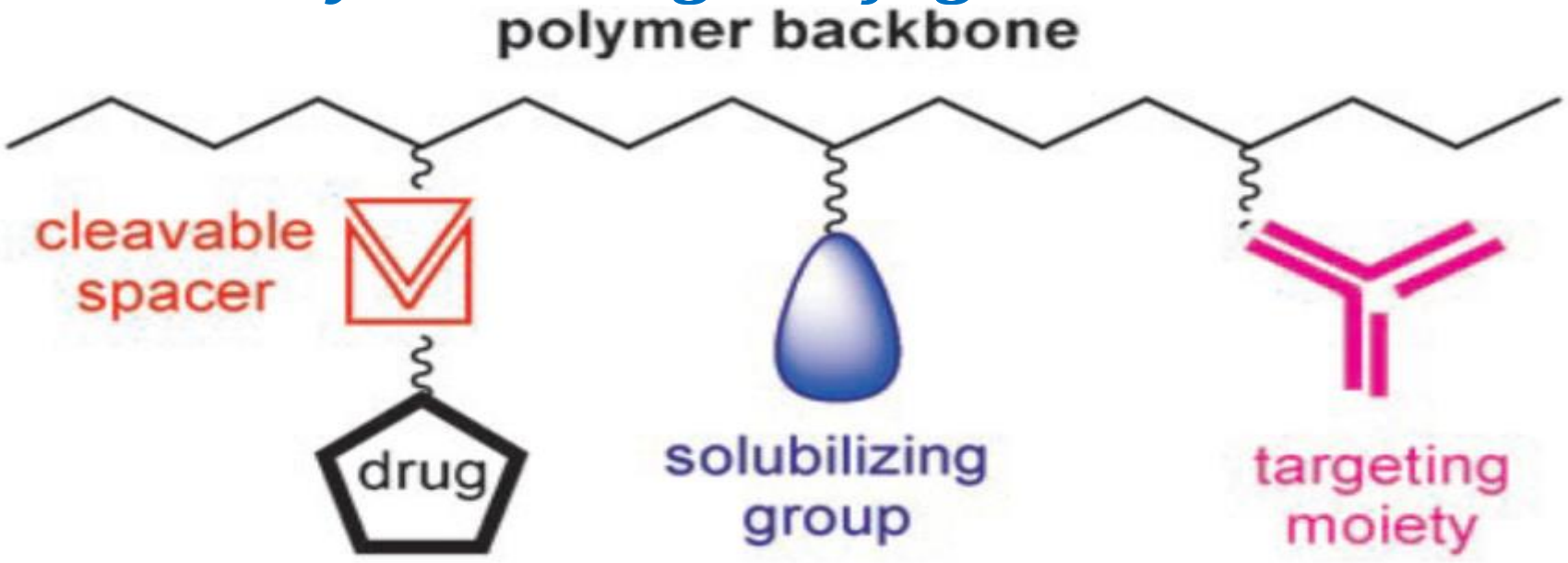
Organic Pharmaceutical Chemistry IV

Lec5: Classification of polymers used for bioconjugation.

Cross-linking Reagents:

Polymer-Drug Conjugates

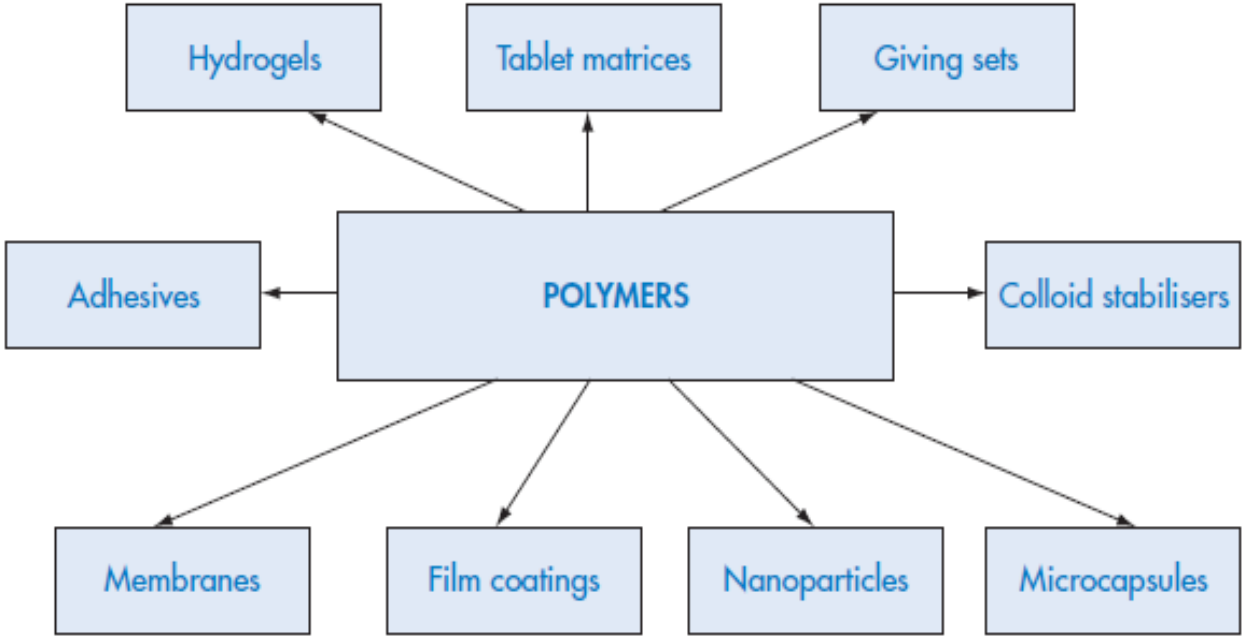


TAMMAR HUSSEIN ALI

Pharmaceutical Polymers:

Polymers are used widely in pharmaceutical systems as:

- Suspending and emulsifying agents
- Flocculating agents
- Adhesives
- Packaging and coating materials
- Controlled and site-specific drug delivery systems



Definitions

Polymers are substances of high molecular weight made up of repeating *monomer* units.

Substances with short chains containing relatively few monomers are called ***oligomers***.

Polymers owe their unique properties to **their size, their three-dimensional shape and sometimes to their asymmetry**.

The **chemical reactivity** of polymers depends on the **chemistry of their monomer units**, but **their properties** depend to a large extent on **the way the monomers are put together**; it is this fact that leads to the versatility of synthetic polymers.

Properties of polymer (Macromolecule)

1. Water soluble
2. Safe (non toxic),non immunogenic, non carcinogenic, not induce allergic reaction.
3. Biocompatible with blood components or other component in the body.
4. Don’t form complication reactions with blood component.
5. Should be elimination from the body within reasonable time (not accumulative).
6. Biodegradable.
7. Molecular weight not exceed 7500 unit.

Classification of polymers used for bioconjugation:

A. Synthetic polymers:

1. **Polyethylene glycol (PEG)**:
2. **Vinyl polymers:**
   a) ***N-(2-hydroxypropyl)methacrylamide (HPMA)***
   b) ***Poly(styrene-co-maleic acid/anhydride) (SMA)***
3. **Divinylethermaleic anhydride/acid copolymer (DIVEMA)**
4. **Polyethylenimine (PEI) or polyaziridine.**

Classification of polymers used for bioconjugation:

B. Natural polymers:

1. Dextran.
2. Chitosan.
3. Proteins.
4. Pullulan.

Synthetic polymers:

1. Polyethylene glycol (PEG) {H-(O-CH_2-CH_2)n-OH}

- ***Advantages of PEG***
  - Non-toxic and non-immunogenic.
  - Flexible, highly water-soluble
  - Site-specific conjugation to a drug
  - Available in wide range m.wt (300-10,000,000 da)

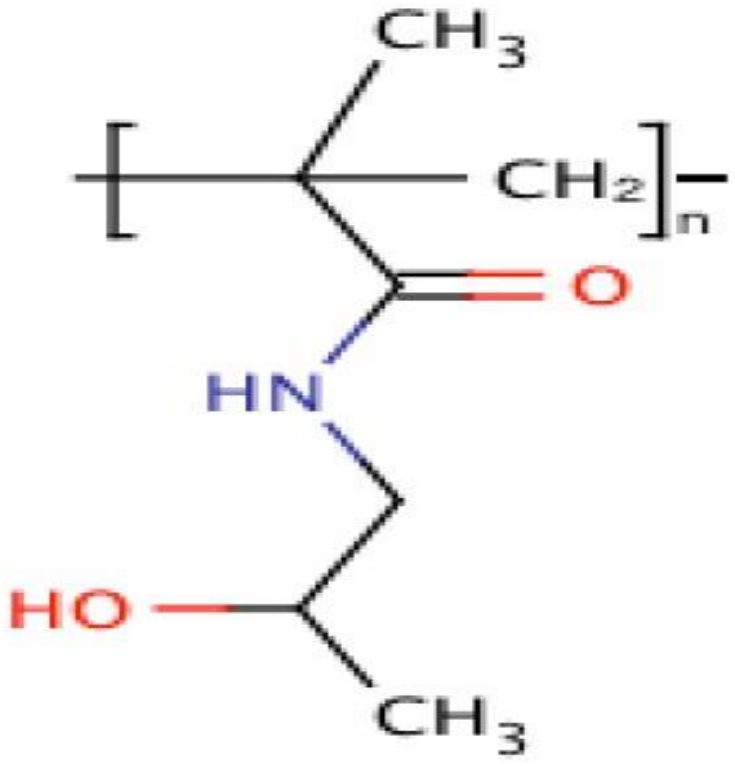
Synthetic polymers:

1. Polyethylene glycol (PEG) {H-(O-CH_2-CH_2)n-OH}

- ***Disadvantage***
  - PEG molecule has only two reactive groups, therefore at most only two drug molecules can be attached to a bulky PEG molecule.

2. Vinyl polymers

- *a) N-(2-hydroxypropyl)methacrylamide (HPMA)*



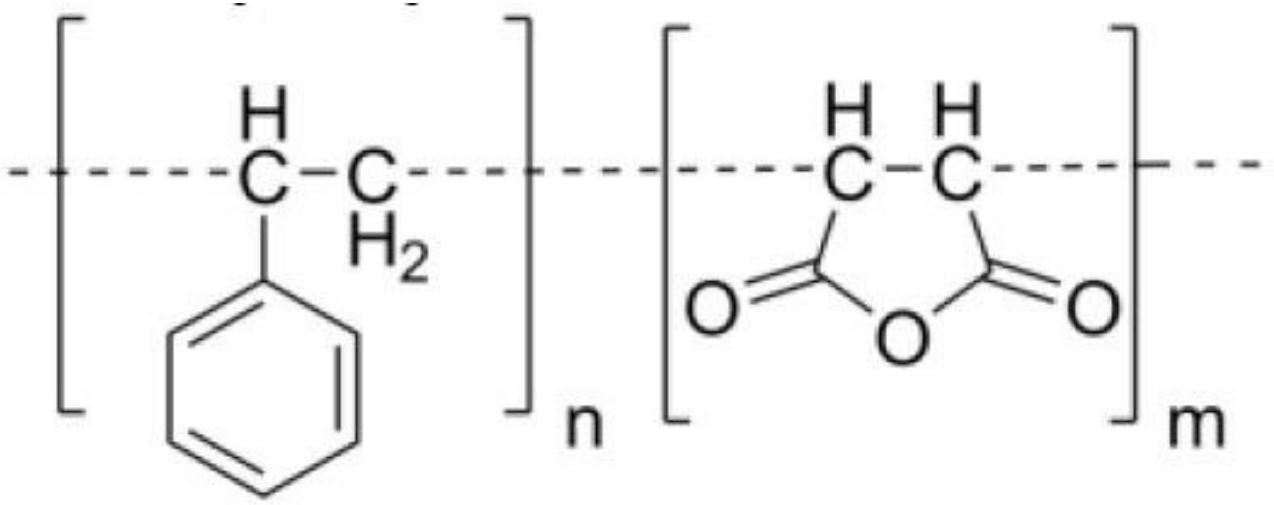
a) N-(2-hydroxypropyl)methacrylamide (HPMA)

- ***Advantages:***
  - Non immunogenic.
  - Nontoxic.
  - Well resides in blood circulation.
  - It is frequently used as macromolecular carriers for low molecular weight drugs (especially anti-cancer chemotherapeutic agents) to enhance therapeutic efficacy and limit side effects.

b) Poly(styrene-co-maleic acid/anhydride) (SMA)

Advantage: Ampiphillic nature of SMA is utilized in stable micelle formation.

- It forms conjugate with Neocarcinostatin (NCS) to form a polymeric prodrug 'SMANCS'.
- This prodrug has been successfully marketed in Japan for the treatment of hepatocellular Carcinoma.



