Solid Phase Peptide Synthesis

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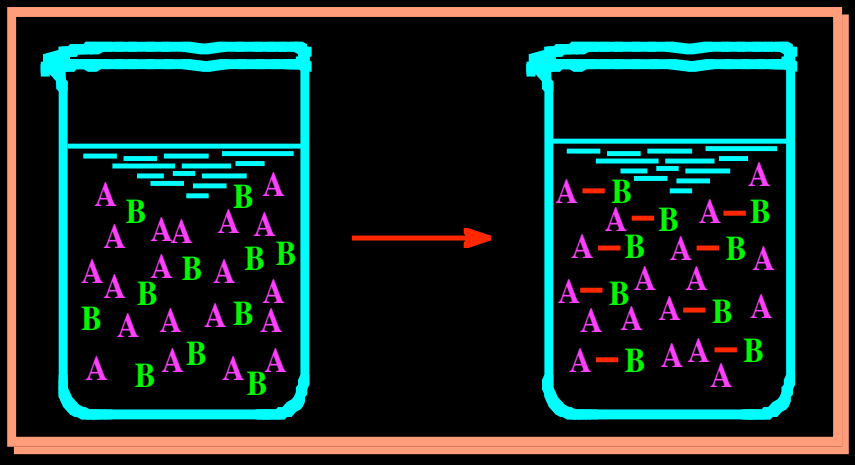
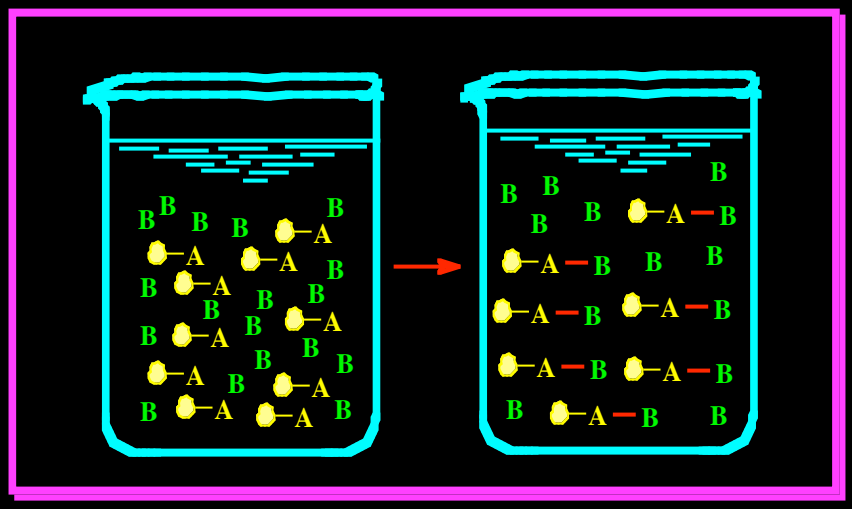
Conventional synthesis



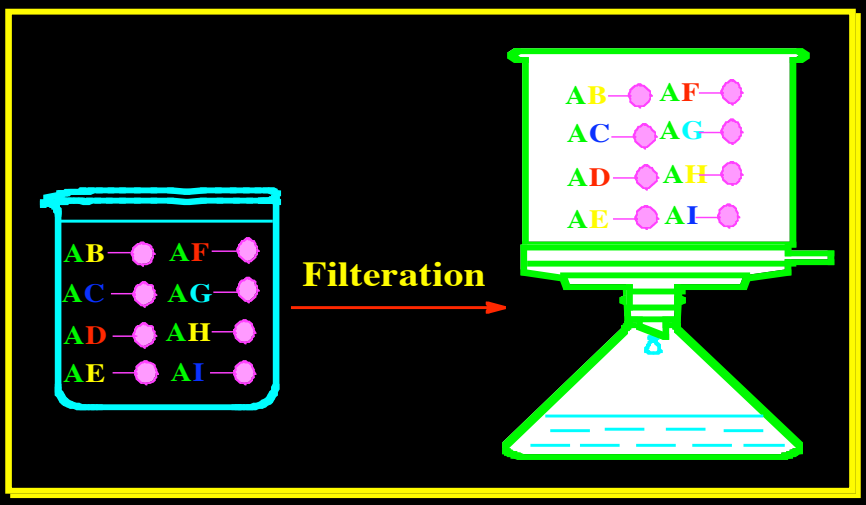
Synthesis of peptide by conventional method

