Solid Phase Peptide Synthesis

Current Pure and Applied Chemistry

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Outline

- History
- Introduction to solid phase peptide synthesis
- Resins and linkers
- Protecting groups
- Activation
- Deprotection
- Cleavage
- Mechanisms
  - Boc and Fmoc
- Purification and characterisation

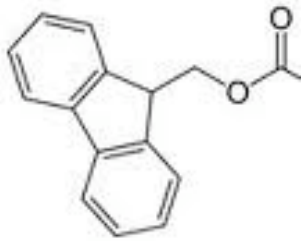
- Leu-Enkephalin

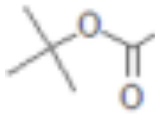
Bruce Merrifield

- Pioneered the field
- Published the synthesis of a tetrapeptide in 1963
  (article can be found on Moodle)
- Nobel prize in chemistry 1984

"for his development of methodology for chemical synthesis on a solid matrix"

- Boc or t-Boc (tert-butyloxycarbonyl)
- Fmoc (9-fluorenylmethyloxycarbonyl)

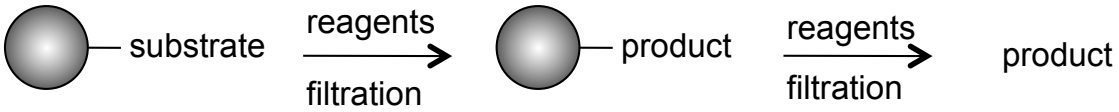




Introduction to SPPS

What is SPPS?

- Organic reactions carried out on substrates that are covalently attached to a polymeric resin



Traditional solution phase synthesis

- Important with good protecting groups

Advantages with SPPS

Why is solid-phase (often) nicer than “traditional synthesis”?

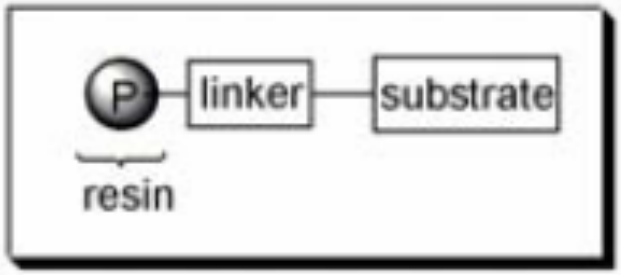
- Synthetic intermediates don’t have to be isolated
- Excess reagents are used ⟶ drive reactions to completion
- Reagents are simply washed away each step
- Overall quicker
- Can be automated with robots

General mechanism SPPS

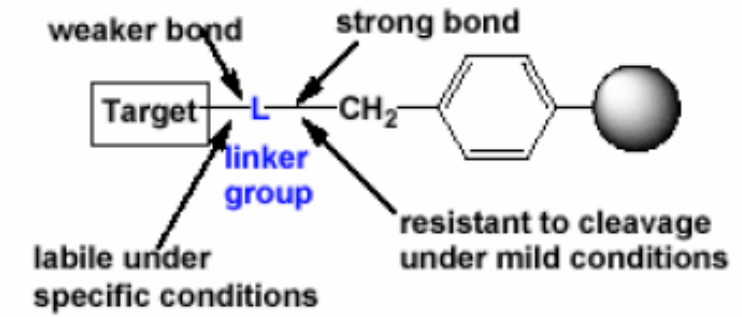
On white board

Resins and linkers

- Synthesis occurs on multiple surface of the bead and inside the bead



Resins and linkers

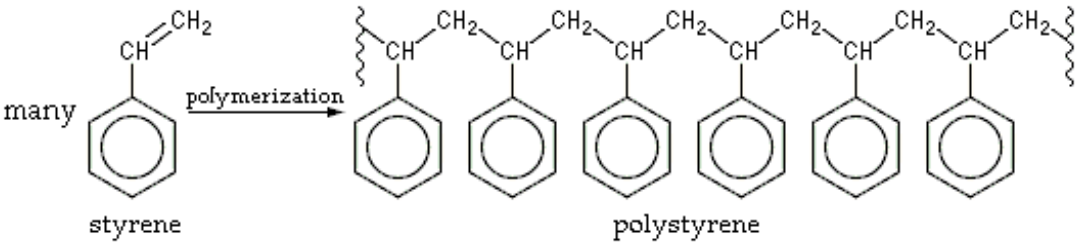


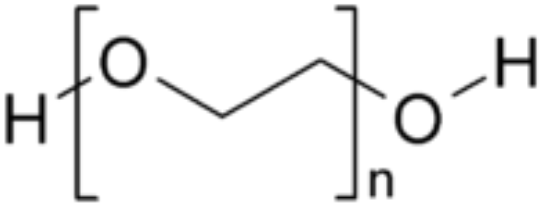
What is a resin?

- Polymeric backbone that substrate is anchored to
- Different resins have different swelling properties

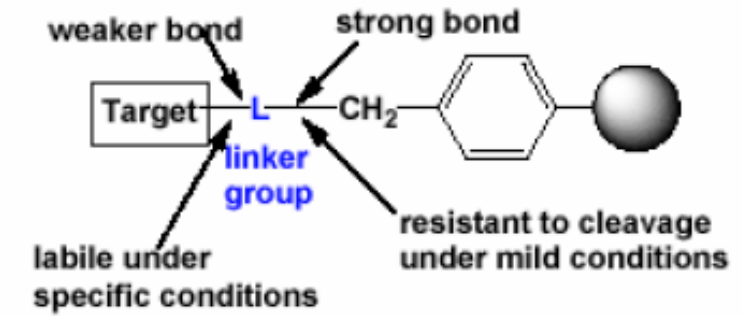
Examples:

- Polystyrene (PS): Swells in non-polar solvents
- Polyethylene glycol (PEG): Swells in polar and non-polar solvents
- Many others exist





Resins and linkers



What is a resin?

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- Different resins have different swelling properties

Examples:

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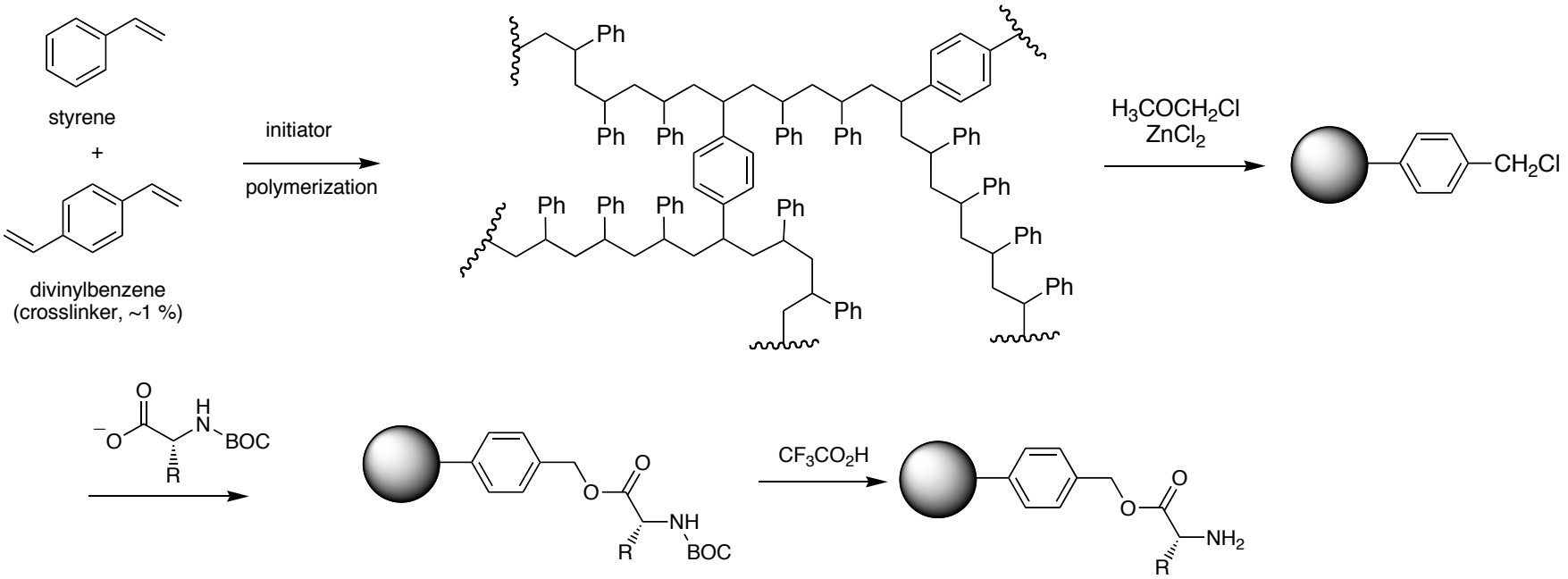
- Polyethylene glycol (PEG): Swells in polar and non-polar solvents
- Many others exist

What is a linker?

