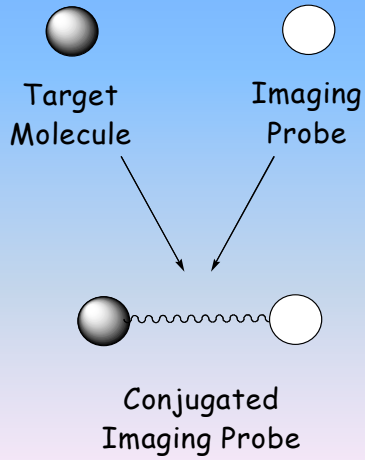


CHEMISTRY OF CONJUGATION

Conjugation (of imaging probes) to specific targets

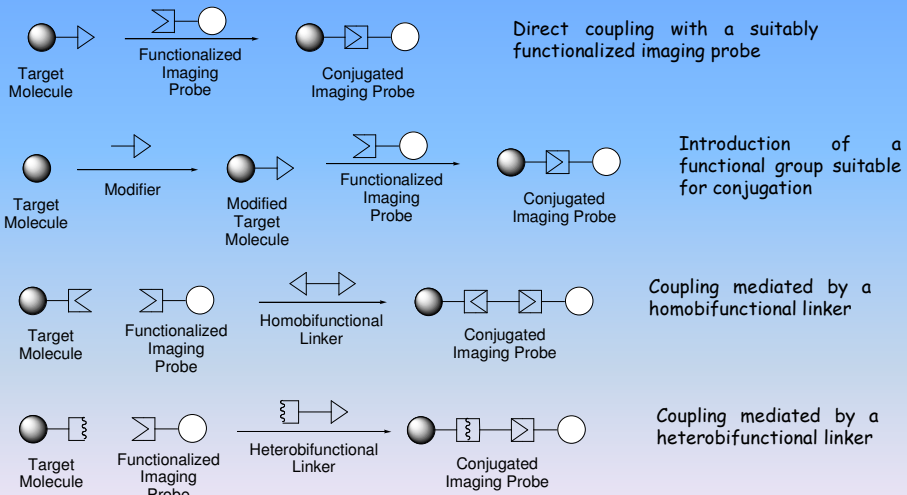


Targets

Peptides
Proteins
Carbohydrates
Nucleic Acids
Lipids
Other organic molecules

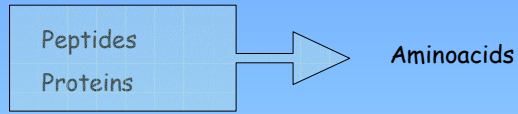
CHEMISTRY OF CONJUGATION

Strategies

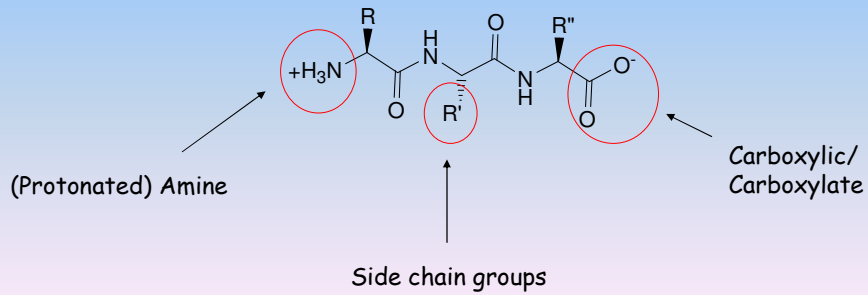


CHEMISTRY OF CONJUGATION

Direct Coupling

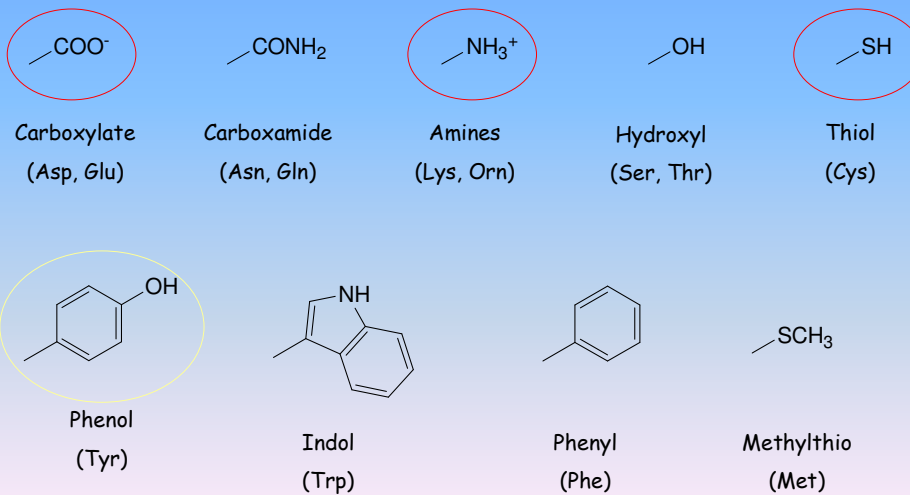


Functional groups amenable to conjugation



CHEMISTRY OF CONJUGATION

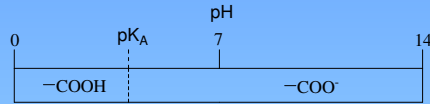
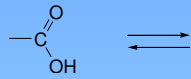
Side chain groups



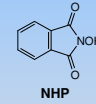
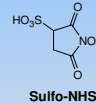
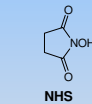
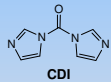
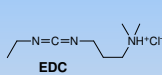
CHEMISTRY OF CONJUGATION

