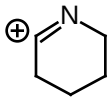
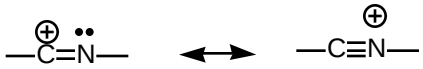
Reading assignment in the Protein Handout: pp 1-13 covered so far, through today's lecture
Note that p 16 is a duplicate of p 14, by mistake.
Also today: handout pp 19-21 [text: 26.3b, 26.3c]

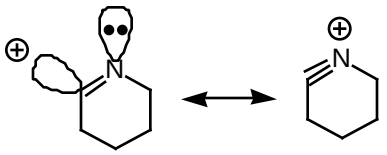
In the Key to PS 8, part C, we refer to the following cation intermediate:



At first this appears to be a "vinyl cation", a very high energy species which would cause you to doubt the reasonableness of the mechanism. However, it is not a simple vinyl cation. If this "imine cation" were in an open chain system, we would draw two resonance structures to show the special stabilizing effect of having the N next door.

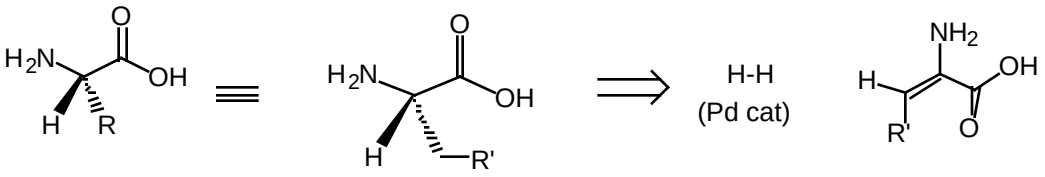


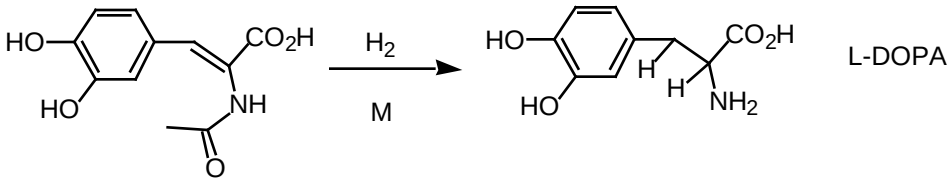
In the cyclic version, the lone pair on N is in the same plane as the empty sp^2 orbital associated with the + charge, but the overlap is not ideal because of the constraints imposed by the ring.



Not as bad as your average vinyl cation

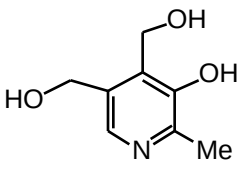
Elaboration from last time:



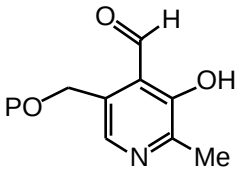


L-DOPA

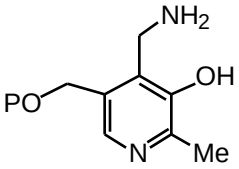
enzyme cofactors for introduction of amino groups:



pyridoxine
(vitamin B_6)

