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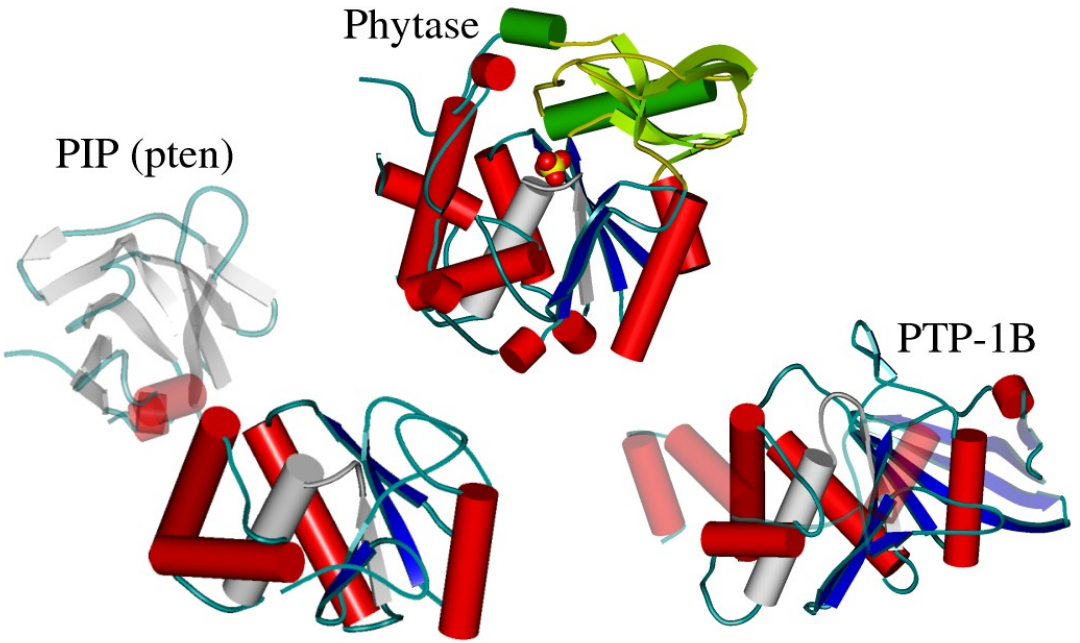
Chapter 7: Covalent Structure of Proteins

Voet & Voet: Pages 161-175, 182-191,203-207



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Chemical Synthesis

Chemical synthesis of 'short' polypeptides has considerable biomedical applications (current and historical)

(1) Study properties of polypeptides by systematically varying side chains .

(2) Produce polypeptides with non-standard and/or labeled amino acid residues

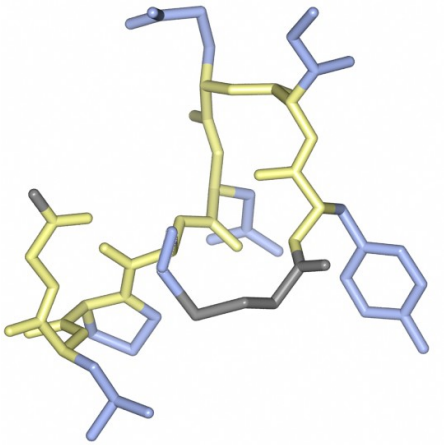
(3) Produce bioactive polypeptides that are scarce or non-existent

First synthetic polypeptides were homopolymers

Early model compound in biochemistry

Current applications

eg. Production of synthetic vaccines, rare peptides, ...



Oxytocin
(peptide hormone)

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Chemical Synthesis

1953 - Chemical synthesis of biologically active polypeptide

Oxytocin - nine residue peptide hormone that stimulates uterine contractions

Synthesized completely in liquid phase

Modern methods have synthesized thousands of biologically active polypeptides and several small proteins

Oxytocin

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Chemical Synthesis

Requires two types of reactions

(1) Coupling reactions

Formation of the peptide bond (or initial attachment to resin)

- To successfully couple two amino acids (*eg.* Ala and Ser), we must prevent the formation of Ala-Ala and Ser-Ser
- Residues used in chemical synthesis of polypeptides are “blocked” amino acids as opposed to free amino acids