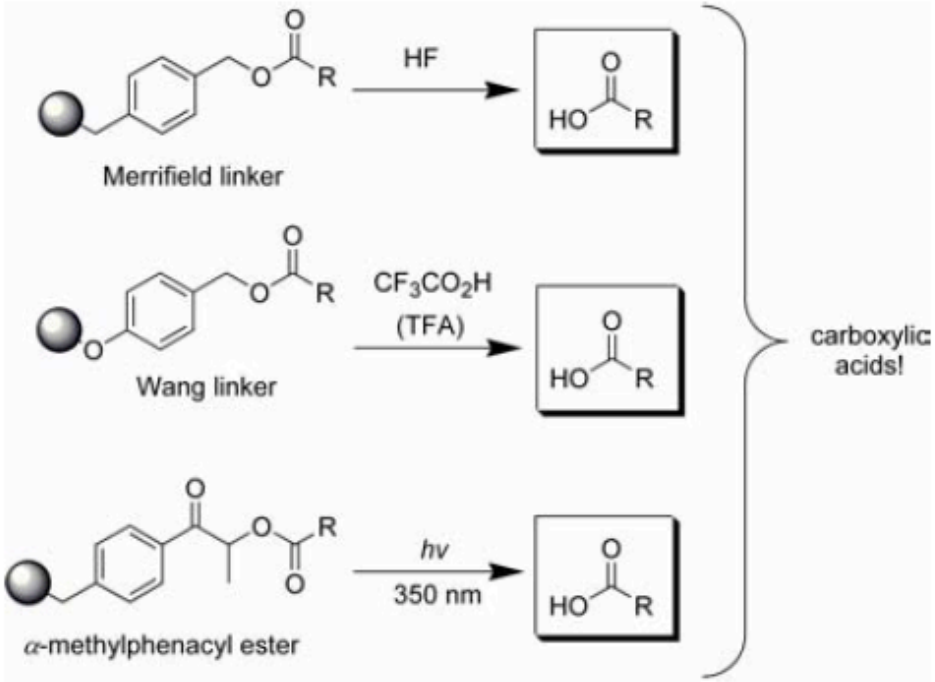
- An intermediate structure between resin and substrate
- Different linkers can be used to unmask different functional groups on the substrate

The solid support (Merrifield resin): polystyrene polymer



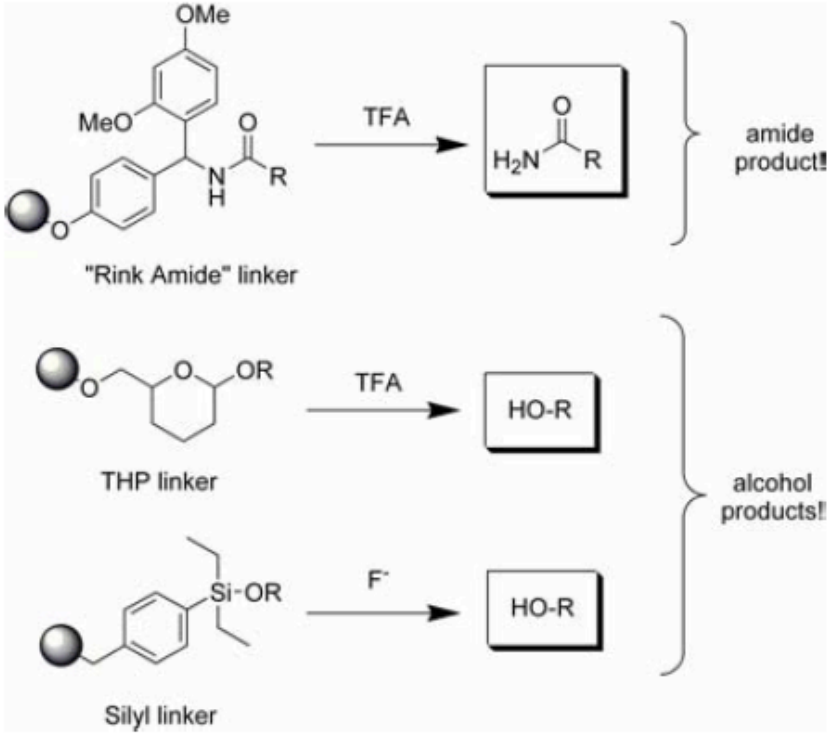
Linkers

Examples of linkers commonly used in solid phase peptide synthesis:

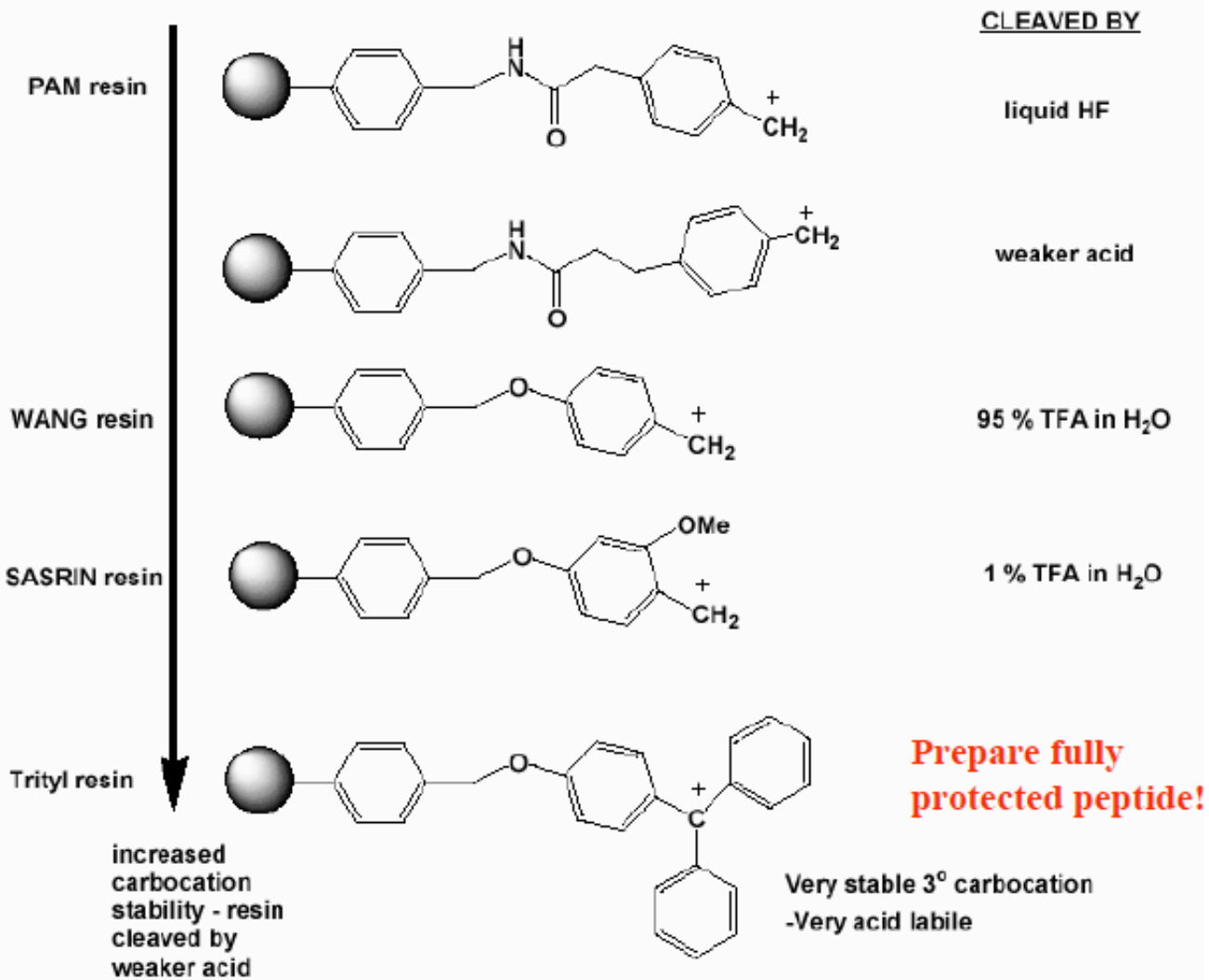


Linkers

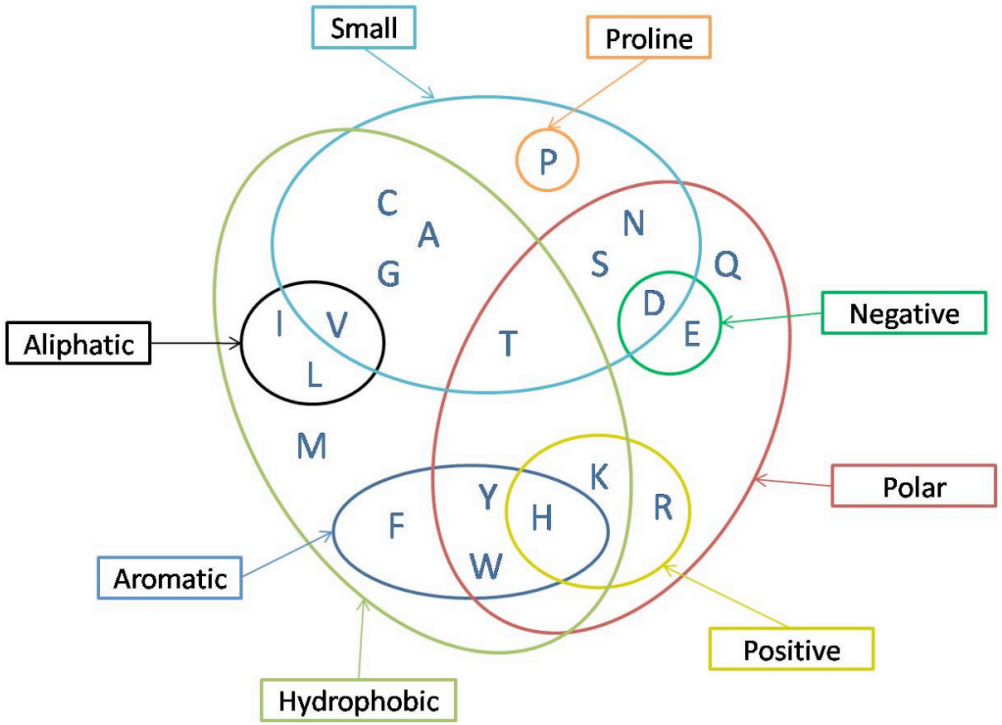
Some more linkers:



The ease of cleavage from the resin depends on the linker used and the stability of the final carbocation:



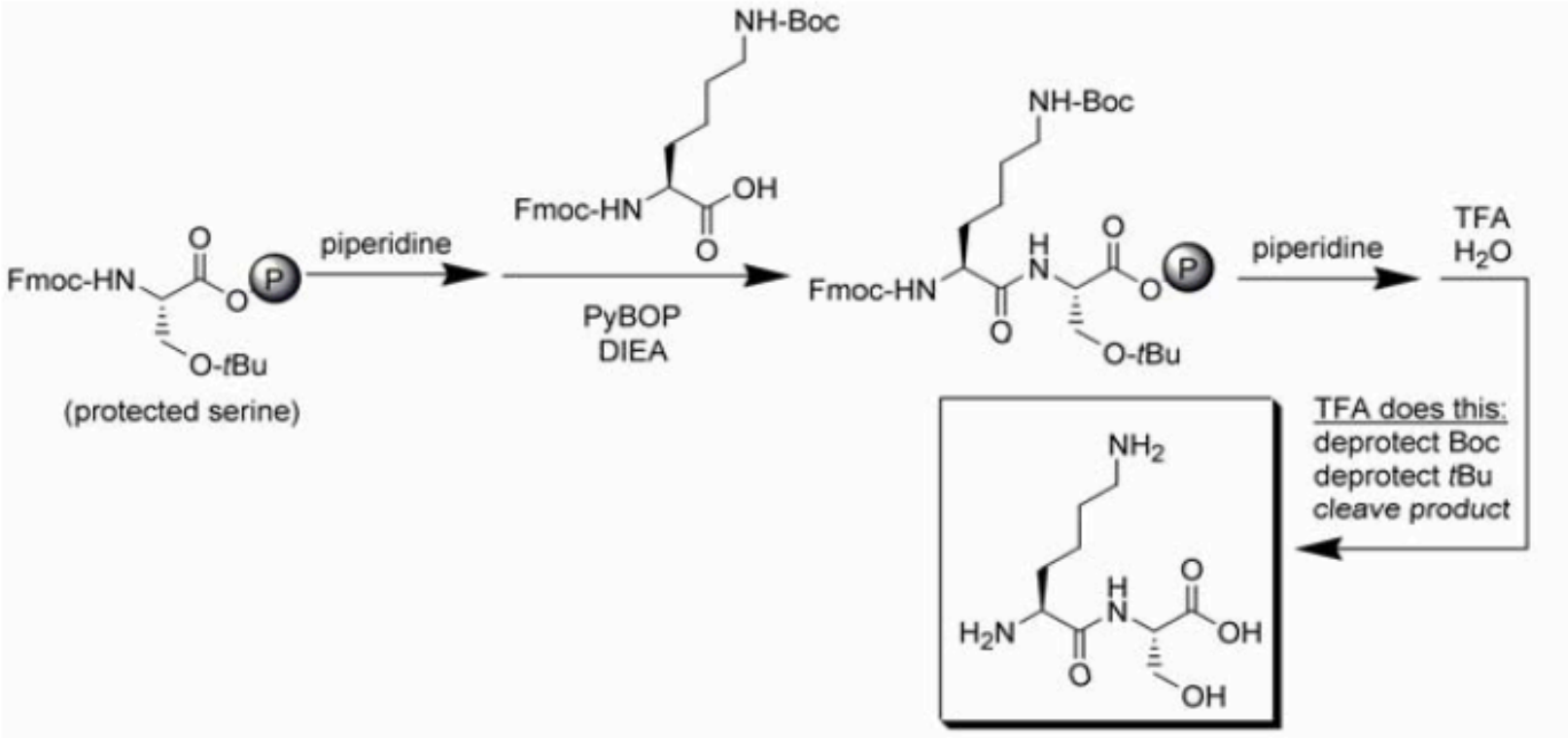
Properties of amino acids



Protecting groups (PG)

How does we deal with reactive side chains of amino acids?

- They are masked/protected with “semi-permanent” PG until cleavage
- Example: H-Lys-Ser-OH



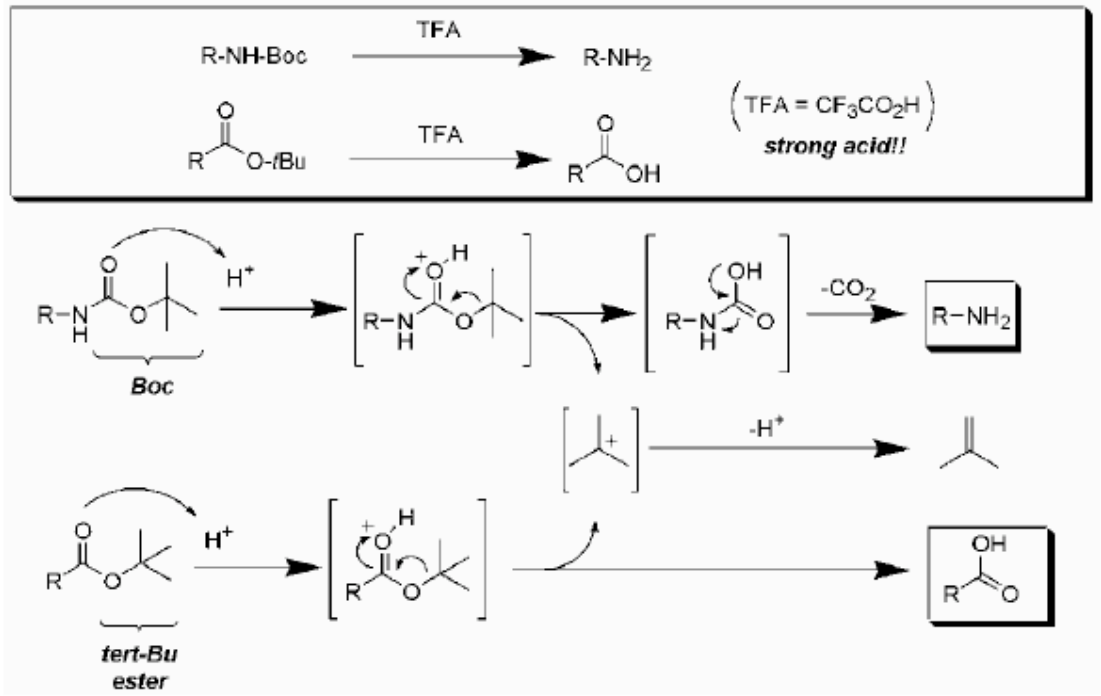
Protecting groups

What is a protecting group?