Carboxylic group

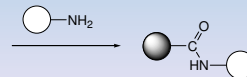
pK_a 2.0-4.5



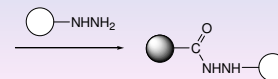
← "Activated form"



Ester



Amide

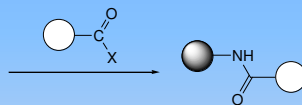
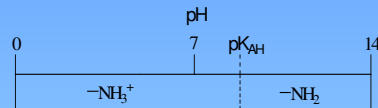


Hydrazide

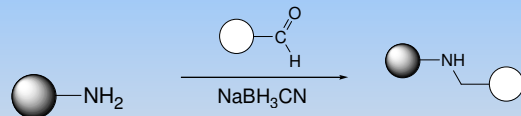
CHEMISTRY OF CONJUGATION

Amines

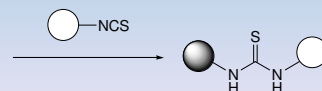
pK_{aH} = 7.5-9.5



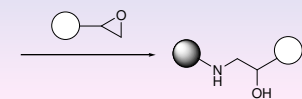
Acylation with "activated" carboxylic acids (anhydrides, chlorides, imidazolides, carbodiimide adducts)



Reductive amination with aldehydes



Reaction with isothiocyanates to give thioureas



Alkylation with epoxides

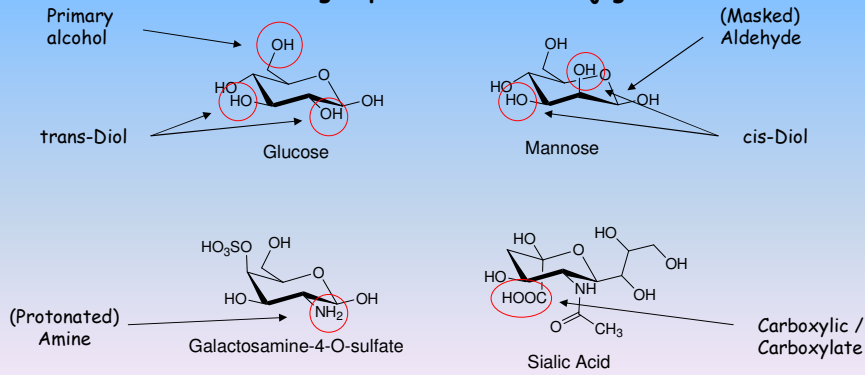
CHEMISTRY OF CONJUGATION

Direct Coupling

Carbohydrates

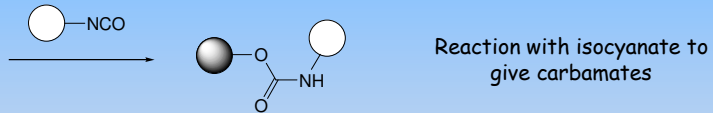
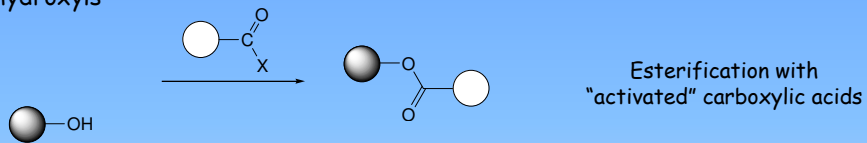
Saccharides