(2) Deblocking reactions

Removal of blocking or protecting groups

- Prepares product for subsequent coupling step in the synthesis

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Solid Phase Peptide Synthesis

Liquid phase (normal chemical) synthesis requires products to be purified after each step

- **Huge problem as unreacted materials have very similar chemical properties**
- **Reduced yields associated with purification make this impractical for all but the smallest polypeptides**

Merrifield (1962) solution to problem

- **Covalently coupled first amino acid to an inert stationary phase (a chromatography resin)**
- **Allows quantitative recovery of the product of each cycle by simply washing away excess reagent in preparation for next cycle of synthesis**

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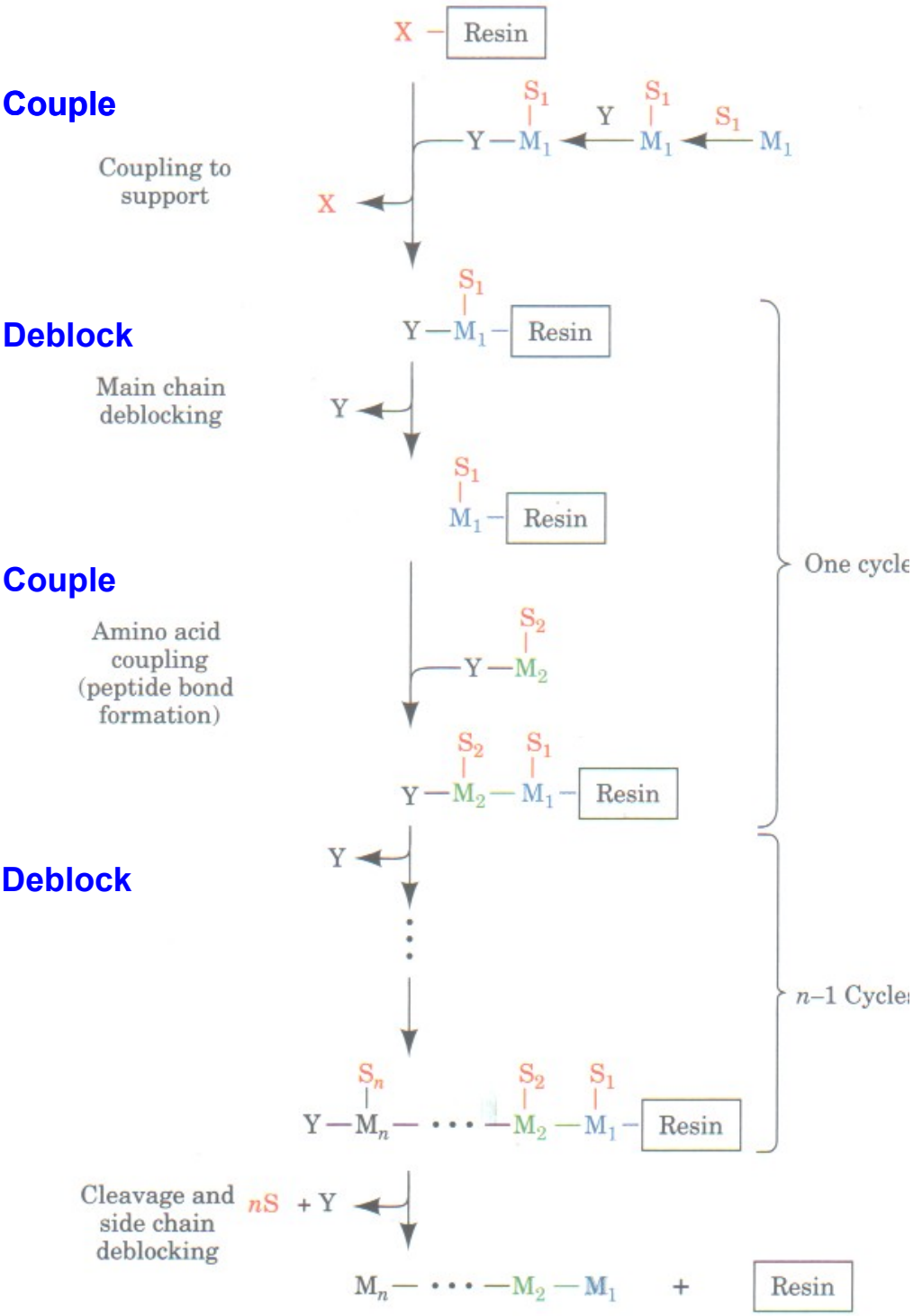
Solid Phase Peptide Synthesis

