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Drug synthesis II
2012

Solid phase peptide synthesis (SPPS)

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Solid phase peptide synthesis (SPPS)

- R. Bruce Merrifield, Professor at Rockefeller University, has been awarded the Nobel Prize in chemistry for 1984 for his development of a simple and ingenious method for obtaining peptides and proteins
- In solid-phase synthesis, the starting material is bonded to an inert solid support.
- Reactants are added in solution.
- Reaction occurs at the interface between the solid and the solution. Because the starting material is bonded to the solid, any product from the starting material remains bonded as well.
- Purification involves simply washing the byproducts from the solid support.

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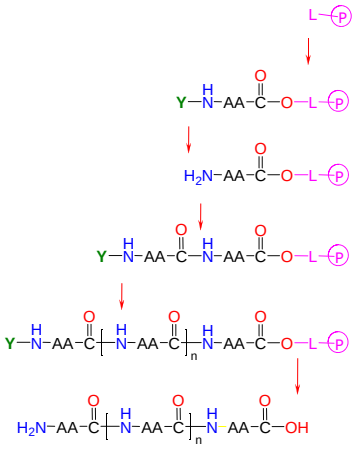
Solid phase peptide synthesis (SPPS)



1) Attaching N-protected amino acid to the solid polymer linker (L)
2) Cleavage of protecting group (Y) of first amino acid
3) Coupling the second protected amino acid
4) Deprotection, coupling cycle is continued until a chain of the required length has been synthesized
5) Polymer removal: Once the desired peptide has been made the bond between the first amino acid and the linkage agent is broken to give the free peptide.

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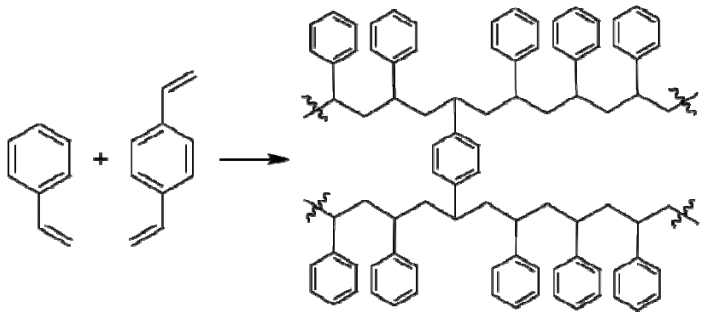
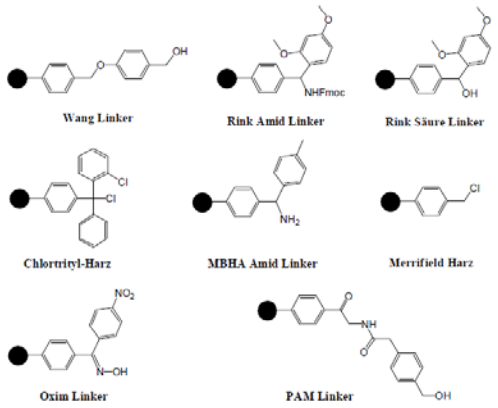
Polystyrene is the Basis for the Solid Support



- The solid support is a copolymer of styrene and divinylbenzene. It is represented above as if it were polystyrene. Cross-linking with divinylbenzene simply provides a more rigid polymer.

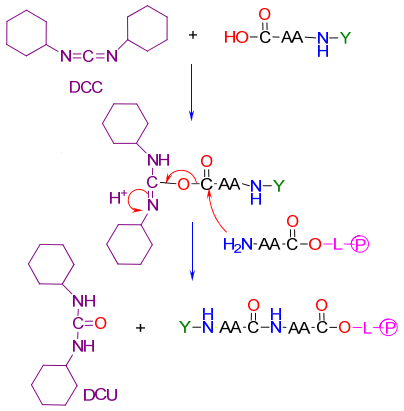
Linkers

- Functionalisation of the resin is usually performed by chloromethylation. Normally a linker is placed between the chloromethyl group and the first amino acid, which allows cleavage of the peptide from the resin at the end of the synthesis.

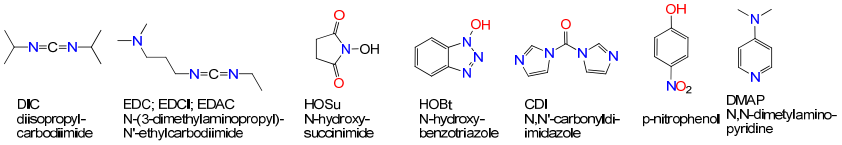


Coupling of protected amino acids using *N,N'*-dicyclohexylcarbodiimide (DCC)

- The protected amino acid will react with the carbodiimide to produce the key intermediate: the O-acylisourea, which can be viewed as a carboxylic ester with an activated leaving group. The O-acylisourea will react with aminoacid to give the desired peptide bond. The addition of an amine results in the formation of a peptide bond. Dicyclohexylurea (DCU), the byproduct formed from DCC, is nearly insoluble in most organic solvents and precipitates from the reaction mixture.



