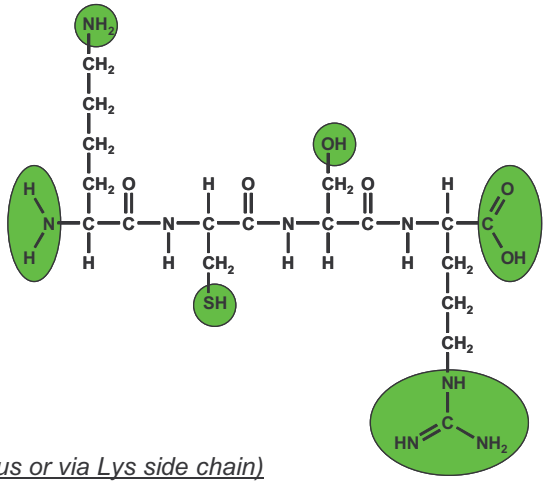
Modifications of peptides

Among common standard modifications of peptides you can find:

- D-amino acids
- unnatural amino acids (6-Aminocaproic acid, Aminobutyric acid, Citrulline, Norleucine, etc.)
- cyclisation
- phosphorylation or sulfurylation (at Ser, Tyr, Thr)
- biotinylation
- conjugation to carrier proteins (BSA, KLH, OVA)
- branching of peptides (MAPs – multiple antigenic peptides)

In general, there are some standard peptide moieties accessible to be modified:

- N-terminal amino group
- amino group of Lys
- thiol group of Cys
- hydroxyl groups of Ser, Thr, Tyr
- guanidine group of Arg
- C-terminal carboxyl group



Amino group modifications (N-terminus or via Lys side chain)

All modifications carrying amine-reactive functional groups can be used.
Among the most commonly used ones, you can find:

- activated esters
- isothiocyanates
- carboxylic acids

Standard modifications that are coupled via amino groups are:

- biotin
- different dyes
- bifunctional linkers
- different acetylating groups

Thiol group modifications (via Cys side chain)

All modifications carrying thiol-reactive functional groups can be used.
Among the most commonly used ones, you can find:

- iodoacetamides
- maleimides
- alkyl halides

Standard modifications that are coupled via thiol groups are:

- different dyes
- KLH or BSA

Carboxyl group modification (C-terminus)
All modifications carrying carboxy-reactive functional groups can be used.
Among the most commonly used ones, you can find:

- amino groups
- amines
- bifunctional aminolinkers

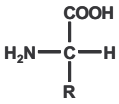
Standard modifications that are coupled via carboxy groups are:

- amides
- chromophores

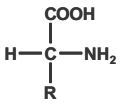
Single modifications – Amino acids

D-amino acids

Amino acids carrying four different groups on their α-C atom (i.e. asymmetric C atom, or C*) are chiral substances. These α-amino acids can be found in respective L- and D-forms (enantiomers):



L-amino acid

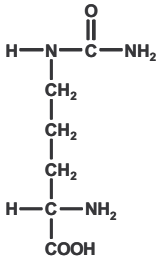


D-amino acid

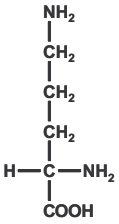
The predominant form in natural proteins is the L-form.
As some enzyme classes are enantioselective, i.e. they can distinguish between L- and D-forms and specifically accept only one of the two forms as substrate, this enantioselectivity makes D-amino acids a valuable tool in medicine (e.g. in peptide antibiotics) and enzyme assays.

Unnatural amino acids, special amino acids and protecting groups

In contrast to the 20 natural amino acids (or proteinogenic), these amino acids are not encoded by the Universal Genetic Code – usually they can be found in nature as metabolic products, especially in plants and bacteria.

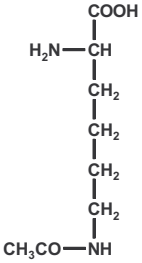


Citrulline

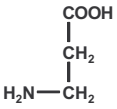


Ornithine

ε-Acetyl-Lysine

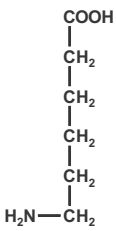


β-Alanine (3-amino-propionic acid)
is the only natural occurring β-amino acid, present e.g in panthothenic acid.

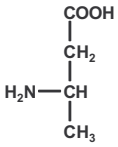


Aminobenzoic acid

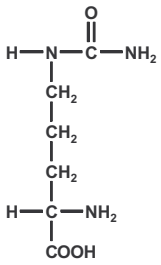
6-Aminocaproic acid (Aca, 6-Aminohexanoic acid)
This amino acid is often used as a linker to increase the distance between the peptide and an additional modification, e.g. a fluorescent dye.



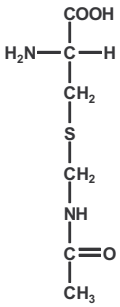
Aminobutyric acid (Abu)
γ-aminobutyric acid (or GABA) is an inhibitory transmitter of the central nerval system. It enhances permeability of postsynaptic membranes for chloride ions, thus leading to hyperpolarisation and consequently to an increase of the membrane's activation potential.