- Time consuming
- Laborious
- Complicated purification needed
- Low yield

Solid Phase Synthesis

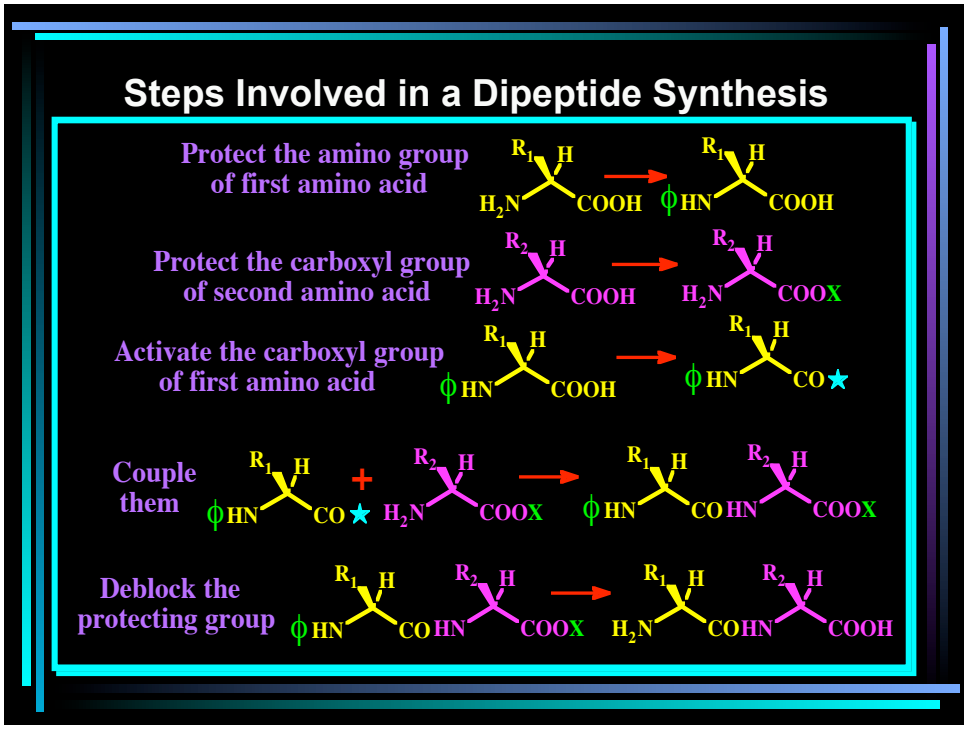


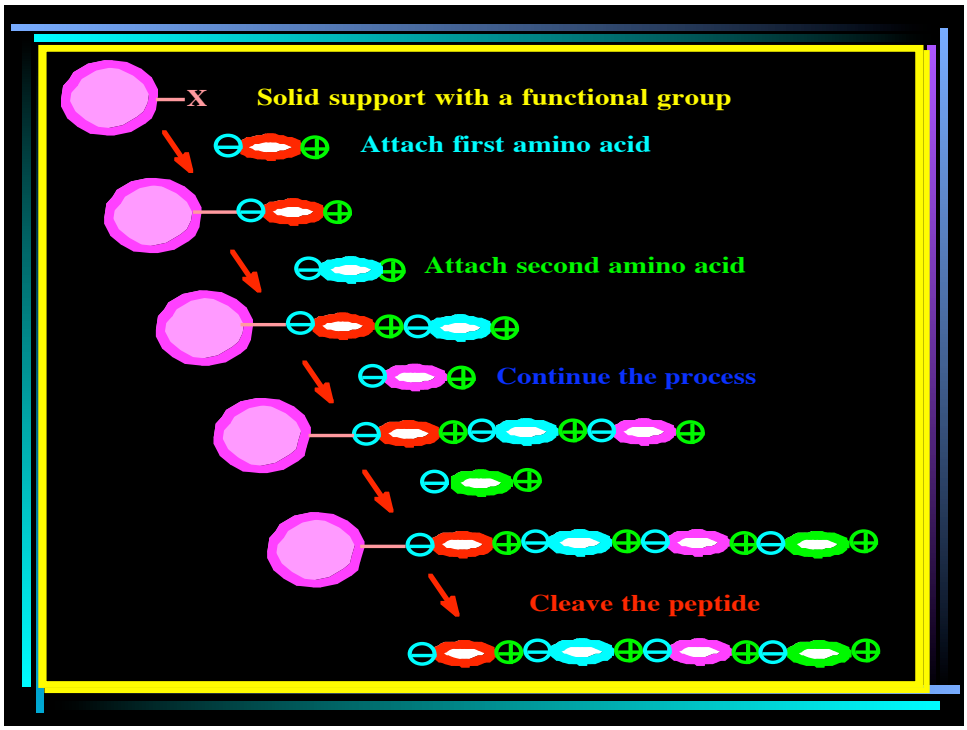
In Solid Phase Synthesis, Products Can Be Isolated Easily By Filtration