Synthesis is from C --> N-terminus

(opposite of protein biosynthesis)



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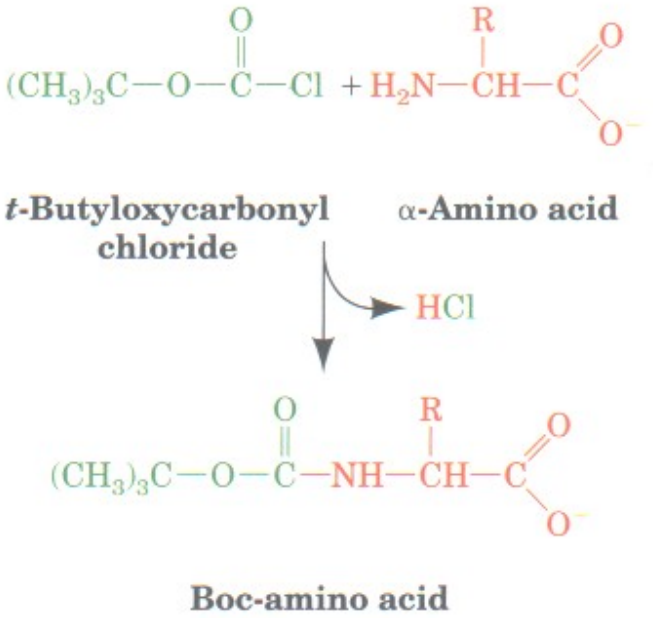
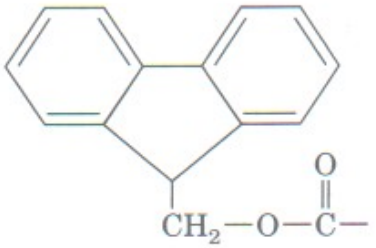
Blocking groups

There are two common (related) main chain (α-amino) blocking groups

Boc	***t*-butyloxycarbonyl chloride**
Fmoc	**9-fluorenylmethoxycarbonyl chloride**

Protect α-amino group of amino acid being added to the polypeptide by forming a carbamate (ROOCNHR)





9-Fluorenylmethoxycarbonyl (Fmoc) group

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Anchoring (coupling & deblocking)

Coupling (mild alkali conditons)