3. Divinylethermaleic anhydride/acid copolymer (DIVEMA)

- ***Advantages:***

1. *It has antitumor activity.*
2. *It induces the formation of interferon.*
3. *It has antiviral, antibacterial, and antifungal activity.*
4. *It is an anticoagulant and an anti-inflammatory agent.*

3. Divinylethermaleic anhydride/acid copolymer (DIVEMA)

- ***Disadvantages:***

1. *Pyrogenicity.*
2. *Thromobocytopenia.*
3. *Inhibition of microsomal enzymes.*
4. *Sensitization to endotoxin.*
5. *Liver damage.*
6. *Organomegaly, and*
7. *Depression of the reticuloendothelial system.*

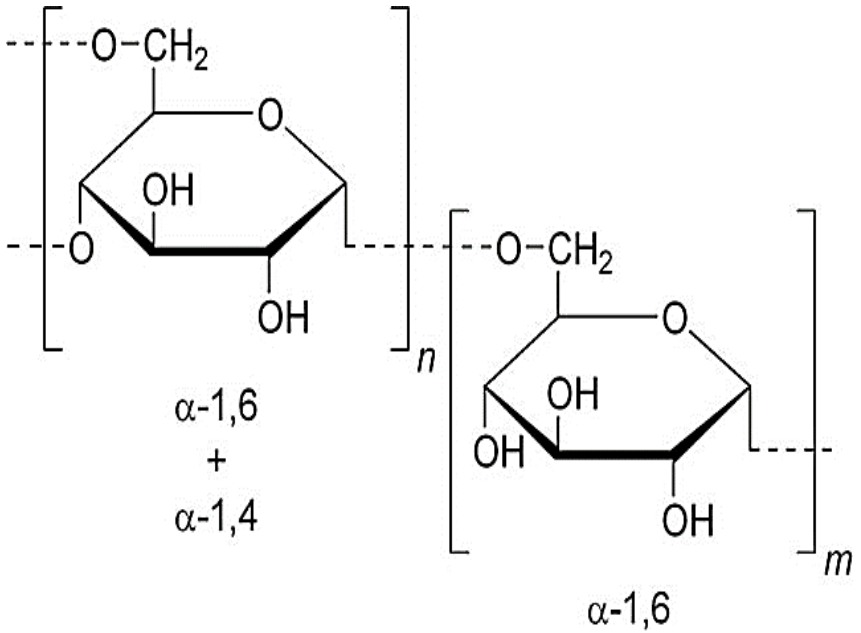
4. Polyethylenimine (PEI) or polyaziridine

- ***Advantage***:
- Linear PEIs of mol. wt. 22,000 are best to overcome nuclear barrier and yields the highest transfection rates.

- ***Disadvantage***:
- It has a limitation of relatively high toxicity and this could prove problematic for repeated systemic use.

Natural polymers:

1) Dextran



- **Advantages:**

i. It is biocompatible and biodegradable.

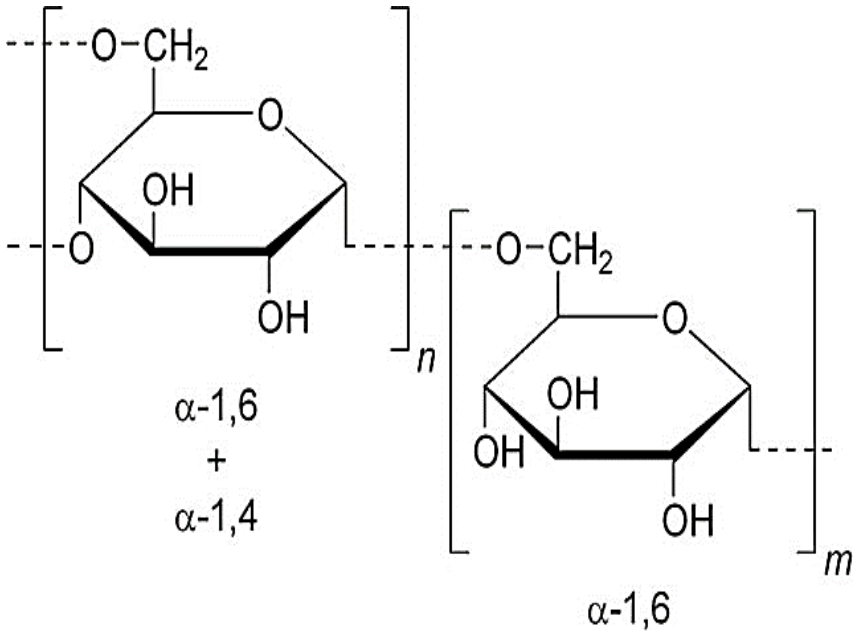
ii. It is biologically active

iii. possesses thrombolytic activity.

iv. It is non-immunogenic and non-toxic.

Natural polymers:

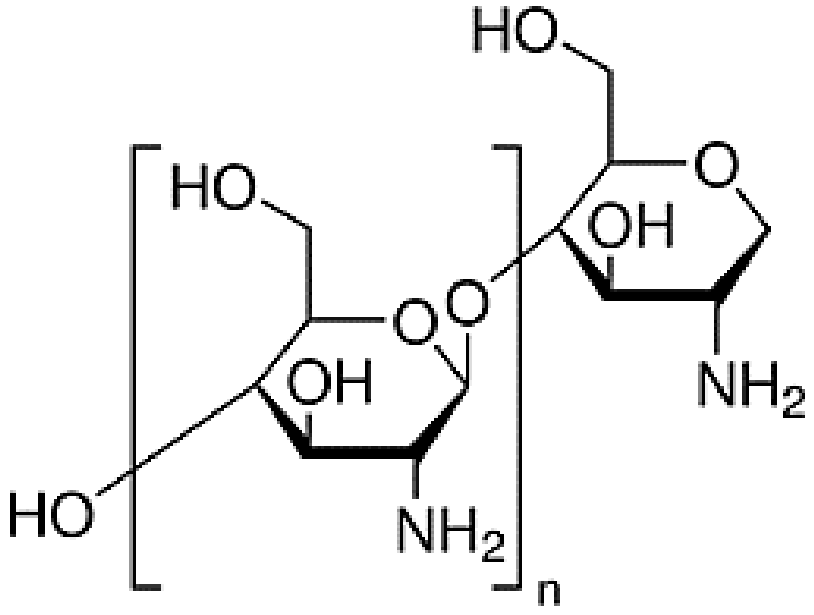
1) Dextran



- **Disadvantages:**
  - i. Anaphylaxis.
  - ii. Volume overload.
  - iii. Pulmonary edema.
  - iv. Cerebral edema, or
  - v. Platelet dysfunction.

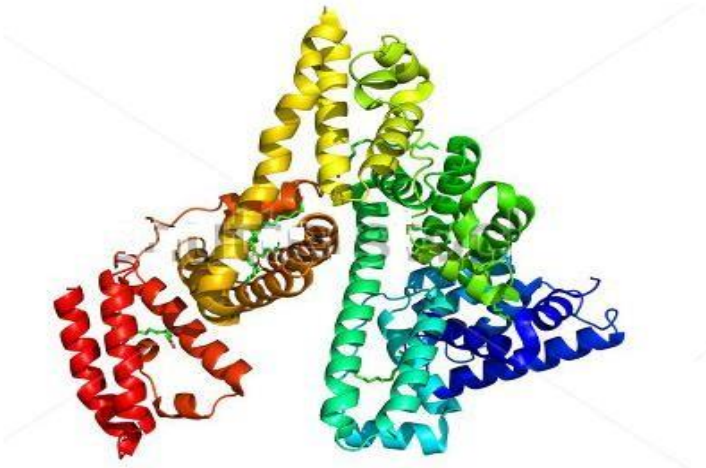
2) Chitosan

Advantages: Chitosan enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable.



3) Proteins: *Albumin*

- ***Advantages:***
  - Albumin gets accumulated within solid tumors and hence is used for drug delivery and tumor targeting.
  - It also increases the stability of attached therapeutic proteins.

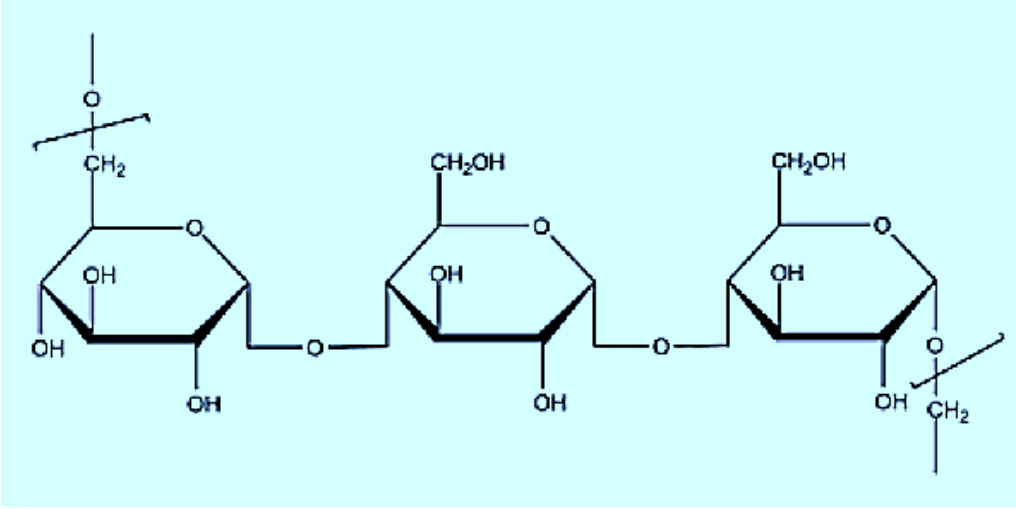


Serum Albumin Molecular Structure

4) Pullulan

- ***Advantages:***

- Biodegradability, low immunogenicity and polyfunctionality and fair solubility in aqueous and few organic solvents, blood compatible, non-toxic, non-mutagenic and noncarcinogenic.



Polymer-Drug Conjugates

- Polymer anticancer-drug conjugates are designed to:
  - *I. Enhance the physico-chemical properties of the drug.*
  - *II. Administer the drug specifically to the tumor site.*
- Polymer anticancer-drug conjugates are prepared by conjugating anticancer drug to a polymeric backbone via covalent linkage.

Cross-linking reagents

- Biodegradable spacer is inserted in the conjugate to:
  a) *Insure stability during systemic circulation.*
  b) *Facilitate specific enzymatic or hydrolytic release of the drug.*
- e.g. Doxorubicin-HPMA (N-(2-hydroxypropyl) methacrylamide) conjugate

Doxorubicin-HPMA conjugate

- The polymer used is N-(2-hydroxypropyl) methacrylamide copolymer.
- The anticancer drug is bound to the polymer backbone using peptidyl spacer (Gly-Phe-Leu-Gly linker).

Doxorubicin-HPMA conjugate

- This linker designed to be cleaved by Lysosomal thiol-dependant proteases.
- The conjugate has a molecular weight of approx. 30,000 Da and a Doxorubicin content of approx. 8.5% (w/w).

Antibody-Directed Enzyme Prodrug Therapy (ADEPT)

Approach	Enzyme	Prodrug	Drug
ADEPT	**Carboxypeptidase A**	**Methotrexate alanine**	**Methotrexate**
ADEPT	**B - Glucuronidase**	**Epirubicin glucoronide**	**Epirubicin**

Gene-Directed Enzyme Prodrug Therapy (GDEPT)

Approach	Enzyme	Prodrug	Drug
GDEPT	**Thymidine kinase**	**Ganciclovir**	**Ganciclovir triphosphate**
GDEPT	**Cytosine deaminase**	**5-Fluorocytosine**	**5-Fluorouracil**
GDEPT	**Nitroreductase**	**4-nitrobenzyloxy carbonyl derivative**	**Actinomycin D**

Some Water-soluble Polymers Used in Pharmacy and Medicine

A- Carboxypolymethylene (Carbomer,Carbopol)

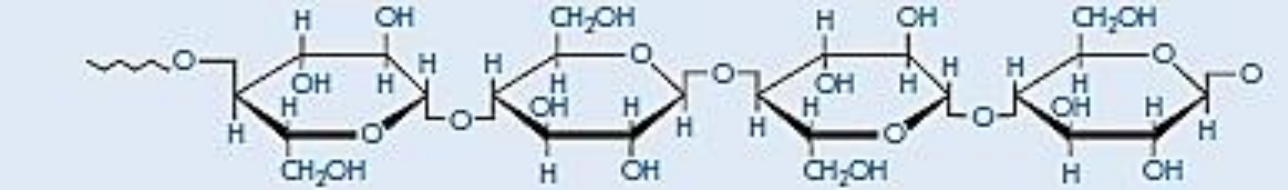
This is used as a suspending agent in pharmaceutical preparations and as a binding agent in tablets, and it is used in the formulation of prolonged-action tablets.

It is a high molecular weight polymer of acrylic acid, containing a high proportion of carboxyl groups.

Its aqueous solutions are acidic; when neutralised the solutions become very viscous with a maximum viscosity between pH 6 and 11. Electrolytes reduce the viscosity of the system and thus high concentrations of the polymer have to be employed in vehicles where ionisable drugs are present.

B- Cellulose Derivatives

Cellulose itself is virtually insoluble in water, but aqueous solubility can be conferred by partial methylation or carboxymethylation.



Ethylcellulose is an ethyl ether of cellulose containing 44–51% of ethoxyl groups.
It is insoluble in water but soluble in chloroform and in alcohol. It is possible to form water-soluble grades with a lower degree of substitution.

Ethylhydroxyethylcellulose is an ether of cellulose with both ethyl and hydroxyethyl substituents attached via ether linkages to the anhydroglucose rings. It swells in water to form a clear viscous colloidal solution. Preparation of solutions of cellulose derivatives requires hydration of the macromolecules, the rate of which is a function of both temperature and pH.

Other cellulose derivatives: *Ethylmethylcellulose, Methylcellulose and Sodium carboxymethylcellulose*

C- Natural Gums and Mucilages

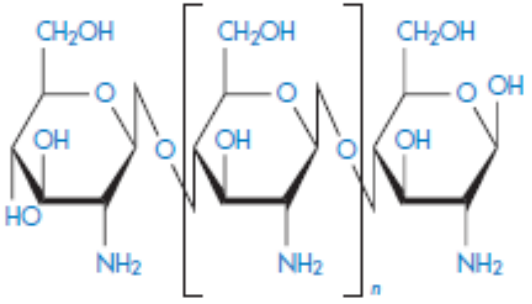
- *Gum arabic* (acacia)
- *Gum tragacanth*
- *Alginates*
- *Pectin*

D- Chitosan

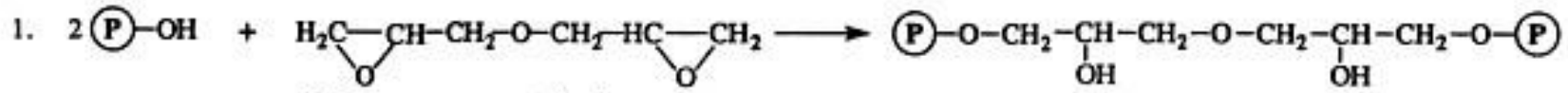
Chitosan is a polymer obtained by the deacetylation of chitin, one of the most abundant polysaccharides.