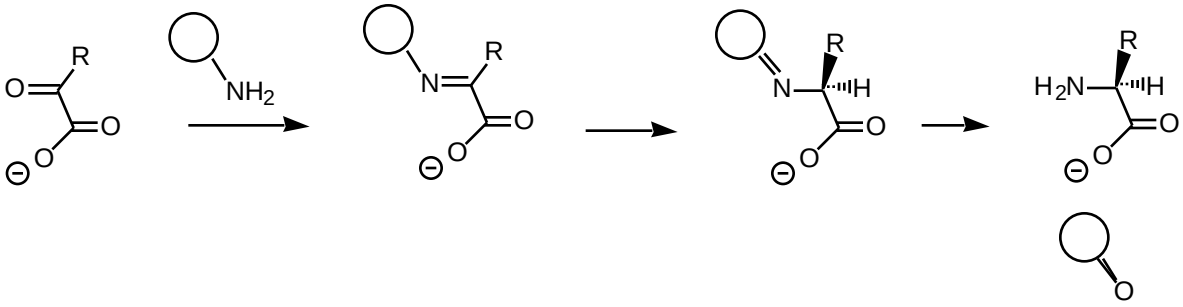


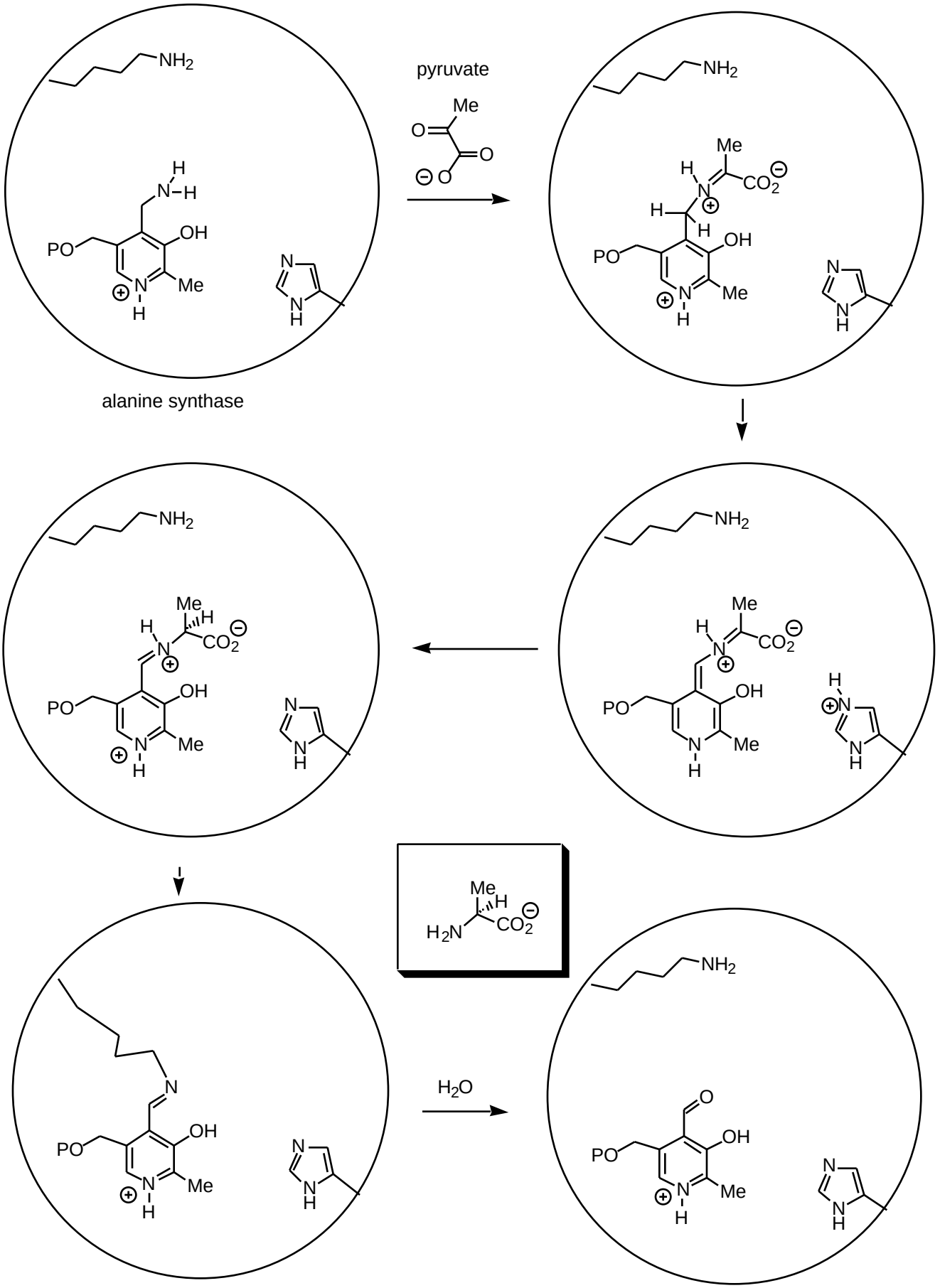
pyridoxal-5'-phosphate
[P = -OPO_2OH (-)]



pyridoxamine-5'-phosphate

Key process:




pyruvate
alanine synthase
H_2O

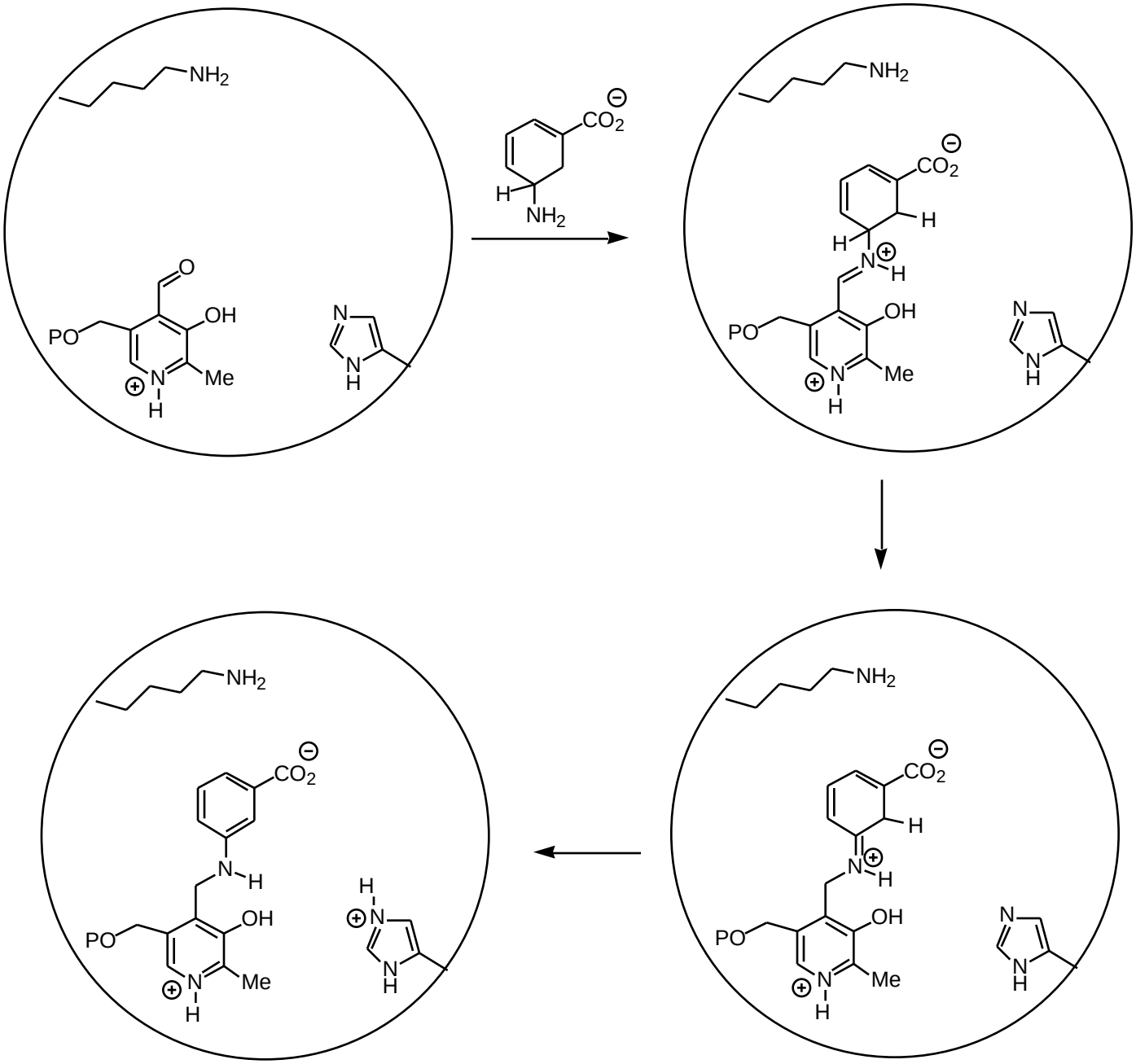
Enzymes are catalysts, doing the same reaction thousands of times before deteriorating and being replaced.