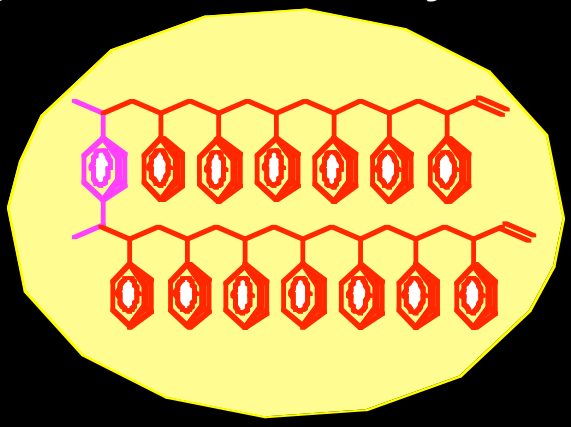
Solid Phase Synthesis -Advantages

- Reaction can be forced to go towards 100% yield by excess reagents.
- Recovery of the products are easy - Simple filtration as opposed to complicated precipitation and recrystallization.
- Purification of products is easy - simple wash.
- Several steps can be accomplished on the same resin.
- In general, large scale saving in time, effort and reagents.


Steps Involved in a Dipeptide Synthesis
Protect the amino group of first amino acid
Protect the carboxyl group of second amino acid
Activate the carboxyl group of first amino acid
Couple them
Deblock the protecting group


X
Solid support with a functional group
Attach first amino acid
Attach second amino acid
Continue the process
Cleave the peptide

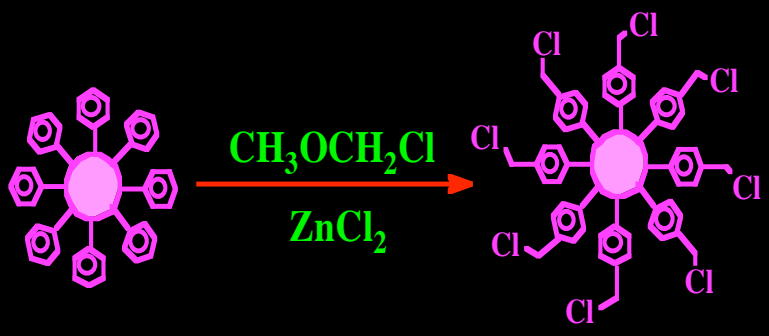
Resin made by copolymerizing styrene and 1% divinylbenzene



Resins (other than polystyrene) used in solid phase synthesis :

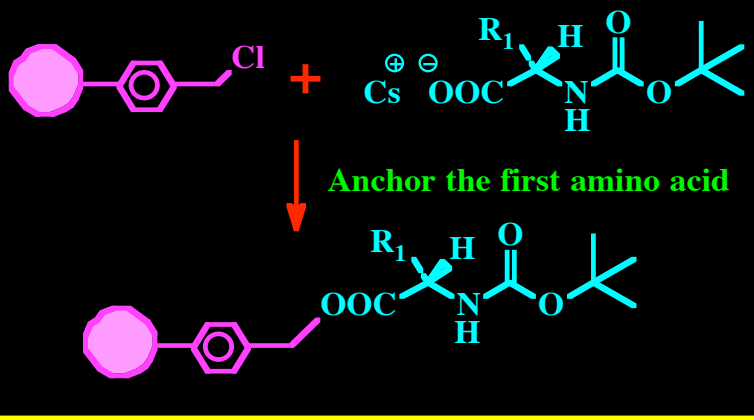
- Polymethylmethacrylates
- polyacrylamides
- Phenolic resins
- Polysaccharides
- Inorganic supports such as Silica and Porous glass