- A fragment bound to a functional group that blocks the reactivity of that group
- Good protecting groups are easily attached and removed using mild reaction conditions

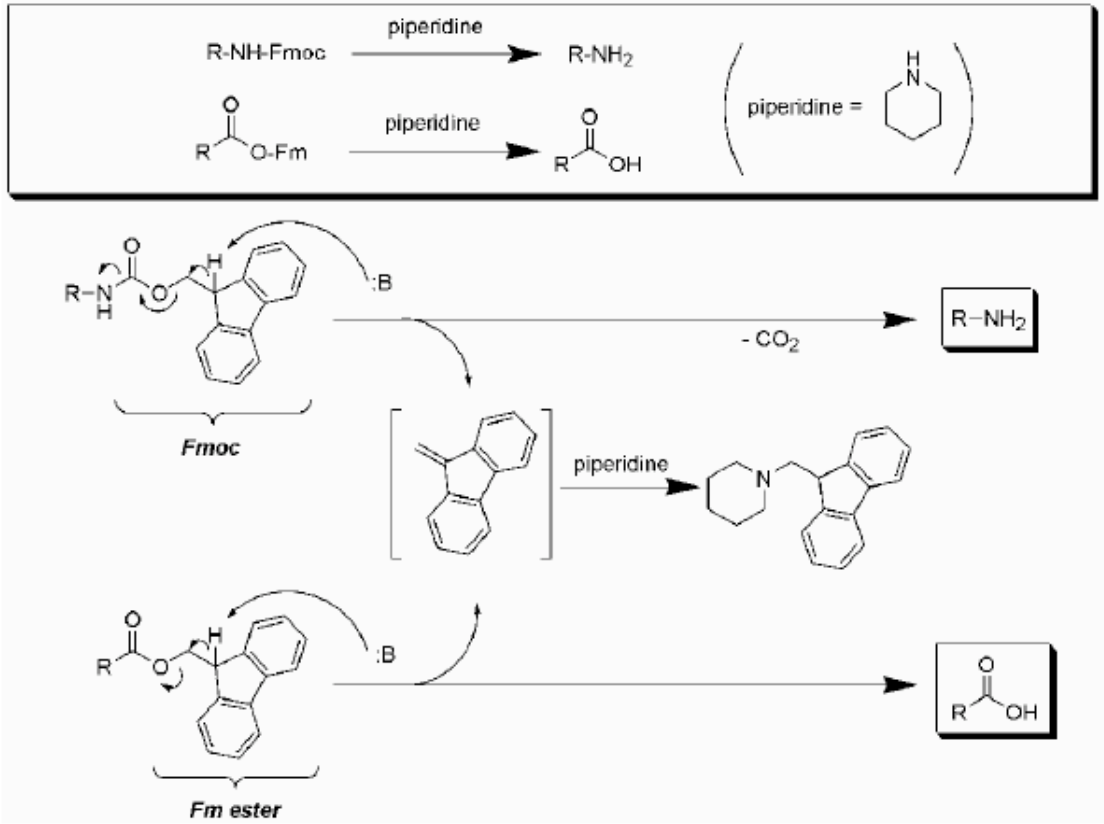
Protecting groups

Acid labile protecting groups: Boc and tert-Bu ester



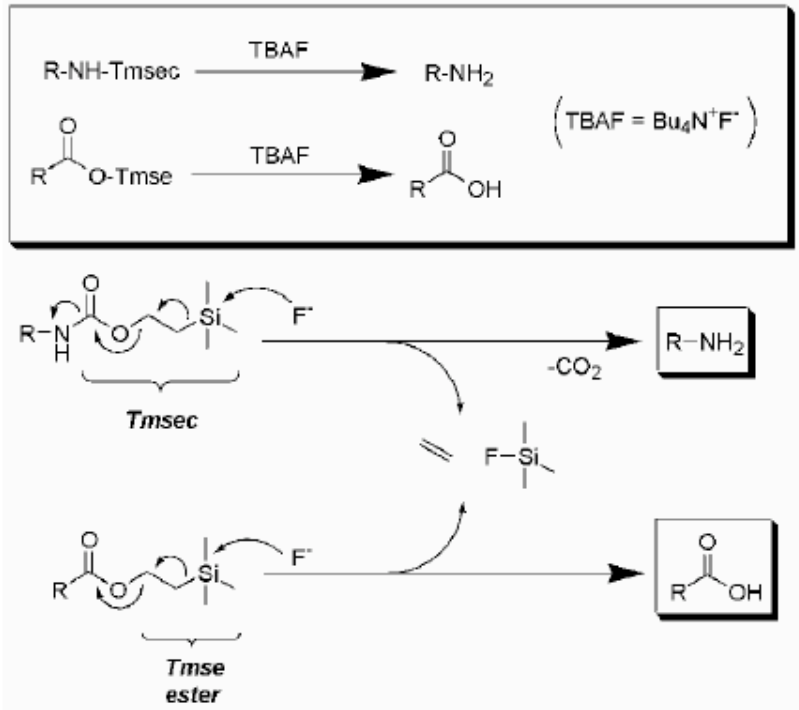
Protecting groups

Base labile protecting groups: Fmoc & Fm ester



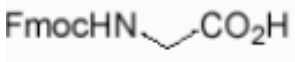
Protecting groups

Fluoride labile protecting groups: Tmsec & Tmse ester

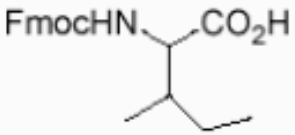


Amino acids

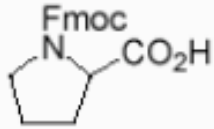
1. side-chain = H, alkyl, aryl –no side-chain protecting groups required



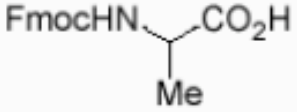
(Glycine, Gly, G)



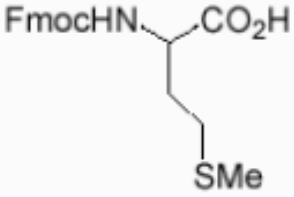
(isoleucine, ile, I)



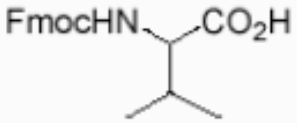
(proline, pro, P)



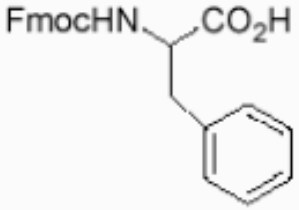
(alanine, ala, A)



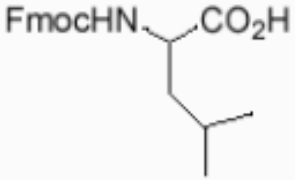
(methionine, met, M)



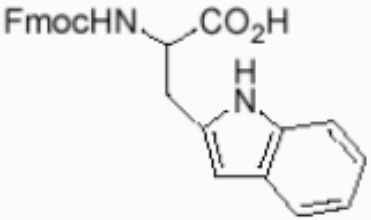
(valine, val, V)



(phenylalaniine, phe, F)



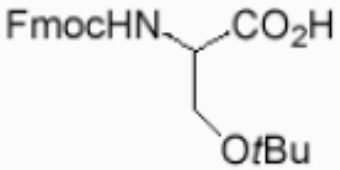
(leucine, leu, L)



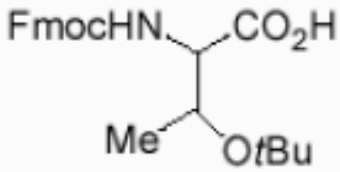
(tryptophan, trp, W)

Amino acids

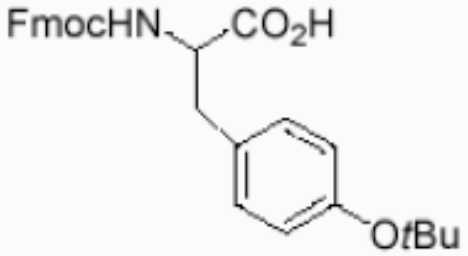
2. side-chain contains OH, protect as *t*Bu ether



(serine, ser, S)

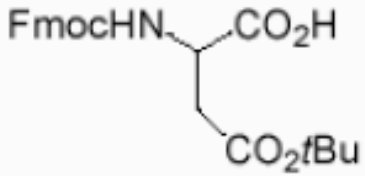


(threonine, thr, T)

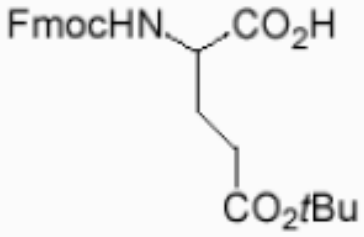


(tyrosine, tyr, Y)

3. side-chain contains CO_2H protected as *t*Bu-ester.



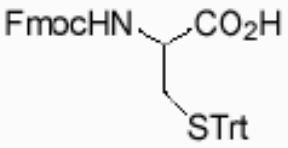
(aspartic acid, asp, D)



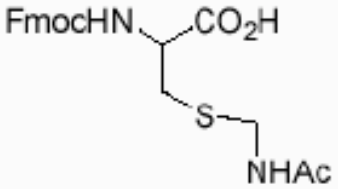
(glutamic acid, glu, E)

Amino acids

4. side-chain contains thiol (SH) group – depends on application.

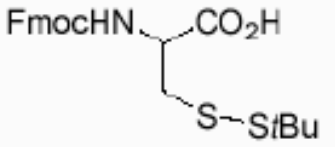


(cysteine, cys, C)



(SAcm)

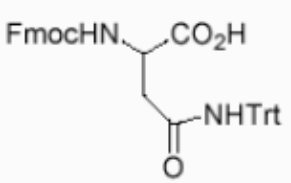
(acid stable, cleaved with Hg^{2+})



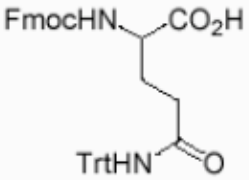
(acid stable, cleaved with thiols)

Amino acids

5. side-chain contains $CONH_2$ group – protect with trityl group.