Functional groups amenable to conjugation

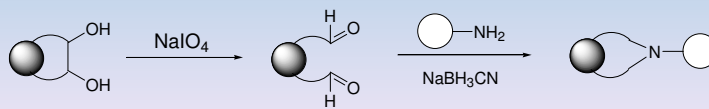


CHEMISTRY OF CONJUGATION

Hydroxyls



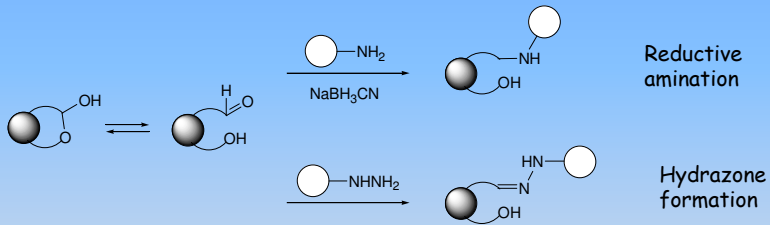
Diols



Oxidative demolition with periodate
followed by reductive amination

CHEMISTRY OF CONJUGATION

"Masked" Aldehydes (hemiacetals)



Amines and Carboxylic Acids

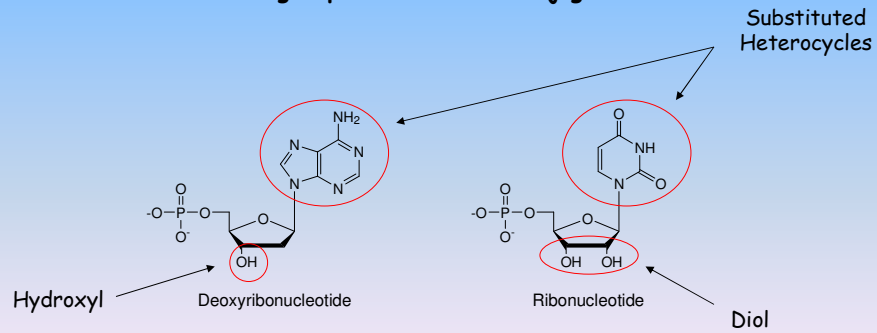
See slides 5-6 for conjugation to amines and carboxylic acids

CHEMISTRY OF CONJUGATION

Oligonucleotides

Nucleotides

Functional groups amenable to conjugation



CHEMISTRY OF CONJUGATION

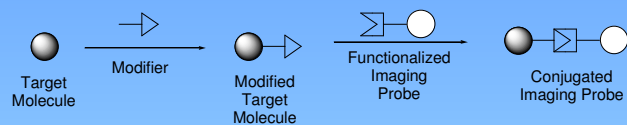
Hydroxyl
Diol

See previous slides for conjugation of alcohols and diols

Substituted Heterocycles

Specific methods of conjugation adapted to the single bases

CHEMISTRY OF CONJUGATION



The modifier introduces a new FG in the target molecule, allowing its conjugation with suitably functionalized imaging probes

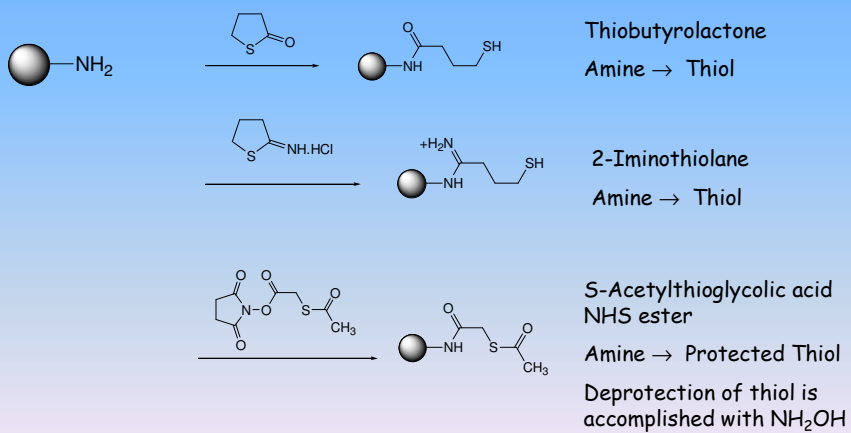
The modifier converts a FG present in the target molecule into another FG, more suitable for conjugation reactions