First: Functionalizing the resin



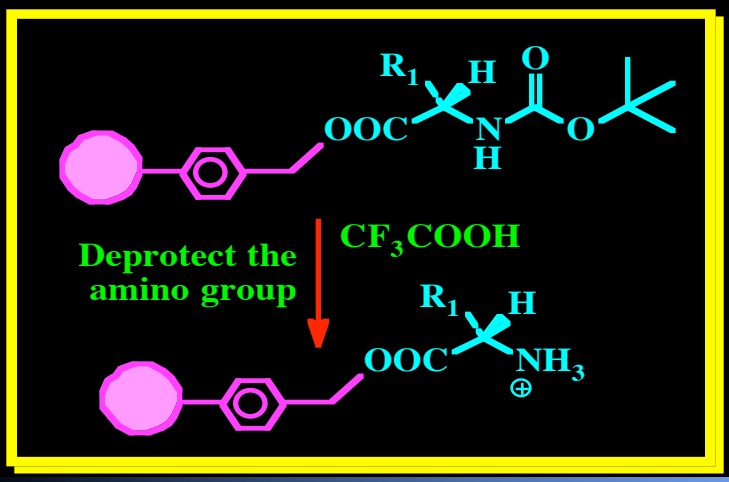
Step -1: Anchoring the first amino acid -

The first t-Boc amino acid is attached *via* benzylester group to the insoluble resin.

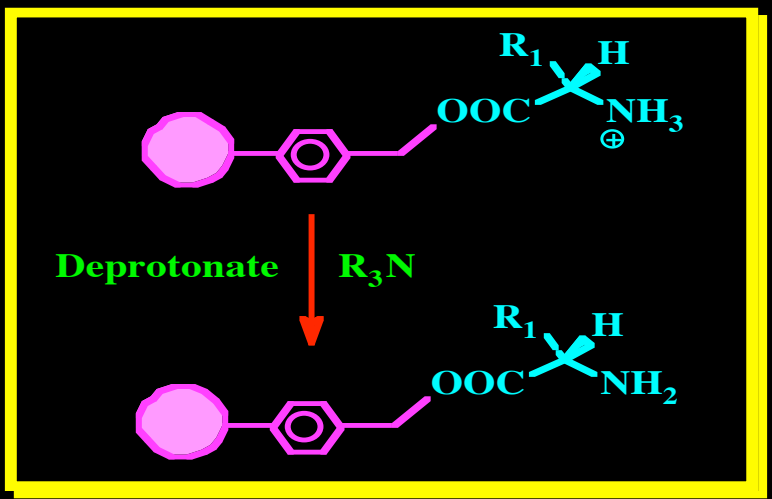


Step-2: Deprotection of the amino group

- t-Boc is completely removed by 50%TFA in dichloromethane, while the benzyl ester is untouched.

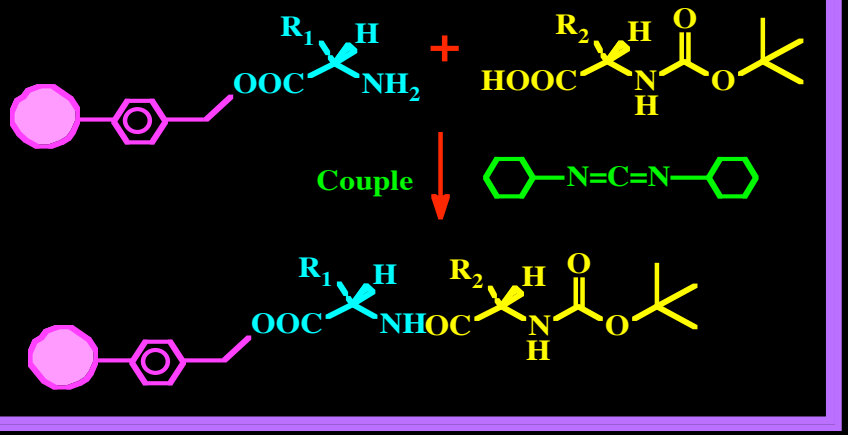


Step 3: Deprotonation - Tertiary amines, (diisopropyl ethyl amine) deprotonate the salt.

