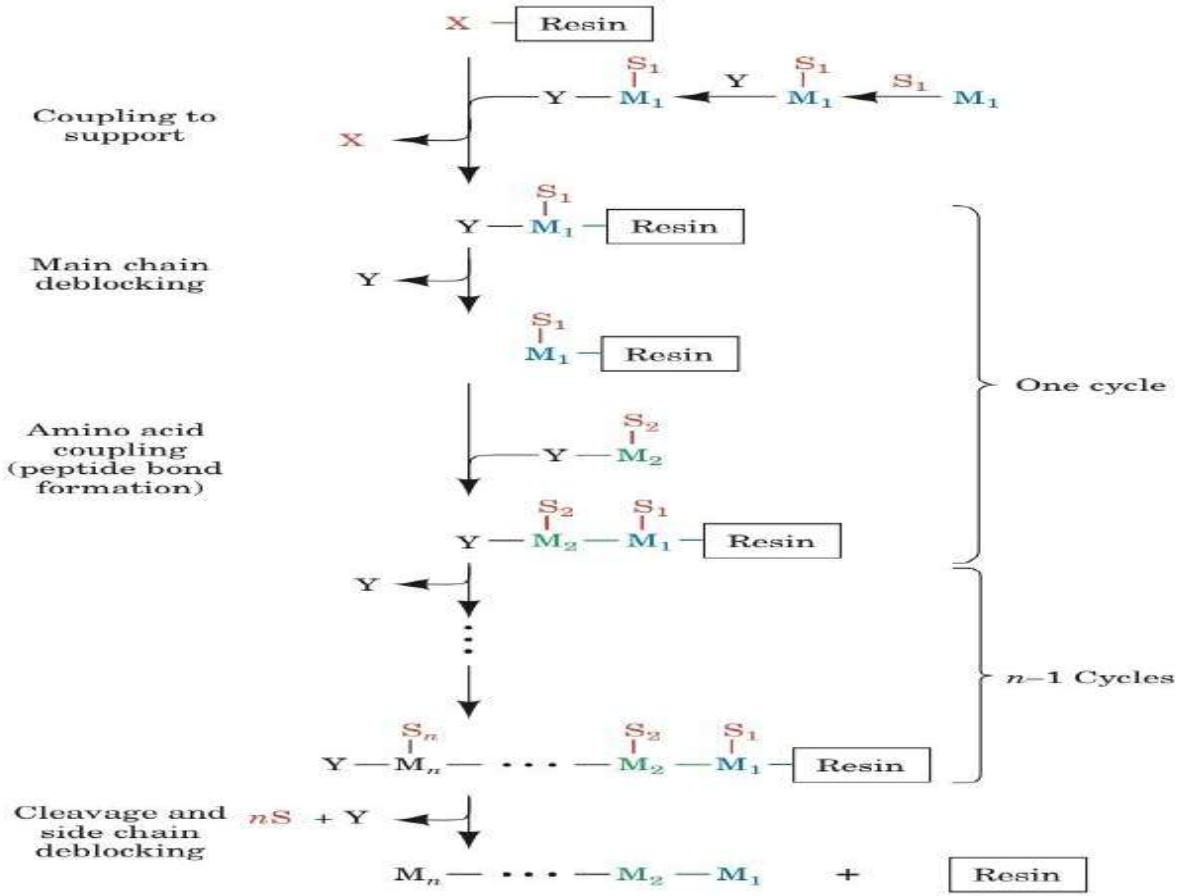
VIVEKANANDA COLLEGE,THAKURPUKUR

TOPIC:SOLID PHASE PEPTIDE SYNTHESIS
COURSE : PROTEIN AND PURIFICATION
PAPER:CC2
SEMESTER:GE-2
NAME OF TEACHER:NINEESHA SEN BANERJEE
DEPARTMENT:BIOCHEMISTRY

Introduction to SPPS

- Polypeptides are chemically synthesized by covalently linking (coupling) amino acids, one at a time, to the termi- nus of a growing polypeptide chain. Imagine that a polypeptide is being synthesized from its C-terminus to- ward its N-terminus; that is, the growing chain ends with a free amino group. Then each amino acid being added to the chain must already have its own □ -amino group chemically protected (blocked) or it would react with other like mole- cules as well as with the N-terminal amino group of the chain. Once the new amino acid is coupled, its now N- terminal amino group must be deprotected (deblocked) so that the next peptide bond can be formed. Every cycle of amino acid addition therefore requires a coupling step and a deblocking step.