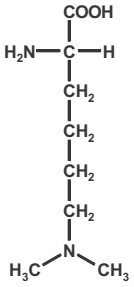
Citrulline
is a metabolic reagent in the urea metabolism pathway of many terrestric vertebrates. In this pathway, unwanted ammonia is being detoxified and eliminated.



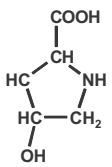
Cysteine, Acm (Acetamidomethyl) protected
this specially protected Cys is used to selectively form disulfide bridges.



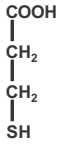
Dimethyl-Lysine



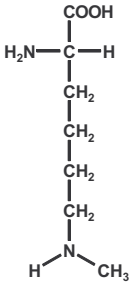
Hydroxy-Proline (Hyp)
is present almost exclusively in structural proteins (e.g. collagens or connective tissues in plant cell walls or mammals). It is formed during a posttranslational modification of proline in cells.



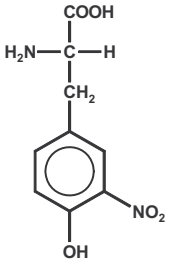
Mercaptopropionic acid



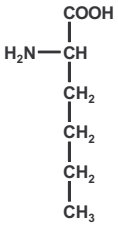
Methyl-Lysine



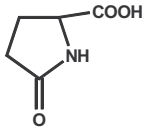
3-Nitro-Tyrosine



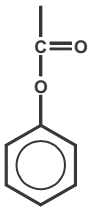
Norleucine (Nle = 2-amino hexanoic acid)



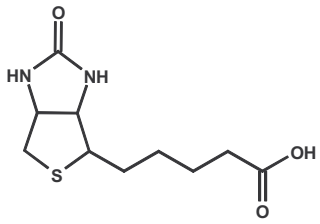
pyro-Glutamic acid (Pyr)
is a common N-terminal amino acid modification in many biologically active peptides (hormones).



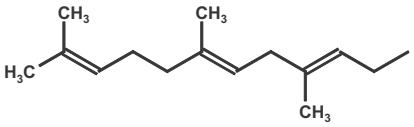
Z (Carbobenzoxyl)
special protecting group for N-terminus.



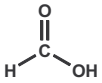
Biotin
Biotin (or vitamin H) is a small biologically active molecule with a molecular weight of 244,31 Da. It acts as a co-enzyme in living cells. With its highly specific affinity towards streptavidin, it is used in various biotechnology assays for quality and quantity testing.



Farnesyl
is a potential substrate to study demethylase activity in enzyme assays.

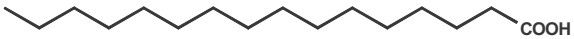


Formic acid (Formyl)

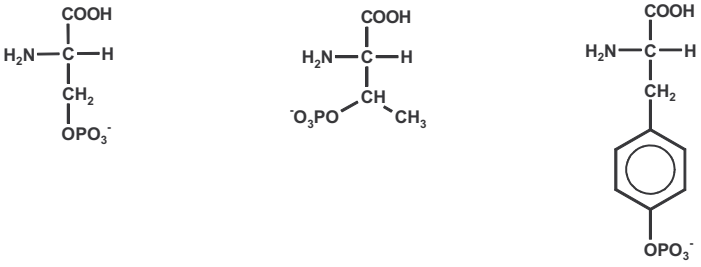


Myristic acid (Myristoyl)

Palmitic acid (Palmitoyl)

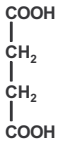


Phosphorylation

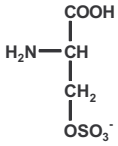


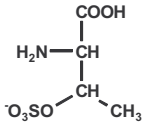
Stearic acid (Stearyl)

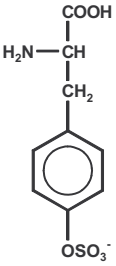
Succinic acid (Succinyl)



Sulfurylation
at Ser, Thr and Tyr is another modifcation of amino acids in nature.
Activity of many enzymes depends on the oxidation state of SH-groups in these residues.







Carboxyl group modification (C-terminus)
All modifications carrying carboxy-reactive functional groups can be used.
Among the most commonly used ones, you can find:

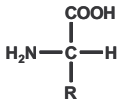
- amino groups
- amines
- bifunctional aminolinkers

Standard modifications that are coupled via carboxy groups are:

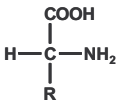
- amides
- chromophores

Single modifications – Amino acids

D-amino acids
Amino acids carrying four different groups on their α-C atom (i.e. asymmetric C atom, or C*) are chiral substances. These α-amino acids can be found in respective L- and D-forms (enantiomers):



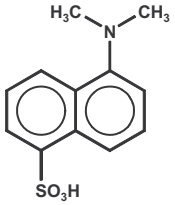
L-amino acid



D-amino acid

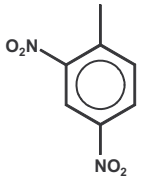
The predominant form in natural proteins is the L-form.
As some enzyme classes are enantioselective, i.e. they can distinguish between L- and D-forms and specifically accept only one of the two forms as substrate, this enantioselectivity makes D-amino acids a valuable tool in medicine (e.g. in peptide antibiotics) and enzyme assays.