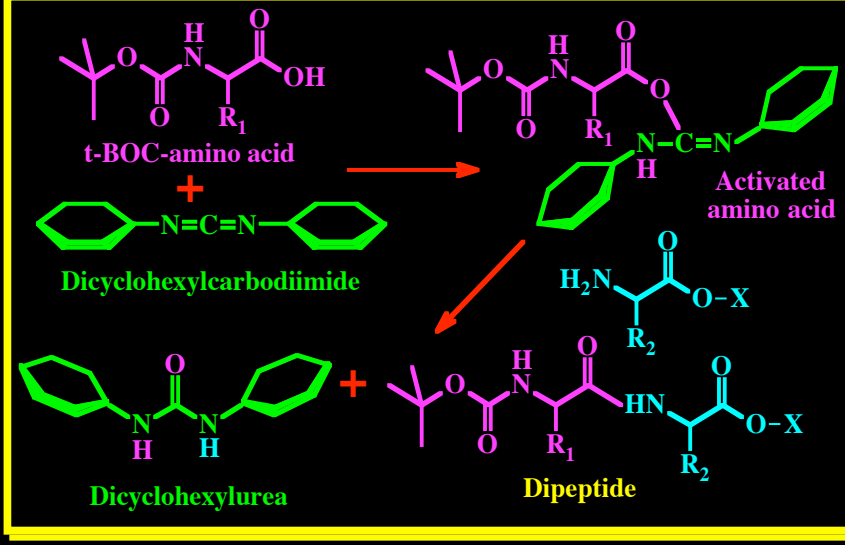


Step 4: Coupling the next amino acid -

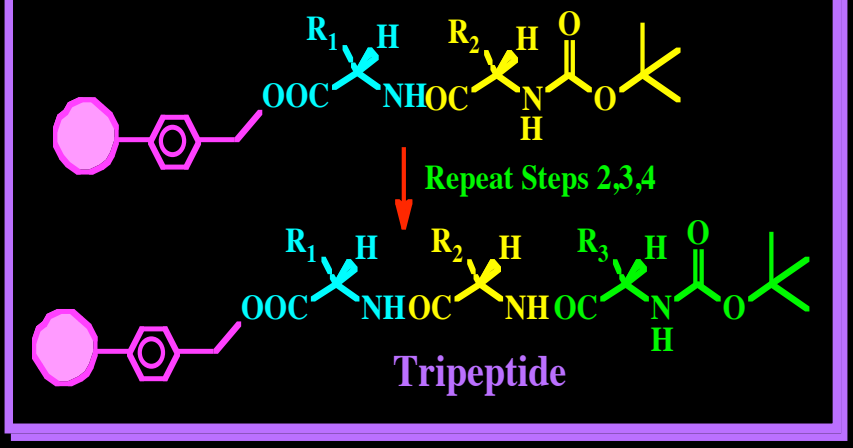
Dicyclohexylcarbodiimide activates the carboxyl group of the second amino acid and allows the coupling to the first amino acid. Water is extracted to form dicyclohexylurea.