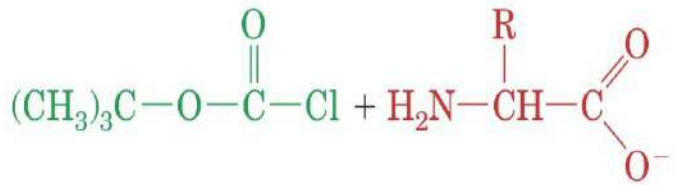
General approach to SPPS



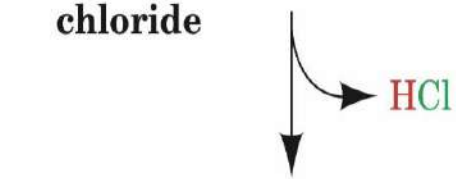
- The losses that are incurred on isolation and purifi-cation of the reaction product in each of the many steps, however, contribute significantly to the low yields of final polypeptide. This difficulty was ingeniously circumvented in 1962 by Bruce Merrifield, through his development of solid phase peptide synthesis (SPPS). In SPPS, a growing polypeptide chain is covalently anchored, usually by its C-terminus, to an insoluble solid support such as beads of poly- styrene resin, and the appropriately blocked amino acids and reagents are added in the proper sequence

Protecting the N-terminal

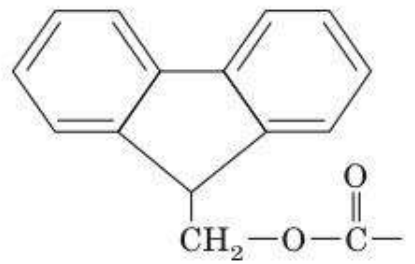
- When polypeptide chains are synthesized by amino acid addition to their N-terminus (the opposite direction to that in protein biosynthesis; Section 5-4Ba), the □ -amino group of each sequentially added amino acid must be chemically protected during the coupling reaction. The tert-butyloxy- carbonyl (Boc) group is frequently used for this purpose,