Inhibiting an enzyme can shut down an important metabolic pathway--

Herbicides: can be designed to interfere selectively with enzymes for amino acid synthesis
which do not appear in humans Kills plants, relatively non-toxic to humans

How inhibit an enzyme? Simply bind tightly (non-covalently): competitive inhibitor
but now consider **"suicide substrate"**

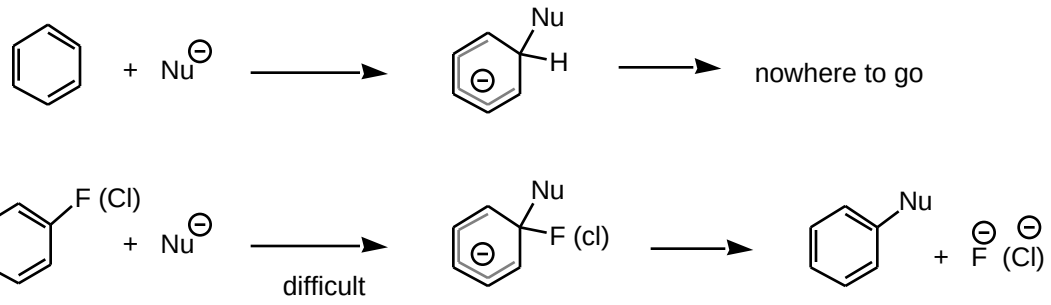
Fool the enzyme into binding onto molecule which then is induced to react in a way to irreversibly bind to enzyme, taking it out of the action.



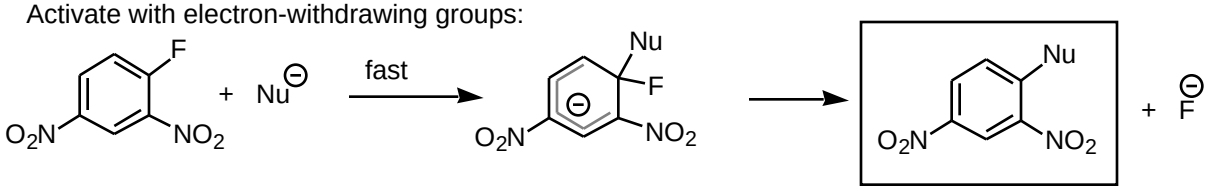
Cannot regenerate active form of enzyme/cofactor complex

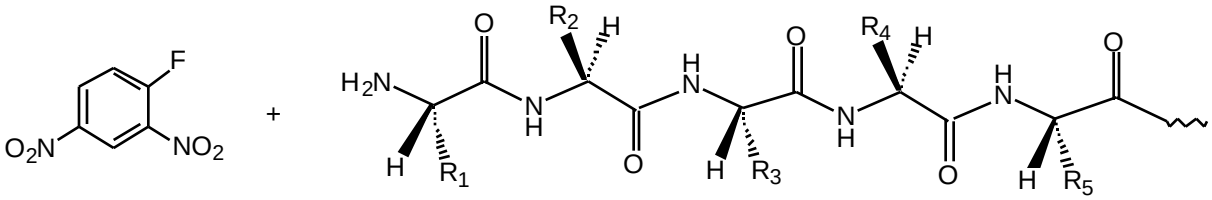
Sanger's reagent:

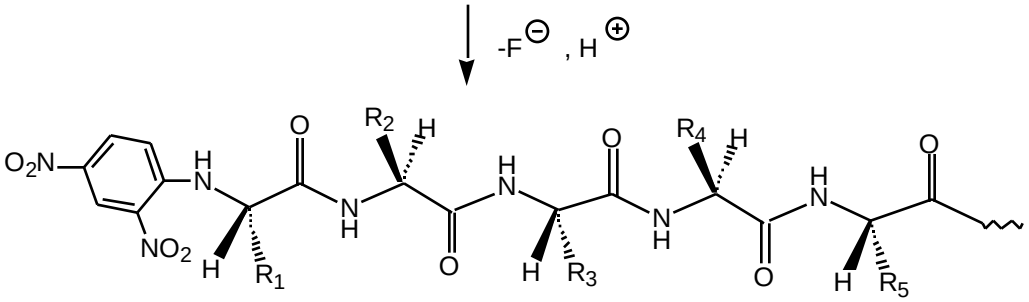
New reaction: Nucleophilic aromatic substitution (text sec 14.13; we skipped this earlier)