Mechanism of coupling reaction

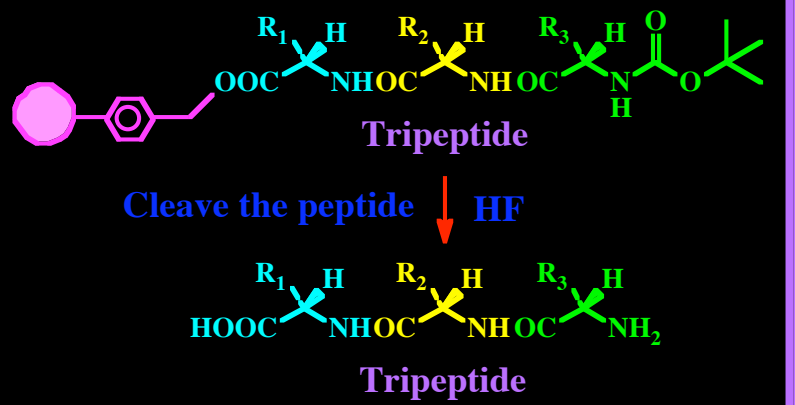


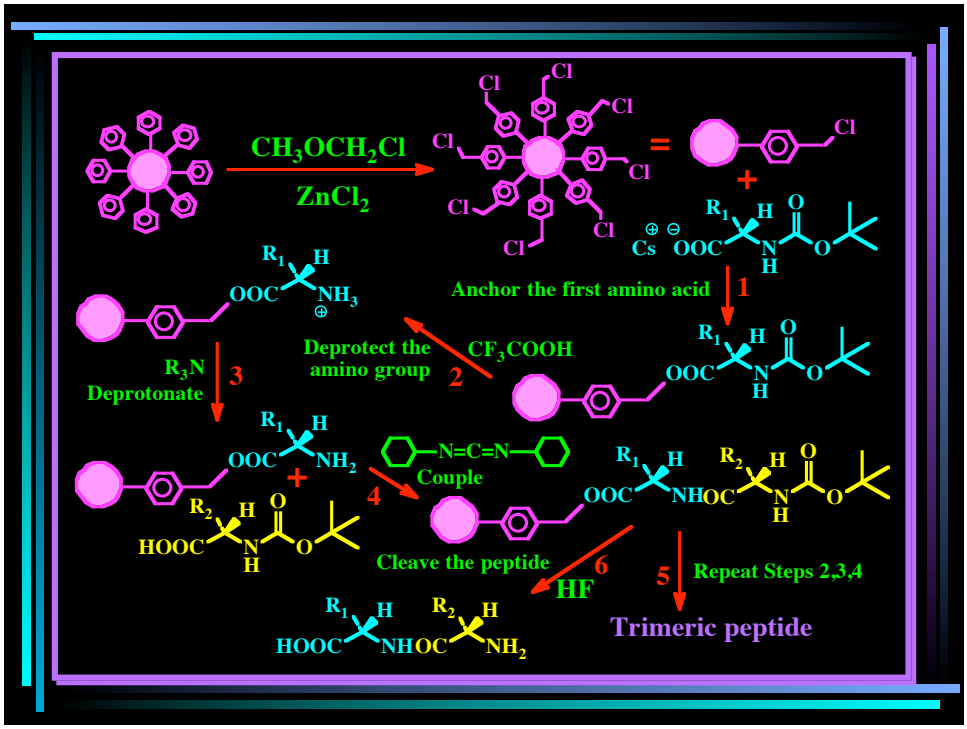
Step 5: Continue peptide synthesis



Step 6: Cleave the peptide from resin -

Finally the free peptide is liberated from the resin by a strong acid such as HF.

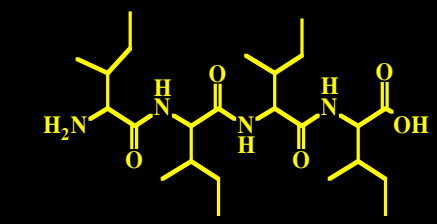




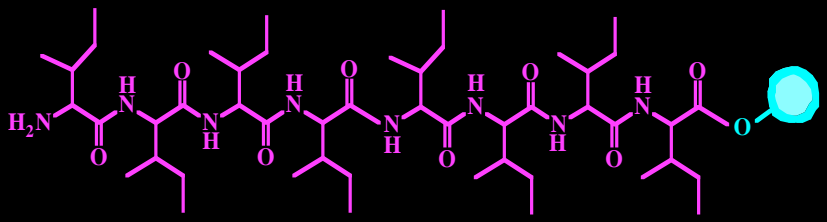
Solid phase synthesis

- Bruce Merrifield synthesized Ribonuclease (124 amino acid) and interferon (155 amino acid) using solid phase peptide synthesis.

ADVANTAGE: SYNTHESIS OF HYDROPHOBIC PEPTIDES



Beyond tetrapeptide aggregation and precipitation prevents further synthesis in solution



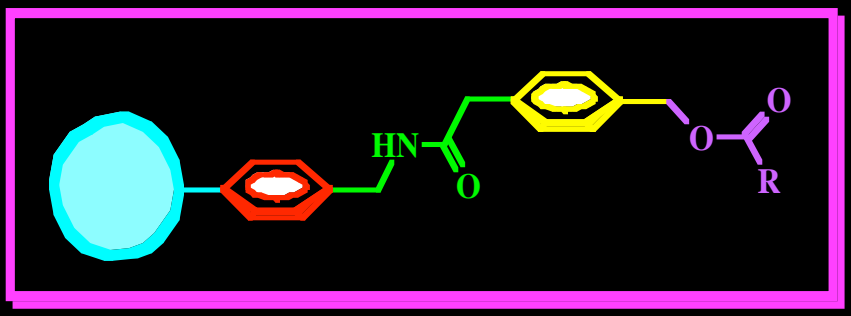
IN SOLID PHASE, OCTAISOLEUCINE AND EVEN DODECYL ISOLEUCINE HAVE BEEN SUCCESSFULLY SYNTHESIZED

For a 50 amino acid peptide synthesis, how coupling efficiency at each step affects the final purity of the product.

