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Fundamental aspects of SPPS and the Green future

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In 1963, Merrifield introduced solid phase peptide synthesis.

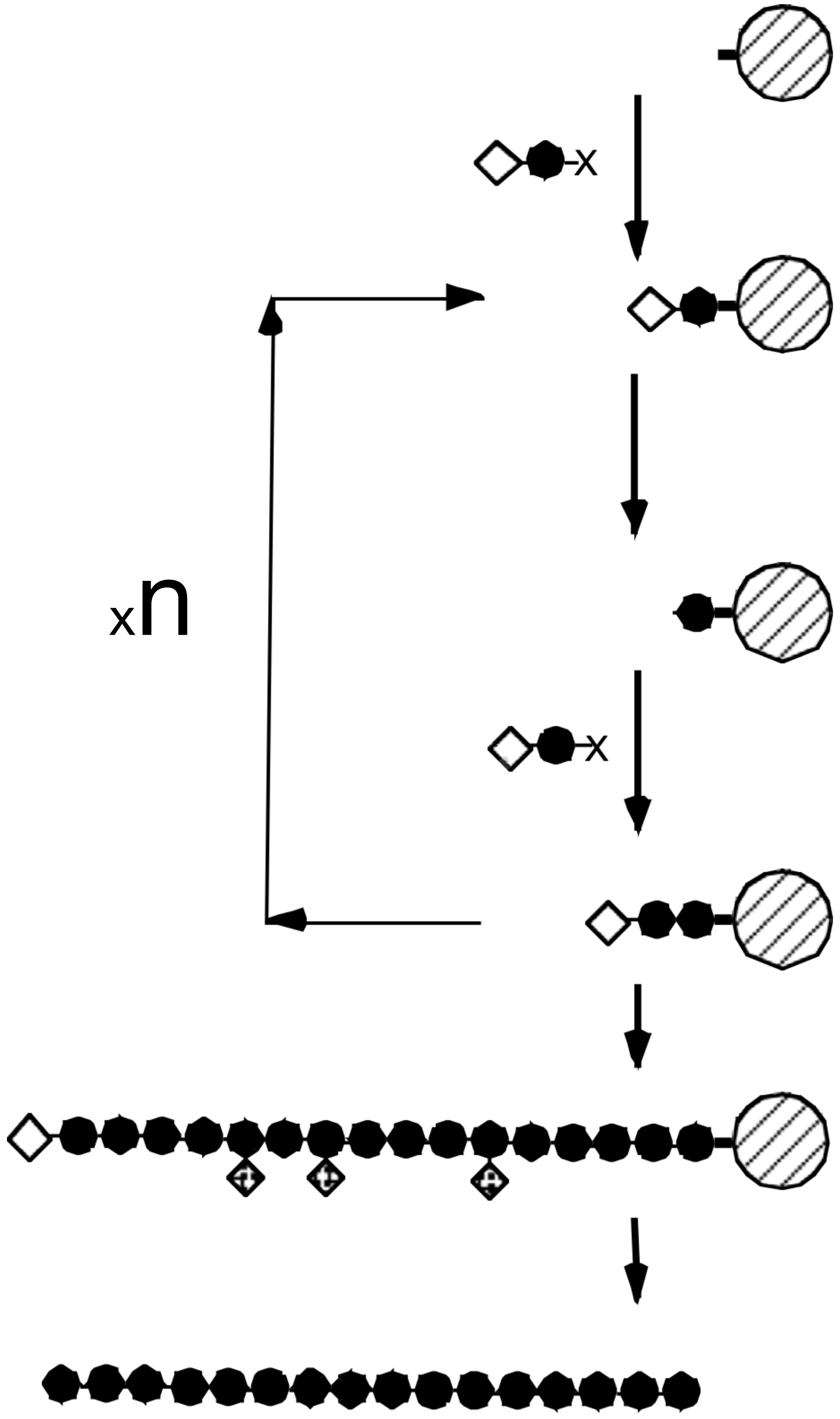
After initial fierce skepticism, SPPS has become almost universally used for the chemical synthesis of peptides, in both academic research and in industry.