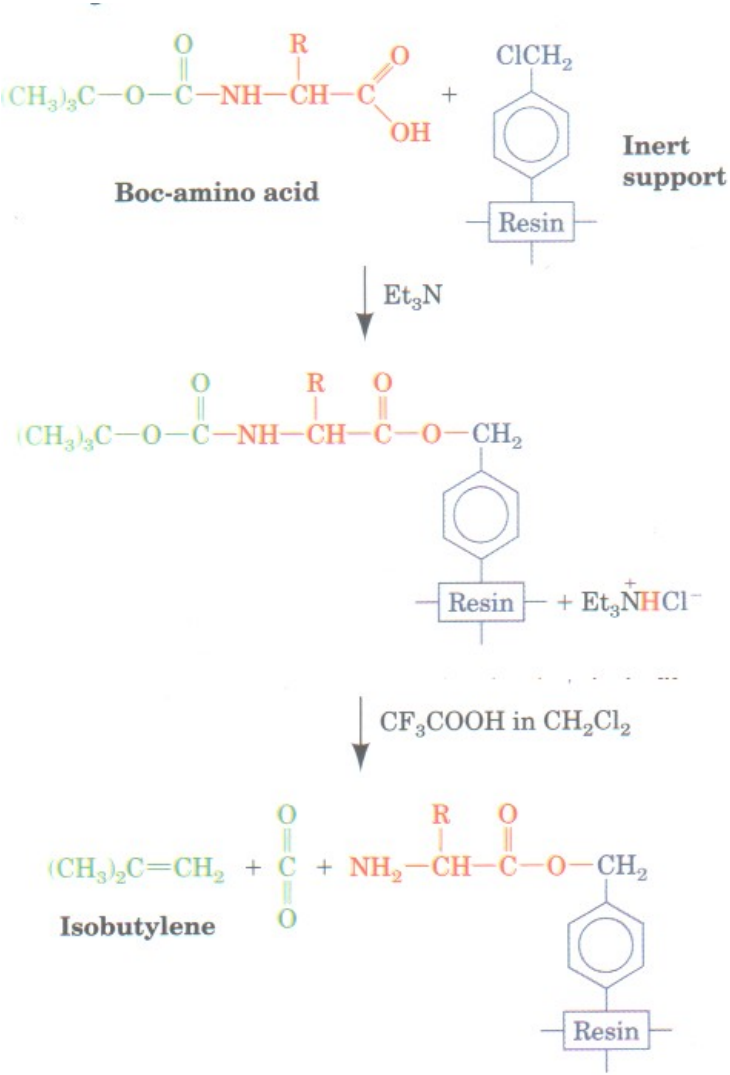
Solid support is typically chloromethyl polystyrene

Blocked amino acid forms ester with alkylbenzyl group (mild alkaline solution)

Resin is filtered and washed to remove blocked amino acids and Et_3N

Deblocking (anhydrous acid)

Anhydrous acid removes blocking group by breaking down Boc leaving the derivatized support



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Amino Acid Coupling

Peptide bond formation is endergonic (requires E)

To drive reaction to completion the carboxylate of protected amino acid must be "activated"

DCCD (dicyclohexylcarbodiimide) activates the carboxylate by creating a better leaving group than -OH

Overall Process

"Activated" and Boc-protected amino acid are reacted with "deblocked" amino acid attached to resin

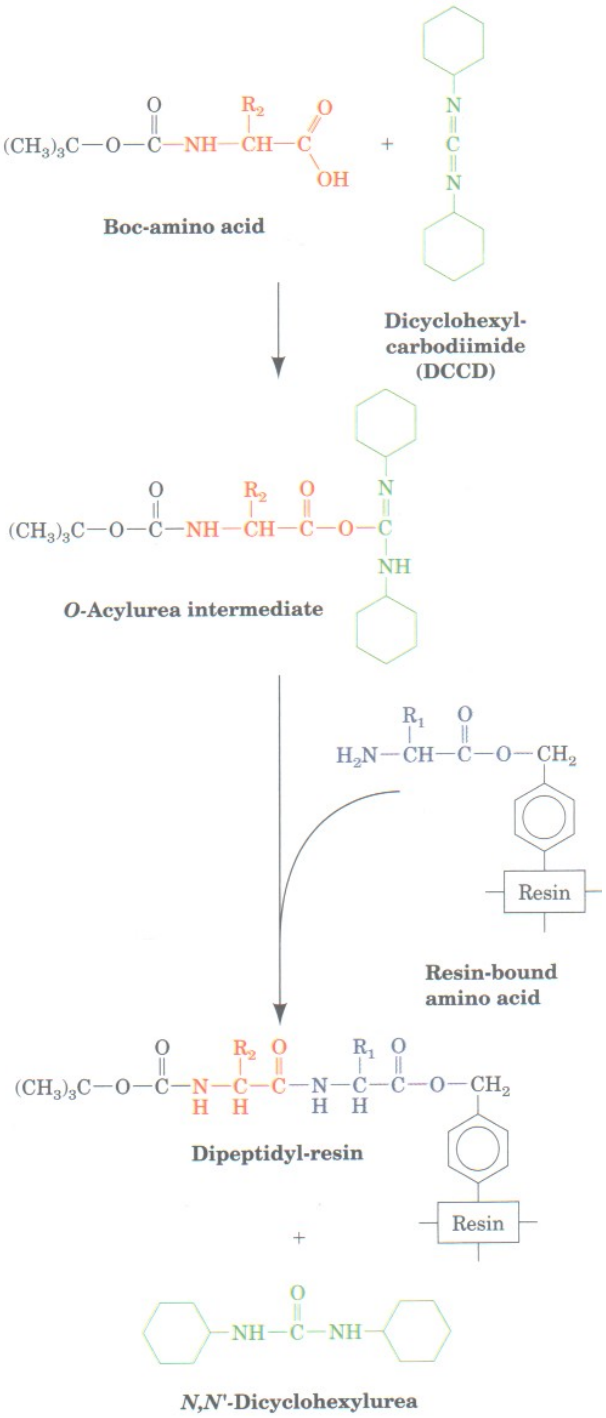
Resin is filtered and washed to remove unreacted solutes