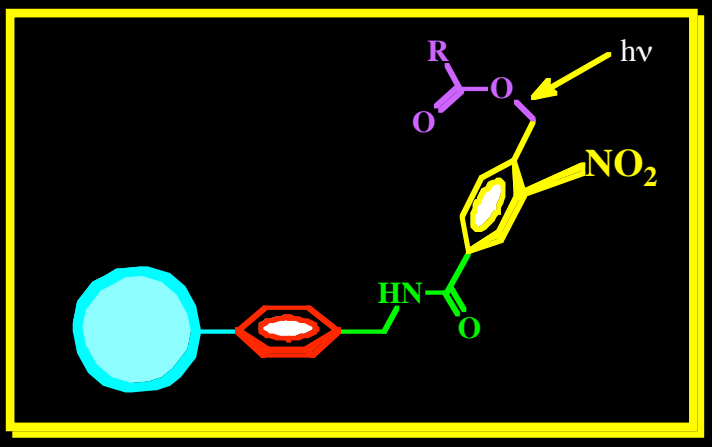
COUPLING EFFICIENCY (%)	PURITY OF PRODUCT (%)
100.0	100.0
99.99	99.15
99.9	94.79
99.5	77.60
99.0	60.44

Acyloxymethyl-Pam-Resin

Insertion of acetamidomethyl group (green) in between the benzyl ester and polystyrene resin increased the stability of benzyl ester to trifluoroacetic acid by about 400 times.