The modifier is a (homo- or hetero-) bifunctional reagent

The modifier acts as a linker and a spacer

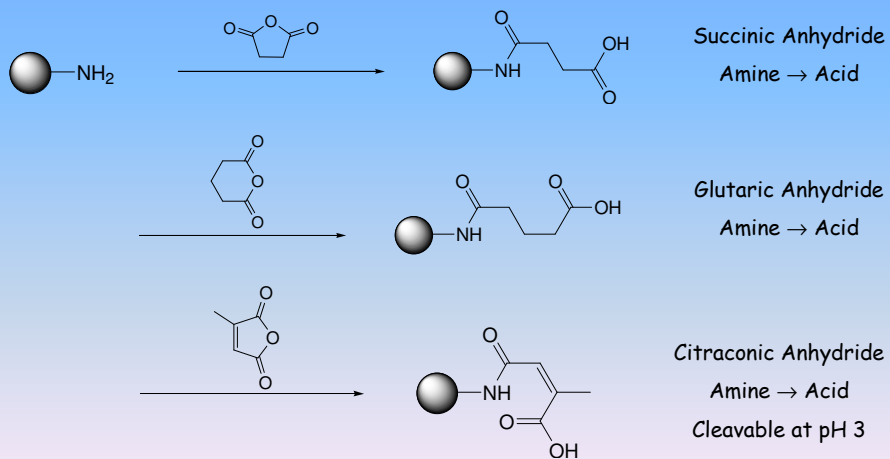
CHEMISTRY OF CONJUGATION

Modification of amines



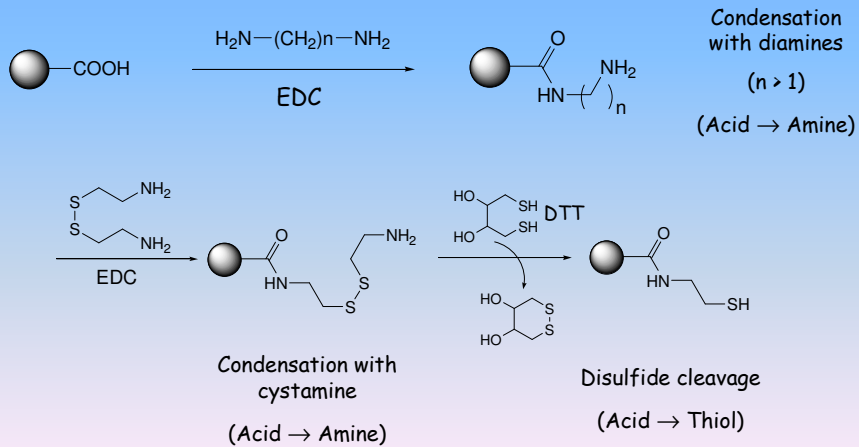
CHEMISTRY OF CONJUGATION

Modification of amines



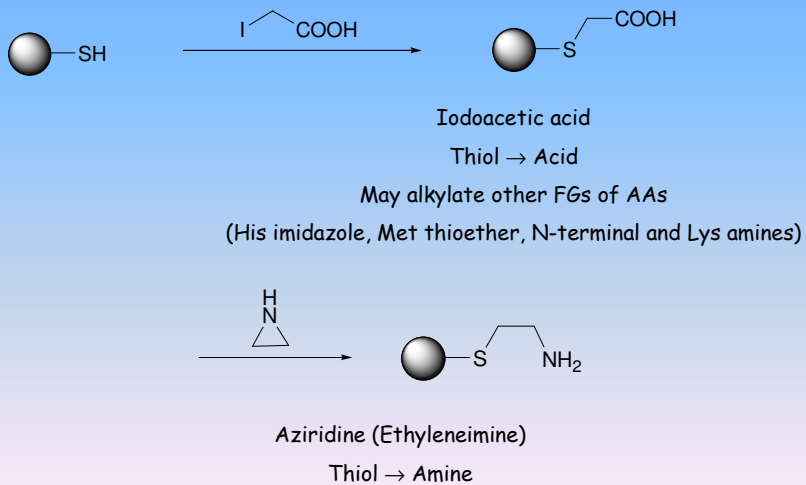
CHEMISTRY OF CONJUGATION

Modification of carboxylic acids



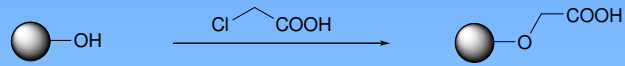
CHEMISTRY OF CONJUGATION

Modification of thiols



CHEMISTRY OF CONJUGATION

Modification of hydroxyls

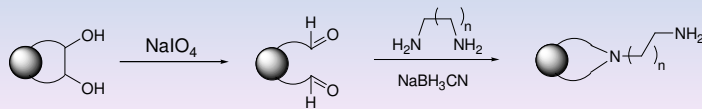
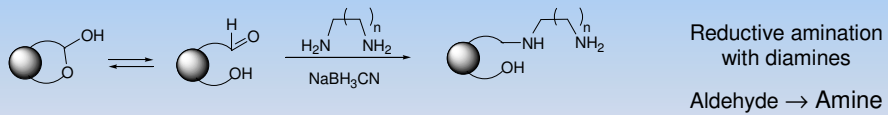


Chloroacetic acid (basic pH)

Hydroxyl \rightarrow Acid

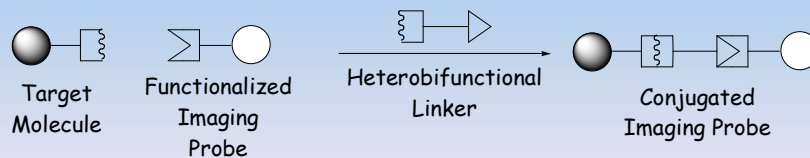
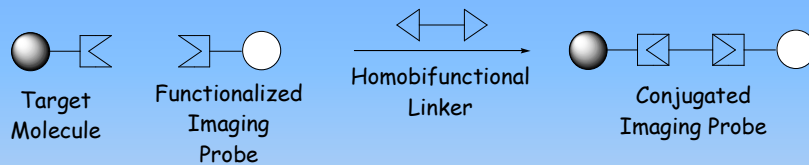
May alkylate other FGs of AAs

Modification of aldehydes (indigenous or periodate-derived)