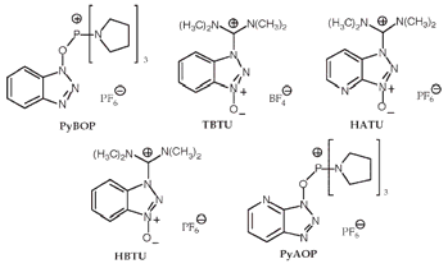
Other coupling reagents



- Alternatively **diisopropylcarbodiimide** (DIC) or **1-ethyl-3-(3-dimethylaminopropyl)carbodiimide** (EDC) can be used instead of DCC, because the urea generated is more soluble and can be separated easier
- **N-Hydroxysuccinimide** (HOSu) is commonly used as an activating reagent for carboxylic acids.
- **Hydroxybenzotriazole** (HOBt) is mainly used to suppress racemization and improve the efficiency of peptide synthesis
- **1,1'-Carbonyldiimidazole** (CDI) is often used for the coupling of amino acids for peptide synthesis and as a reagent in organic synthesis
- **p-Nitrophenyl** ester is an "active ester," better leaving group than alkyl esters and more reactive in nucleophilic acyl substitution.
- **4-Dimethylaminopyridine** (DMAP) is a useful nucleophilic catalyst for a variety of reactions such as esterifications

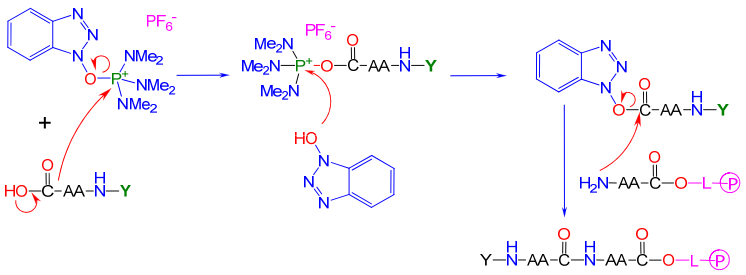
Other peptide coupling reagents

- A more modern alternatives to DCC activation is coupling with a mixture of HBTU/HOBT or with PyBOP.
  - PyBOP = benzotriazol-1-yl-oxytripyrrolidino phosphonium hexafluorophosphate
  - HBTU=N-[1H-benzotriazol-1-yl) (dimeth-ylamino)methylene]-N-methyl-methanaminium hexafluorophosphate N-oxide



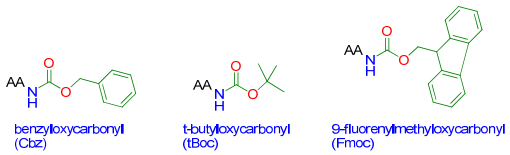
Other peptide coupling reagents

- **BOP reagent** (Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate)
  - Carboxylate anion of the amino acid attacks the phosphorus atom of BOP. The resulting intermediate rearranges to form the hydroxybenzotriazolyl ester, which is then attacked by the amine component to form the amide product.



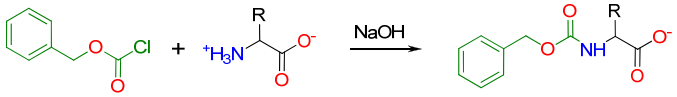
Protecting groups for amino group

- The commonly used protecting groups for amino group are carbamates: Fmoc (9-fluorenylmethyl carbamate), t-Boc (Di-tert-butyloxycarbonyl) and Cbz (benzyloxycarbonyl).



- Deprotection
  - Fmoc aq. NH_3 or 20% piperidine
  - tBoc 100% TFA, rt, 1 h
  - Cbz H_2/Pd-C

Benzyloxycarbonyl (Cbz or Z)



CbzCl
(Benzyl chloroformate)

Cbz-AA
(carbobenzyloxy)

- Cbz-protection is carried out by treating an amino acid with benzyl chloroformate
- An advantage of the benzyloxycarbonyl protecting group is that it is easily removed by:
  a) catalytic hydrogenolysis under extremely mild conditions
  b) cleavage with HBr in acetic acid
- Both reagents cleave the relatively weak benzylic carbon-oxygen ether bond, albeit by different mechanisms

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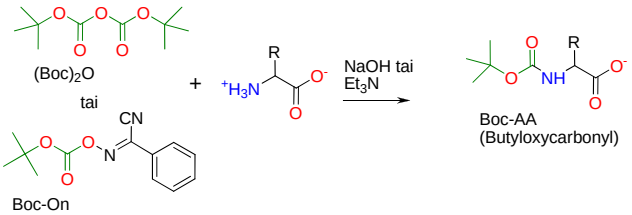
Tert-butyloxycarbonyl (Boc)

- Used in the standard Merrifield system, which is based on graduated acid lability.
- The Boc group is stable to basic conditions and nucleophiles.
- Usually introduced onto an amino acid through Di-tert-butyl dicarbonate, $(Boc)_2O$ or Boc-ON in aqueous 1,4-dioxane and NaOH or TEA.