***t*-Butyloxycarbonyl chloride** + **α-Amino acid**



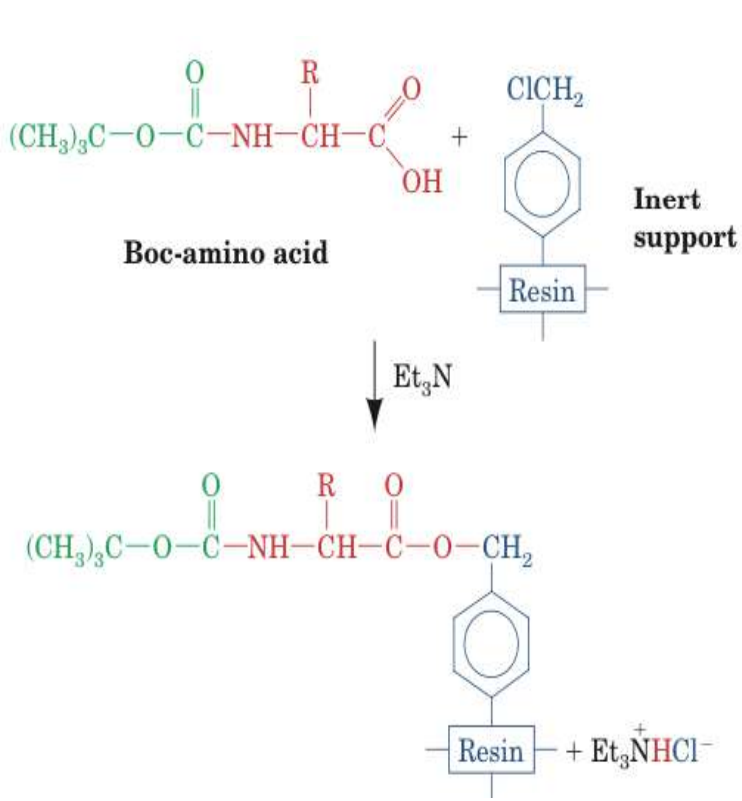
Boc-amino acid

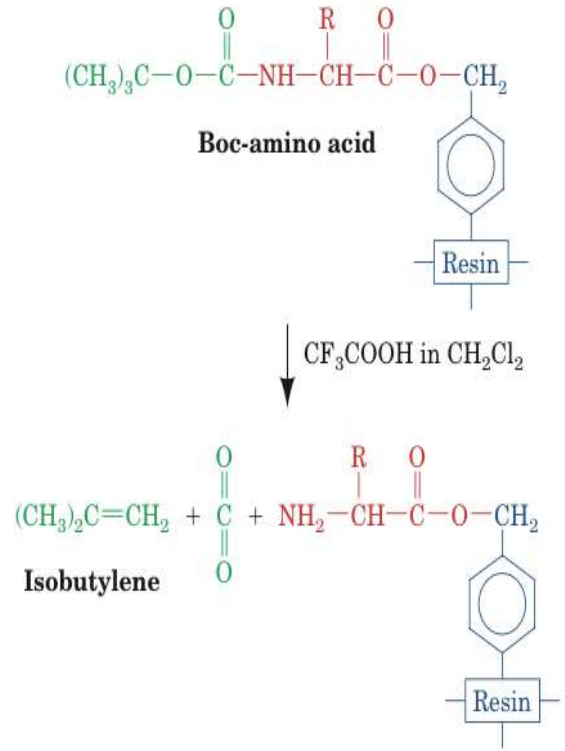


9-Fluorenylmethoxycarbonyl (Fmoc) group

Anchoring the chain to the inert support

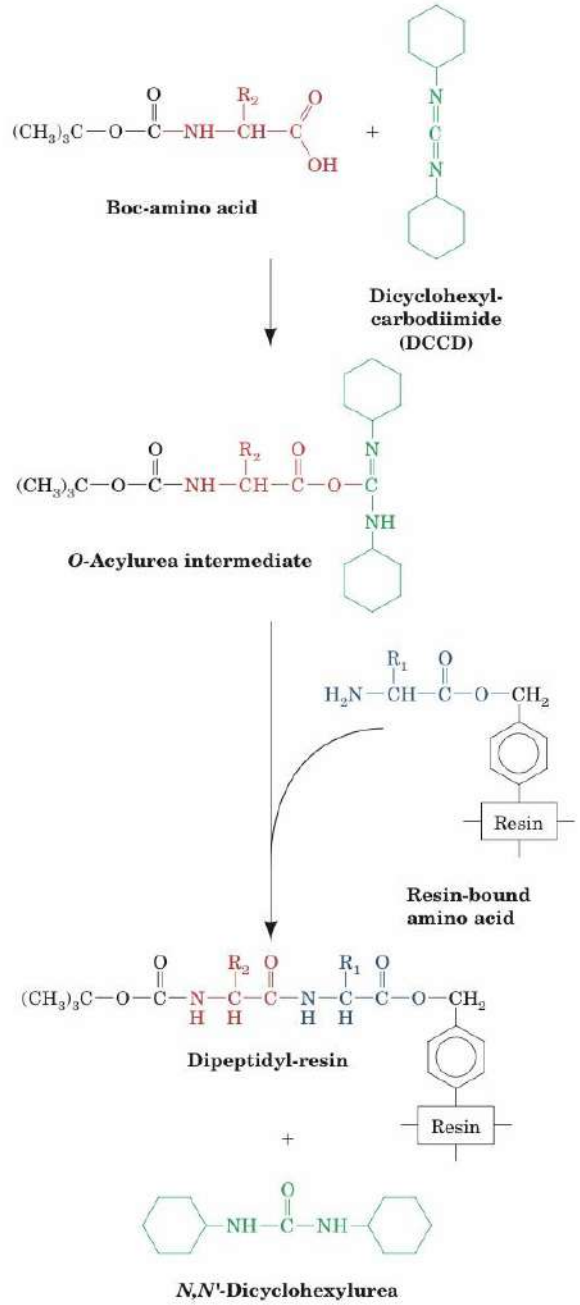
- The first step in SPPS is the coupling of the C-terminal amino acid to a solid support. The most commonly used support is a cross-linked polystyrene resin with pendant chloromethyl groups. Resin coupling occurs through the following reaction:(CH3)3C O C NH CH C Boc-amino acidOH C NDicyclohexyl- carbodiimide (DCCD)