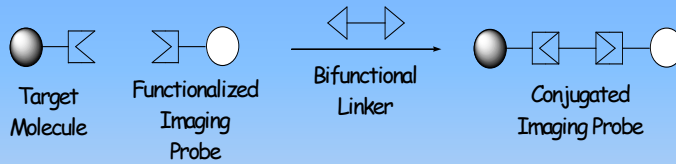
CHEMISTRY OF CONJUGATION

Bifunctional Linkers

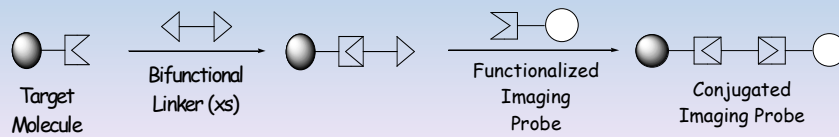


CHEMISTRY OF CONJUGATION

Coupling mediated by a homobifunctional linker



Stepwise procedure \rightarrow avoids self-cross-linking of target molecules

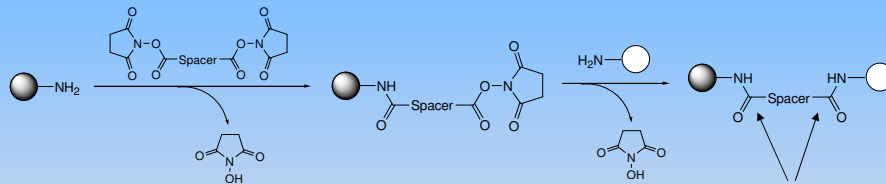


CHEMISTRY OF CONJUGATION

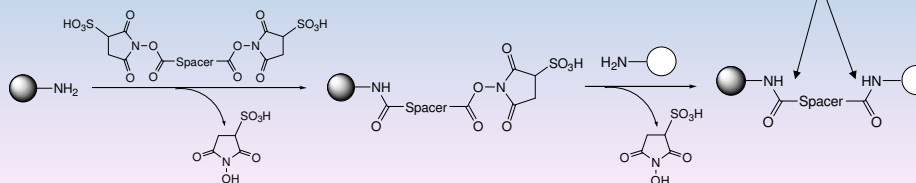
Homobifunctional Linkers

Specific for Amine-Amine

NHS-Diesters



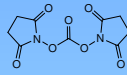
Sulfo-NHS-Diesters



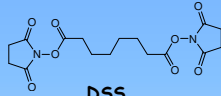
CHEMISTRY OF CONJUGATION

Homobifunctional Linkers for Amine-Amine

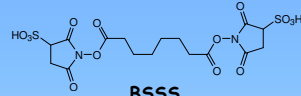
NHS and Sulfo-NHS active diesters



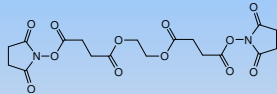
DSC



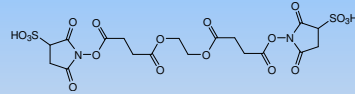
DSS



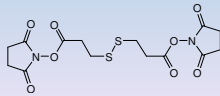
BSSS



EGS

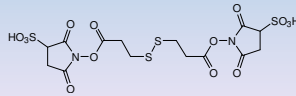


Sulfo-EGS



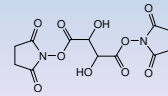
DSP

Cleavable (DTT)



DTSSP

Cleavable (DTT)



DST

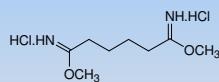
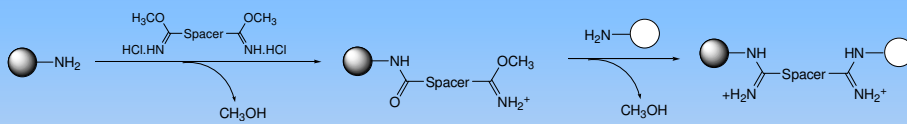
Cleavable (NaIO₄)

CHEMISTRY OF CONJUGATION

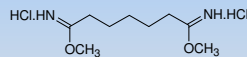
Homobifunctional Linkers

Specific for Amine-Amine

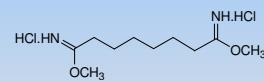
Diimidates