Deblock (remove Boc) with anhydrous acid

Resin is filtered and washed to remove unreacted solutes

… repeat process until synthesis is complete ...

Process is easily repeated and automated



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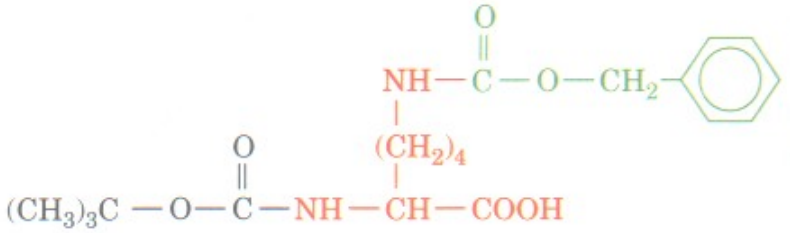
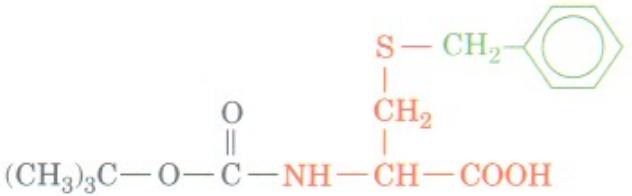
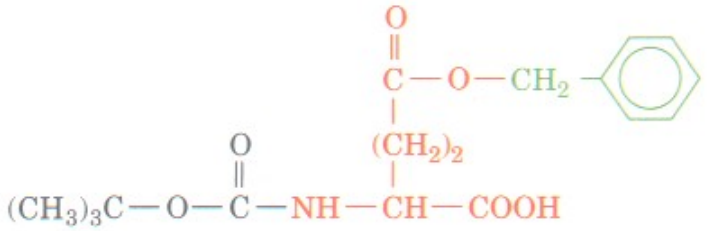
Issues



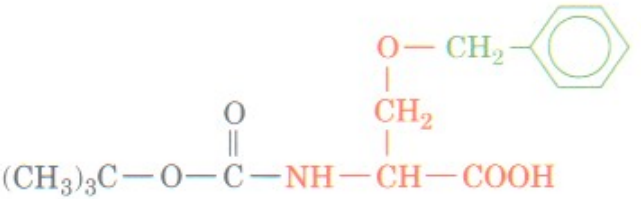
Boc, N^{ε}-benzyloxycarbonyl-Lys



Boc, *S*-benzyl-Cys



Boc-Glu, γ-Benzyl ester



Boc, *O*-benzyl-Ser

Polar and charged amino acids must be protected throughout all synthetic reactions

- **Typically, utilize a protecting group with properties similar to the anchoring group**
- **Ether or ester linkages are common used (for protecting groups) as they are resistant to mild acid and base.**