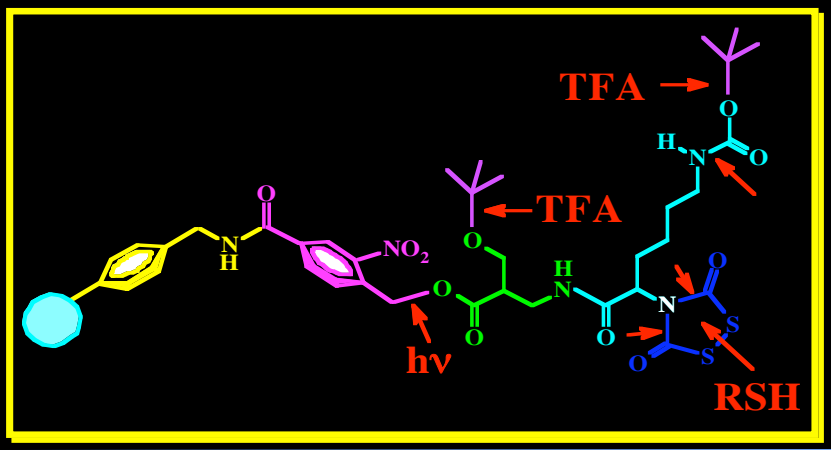


Use of photolabile resin as a mean to remove the synthesized peptide

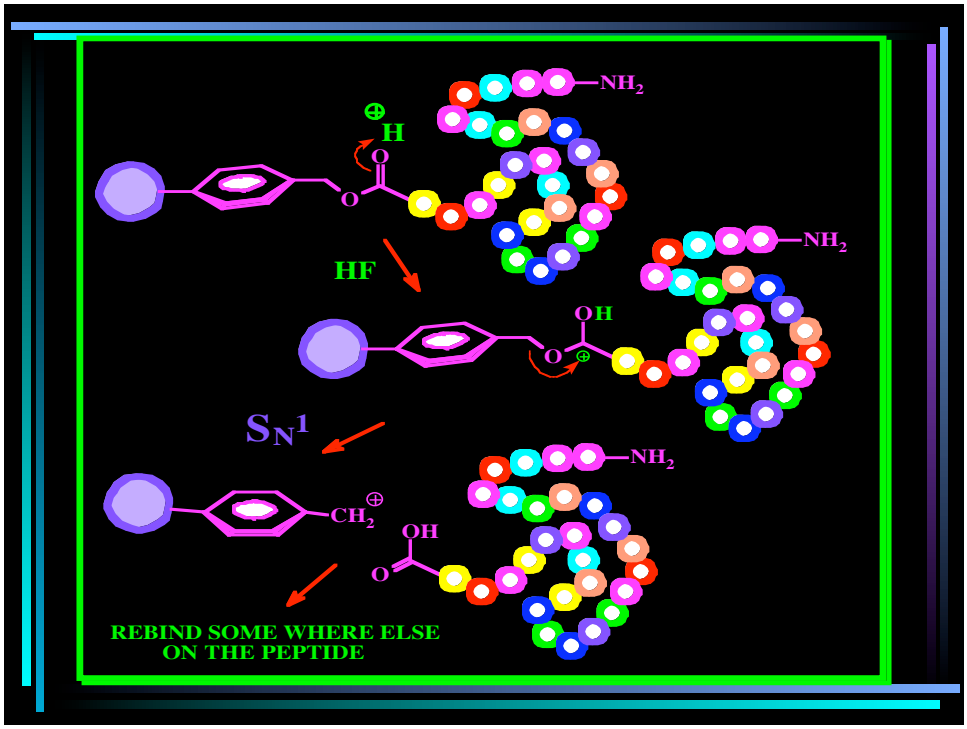


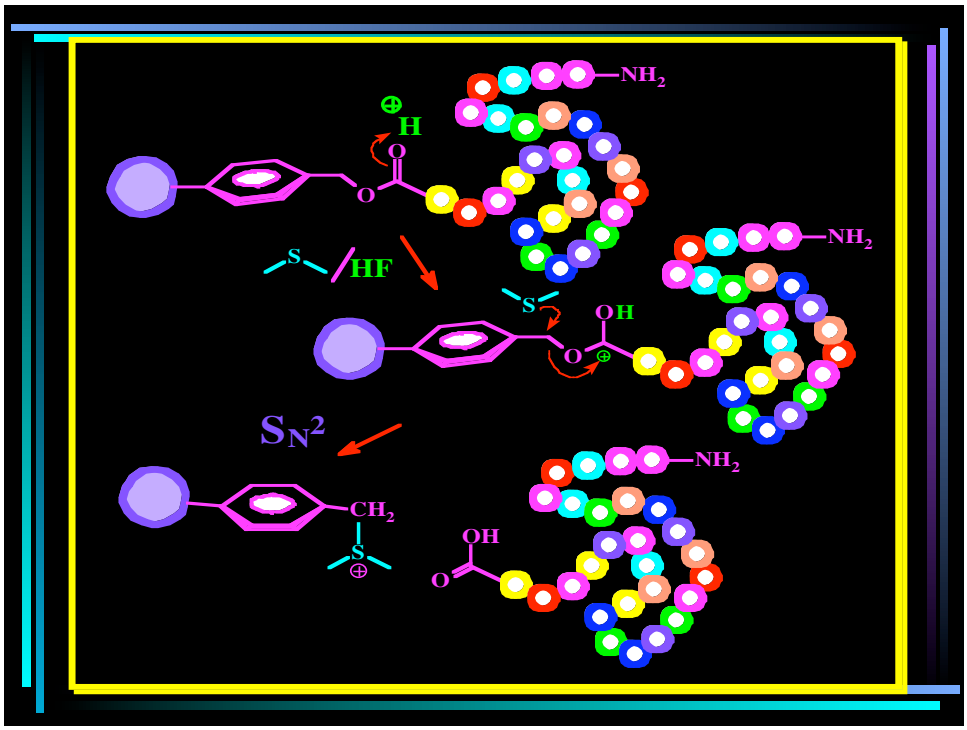
Different protecting groups

Photolabile group at the first linkage site.
t-BOC for protection of side chains.
Thiol labile group for protection of N-terminals.



TO AVOID THE SN1 REACTION, A BASE (DIMETHYLSULFIDE) IS ADDED TO HF IN A 1:1 RATIO. THIS FORCES THE CLEAVAGE TO SN2 REACTION AND PREVENTS THE BENZYL CATION FROM ATTACKING THE PEPTIDE.


NH2
H
O
O
HF
OH
O
NH2
SN1
CH2
OH
O
NH2
REBIND SOME WHERE ELSE
ON THE PEPTIDE


NH2
H
O
O
S
HF
S
OH
O
NH2
SN2
CH2
S
OH
O
NH2