Solid Phase Peptide Synthesis, Strategies and Resins (Fmoc strategy)

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Introduction

Fmoc - base labile $N^\alpha$ protecting group

Protection of the side chains

Resins (Wang and Rink Amide linker)

Cycle:

1. attachment of the first $N^\alpha$ protected amino acid
2. deprotecting $N^\alpha$
3. activation and coupling of the amino acid
4. cleavage steps
Solid Phase Peptide Synthesis (SPPS)

Fmoc

- SPPS is more efficient, faster and the purification is easier
  ⇒ method often used in chemistry and biochemistry
- fully automated peptide synthesizer
- it is possible to synthesize peptides with an length of 50 aa
- synthesis from the C- to the N-terminus
- a label group protects the α-amino group for amines
  ⇒ Fmoc is a base labile Nα protecting group
  ⇒ all permanent protecting groups for the side-chains are acid-labile
Introduction: Fmoc-Cl or Fmoc-Succinimide

- exceptional acid stability
- high ultraviolet absorption ⇒ permits monitoring of acylation and deprotecting reaction
- compatible with tert-butyl-based side-chain protection
- Fmoc protected amino acids are generally easy to prepare
- labile to organic bases, particularly to secondary amines

Fluorenyl-9-methoxycarbonyl group (Fmoc)
Side Chain Protecting Groups

- protects the reactive functional group of the amino acid
  - prevents formation of branched chains
- permanent, orthogonal, compatible with $N^\alpha$-protection
  - remain attached throughout the synthesis
- easy to remove (with acid) after completion of the synthesis

- Ser, Thr, Tyr: Ether
  - tert-Butylether
- Asp and Glu: tert-Butylester
- Lys: boc-protecting group
- His: $N^\alpha$: Boc or trityl
  - $N^\pi$: Benzyloxymethyl
- Cys: Trityl
Resins

- insoluble and chemically unreactive
  ⇒ polystyrol
- the polymer support is constructed of 20 – 50 µm diameter particles
- swell extensively in solvents
  ⇒ linker arms are accessible
- first amino acid is covalently bound
- cleavage after completion of synthesis
1st Step: Attachment of the First N\(^\alpha\)-Protected Amino Acid
2nd Step: Deprotection of the Nα

- Exceptional lability to secondary amines
- Activation of the ring proton β
- Carbanion
- E1cb-mechanism
- Primary products of the cleavage reaction are carbamate salt and dibenzofulvene
- 20% piperidine in DMF
3rd Step: Coupling and Activation of the Second Amino Acid

- a mixture of DCC and HOBt is the classical technique
- DCC activates the carbonacid ⇒ formation of a acylisourea
- HOBt + acylisourea → active ester
4th Step: Deprotection of the Side Chains

- protected amino acid:

- reaction mechanism of the side chain deprotection with trifluoroacetic acid:
4th Step: Cleavage from the Resin

- deprotection with TFA (95 %)
- result: Full-length, deprotected peptide in solution separated from the insoluble resin support by filtration
- cleavage solution contains scavengers to minimize any alkylating side reactions
Literature

- http://www.biotech.uiuc.edu/centers/Proteomics/Proteinscience/spps.htm
- http://www.protein.iastate.edu/synthesis.html