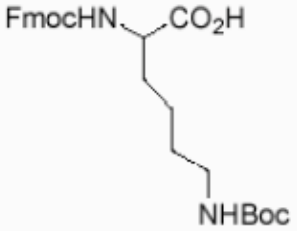


(asparagine, asn, N)

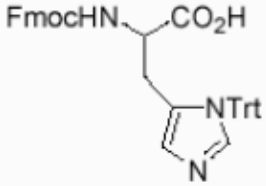


(glutamine, gln, Q)

6. side-chains contain basic or imidazole groups.

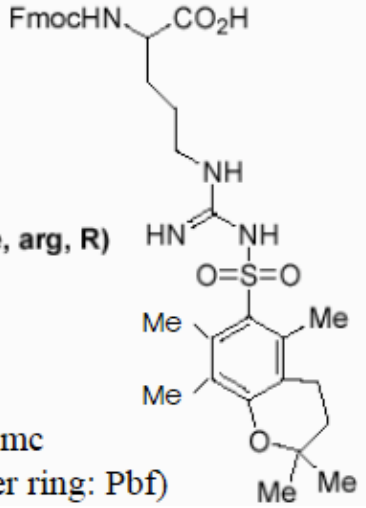


(lysine, lys, K)



(histidine, his, H)

(arginine, arg, R)

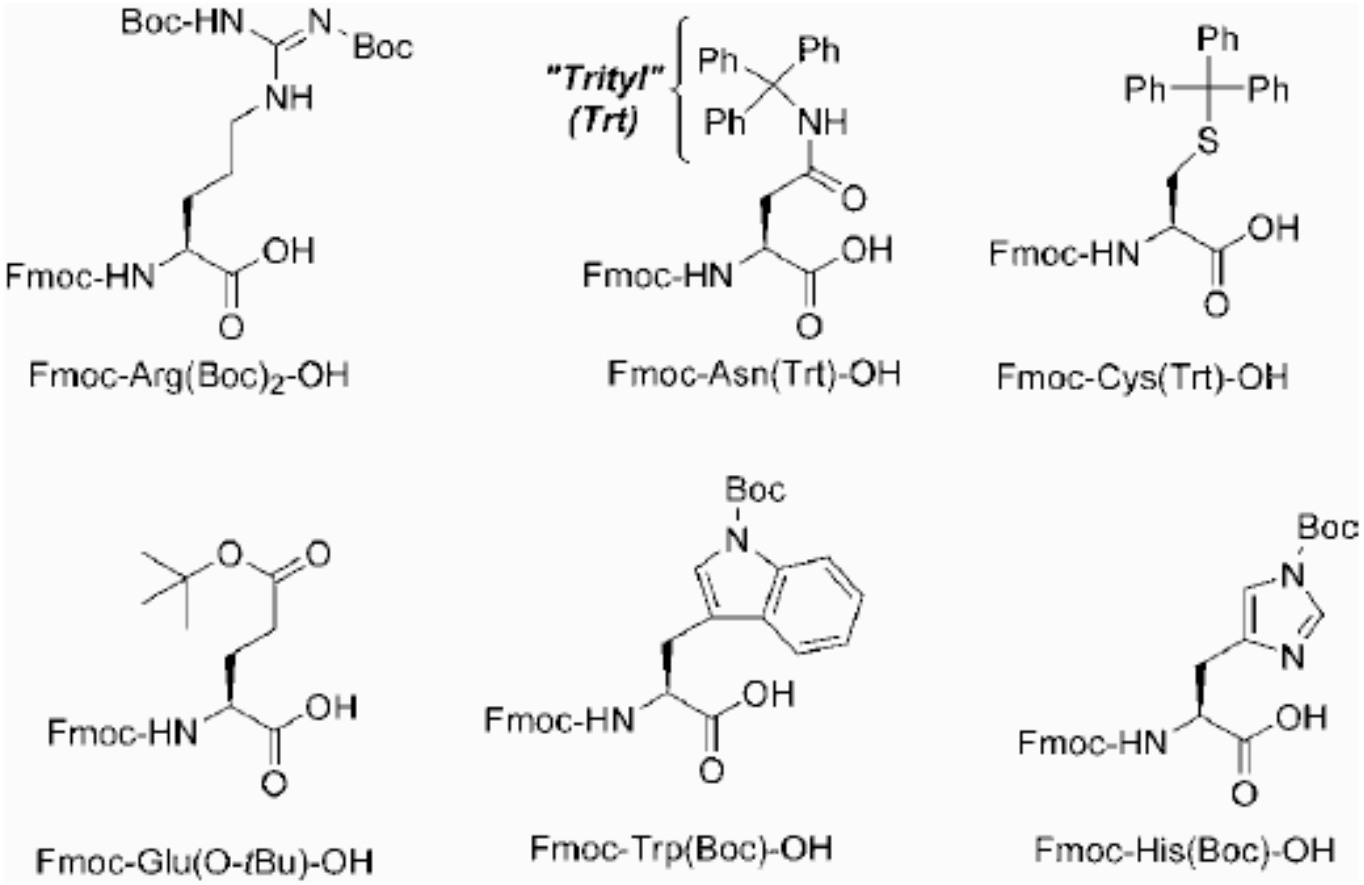


Pmc

(5-member ring: Pbf)

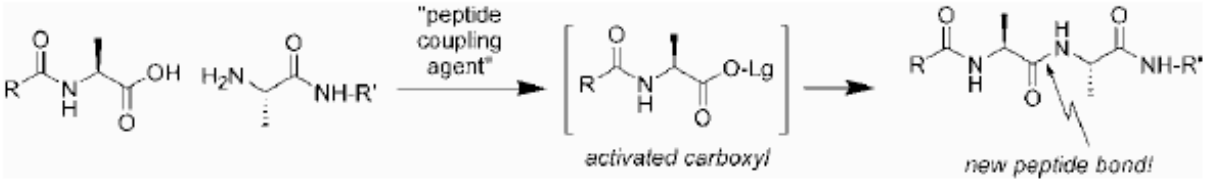
Solid phase synthesis: to make peptides

- Examples of protected AAs with reactive side-chains:
  - Protected side chains cleaved during resin cleavage with TFA!

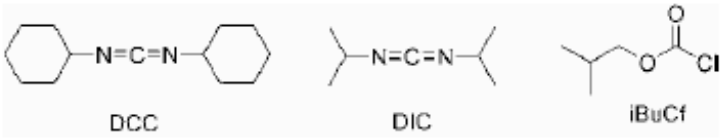


Solid phase synthesis: to make peptides

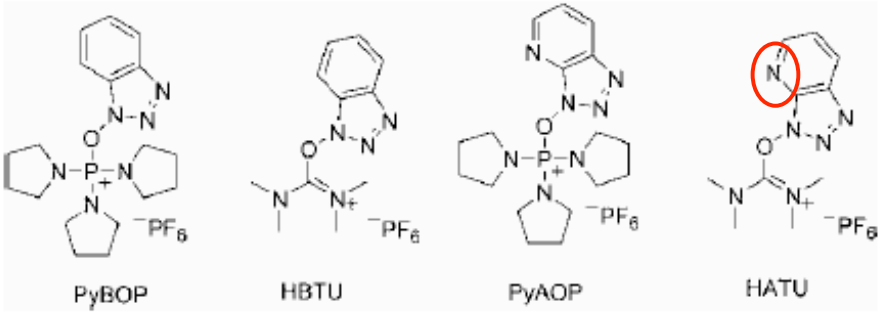
- Peptide bonds created with peptide coupling agents:



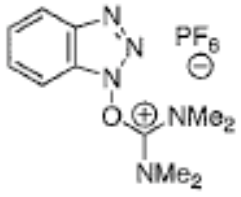
- First generation coupling agents:



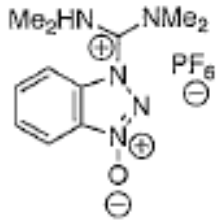
- Later generation coupling agents: (improved in many ways…)