Boc-On
(t-butoxycarbonyloximino-phenylacetonitrile)

Boc-AA
(Butyloxycarbonyl)

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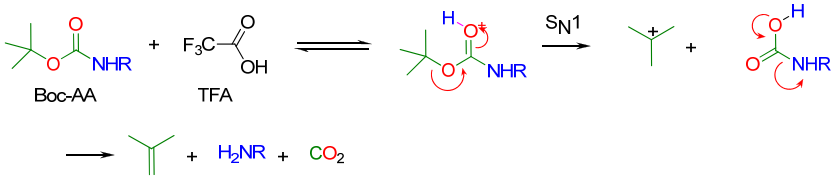
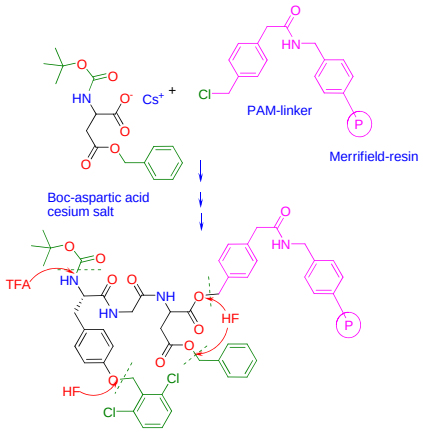
Cleavage of Boc Groups

- The *tert*-butyloxycabonyl protecting group is readily removed by treatment wit strong, anhydrous BrØnsted acids:
  a) cleavage with trifluoroacetic acid in methylene chloride
  b) cleavage with HBr in acetic acid
  c) 4N HCl in dioxane
- Both reagents cleave the quaternary carbon-oxygen ether bond by an acid-mediated elimination reaction.

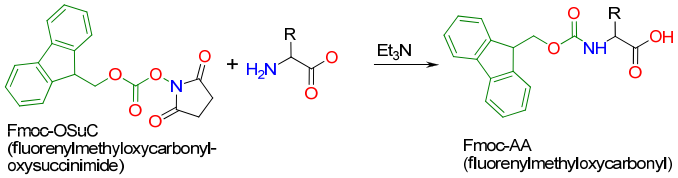


Boc peptide synthesis strategy using Merrifield linker

- Amino acid is protected with tert-Butoxycarbonyl (tBoc)
- Side chain protecting groups are usually ether, ester and urethane derivatives of benzyl alcohols (Bn)
- Boc is removed using TFA in DCM
- 4-(hydroxymethyl)phenylacetic acid (PAM) linker is typical in Boc synthesis and is resistant to TFA.
- Synthesized peptides are cleaved from solid support and side chain protecting groups are removed using strong acids such as HF



Fluorenylmethoxycarbonyl (Fmoc)



- The Fmoc method allows for a milder deprotection by base, usually piperidine (20-50%) in DMF and thus the exposed amine is neutral.
- Deprotection by Fmoc can be monitored by UV absorbance
- Fmoc is stable under acidic conditions. This allows mild acid-labile protecting groups, such as Boc and benzyl groups, to be used on the side-chains of amino acid residues.
- Final cleavage of the protein from the resin and removal of permanent protecting groups is performed with TFA.
- The resulting final product is a TFA salt.

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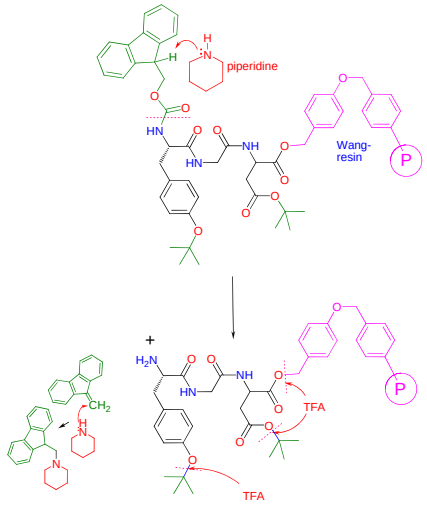
Fmoc peptide synthesis strategy using Wang linker

- 9-Fluorenylmethoxycarbonyl (Fmoc) is a base labile protecting group that is usually removed using 20-50% piperidine in DMF
- Side chain protecting groups are usually based on ether, ester and uretheane derivatives of tert-butanol
- 4-alkoxybenzyl alcohol resin (Wang) is used
- The peptide and side chain protecting groups can be cleaved using TFA



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Protection of amino acid side chains

- Amino acid side chains that are acidic, basic or highly polar must be protected
- Side chains of Gly (glycine), Ala (alanine), Val (valine), Phe (phenylalanine), Leu (leucine) Ile (isoleucine), Met (methionine), Pro (proline), Trp (tryptophan), Asn (asparagine) ja Gln (glutamine) does not usually need protection
- The amino group of lysine, guanidine group of arginine and thiol side chain of cysteine are strongly nucleophilic and must be protected.