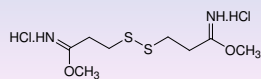
**Dimethyl
adipimidate**



**Dimethyl
pimelimidate**



**Dimethyl
suberimidate**

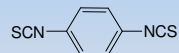
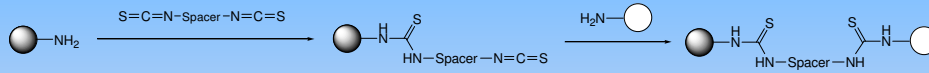


DTSSP
Cleavable (DTT)

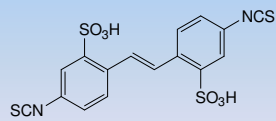
CHEMISTRY OF CONJUGATION

Homobifunctional Linkers
Specific for Amine-Amine

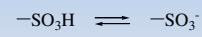
Diisothiocyanate



1,4-Phenylene
diisothiocyanate



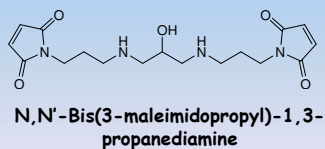
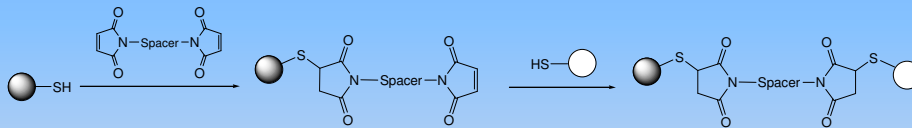
4,4'-Diisothiocyanato
stilbene-2,2'-disulfonic acid



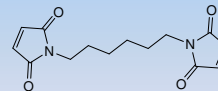
CHEMISTRY OF CONJUGATION

Homobifunctional Linkers
Specific for Thiol-Thiol

Bis-Maleimides



N,N'-Bis(3-maleimidopropyl)-1,3-
propanediamine



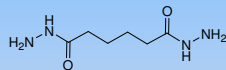
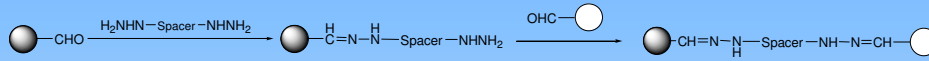
BMH

CHEMISTRY OF CONJUGATION

Homobifunctional Linkers

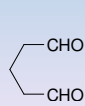
Specific for Aldehyde-Aldehyde

Bis-Hydrazides



Adipic acid dihydrazide

Other Homobifunctional Linkers

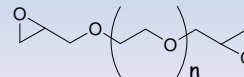


Glutaraldehyde

Aldehyde groups react with amines to form imine or emiaminal or aiminal linkages

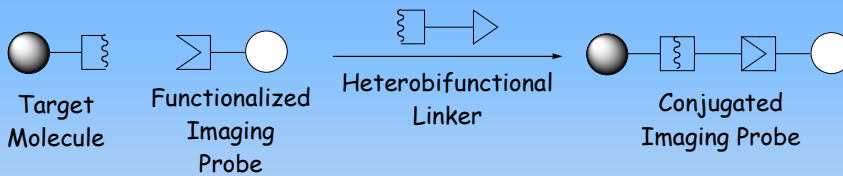
Oligo(Poly)ethylenglycol diglycidyl ether