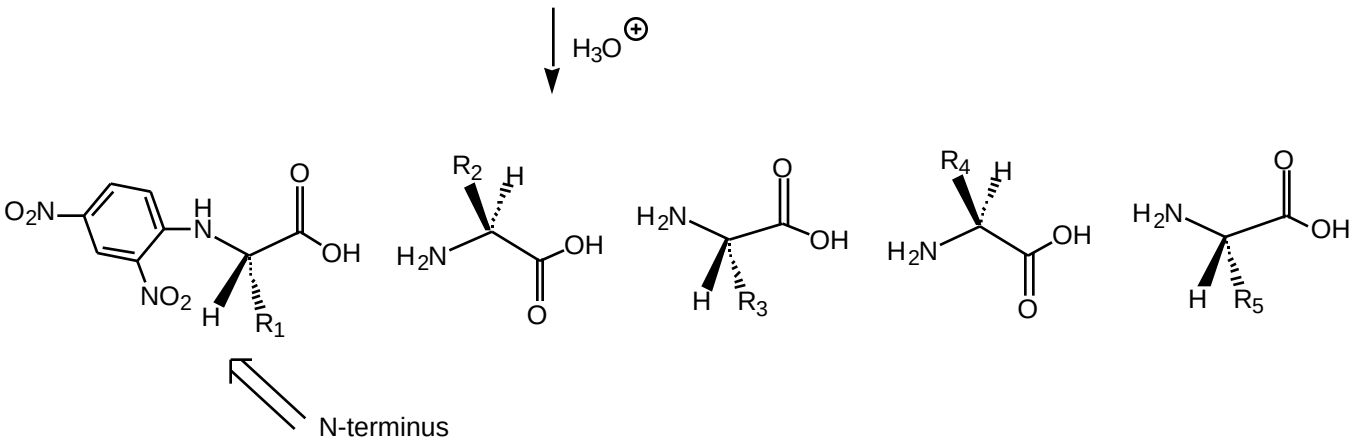


Activate with electron-withdrawing groups:



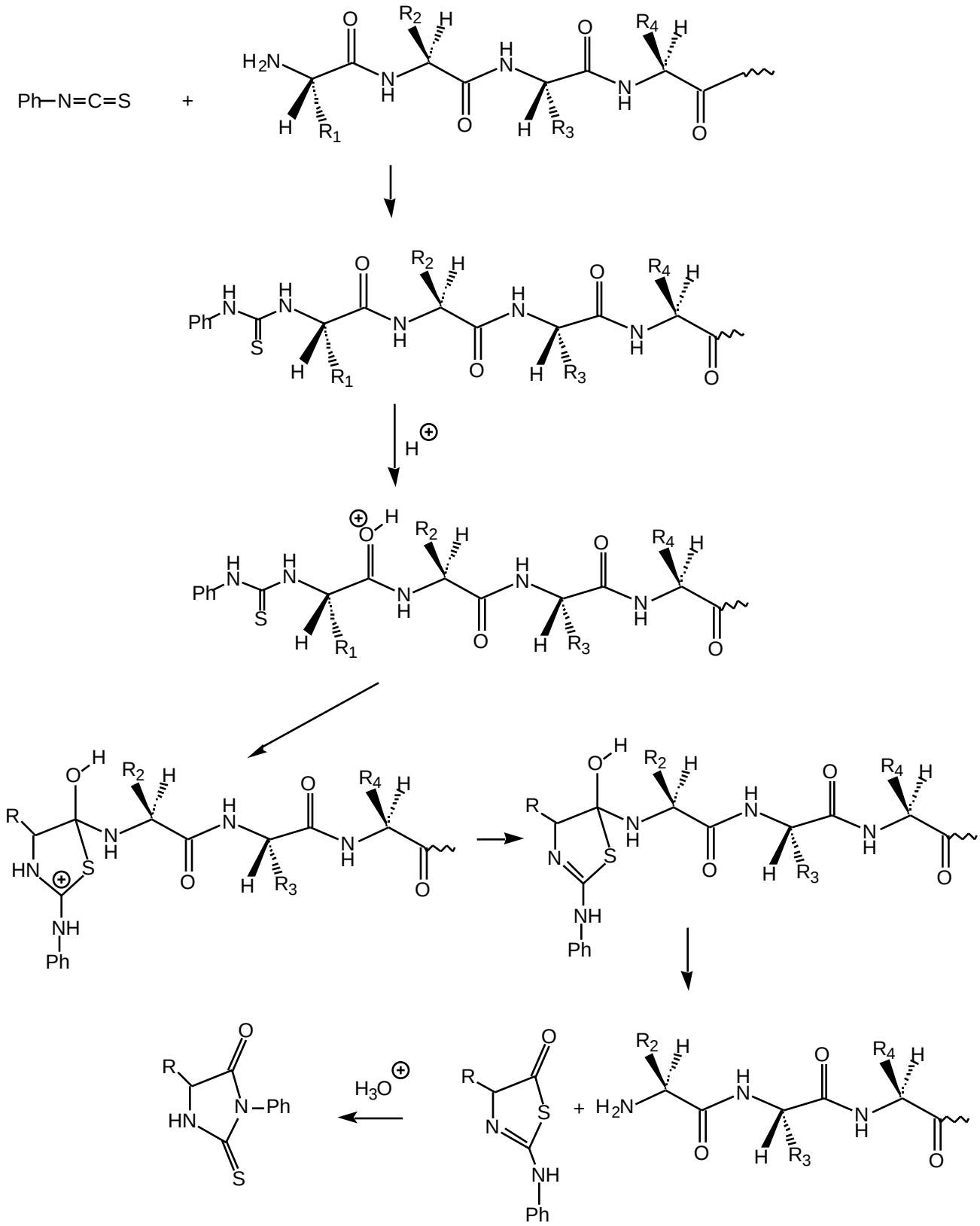






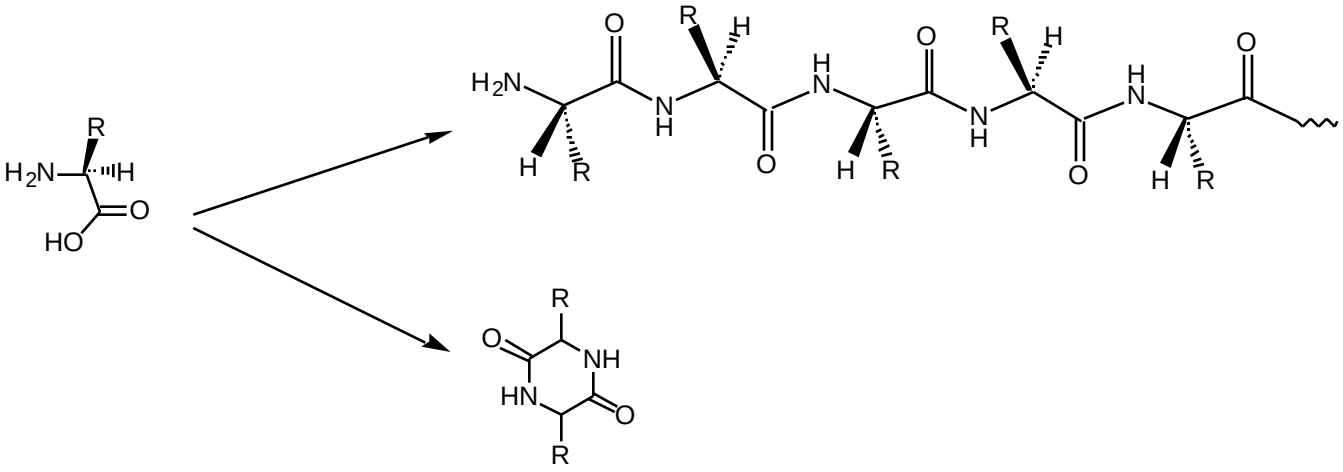
Better to unzip the protein one amino acid at a time, from one end to the other:

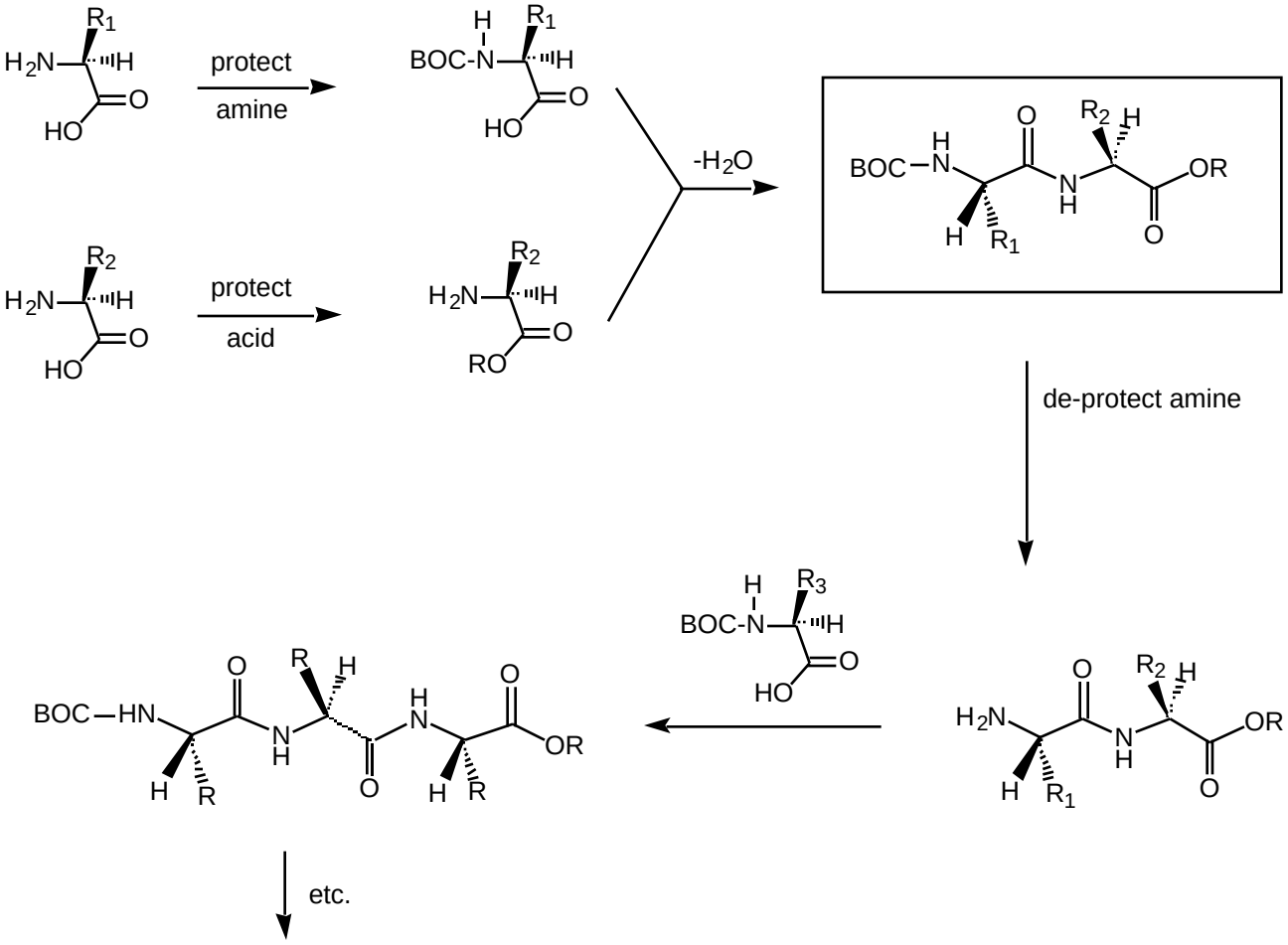
Edman degradation: phenyl isothiocyanate R-N=C=S