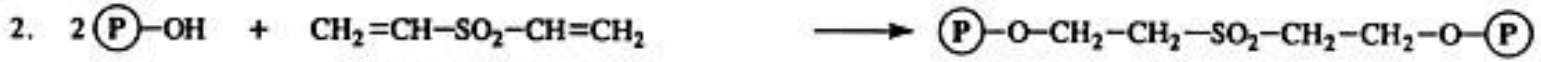
Chitosan also has film forming abilities and its gel- and matrix forming abilities make it useful for solid dosage forms, such as granules or micro particles.

The molecular weight, crystallinity and degree of deacetylation are all factors that can be varied to control the release rates from chitosan-based granules.



Cross-linking Reagents

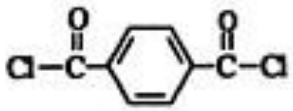
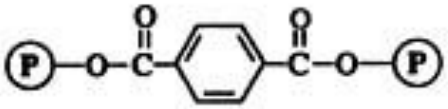
1. 

2. 

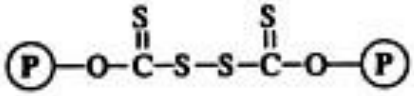
3. 

4. 

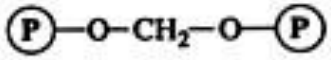
5. 

6. $2\,P\text{–}OH$ +  → 

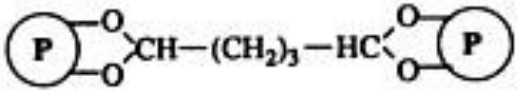
Terephthaloyl chloride

7. $2\,P\text{–}OH + CS_2$ → 

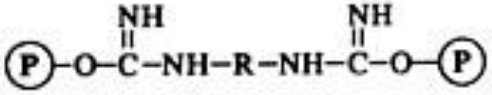
Carbon disulfide

8. $2\,P\text{–}OH + HCHO$ → 

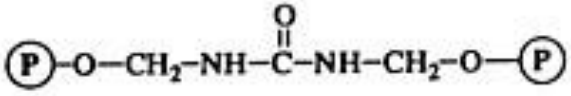
Formaldehyde

9. $2\,P\text{–}OH + OHC\text{–}(CH_2)_3\text{–}CHO$ → 

Glutaraldehyde

10. $2\,P\text{–}OH \xrightarrow[\text{Cyanogen bromide}]{CNBr} 2\,P\text{–}O\text{–}C{\equiv}N \xrightarrow[\text{Diamine}]{H_2N\text{-}R\text{-}NH_2}$ 

Cyanate ester

11. $2\,P\text{–}OH + HO\text{–}CH_2\text{–}NH\text{–}C(=O)\text{–}NH\text{–}CH_2OH$ → 

Dimethylolurea

Why the polymeric prodrugs? What are the advantages of them over their low molecular weight precursors ?!

The advantages are:

1. An increase in water solubility of low soluble or insoluble drugs, and therefore, enhancement of drug bioavailability.
2. Protection of drug from deactivation and preservation of its activity during circulation, transport to targeted organ or tissue and intracellular trafficking.
3. An improvement in pharmacokinetics.
4. A reduction in antigenic activity of the drug leading to a less pronounced immunological body response.
5. The ability to provide targeting of the drug specifically to the site of its action.
6. The possibility to form an advanced complex drug delivery system, which, in addition to drug and polymer carrier, may include several other active components that enhance the specific activity of the main drug.

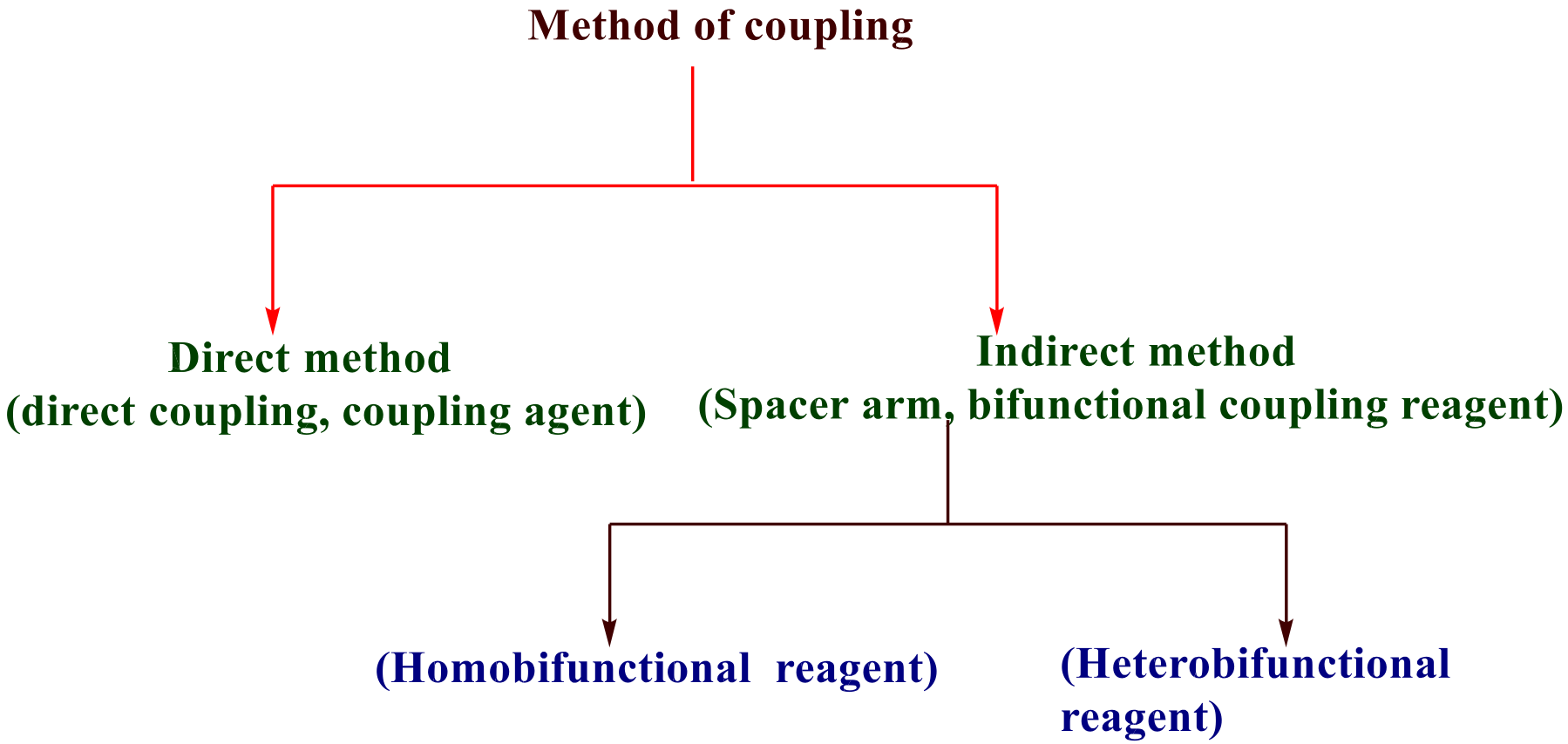
References:

1. Wilson and Gisvold Textbook of Organic Medicinal and Pharmaceutical Chemistry; Delgado JN, Remers WA, (Eds.); 12th ed., 2011.
2. http://www.pcb.ub.edu/fama/pdf/Current%20Drug%20Delivery,%202012,%209,%20000-000.pdf
3. http://omicsonline.org/polymeric-prodrugs-recent-achievements-and-general-strategies-jaa.S15-007.pdf
4. http://www.shutterstock.com/pic-108250010/stock-photo-serum-albumin-molecular-structure-on-a-white-background.html

Modification of a polymer to form a conjugate with a drug molecules depend on two factors:-

1- Reactive functional groups present in the polymer

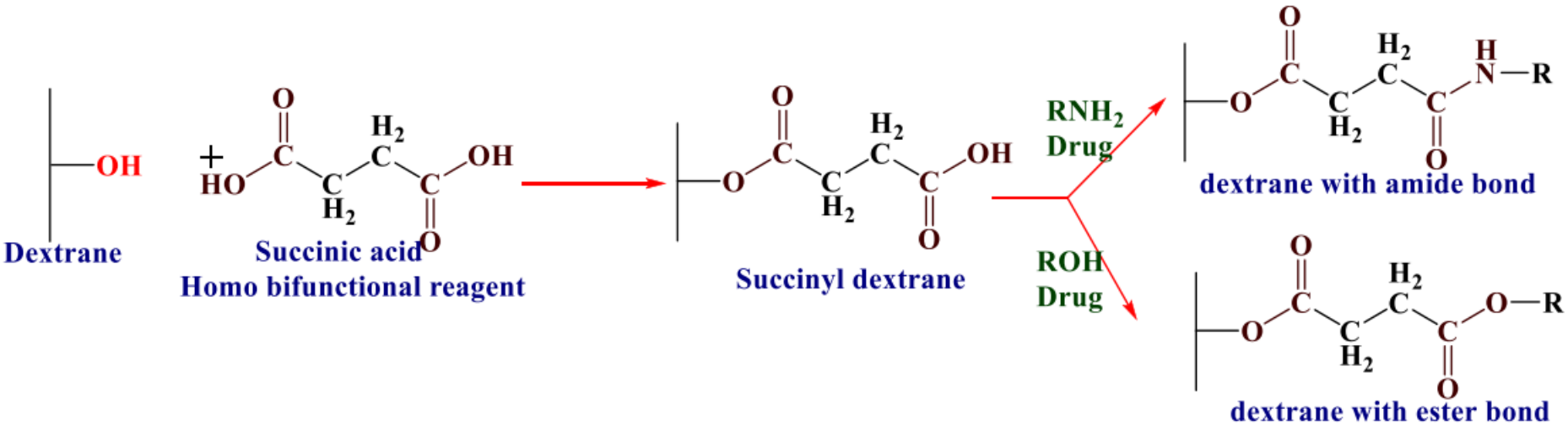
2- Functional groups present on the drug.



1- Spacer arm (bifunctional coupling reagent)

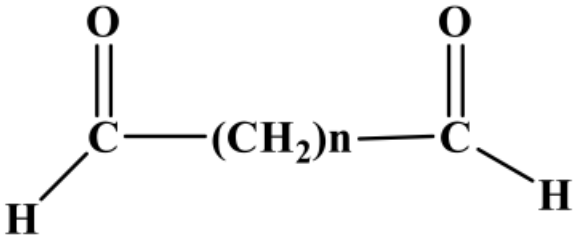
a- Homobifuctional coupling reagent $X-X$

1- Succinic acid with dextrane

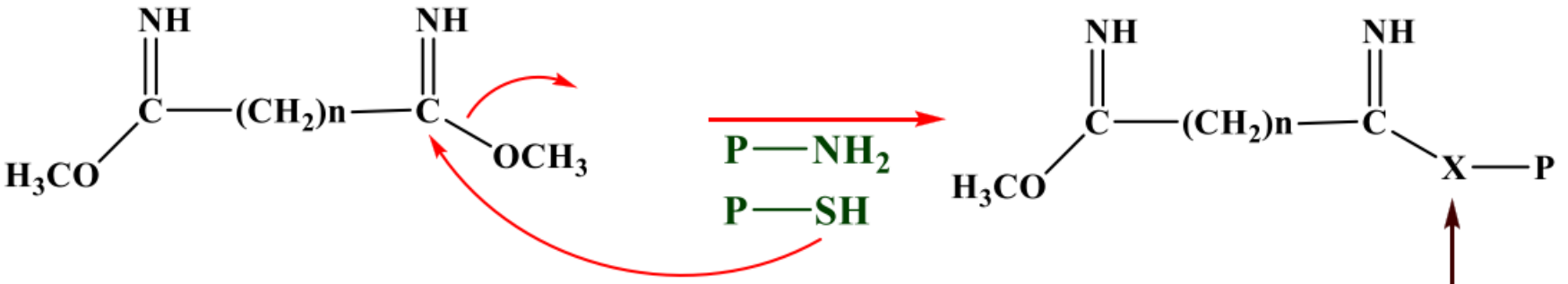


Succinic acid with PEG (Homework)

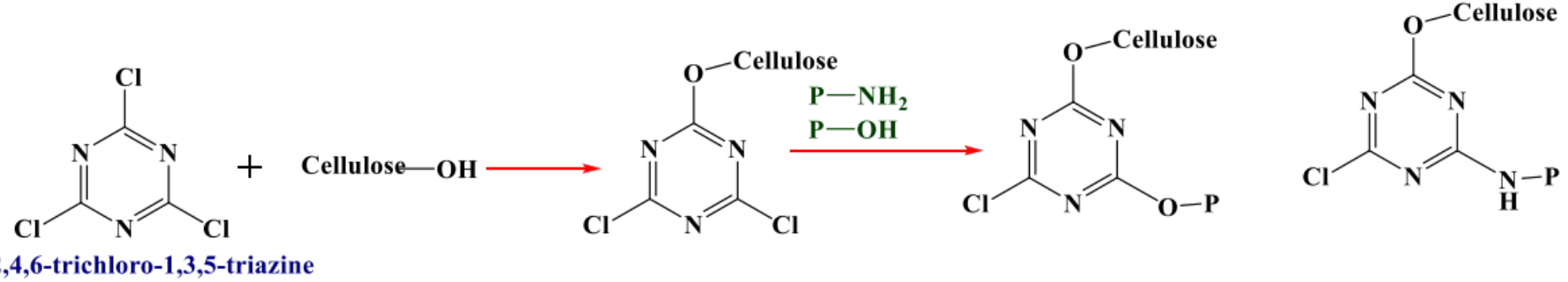
2-



3- HS—(CH$_2$)n—SH



S, N

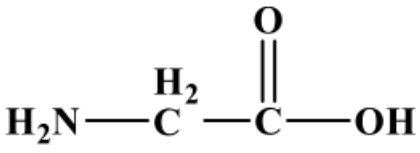


2,4,6-trichloro-1,3,5-triazine

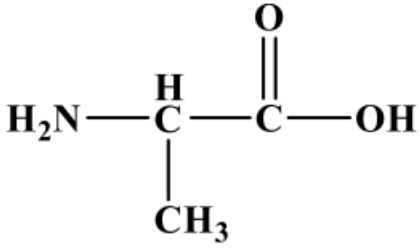
Heterobifunctional coupling reagents X ∿ Y

•**Amino acid spacers** such as glycine, alanine, and small peptides are preferred due to their chemical versatility for covalent conjugation and biodegradability.