Boc-amino acid
Inert support
Resin
Et3N
Resin
+ Et3NHCl−

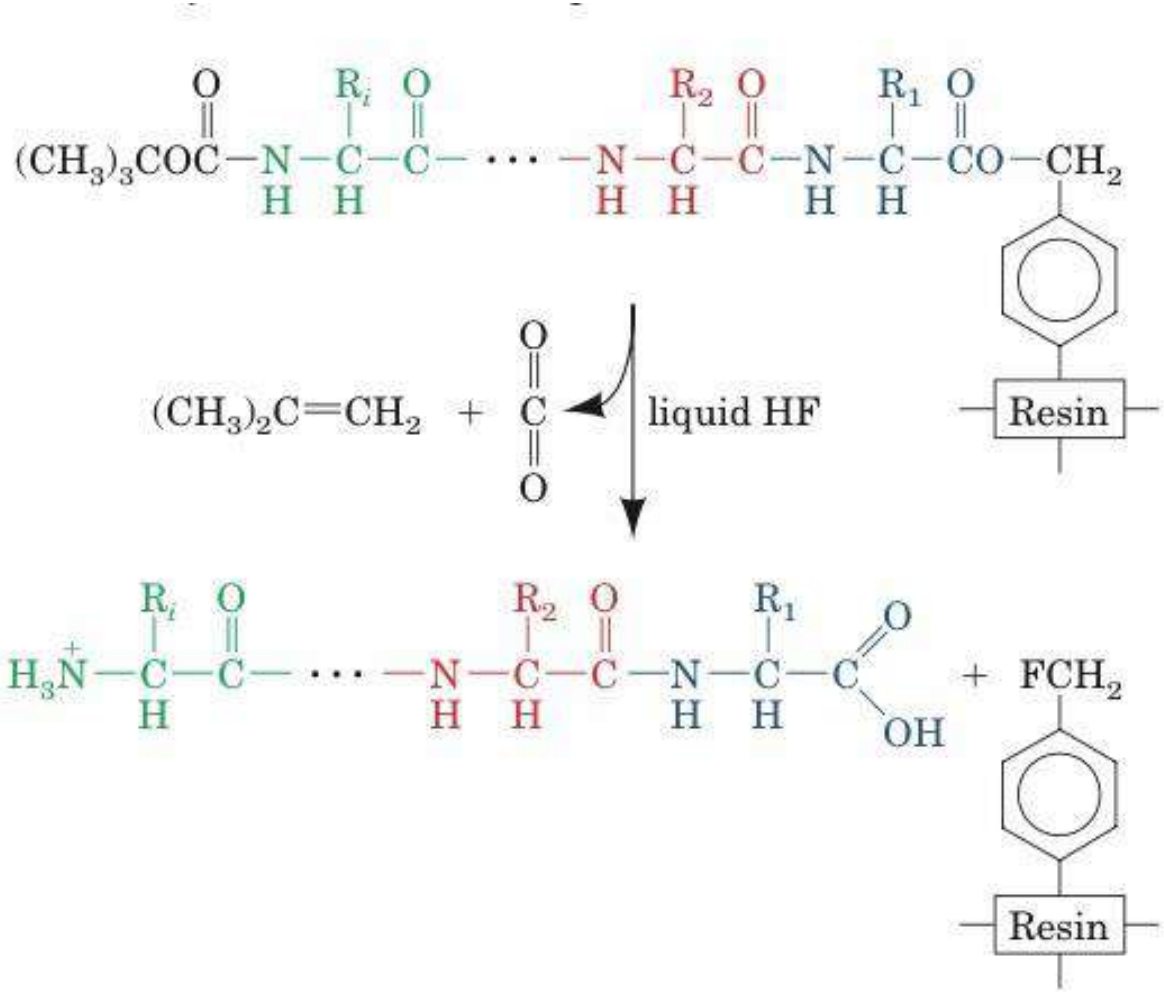

Boc-amino acid
Resin
CF3COOH in CH2Cl2
Isobutylene
Resin

Coupling the chain

- The reaction coupling two amino acids through a peptide bond is endergonic and therefore must be activated to obtain significant yields. Carbodiimides (R¬N“C“N¬R¿) such as dicyclohexylcarbodiimide (DCCD) are commonly used coupling agents:


Boc-amino acid
Dicyclohexyl-carbodiimide (DCCD)
O-Acylurea intermediate
Resin
Resin-bound amino acid
Dipeptidyl-resin
Resin
N,N'-Dicyclohexylurea

Releasing the polypeptide from the resin



Advantages of SPPS

1. During the synthesis, the peptide remains attached to the insoluble polymer support. Therefore, it is easy to separate the soluble byproducts by filtration. Intermediates can thus be purified after each step avoiding the time consuming purification methods.
2. Excess reagents can be used to force the reaction to completion. This excess reagent can be removed by filtration.
3. Mechanical loss of the material is avoided because the series of reaction steps are carried out in the same reaction vessel.
4. The method is very fast and simple.
5. The ability to purify, after each reaction by simple filtration and washing and the fact that all reactions can be conducted within a single reaction vessel, made this method ideal for automation.