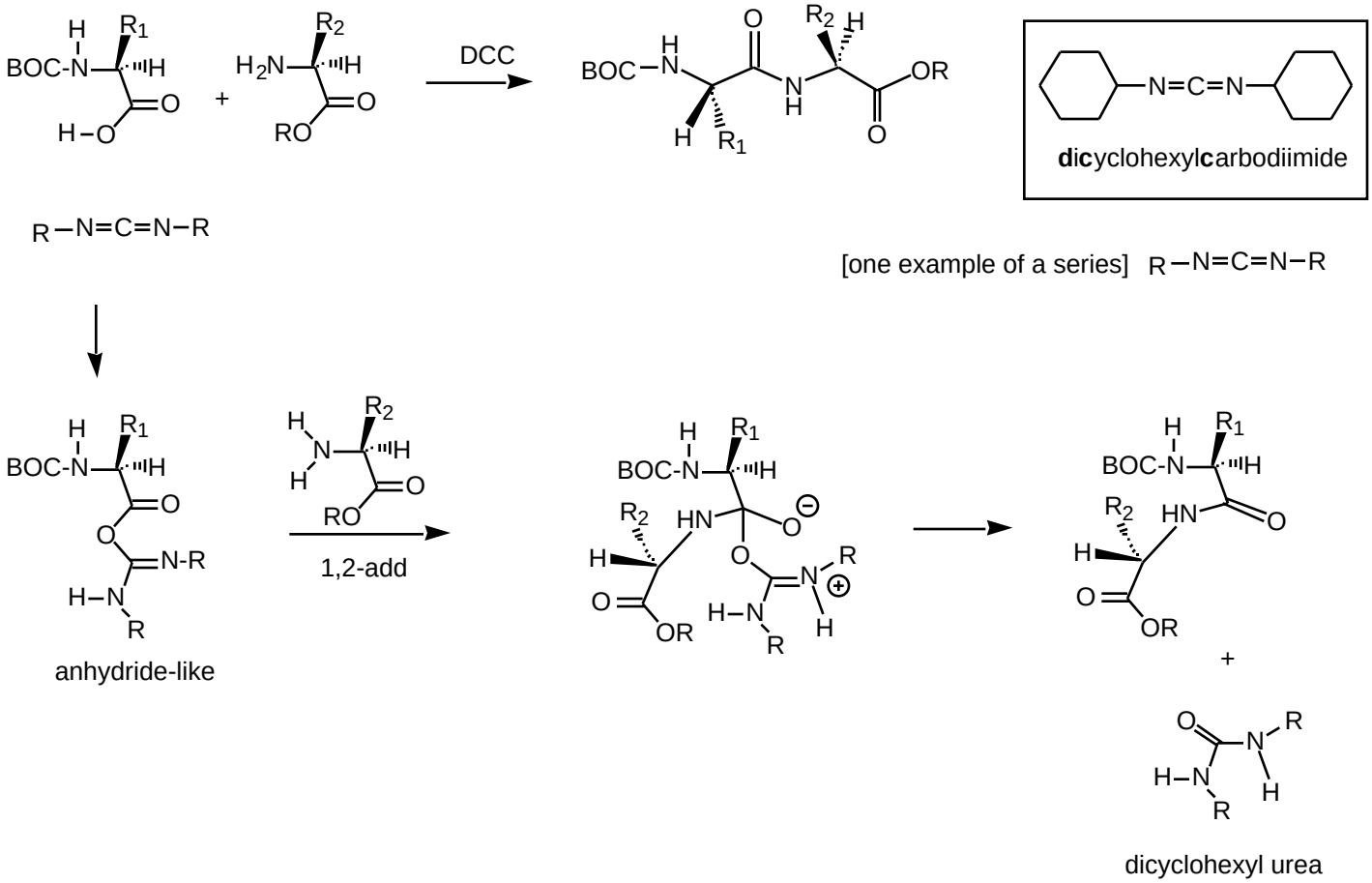
The chemical synthesis of proteins:

Problems: protecting group needed.
create amide bonds.





How induce loss of water and amide bond formation? Many reagents. Most common is complicated: DCC

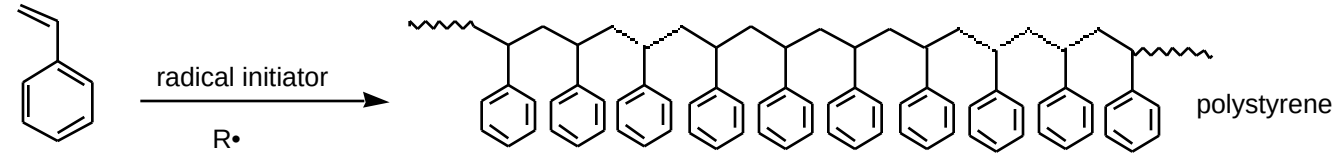


The "Peptide Synthesizer" Automated peptide synthesis. Repeated amide bond formation with different AA
Technical problem: how separate the byproducts from DCC coupling and deprotection steps?