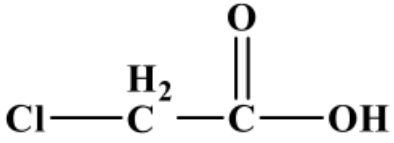
a- Glycine (Gly)



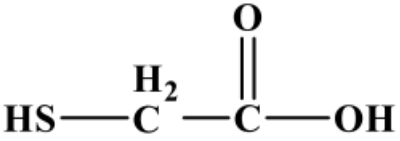
b- Alanine



2- Chloroacetic acid

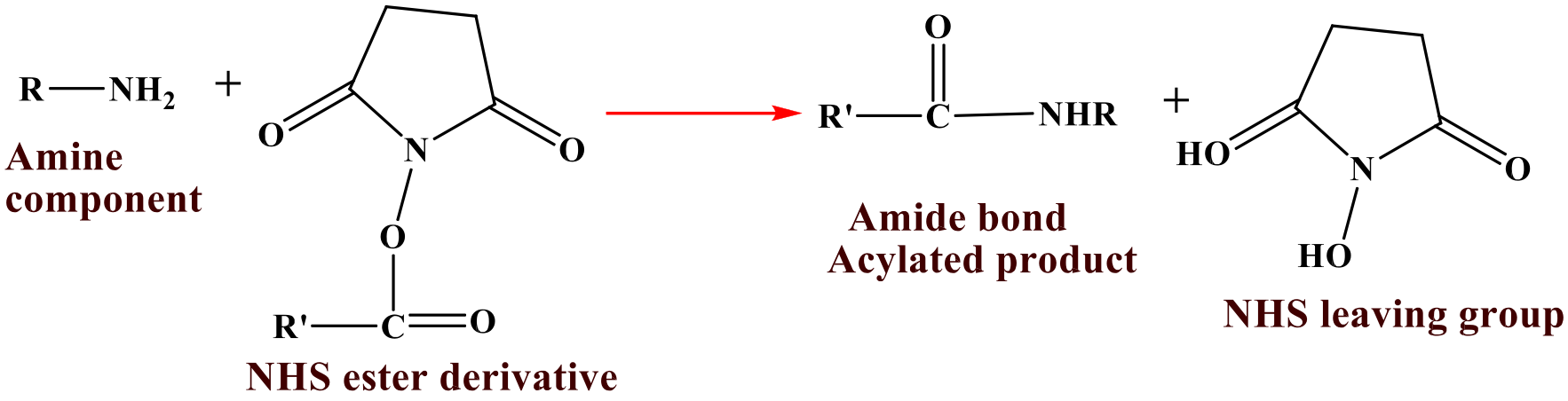


3- Mercapto acetic acid

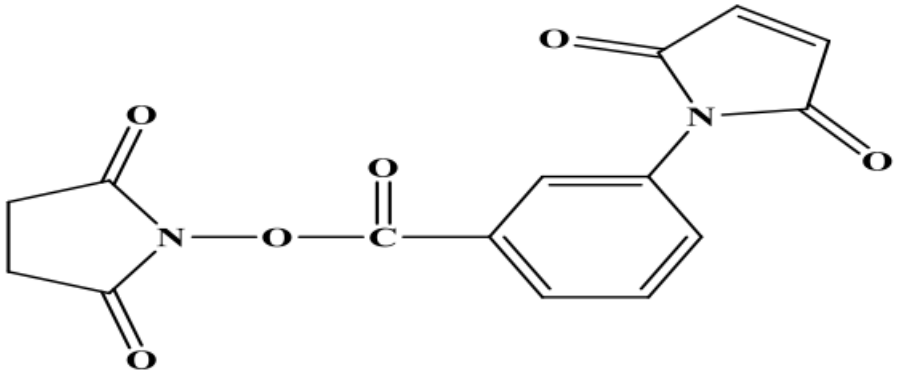


N-hydroxysuccinimidyl (NHS) ester and coupling methods•

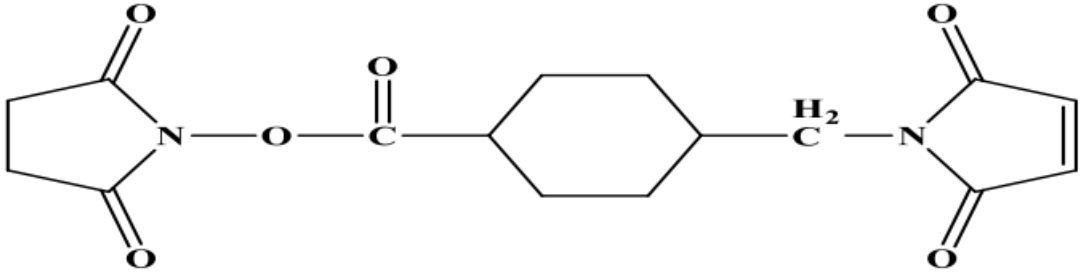
NHS is widely used as an acylating agent and is preferred for conjugation with amine terminal compounds. NHS ester compounds react with nucleophiles to release the NHS leaving group and form an acylated product.



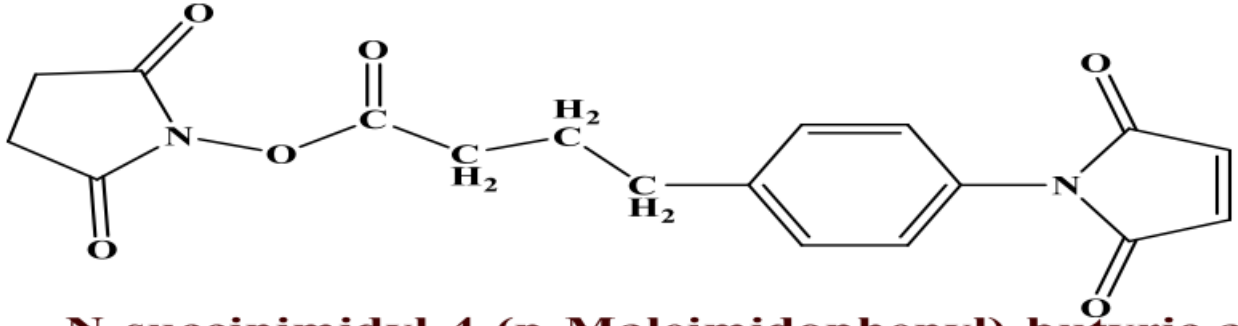
Examples of NHS



N-maleimidobenzoyl-N-hydroxysuccinimide
(MBS)

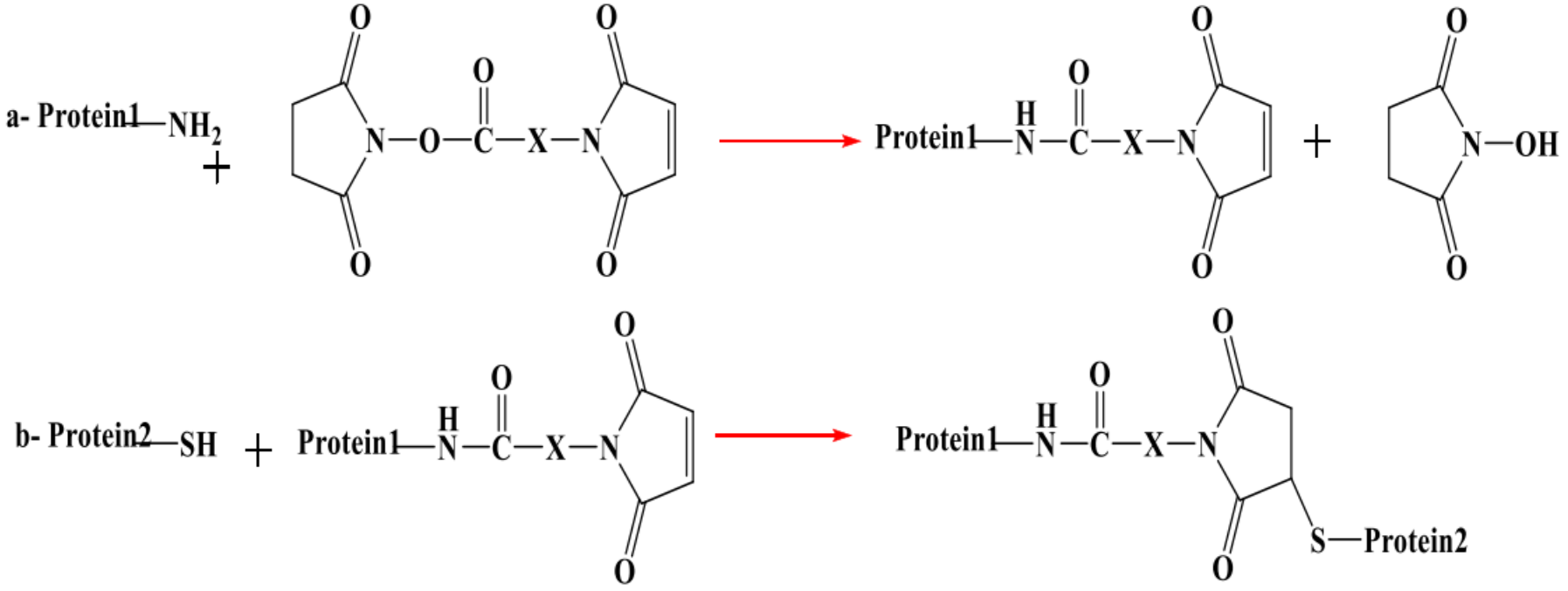


N-succinimidyl-4-(N-Maleimidomethyl)-cyclohexane-1-carboxylate
(SMCC)



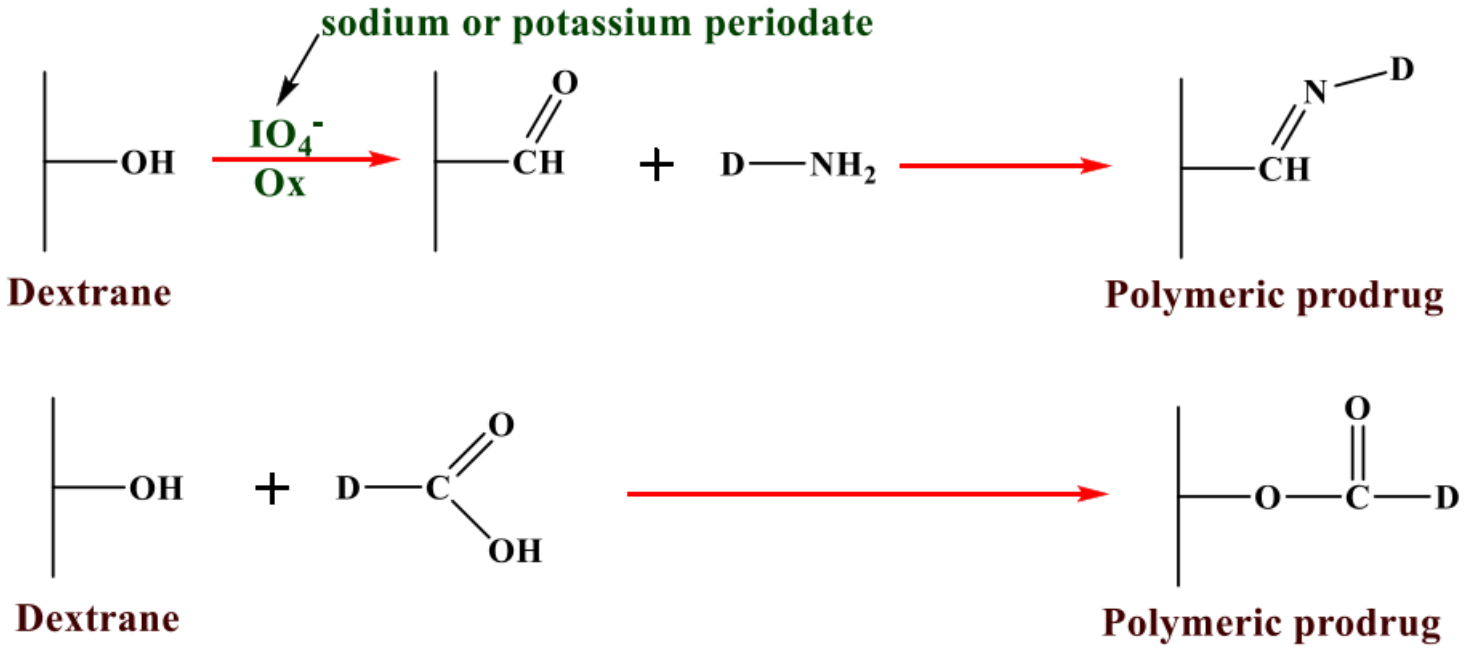
N-succinimidyl-4-(p-Maleimidophenyl)-butyric acid
(SMPB)

In most of the bioconjugates, the NHS ester anhydride is reacted with primary $-NH_2$ of the peptide at slightly higher pH (7.5) to form an amide bond which links the maleimide group to the protein and releases NHS. Thereafter, the maleimide group can be further reacted with the thiol containing moieties or proteins to form a thioether bond in the presence of a slightly acidic or neutral pH.