Epoxides react with nucleophilic groups (amines, thiols, alcohols)



CHEMISTRY OF CONJUGATION

Heterobifunctional Linkers



The most reactive FG of heterobifunctional linker is usually reacted first
Different functional groups in the linker prevent self-cross-linking of target molecules

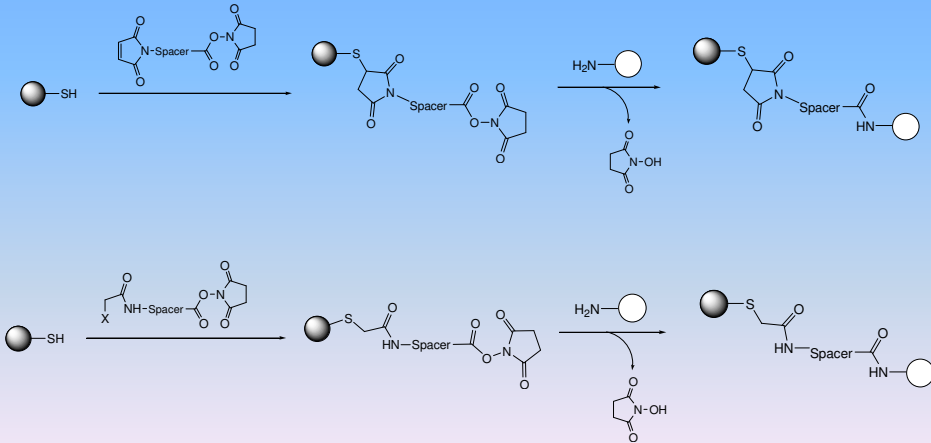
Higher specificity

Higher yields

CHEMISTRY OF CONJUGATION

Heterobifunctional Linkers

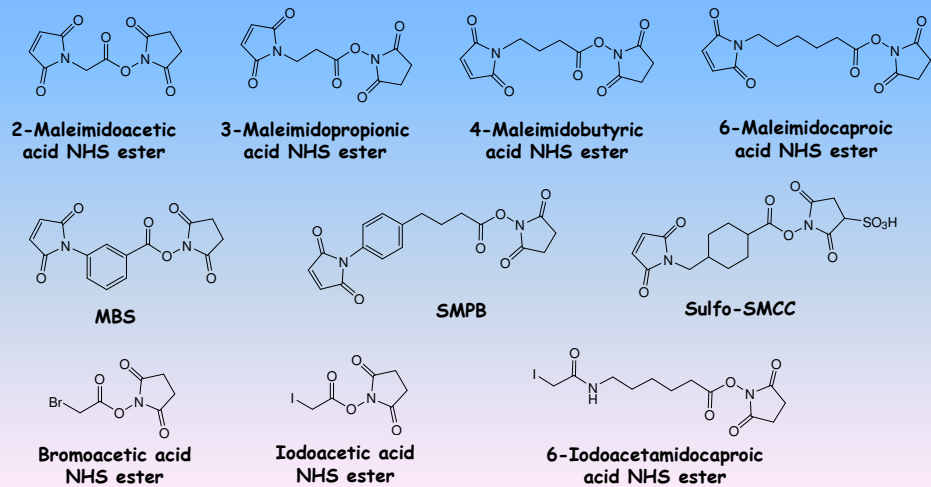
Specific for Amine-Thiols



CHEMISTRY OF CONJUGATION

Heterobifunctional Linkers

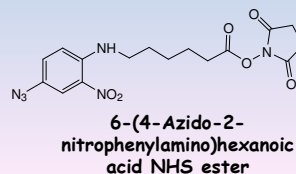
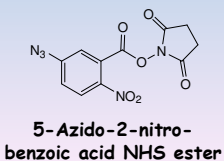
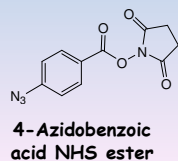
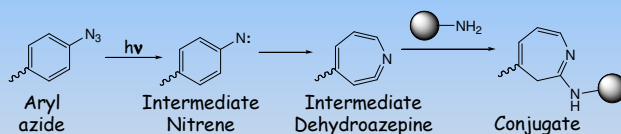
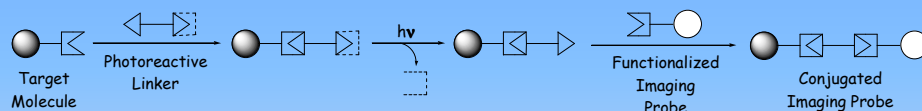
Specific for Amine-Thiols