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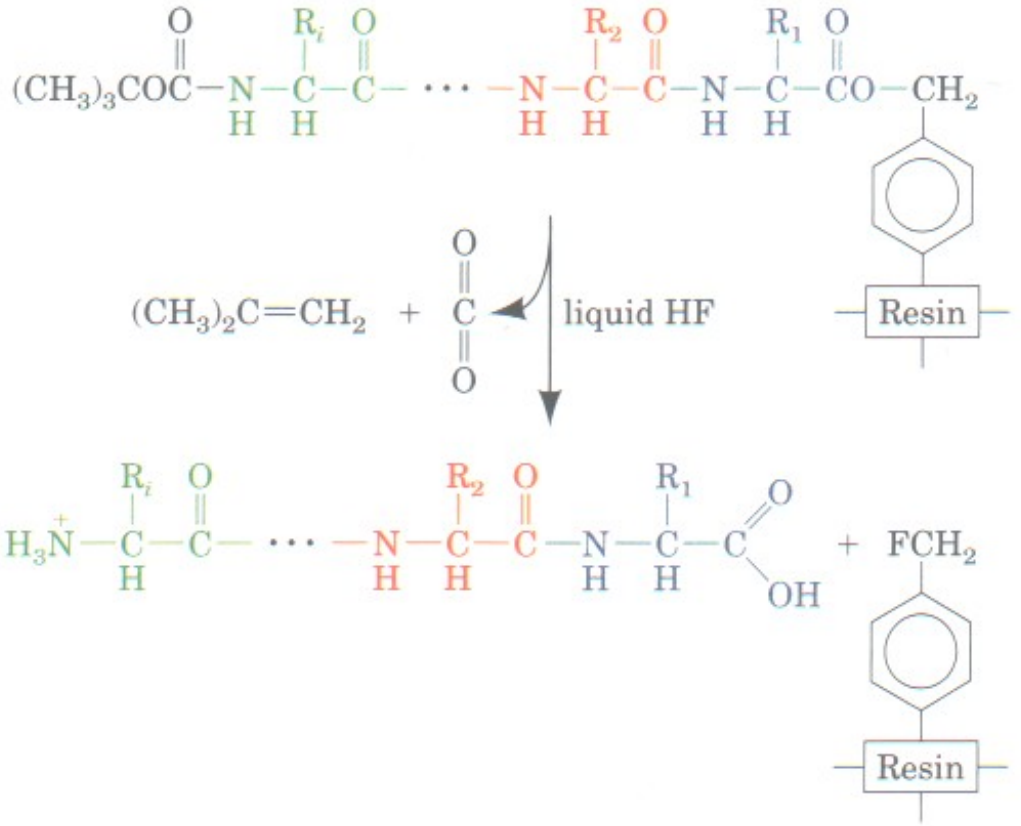
Polypeptide Release

Completely synthesized polypeptide can be released using HF

Cleaves all protecting group and ester linkage to resin



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Problems

Synthesis of longer polypeptides requires *exceptional* yields at each step of the reaction

Purification of released polypeptide from incomplete reactions is still a problem due to similarity

- **Reverse Phase HPLC greatly facilitates purification**

Length	Coupling Efficiency				
1	0.995	0.99	0.98	0.97	0.96
5	0.98	0.95	0.92	0.89	0.85
10	0.96	0.91	0.83	0.76	0.69
15	0.93	0.87	0.75	0.65	0.56
20	0.91	0.83	0.68	0.56	0.46
25	0.89	0.79	0.62	0.48	0.38
30	0.86	0.75	0.56	0.41	0.31
35	0.84	0.71	0.50	0.36	0.25
40	0.82	0.67	0.45	0.30	0.20
45	0.80	0.63	0.41	0.26	0.17
50	0.78	0.60	0.37	0.22	0.14
55	0.76	0.58	0.34	0.19	0.11
60	0.74	0.55	0.30	0.17	0.09
65	0.73	0.53	0.27	0.14	0.07
70	0.71	0.50	0.25	0.12	0.06

99.5% efficient synthesis steps – 78% yield on 50mer
96.0% efficient synthesis steps – 14% yield on 50mer

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Side Chain Modification

Chemical modification of side chains can be separated into three general classes:

(1) Reductions (almost exclusively target sulfur containing residues)

(2) Additions (multiple compounds targeting a variety of residues)

(3) Cleavage (typically a specific enzymatic reactions)

Why modify a protein?