Dansyl
Dansyl is also used as a fluorophore quencher. Unlike Dabcyl, it inherits own fluorescence and thus might not be as useful for highly sensitive assays.



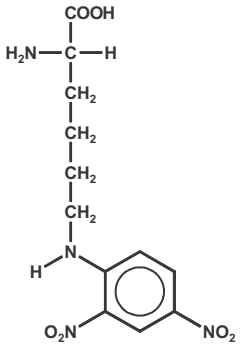
Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
Dansyl	335 nm	526 nm	4.600

2,4-Dinitrophenyl (DNP)
is a non-fluorescent dye that can be used as a fluorophore quencher (see Dabcyl for more details).



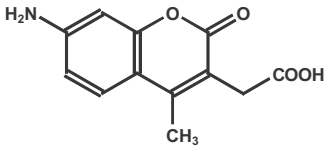
Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
DNP	348 nm	none	18.000

DNP-Lysine
is a non-fluorescent dye that can be used as a fluorophore quencher (see Dabcyl for more details).



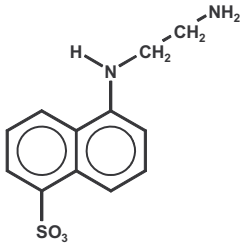
Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
DNP-Lysine	348 nm	none	18.000

AMC (7-Amino-4-methyl-coumarin)
UV-excitable dye, used in enzyme assays using cuvettes or flow cytometry.



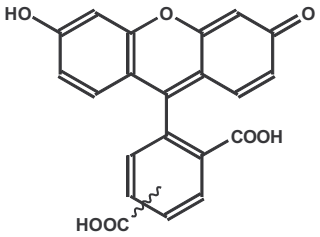
Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
AMC	353 nm	422 nm	19.000

EDANS (5-((2-aminoethyl)amino)napthalene-1-sulfonic acid)
a commonly used dye in FRET (fluorescence resonance energy transfer) peptides in combination with Dabcyl as quencher.



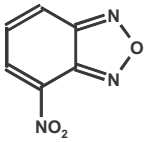
Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
EDANS	335 nm	493 nm	5.900

Fluorescein
the commonly used fluorescent dye in confocal laser-scanning microscopy and flow cytometry applications.



Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
Fluorescein	495 nm	520 nm	83.000

NBD (7-nitrobenz-2-oxa-1, 3-diazole)
a fluorescent dye, used for amine modification.

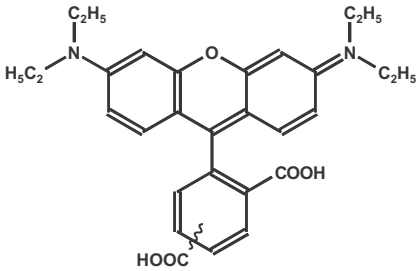


Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
NBD	486 nm	543 nm	27.000

p-Nitro-Aniline
a chromogen used as colorimetric enzyme substrate in many standard enzyme assays in cuvettes.

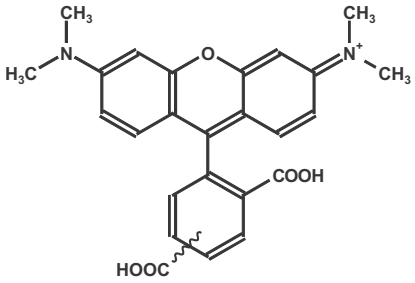
Dye	Excitation maximum	Emission maximum
p-Nitro-Aniline	410 nm	none

Rhodamine B
represents one among a numerous range of rhodamine dyes, used in fluorescence assays.



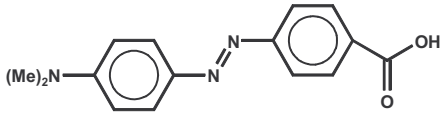
Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
Rhodamine B	550 nm	580 nm	90.000

Tamra
the most commonly used rhodamine dye in fluorescence assays.



Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
Tamra	544 nm	576 nm	90.000

Dabcyl
Dabcyl is a non-fluorescent dye predominantly used as a quencher for other fluorophores (esp. Fluorescein type dyes, EDANS..).
If Dabcyl is coupled to a peptide in close proximity to a fluorophore, it absorbs the emitted light of the fluorophore. Enlarging this distance (i.e. by enzymatic cleavage of the peptide) results in excitation of the fluorophore with an emission signal that can be detected.



Dye	Excitation maximum	Emission maximum	Molar Extinction Coefficient
Dabcyl	453 nm	none	32.000