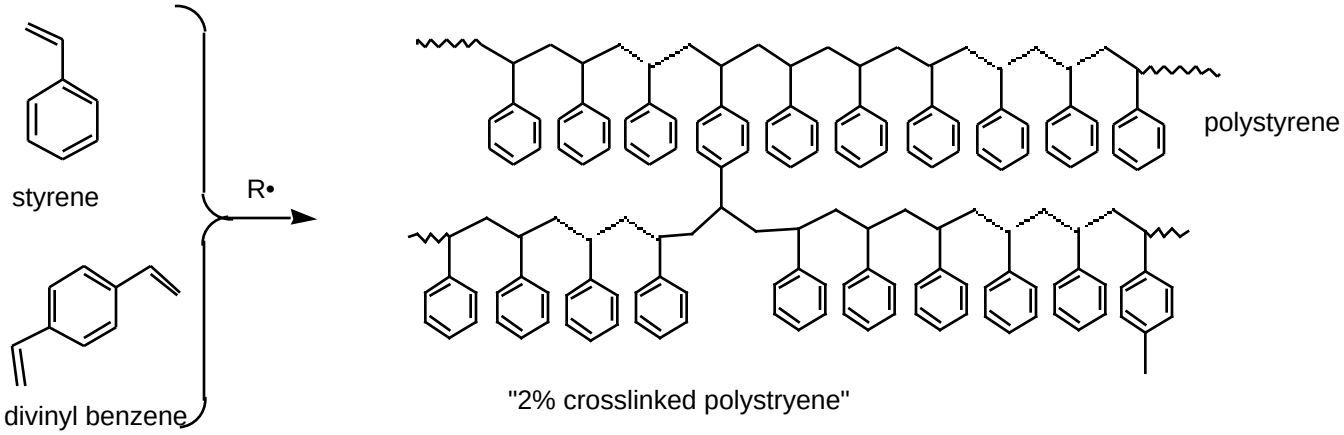
Solid phase synthesis: Easy separation of byproducts by filtration R. Bruce Merrifield, Nobel prize 1986

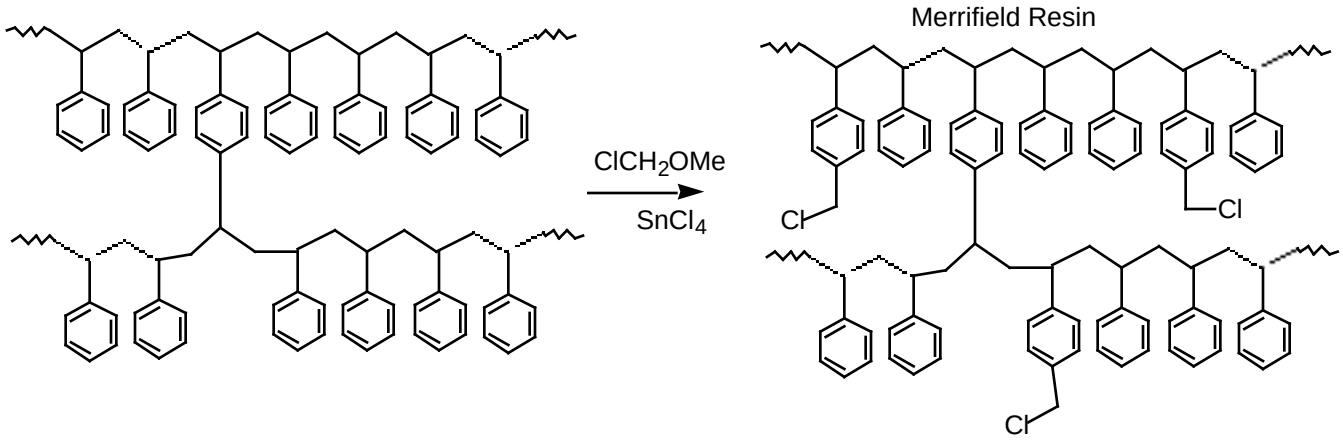
1. Attach AA_1-NH-BOC to an insoluble polymer via carboxylate (as ester). Deprotect the amino group
2. Add AA_2 (free -CO_2H) + DCC in solution.
3. Reaction occurs to couple AA_2 to AA_1 on the polymer
4. Filter away the solution and byproducts
5. Add CF_3CO_2H to deprotect amino group on AA_2, filter, rinse
6. Add AA_3 (free CO_2H) DCC in solution
7. Reaction occurs to couple AA_3 with AA_1-AA_2 at AA_2 amino group
8. Filter
9. Cleave ester linkage to polymer: HO_2C-AA_1-AA_2-AA_3-NH-BOC

Details:

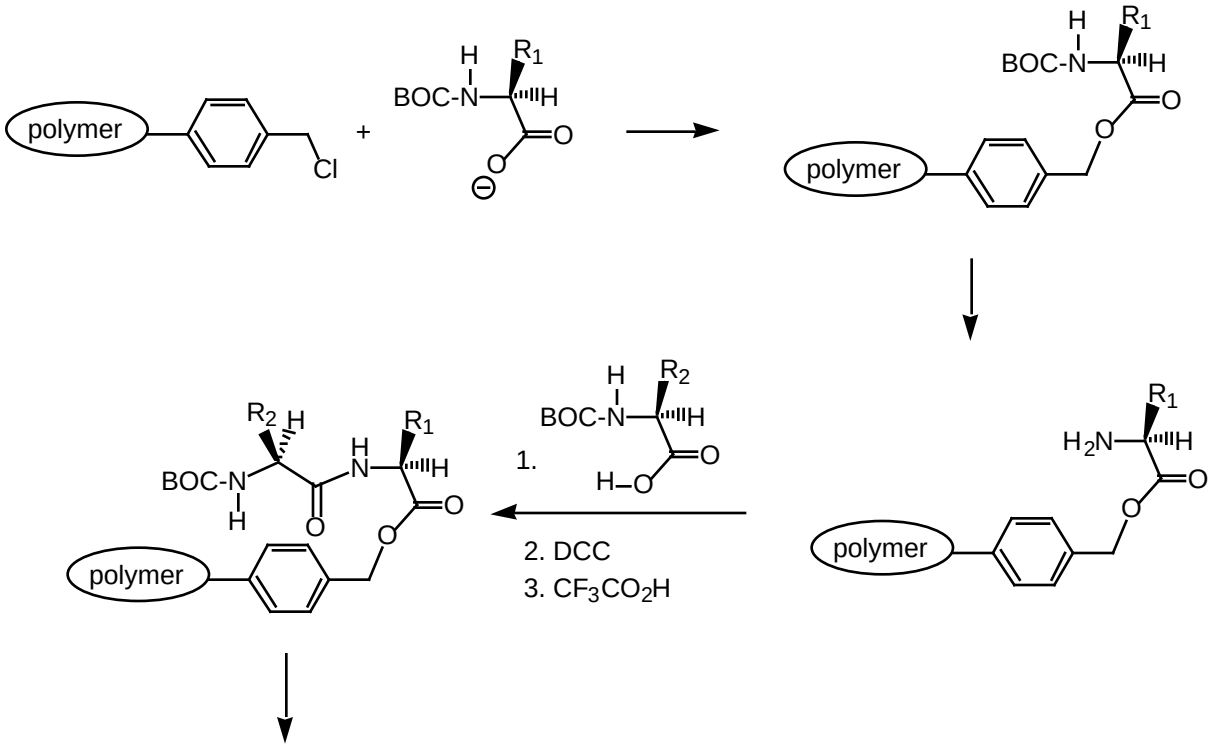


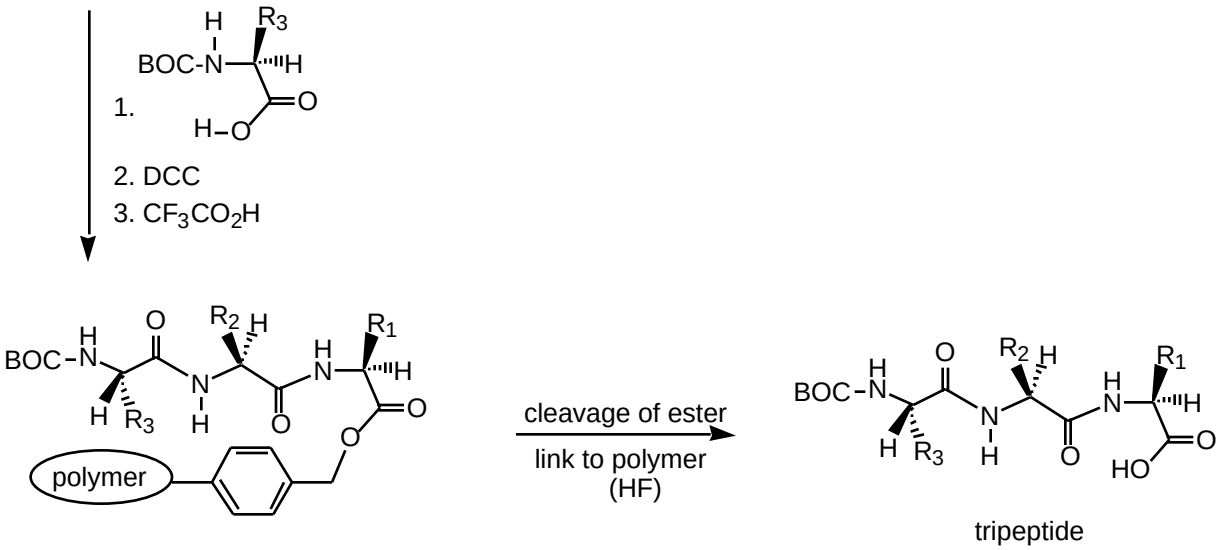
[p 471, text]

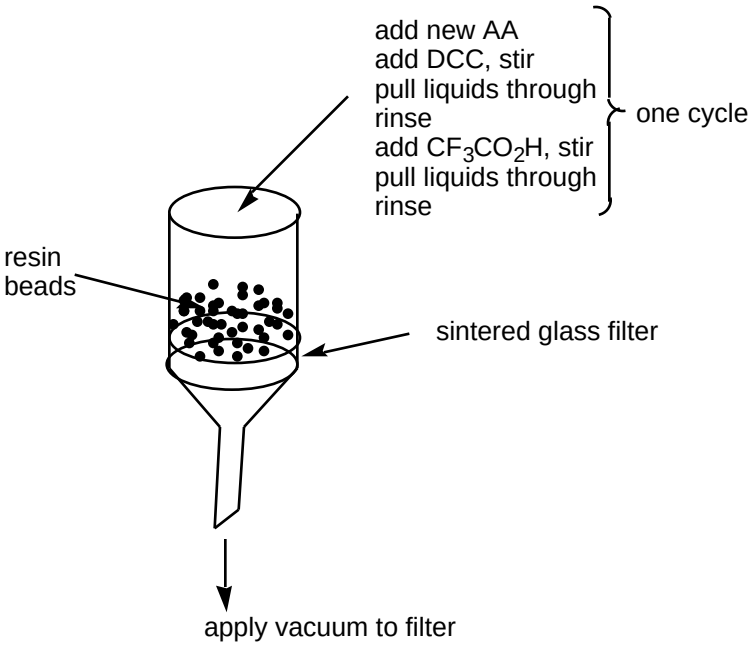




Polypeptide Synthesis:







Very effective for polypeptides of 10-30 size.
Enzymes of 100-150 AA have been synthesized by techniques such as this.