In this brief talk, I will describe the fundamental physicochemical properties of peptide-resins that make SPPS so useful.

I will suggest ways to retain that utility in a 'Green' SPPS.

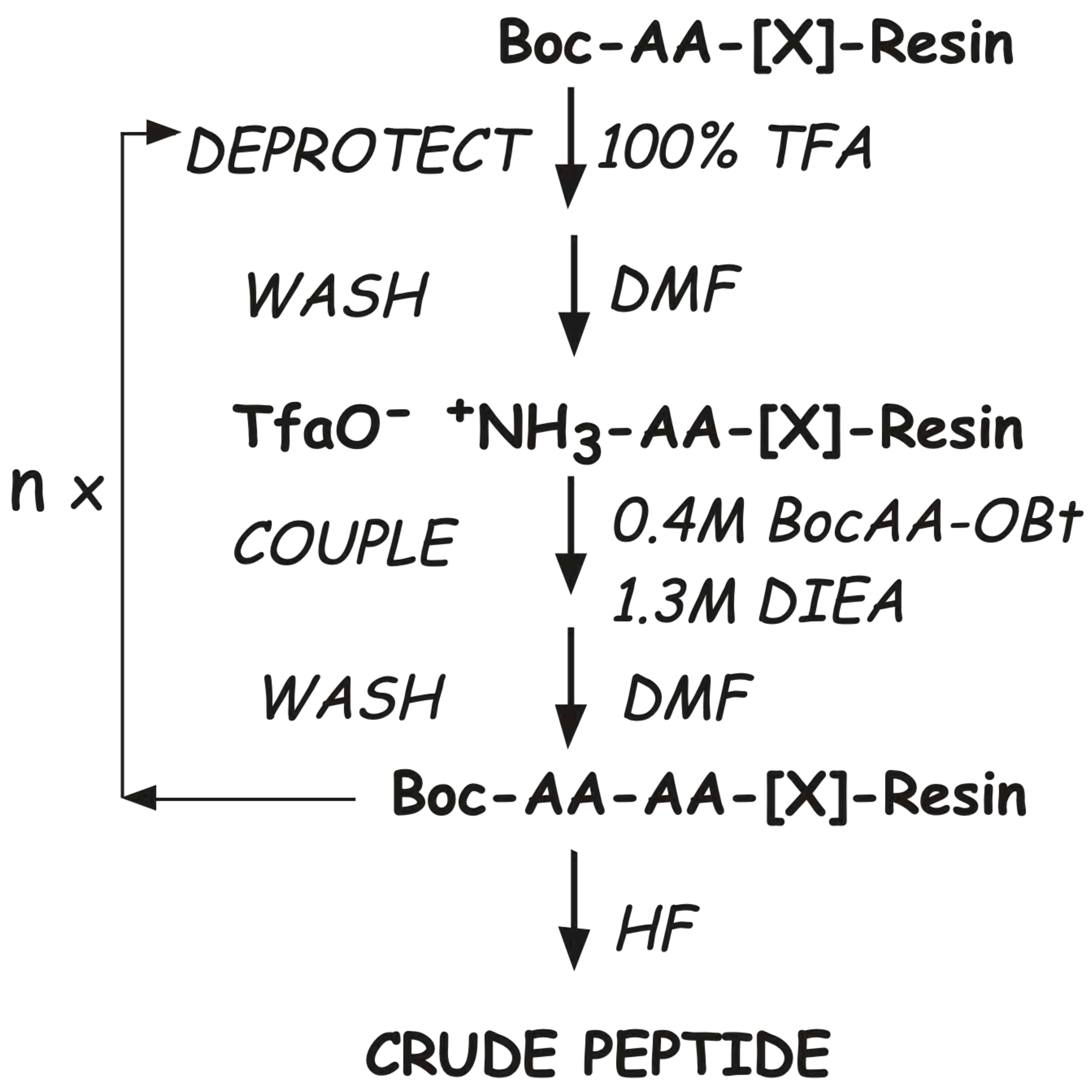
Then I will explain how to combine Green SPPS with chemical ligation for more efficient production of peptide molecules.