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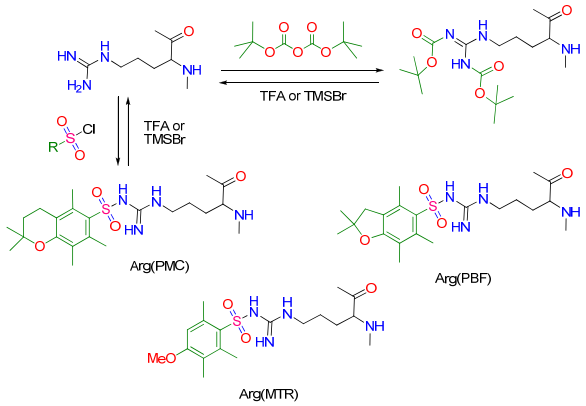
Side Chain protection in Fmoc-chemistry

- In Fmoc chemistry guanidine group of arginine is protected with BOC or arylsulfonyls (e.g. PMC = 2,2,5,7,8-pentamethylchroman-6-sufonyl), which can cleaved with TFA or trimethylsilylbromide (TMSBr):



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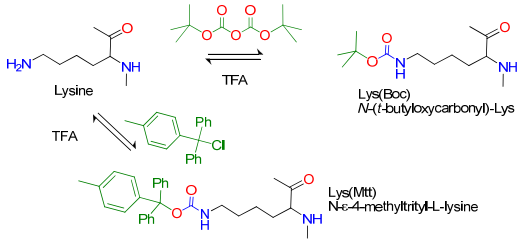
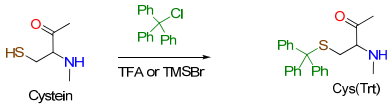
Side Chain protection in Fmoc-chemistry

– Lysine:



- Cystein:



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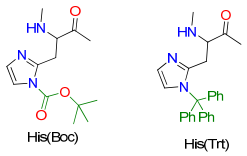
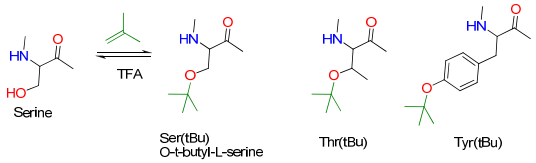
Side Chain protection in Fmoc-chemistry

- Histidine:



- Serine, treonine and tyrosine:



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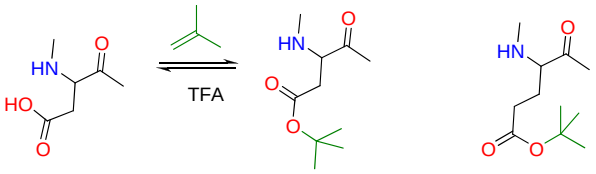
Side Chain protection in Fmoc-chemistry

– **Aspartic acid/glutamic acid carboxyl groups are protected as *tert*-butylesters.**



Aspartic acid

Asp(OtBu)
L-aspartic acid
4-tert-butyl ester

Glu(OtBu)

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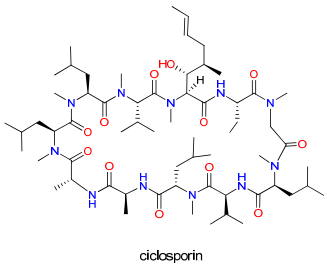
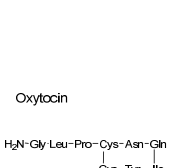
Peptide drugs

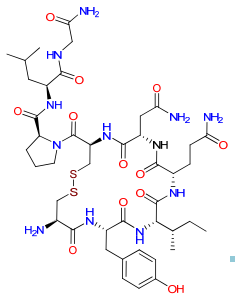
- Peptide drugs are either naturally-occuring peptides or altered natural peptides. .
- The amino acids in an active peptide can be altered to make analogues of the original peptide
- Oxytocin and vasopressin are important peptide hormones
- Ciclosporin is an immunosuppressant drug widely used in organ transplantation to prevent rejection
- Enalapril is a tripeptide based drug, an angiotensin converting enzyme (ACE) inhibitor

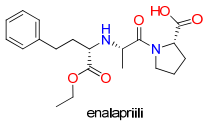


ciclosporin

Oxytocin







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