Coupling agents – Activation of amino acids

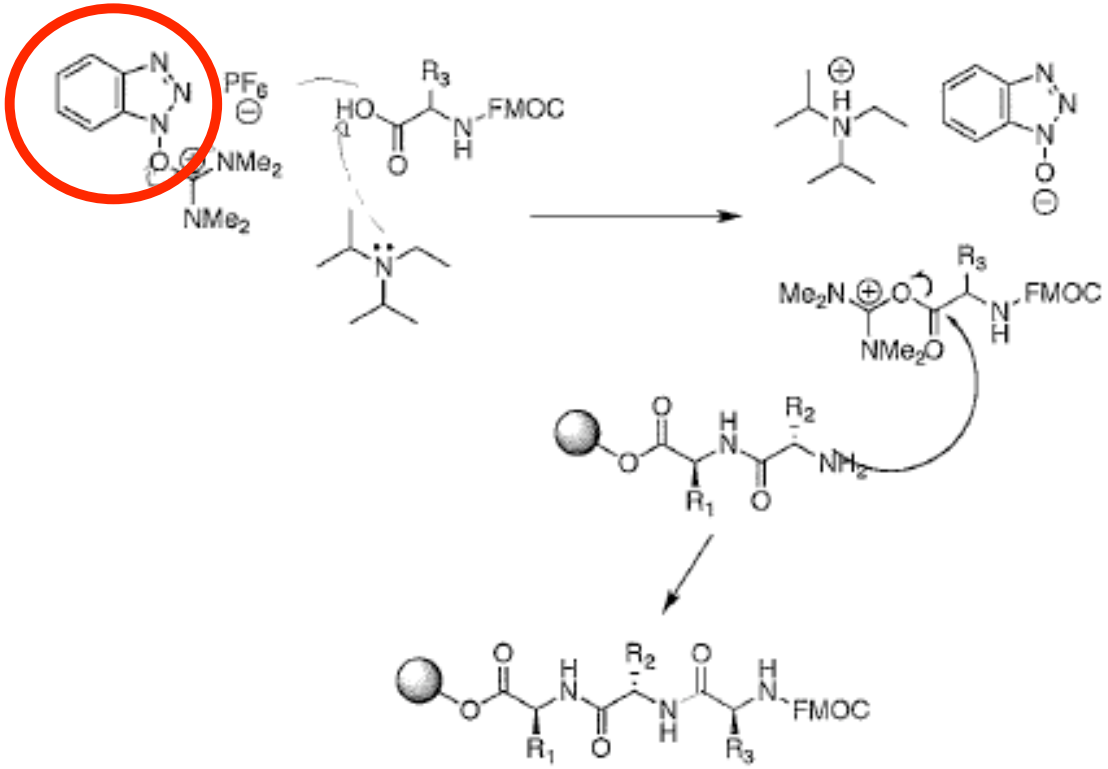


uronium isomer

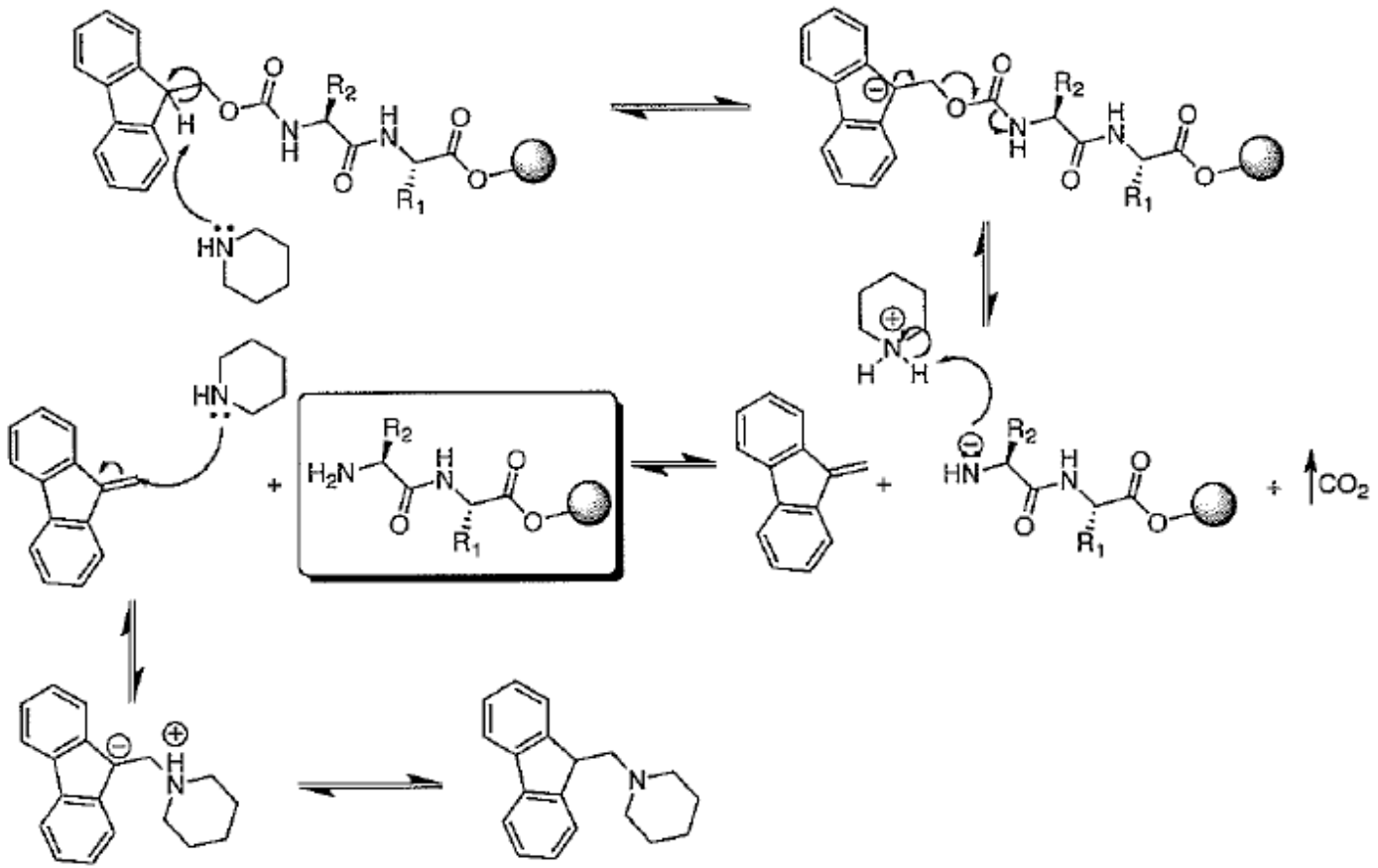


guanidinium isomer

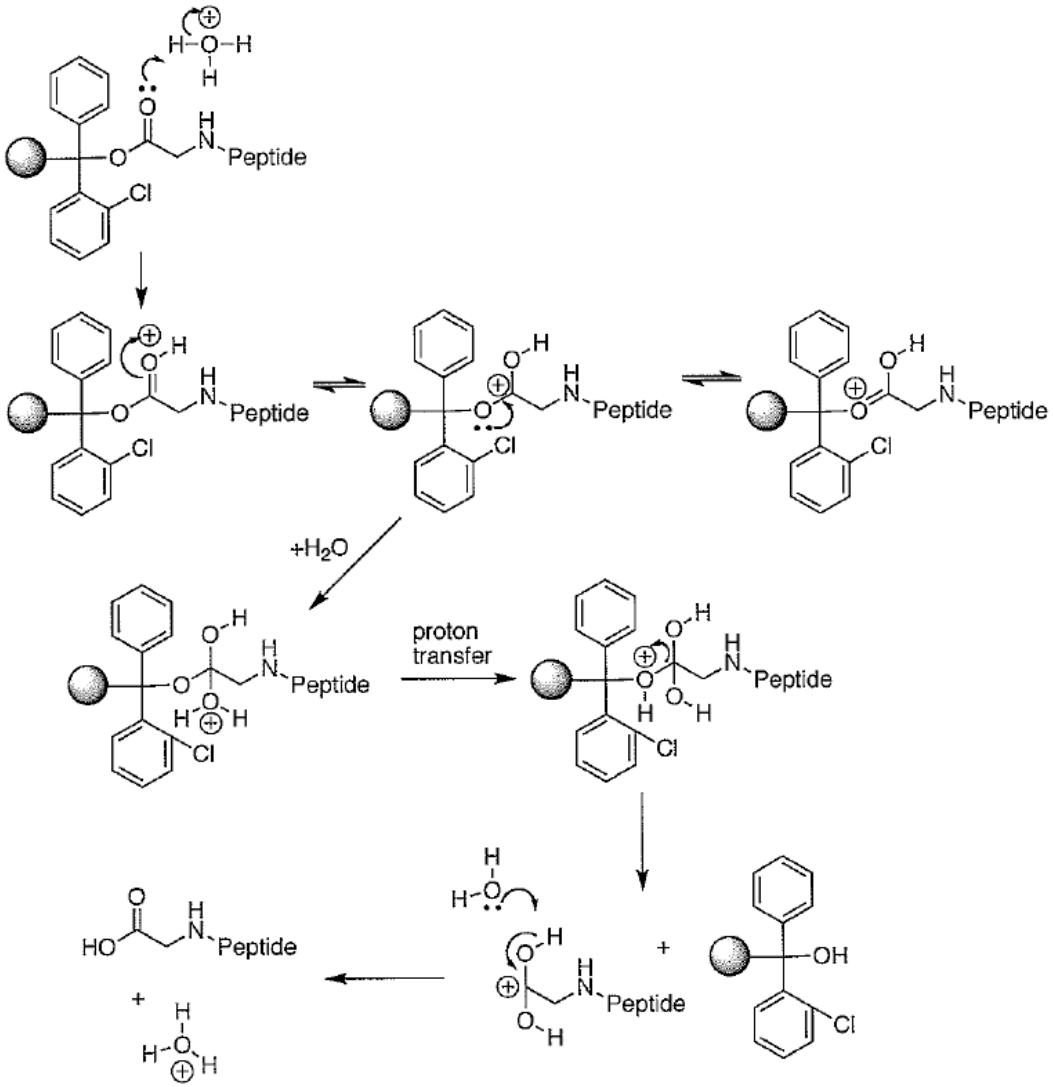
Active ester - good leaving group



Deprotection of Fmoc by using piperidine



Cleavage reaction using TFA



Cleavage reaction

Mild cleavage – PGs are not removed

Scavengers:

- EDT (ethanedithiol) – scavenger for t-butyl cation, help to remove Trt from Cys
- EDT and thioanisole – avoid Met oxidation
- Phenol – protect Tyr and Trp
- TIS (Triisopropylsilane) – quenching high stable Trt cation

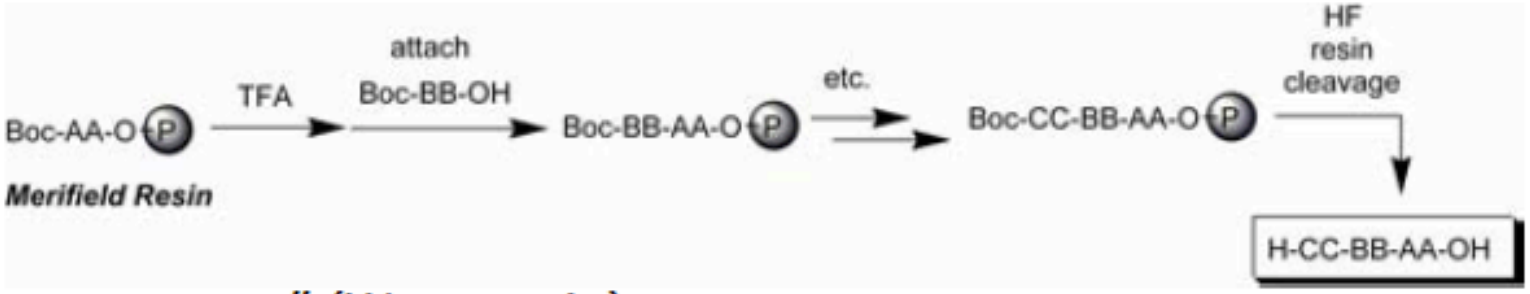
Cleavage

Side reactions during cleavage:

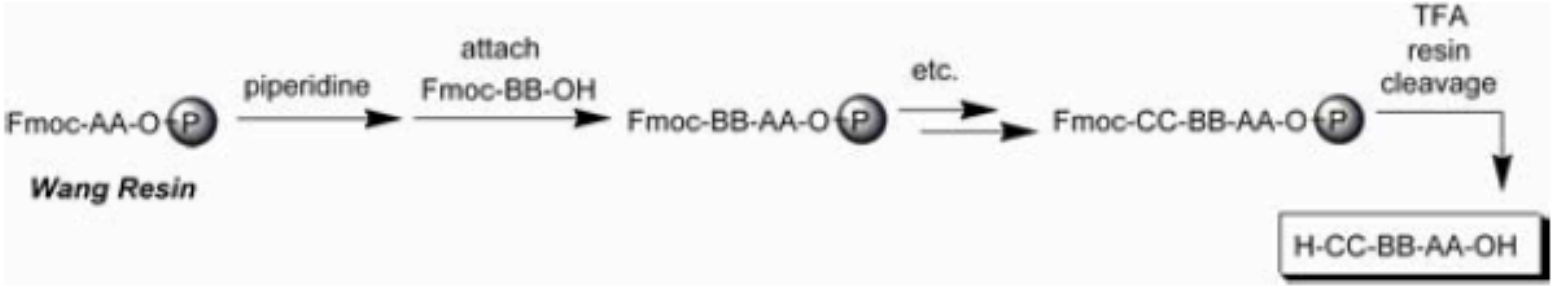
- Alkylation for Met, Cys and Trp (by t-Butyl action)
- Sulfination for Trp (by Mtr and Pmc)
    - Use Trp(Boc)

Summary - SPPS

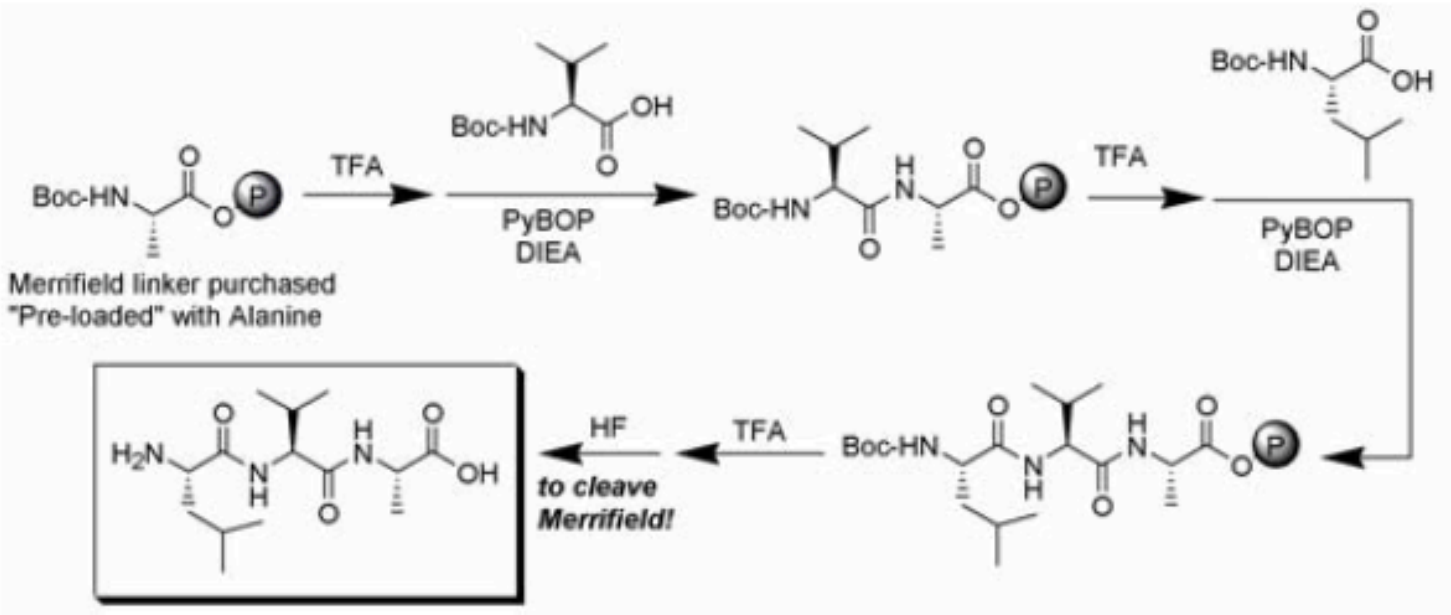
- "Boc-strategy" (Merifield resin)



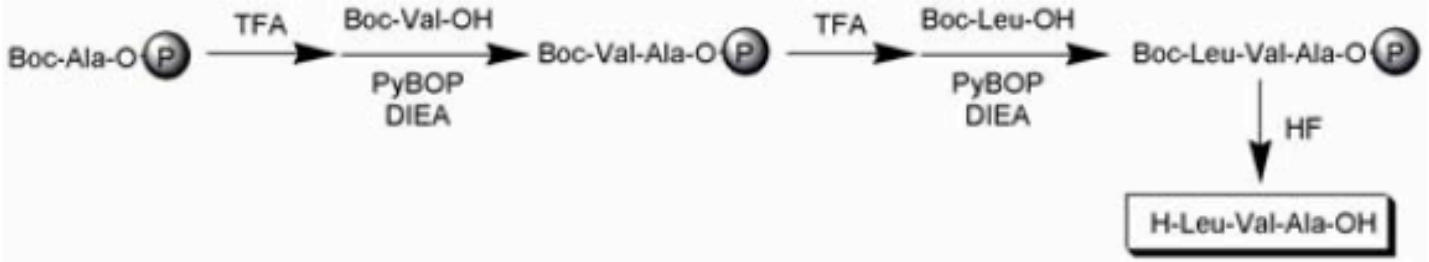
- "Fmoc-strategy" (Wang resin)