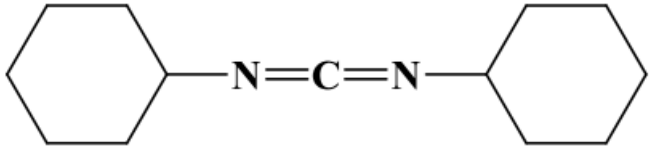
Scheme for protein coupling using N-hydroxysuccinimide ester/maleimide heterobifunctional agents. X represents the spacer groups of varying chain lengths

Direct coupling (no spacer) :-

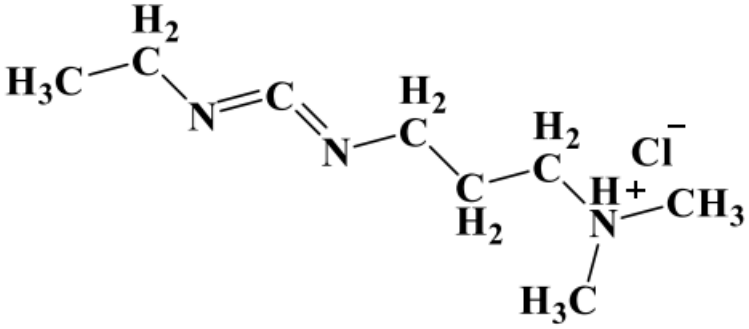


Zero lengths cross- linkers:- Coupling agents mediate the conjugation of the two molecules by forming a bond with no additional spacer atom. Therefore, one atom of the molecule is covalently linked to an atom of the second molecule with no additional linker or spacer needed.

a) Carbodiimides

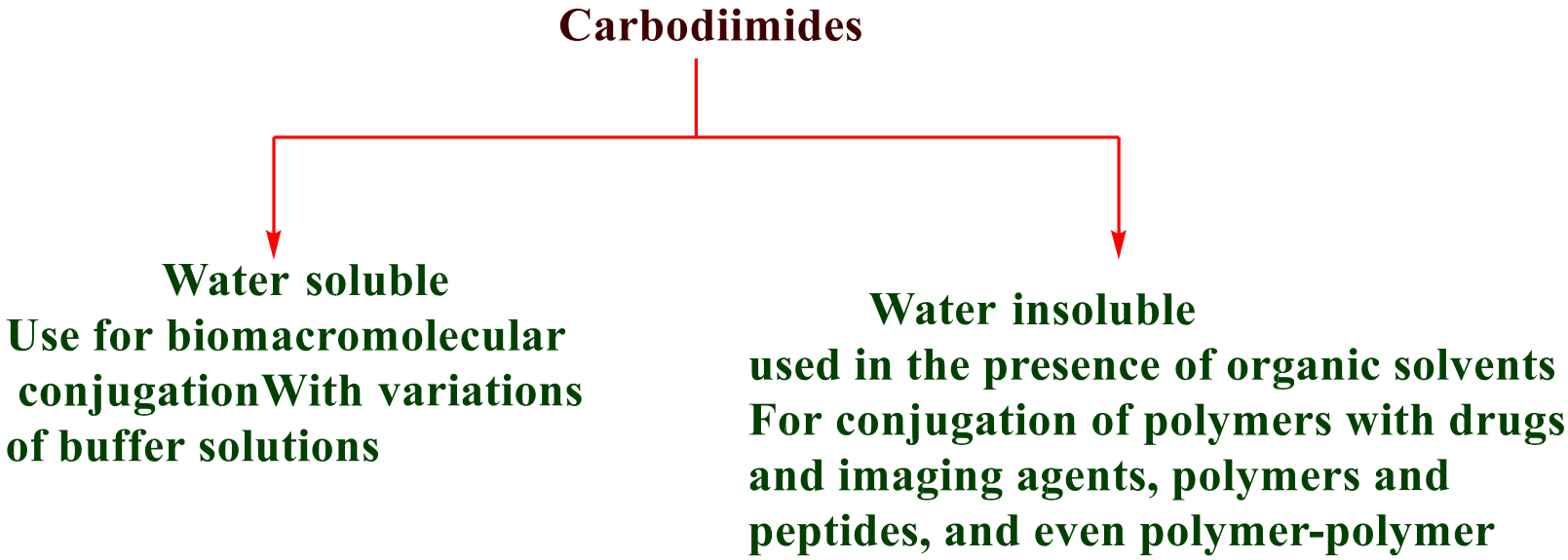


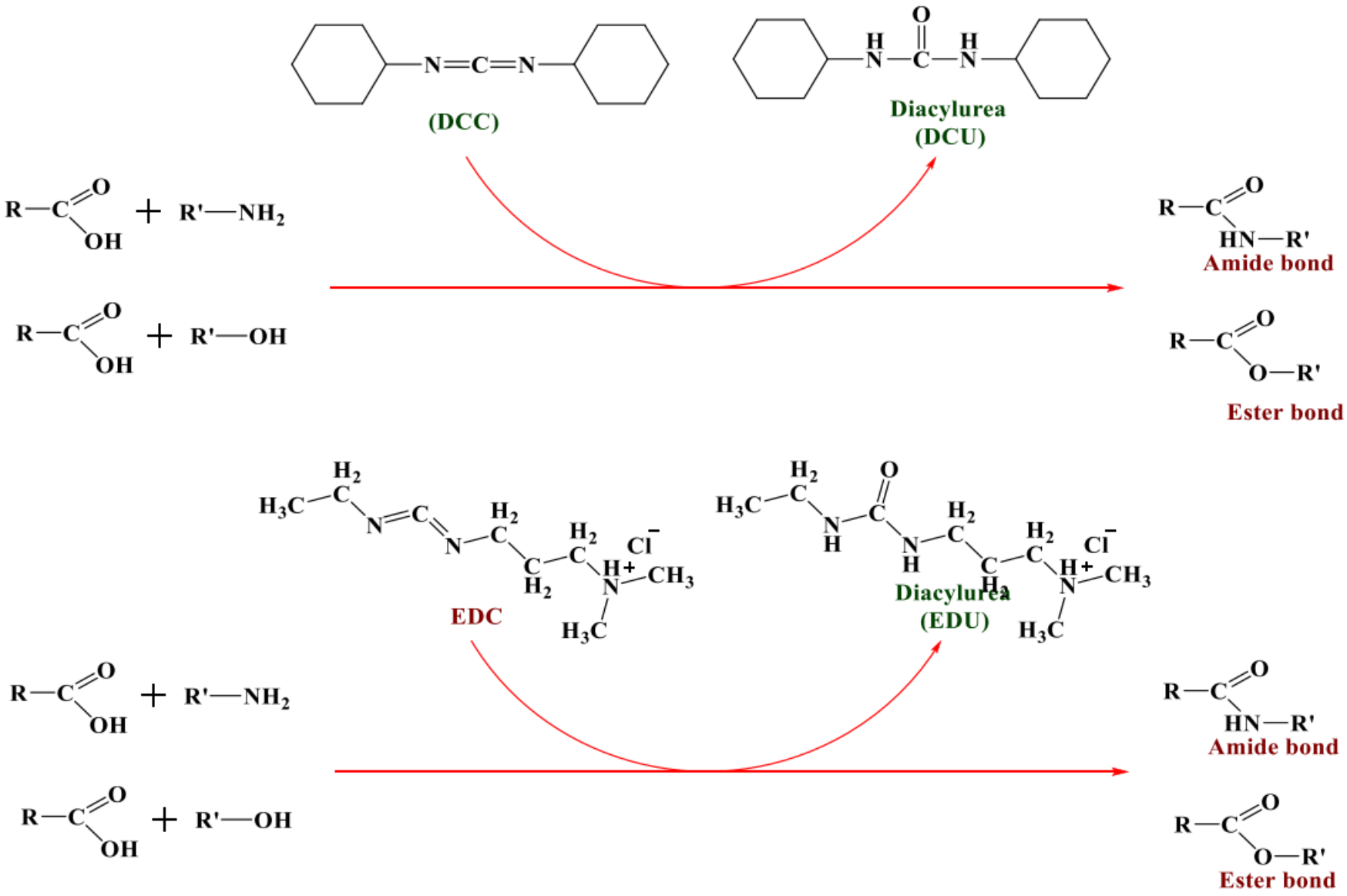
Dicyclohexyl carbodiimide (DCC)
soluble in organic solvent



1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)
Soluble in water

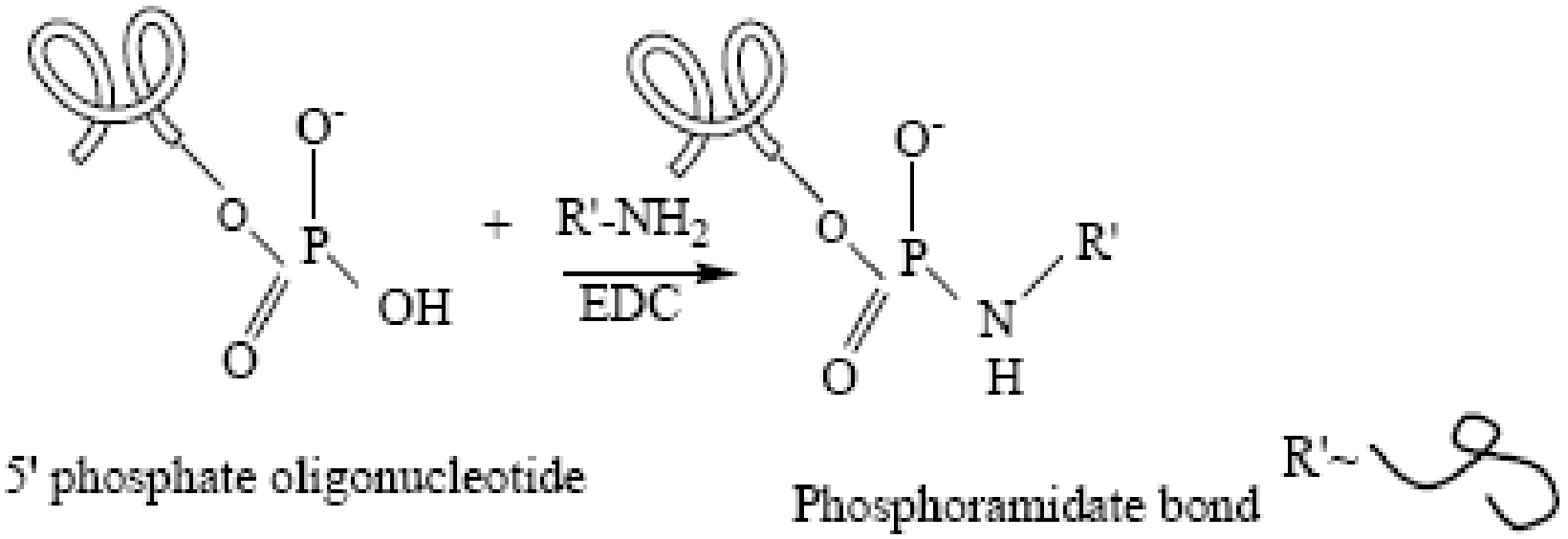
They are most commonly used as coupling reagents to obtain amide linkage between a carboxylate and an amine or phosphoramidate linkage between a phosphate and an amine. They are unique due to their efficiency and versatility to form a conjugate between two polymers, between protein molecules, between a peptide and a drug molecule, or between a peptide and a protein plus any combination of these small molecules.