(For the same reasons you may create a mutant protein)

- alter structure and function (activate or inactivate)
- introduce reporter group (fluorescent or radioactive probe)
- basic characterization of protein isolated from natural sources

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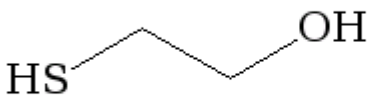
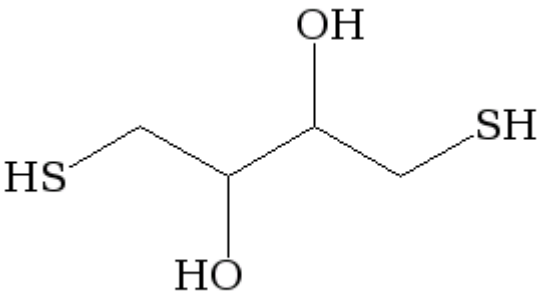
Reductions

Primarily target disulfides and occasionally oxidized cysteine or methionine residues

- **reduced thiols are excellent sites for labels (Fluorescent tag for binding/kinetic studies; Fluorescent Resonance Energy Transfer for distance measurements)**
- **reduction of disulfides important in oligomer separation, protein solubility and activity (*eg.* insulin folding and activity)**



2-mercaptoethanol (BME)



dithiothreitol (DTT)

Common disulfide reducing agents

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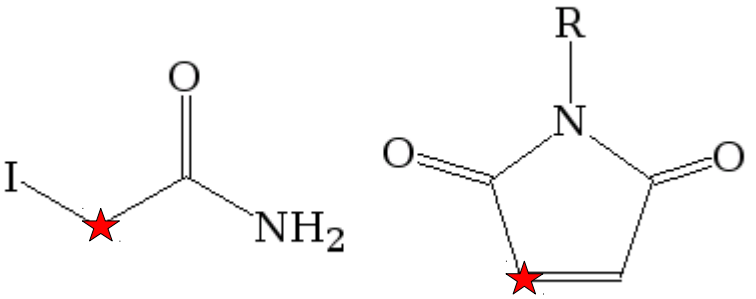
(Specific) Additions

Cysteine

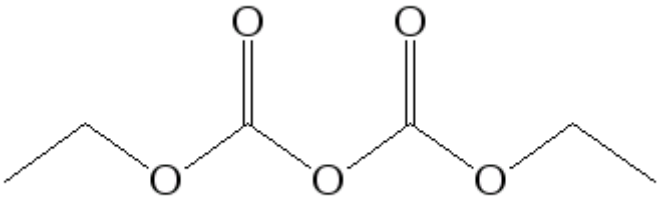
iodoacetamide and maleimides

irreversible addition



Histidine

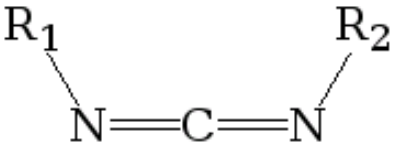
pyrocarbonates (*eg.* DEPC)



Carboxylates (Asp/Glu)

carbodiimides

difficult to achieve specificity



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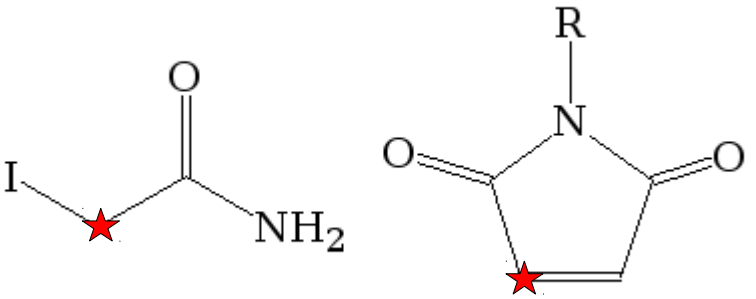
(Specific) Additions

Cysteine

iodoacetamide and maleimides

irreversible addition



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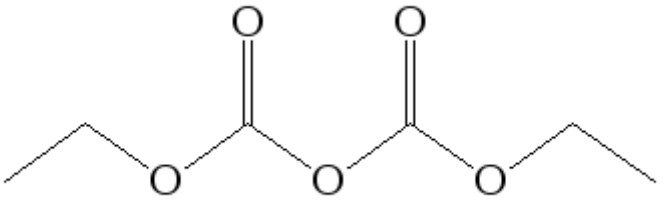

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