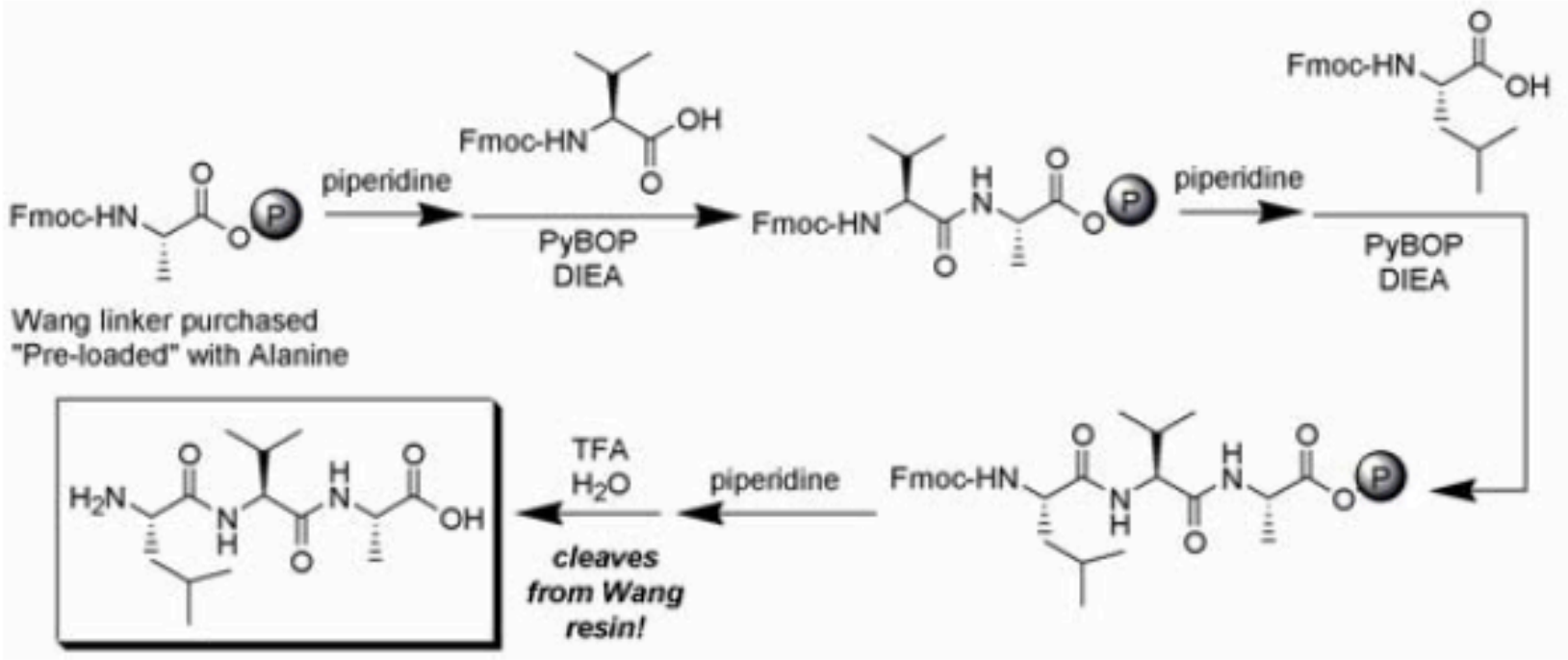
- "Boc strategy" of SPSS: (making H-Leu-Val-Ala-OH):



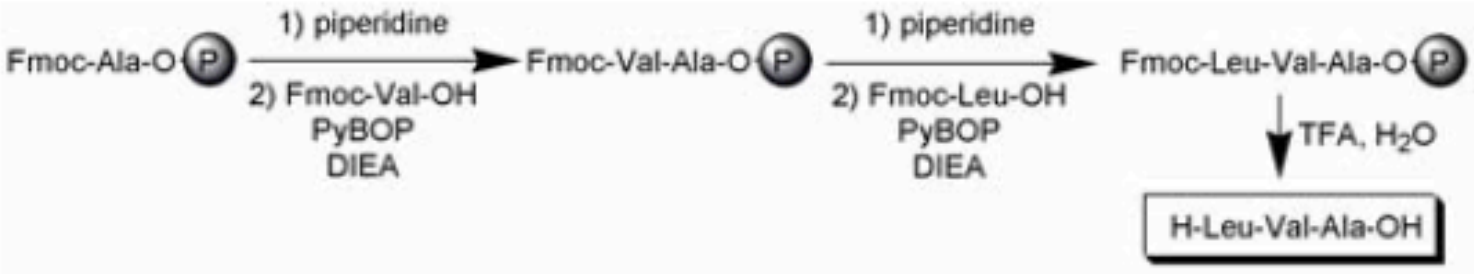
- The sequence can be abbreviated like this:



- And here is the "Fmoc strategy" of SPSS: (H-Leu-Val-Ala-OH):



- The sequence can be abbreviated like this:

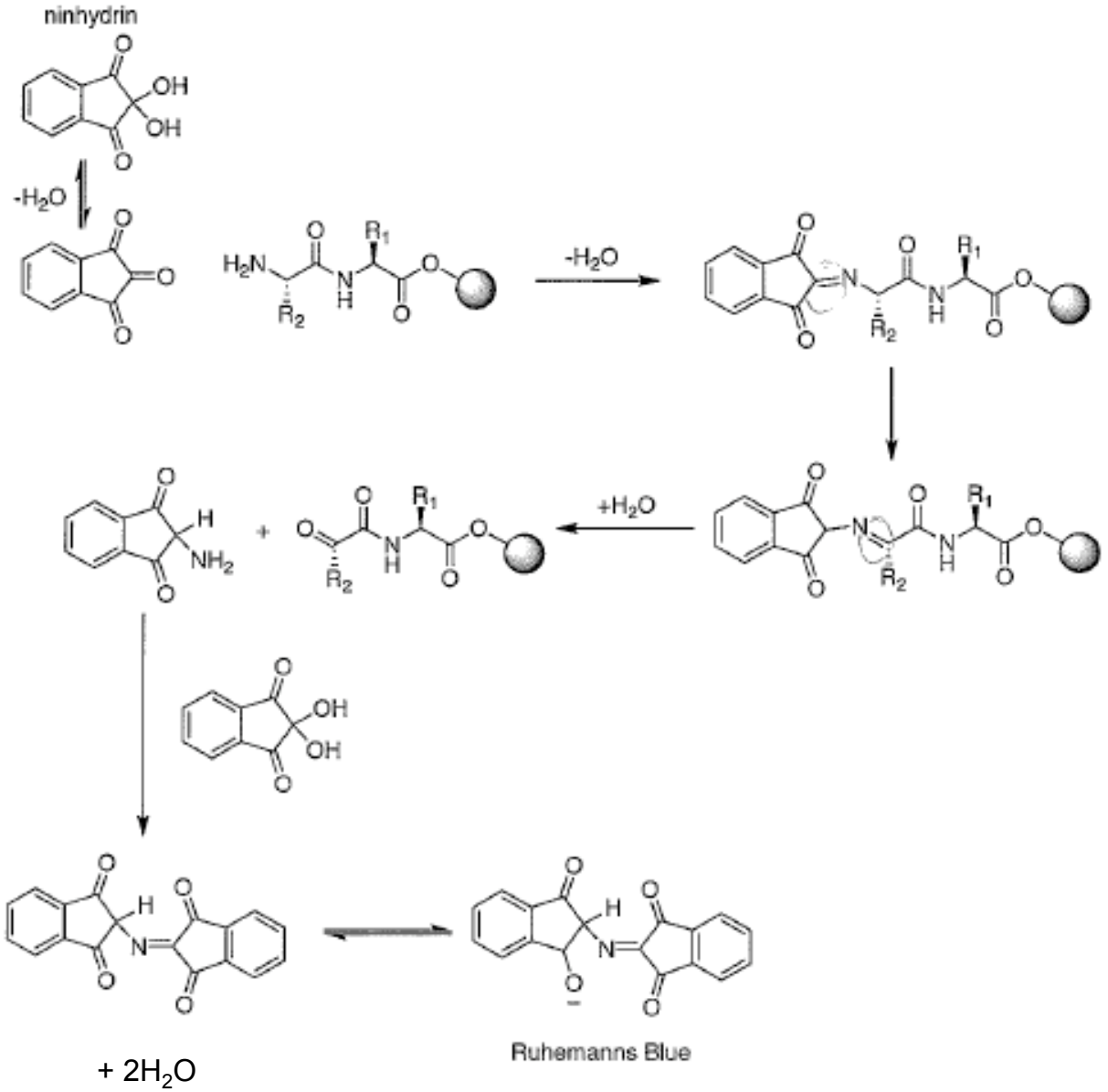


Coupling efficiency and final yield

Efficiency (%)	10-mer	20-mer	30-mer	40-mer	50-mer	60-mer
99	90	82	74	67	61	55
98	82	67	55	45	36	30
95	60	36	21	13	8	5
80	11	1	0	0	0	0

Ninhydrin test

- To detect a free pri
  - Blue colour – fr
  - Yellow colour –



Purification and characterisation

- RP-HPLC
- Mass spectroscopy
- NMR

Leu-Enkephalin

- Pentapeptide
  - NH_2 - Tyr - Gly - Gly - Phe - Leu – COOH
- Using Fmoc based SPPS
- Characterise the peptide by RP-HPLC, NMR and maybe SELDI-MS
- Read “Guide to SPPS” before 1:00 pm
- Check MSDS for all chemicals needed for SPPS before 1:00 pm
  - DMF/Pregnancy
- Two groups
- We’ll meet in N120
- Report