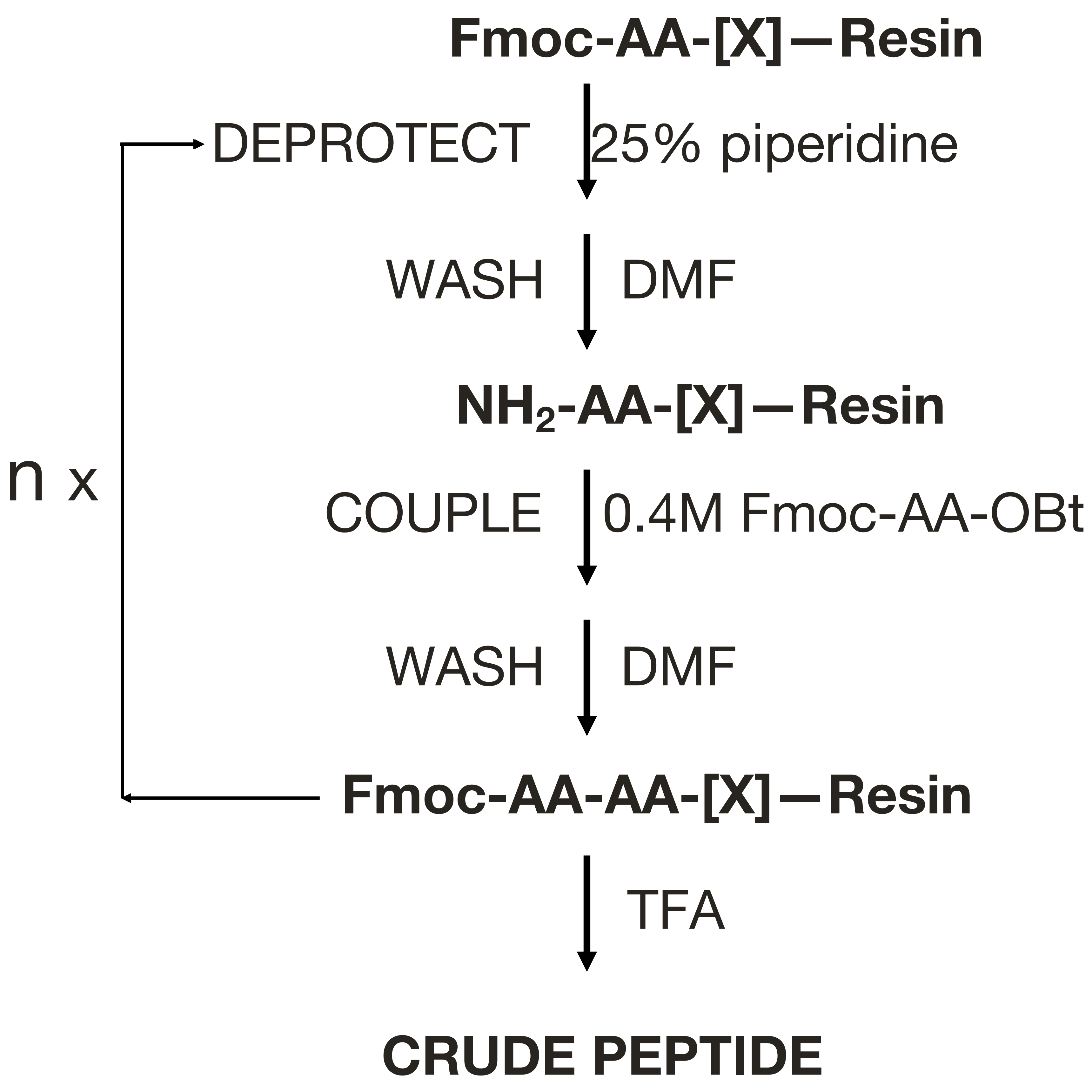
Solid Phase Peptide Synthesis (Merrifield 1963)



- C-terminal Xaa attached to resin beads
- stepwise chain elongation
- maximal protection of side chains
- global deprotection/release from resin

Is SPPS ‘Green’?





Is SPPS ‘Green’?

Boc-AA-[X]-Resin

NO!