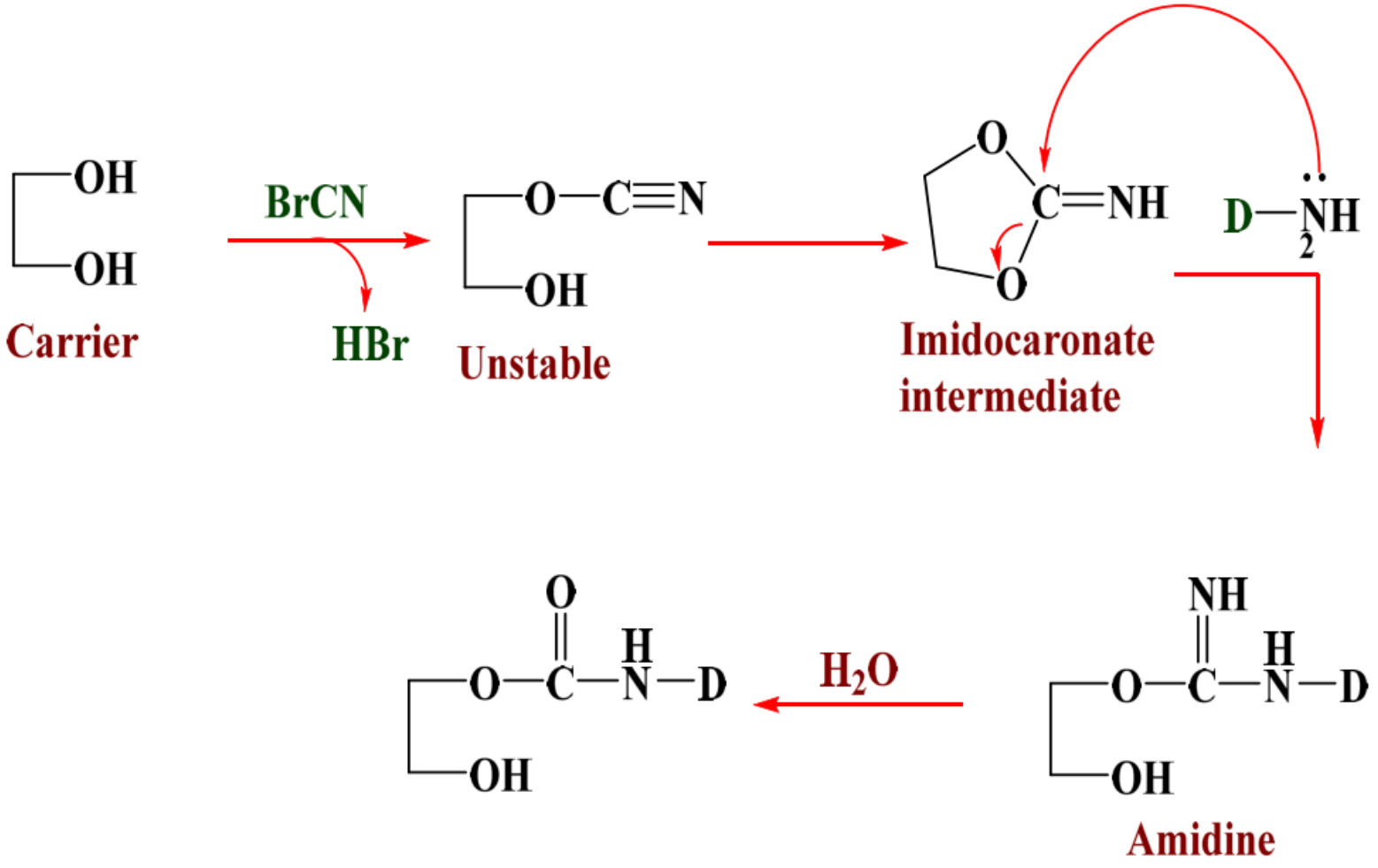


Mechanism of action of DCC and EDC→ Homework

Carbodiimide activates the phosphate to an intermediate phosphate ester, identical to its reaction with carboxylates. Further, in the presence of an amine on a polymer containing – NH_2 terminal groups, carbodiimide can be conjugated to form a stable phosphoramidate bond.

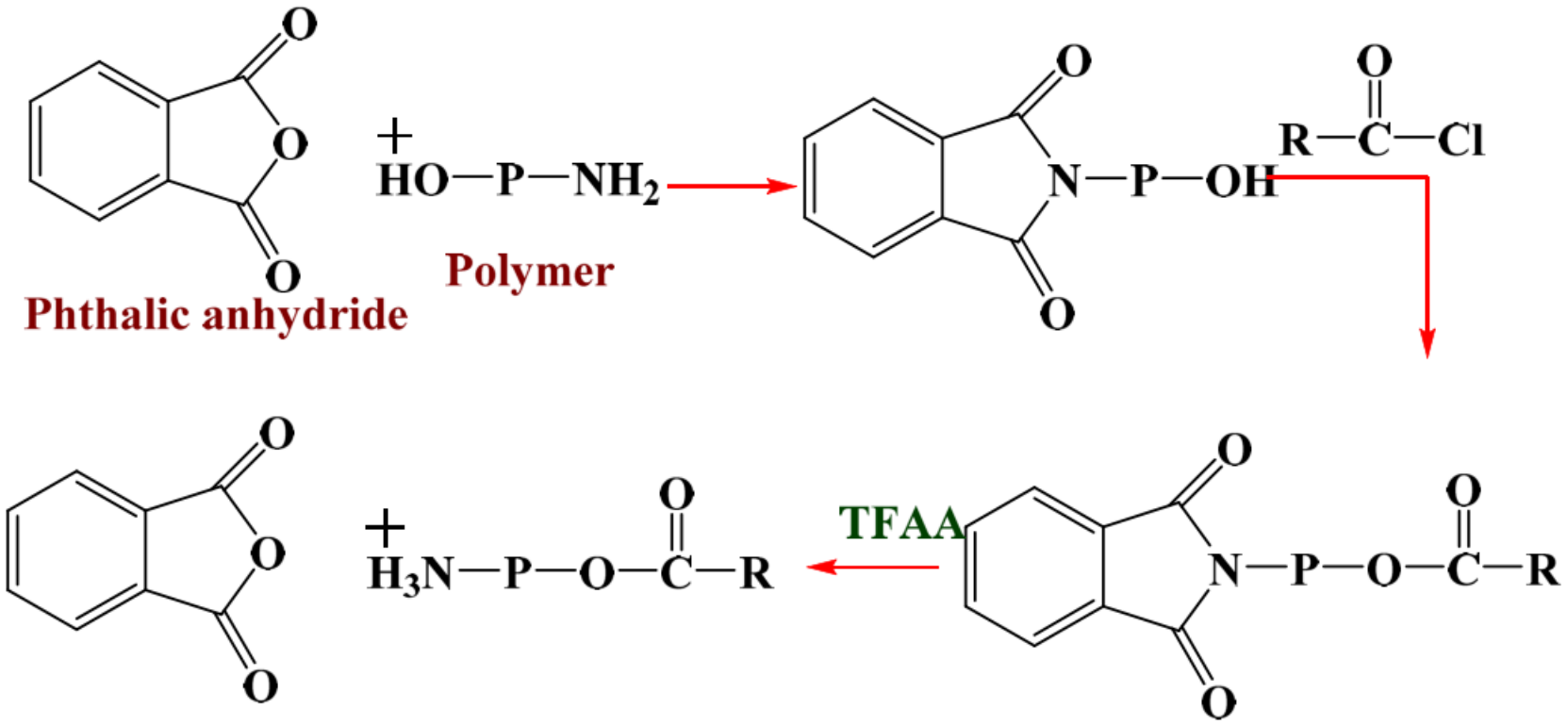


Cyanogens bromide (BrCN) → (activation to OH group)

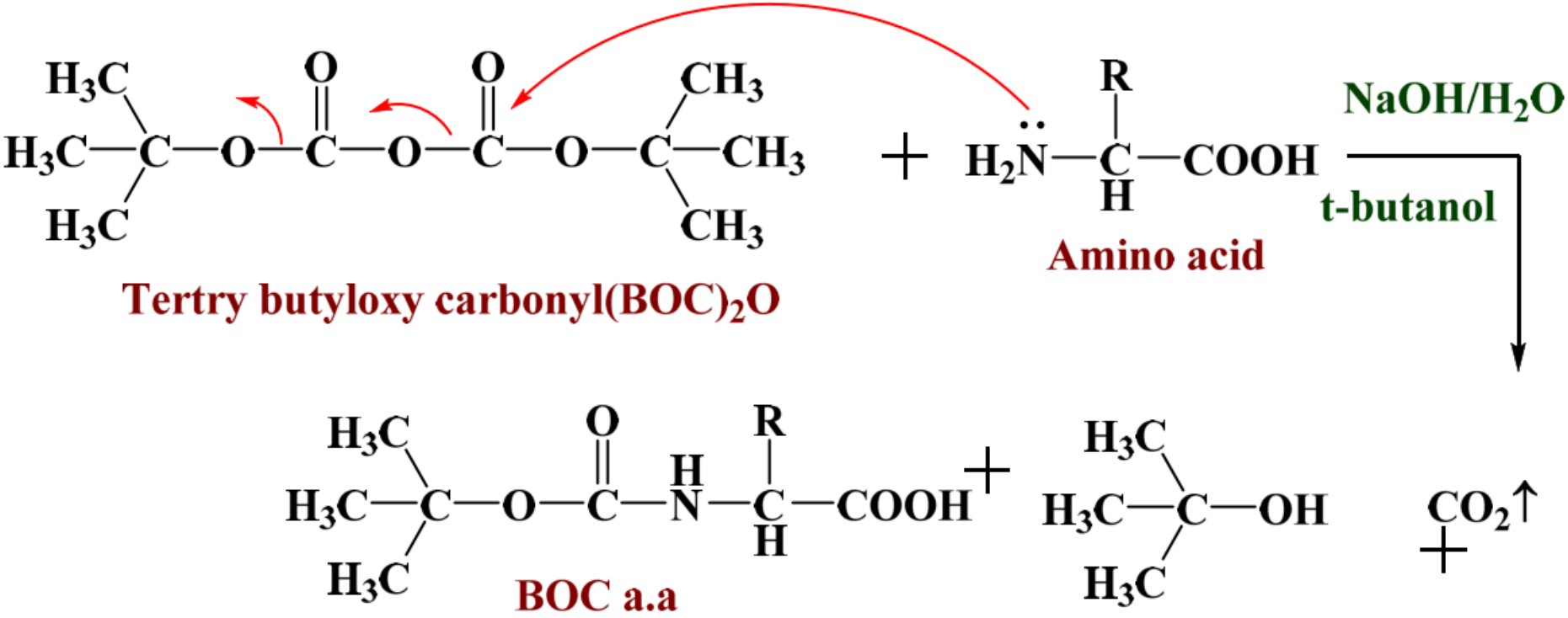


N- protection

- Phthalic anhydride



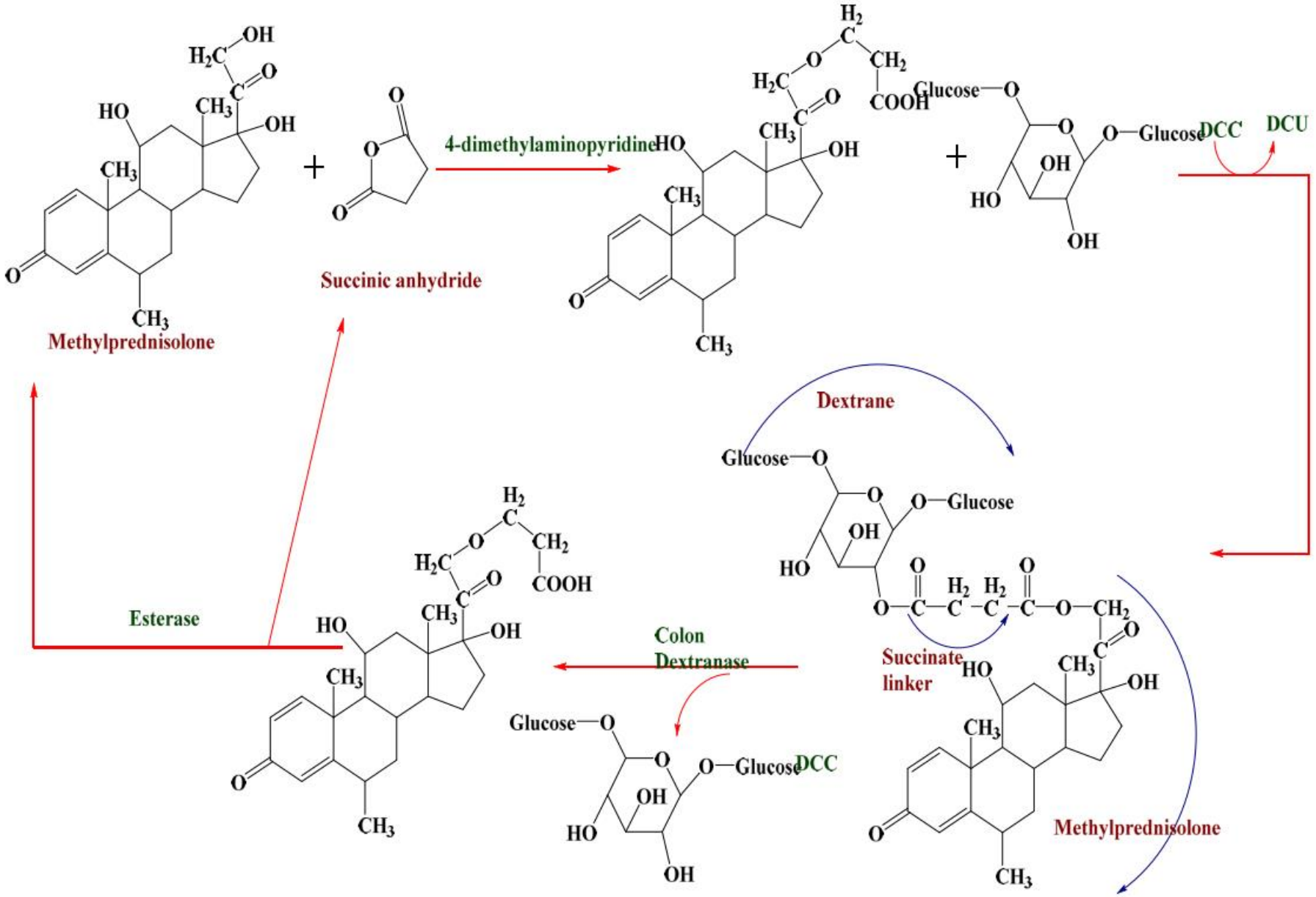
Tertry butyloxy carbonyl$(BOC)_2O$•



Example of polymeric prodrug

Dextran possesses multiple hydroxyl groups and therefore can be easily conjugated with drugs and proteins with reactive groups either by direct conjugation or by incorporation of a spacer arm. After oral administration, the polymer is not significantly absorbed. Therefore, most of the effective applications of dextran as polymeric carriers are through injections.