CHEMISTRY OF CONJUGATION

Heterobifunctional Photoreactive Linkers

One (or both) FG react only after irradiation with UV-light



CHEMISTRY OF CONJUGATION

Recent Trends: Click Chemistry

be useful in this context. The reaction must be *modular*, *wide in scope*, give *very high yields*, generate only *inoffensive byproducts* that can be removed by nonchromatographic methods, and be *stereospecific* (but not necessarily enantioselective). The required process characteristics include *simple reaction conditions* (ideally, the process should be insensitive to oxygen and water), *readily available starting materials and reagents*, the use of *no solvent or a solvent that is benign* (such as water) or *easily removed*, and *simple product isolation*. Purification—if required—must be by nonchromatographic methods, such as crystallization or distillation, and the product must be stable under physiological conditions.

Sharpless, K.B. et al
ACIEE 2001, 40, 2004

Click Chemistry

Azide-Alkyne

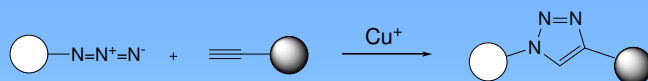
Thiol Click

Diels-Alder

Photo-Click

CHEMISTRY OF CONJUGATION

Cu-Catalyzed Azide Alkyne Cycloaddition (CuAAC)



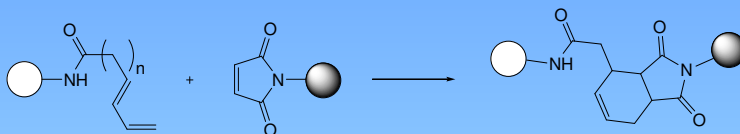
Very efficient reaction
Compatible with several functional groups
(Bio)Orthogonal reaction
Operational simplicity
Stable linkage



Copper ion issues
FGs not present in biomolecules
Chelating agents bind Cu⁺
Triazole ring effect unpredictable

CHEMISTRY OF CONJUGATION

Diels-Alder Cycloaddition



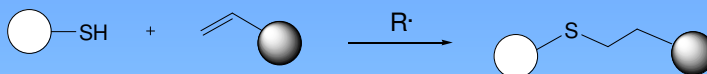
Efficient reaction
Compatible with several functional groups
(Bio)Orthogonal reaction
Operational simplicity
Stable linkage



FGs not present in biomolecules
Limited stability of the diene moiety
Bulky Adducts

CHEMISTRY OF CONJUGATION

Thiol-Ene Radical



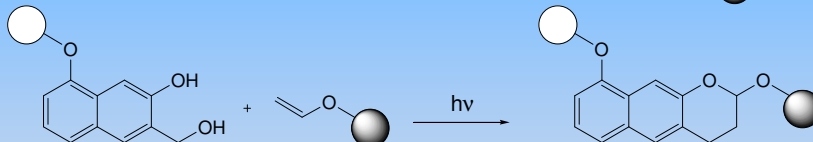
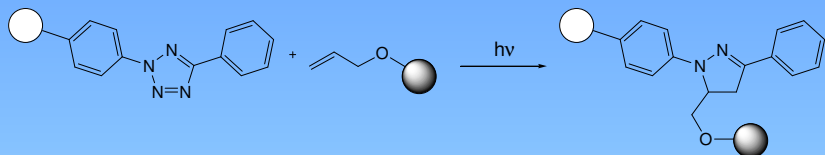
Very efficient reaction
 (Bio)Orthogonal reaction
 Operational simplicity
 Quite Stable Linkage
 Thiol group frequently available



Need for radical initiator
 Presence of byproducts
 Possibility of side reactions
 Alkene not present in biomolecules

CHEMISTRY OF CONJUGATION

Photo-Click Cycloaddition



Very fast reactions
 (Bio)Orthogonal reactions
 Triggered by irradiation
 Stable Linkage
 No byproducts



FGs not present in biomolecules
 Photochemical hardware needed
 Possibility of side reactions
 Bulky adducts

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