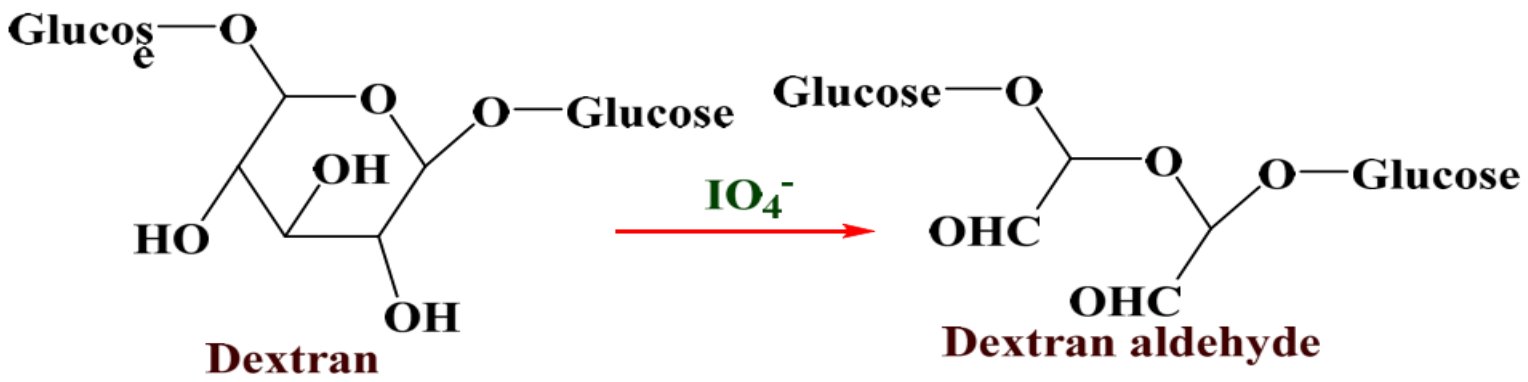
Conjugates of dextrans with corticosteroids have been evaluated previously for the local delivery of steroids in colon as anti-inflammatory agents.



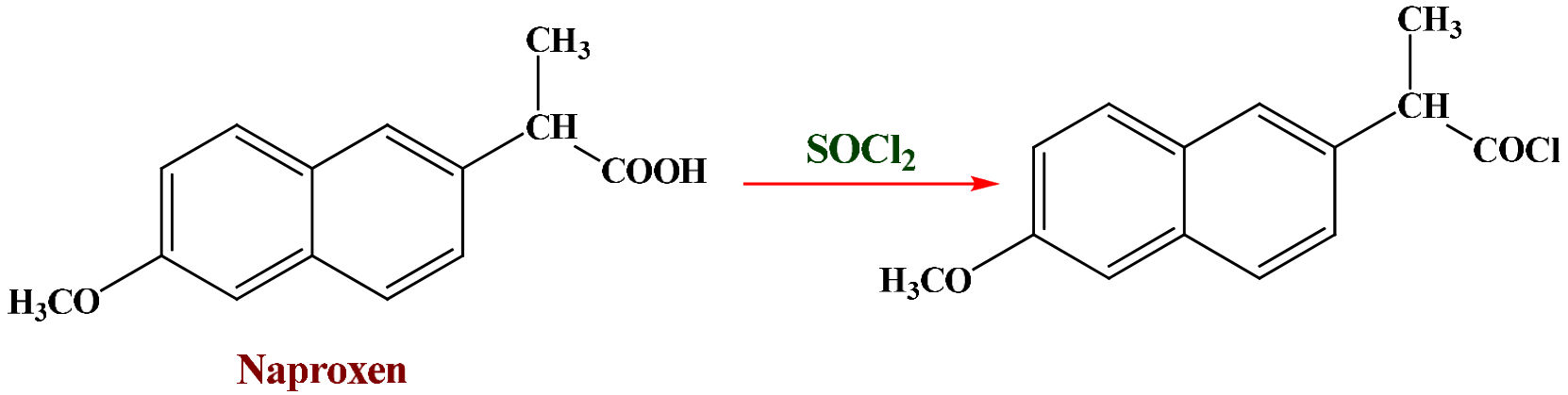
methylprednisolone -succinate-dextran conjugate is more hydrophilic and has a larger molecular weight, which may decrease its possibility of being absorbed into the systemic circulation through the small intestinal epithelial cells. When it arrives to the colon, the dextran structure is hydrolyzed quickly by endogenous dextranase and then the esterase breaks the ester bond to release the methylprednisolone.

Naproxen-Dextran prodrug (long duration of action and reduced ulcerogeneicity)

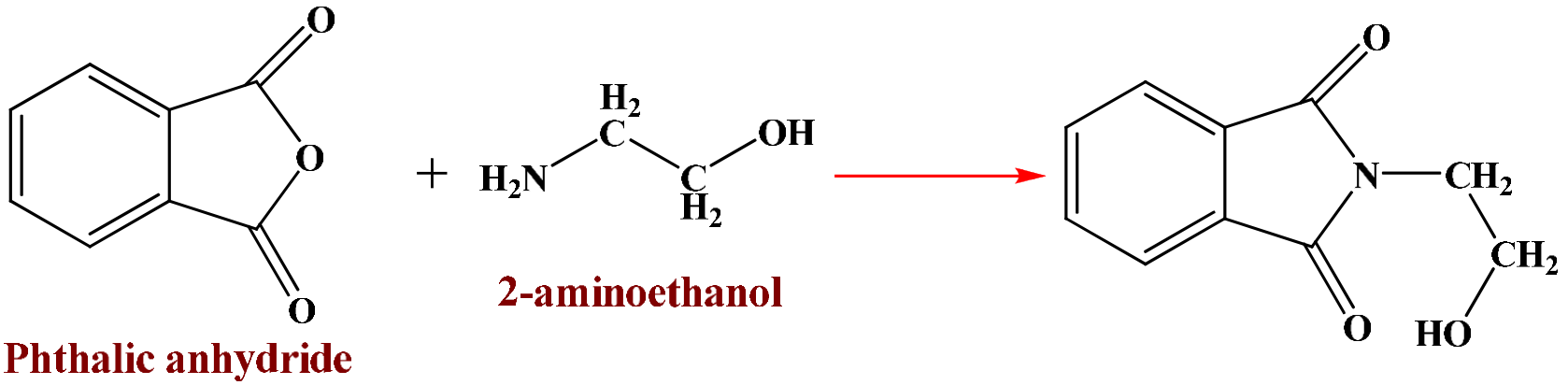
1) Activate the hydroxyl groups of dextran (periodate oxidation)

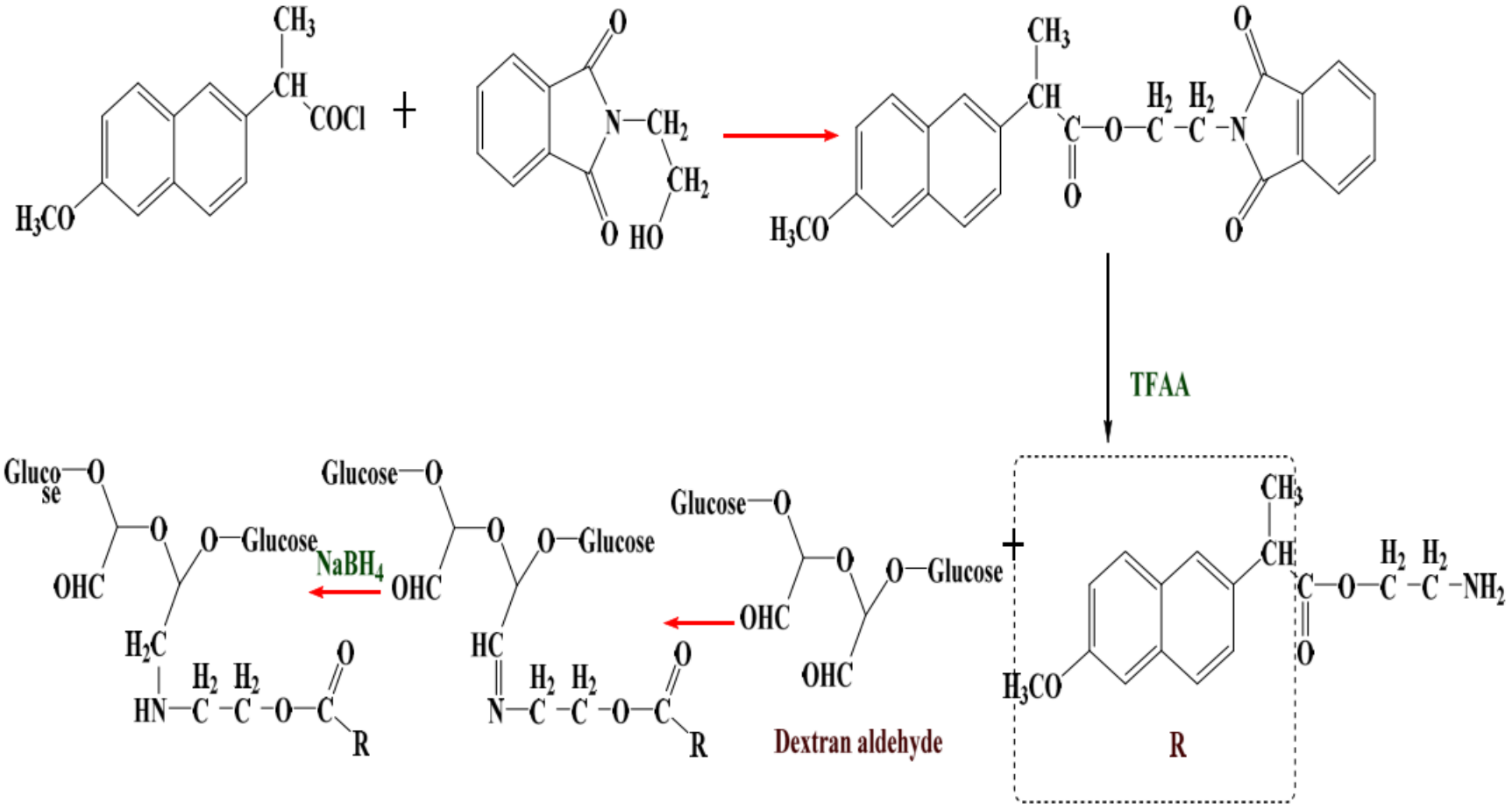


2) Carboxyl group of the active ingredient Naproxen was used for coupling.

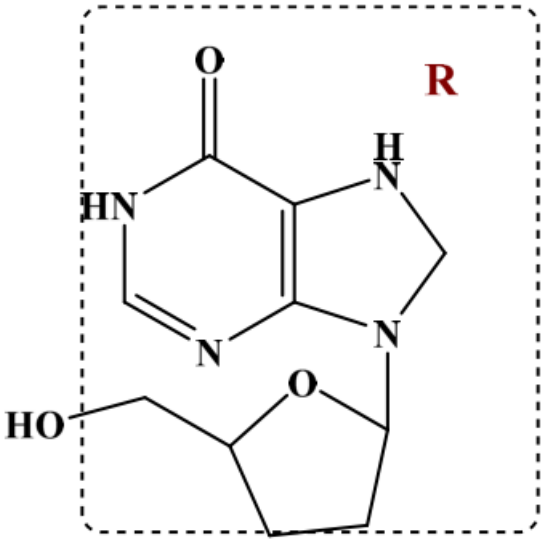


3) Spacer arm Ethanol amine (N-protection)




TFAA
NaBH4
Dextran aldehyde
R

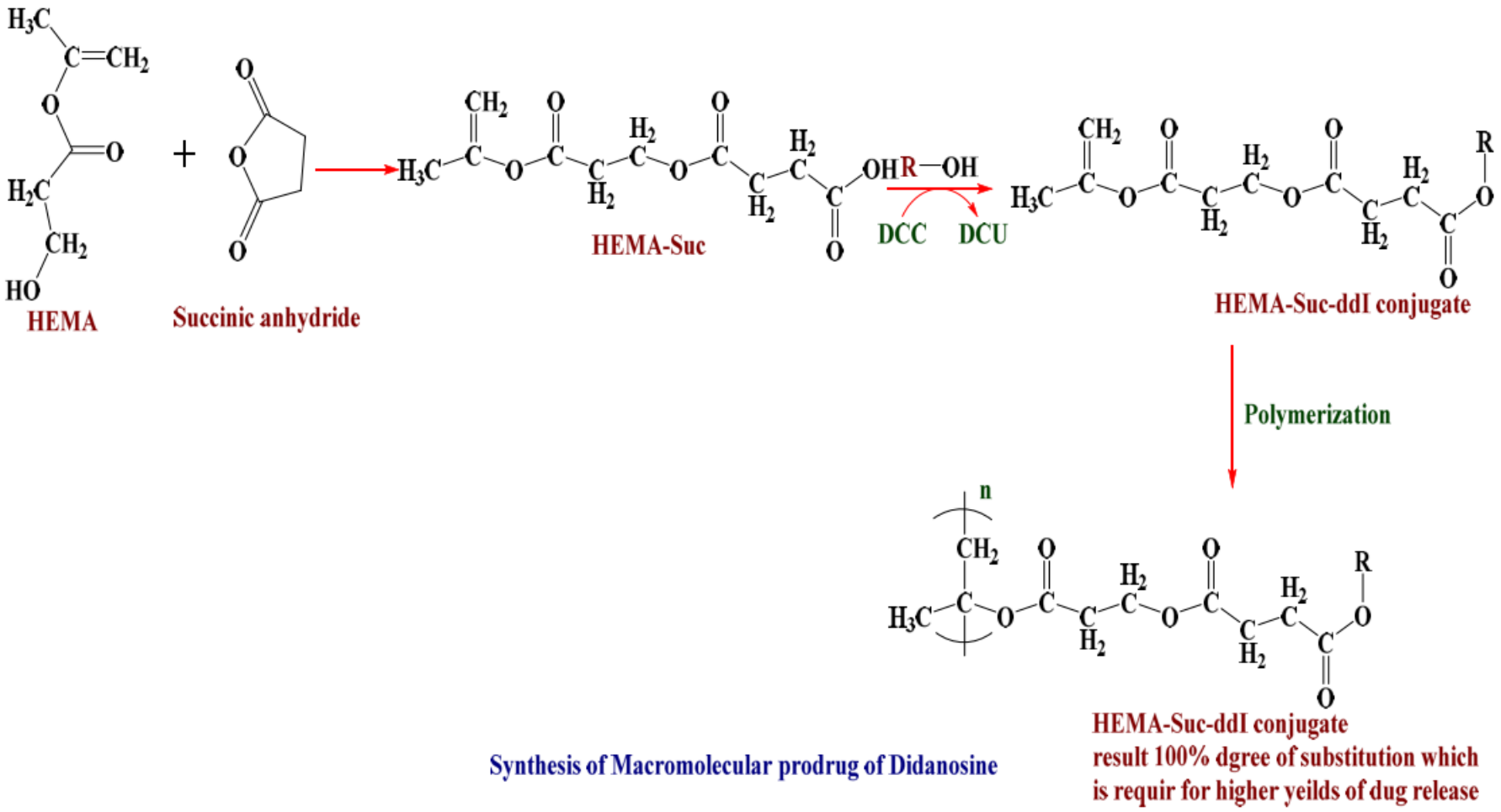
Synthesis, of a macromolecular prodrug of Didanosine(ddI)



Didanosine (ddI) is effective against HIV

Didanosine is associated with several limitations like short plasma half life (1-1.5 h), relatively low bioavailability (42 %) and severe dose dependent cellular toxicities.Didanosine is a highly acid labile compound which is quite stable in alkaline environment

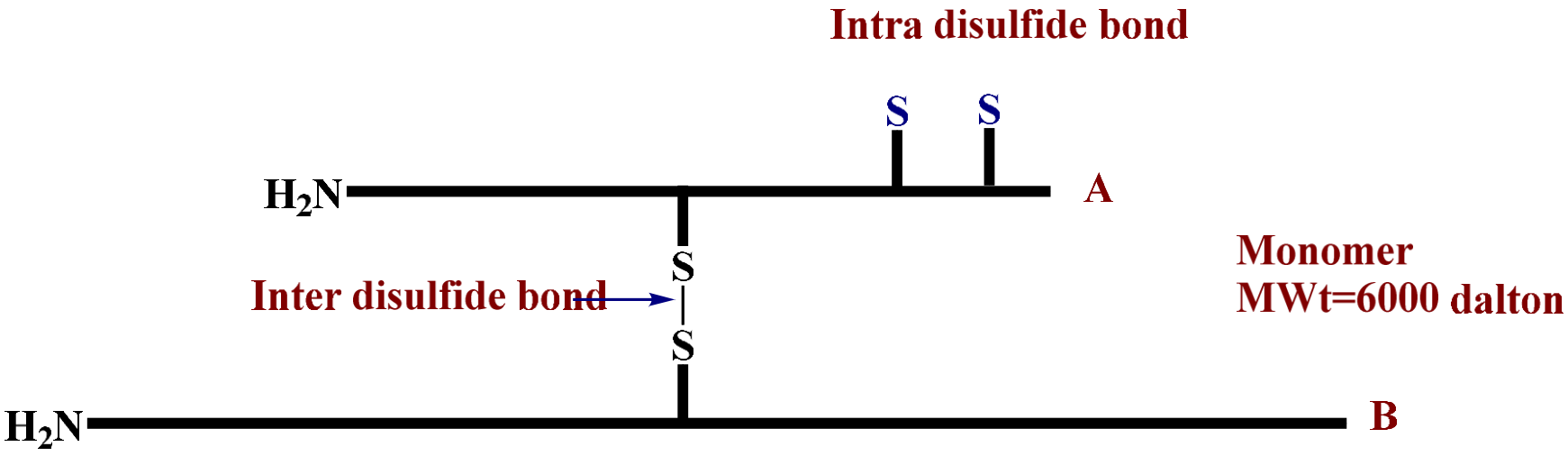
It is easily damaged by stomach acid which is the major reason for its low bioavailability. It was proposed therefore, to synthesize a macromolecular prodrug of ddI for oral administration by coupling the drug to Poly (2-hydroxy ethyl methacrylate, HEMA) through a succinic spacer by ester linkages which would undergo pH dependent hydrolysis to cleave the parent drug in a sustained manner in the alkaline environment of the lower GI tract rather than the acidic environment of the stomach. This pH dependent and sustained release of ddI may result in increasing the bioavailability, t1/2 and maintaining the plasma drug level within the therapeutic range.



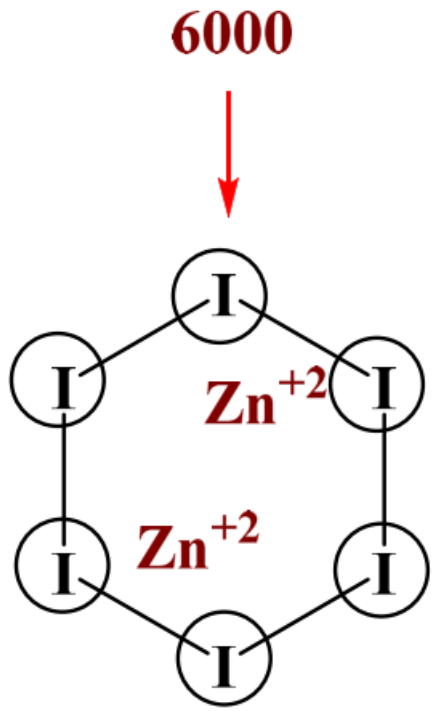
Synthesis of Macromolecular prodrug of Didanosine

Insulin example for chemical modification.

Insulin molecule consist of two chains A and B, with 21 and 31 amino acid residues respectively. These two chains are connected by two disulfide linkages, with an additional disulfide linkage within chain A.

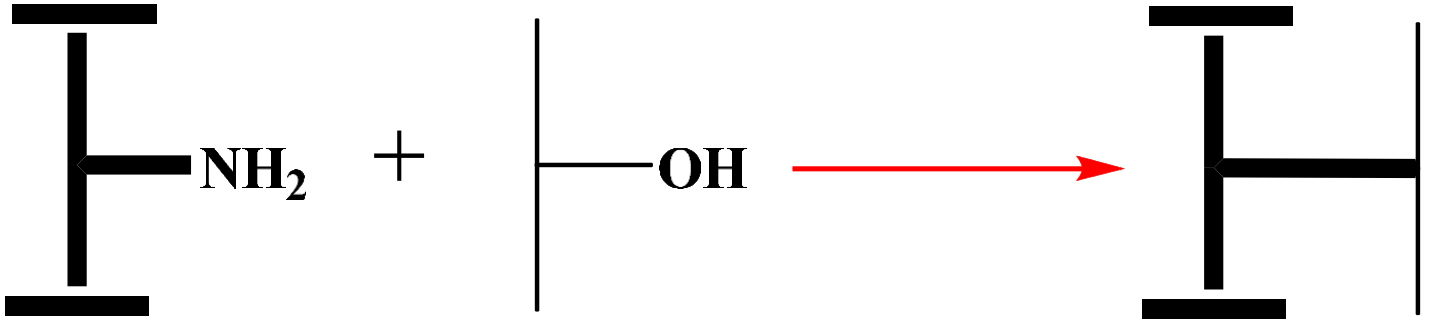


Insulin exist as hexoses "hexane form" physiologically attached to the Zn^{+2} to provide large duration of action.



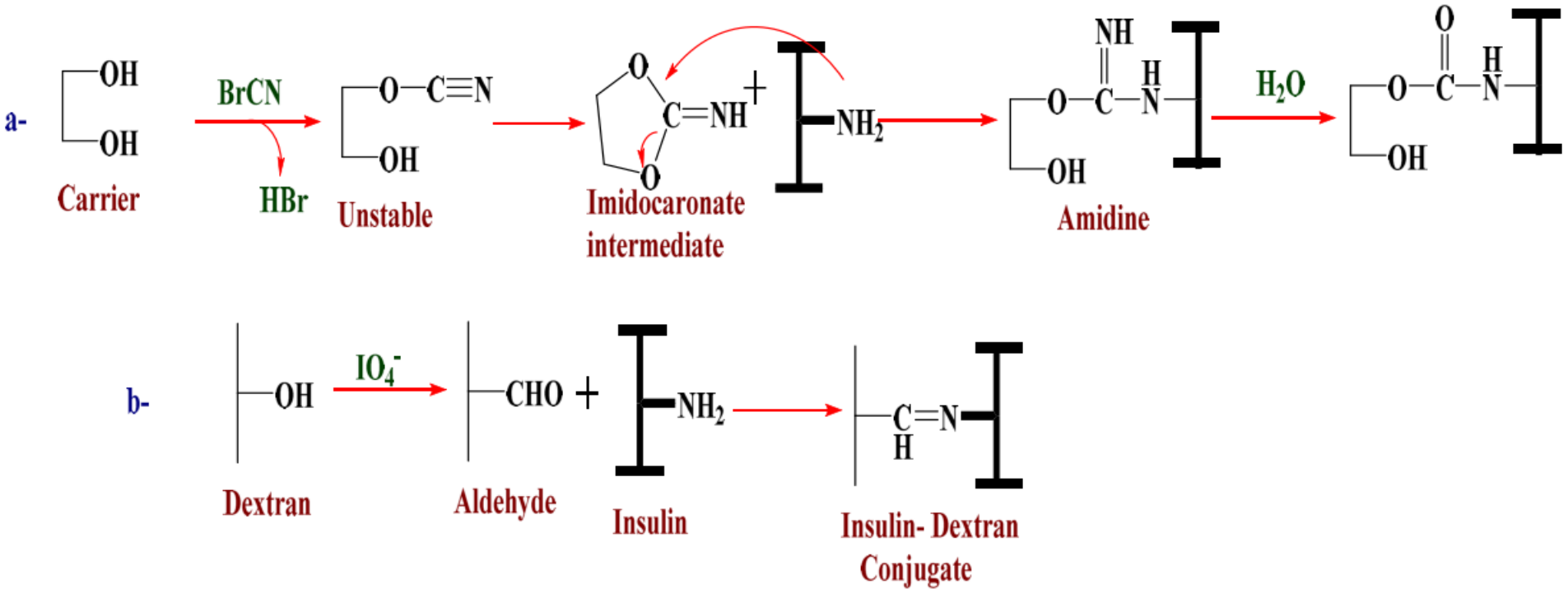
Hexamer of insulin
[Wt: 6 * 6000 =36000 dalton

Chemical modification of insulin → gives long acting insulin

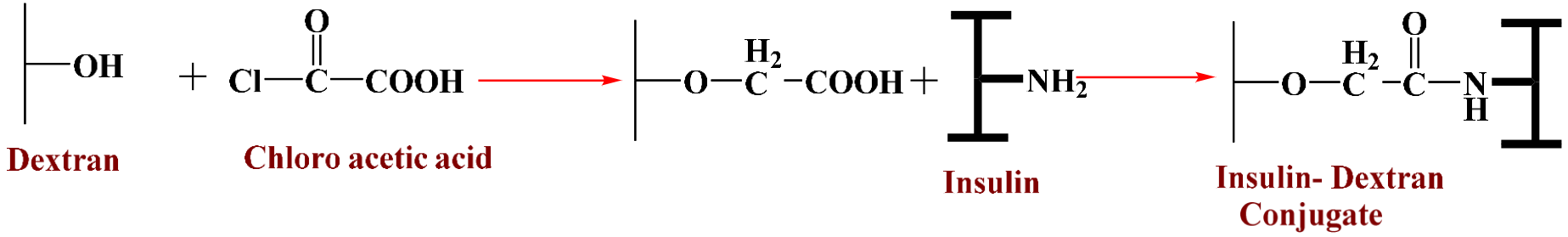


Insulin **Dextran** **Insulin-Dextran conjugate**

Direct reaction•

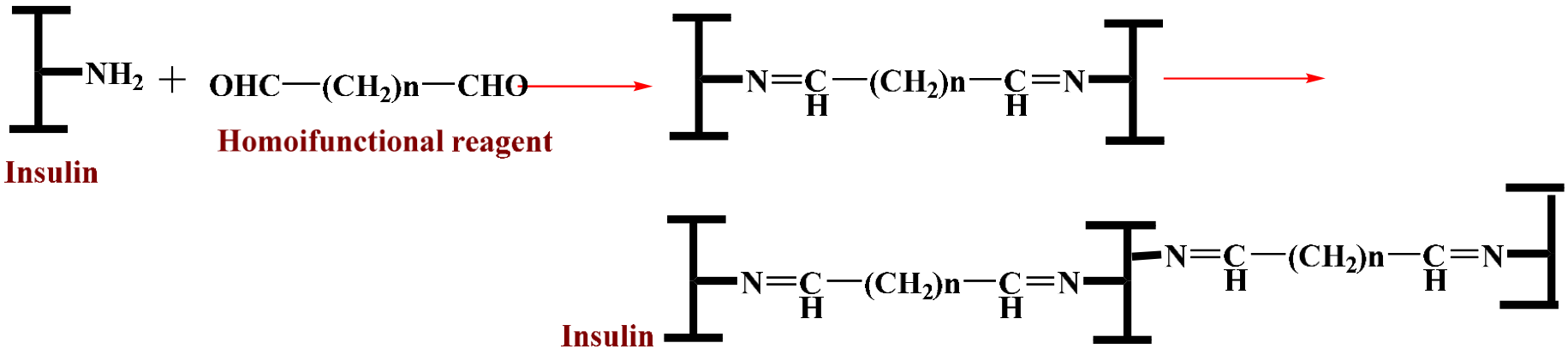


Indirect method (Spacer arm, chloroacetic acid)•



Polymerization of insulin (I-I-I-I)

- Homo bifunctional



If the reaction persists for long time → ppt will occur because of high MWt. In this case all the NH_2 group will react which lead to loss activity and solubility. So we must determine the:-

- Degree of modification.
- Limitation 50%.

Heterobifunctional•