Fmoc-AA-[X]—Resin

Poor atom economy:

* maximal use of protecting groups
* large excesses of reactants

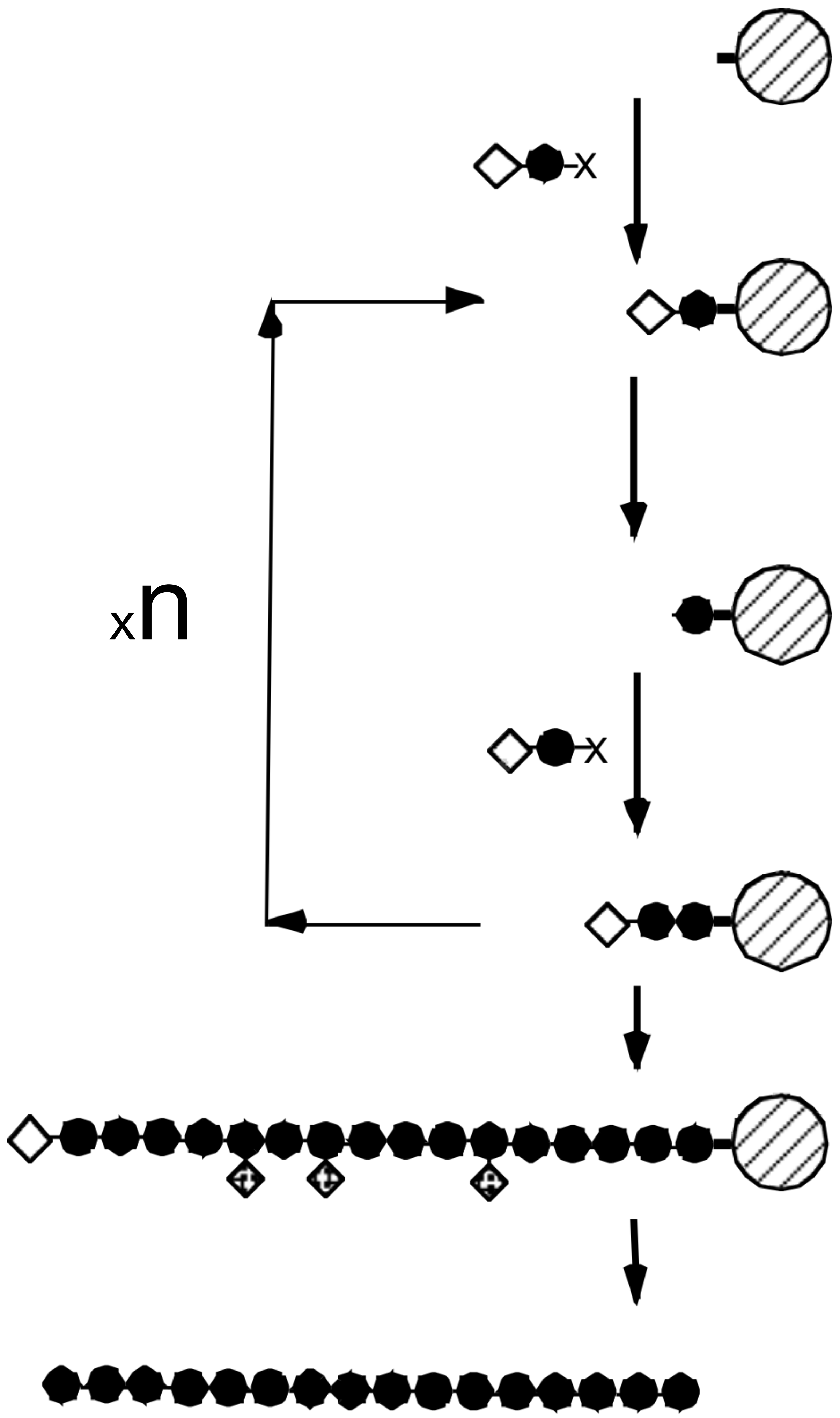
Process mass intensive (PMI):

* corrosive/toxic chemicals
* inefficient purification
* high volume waste streams

Can we preserve the inherent advantages of SPPS

for 'Green' peptide synthesis?

Advantages of SPPS



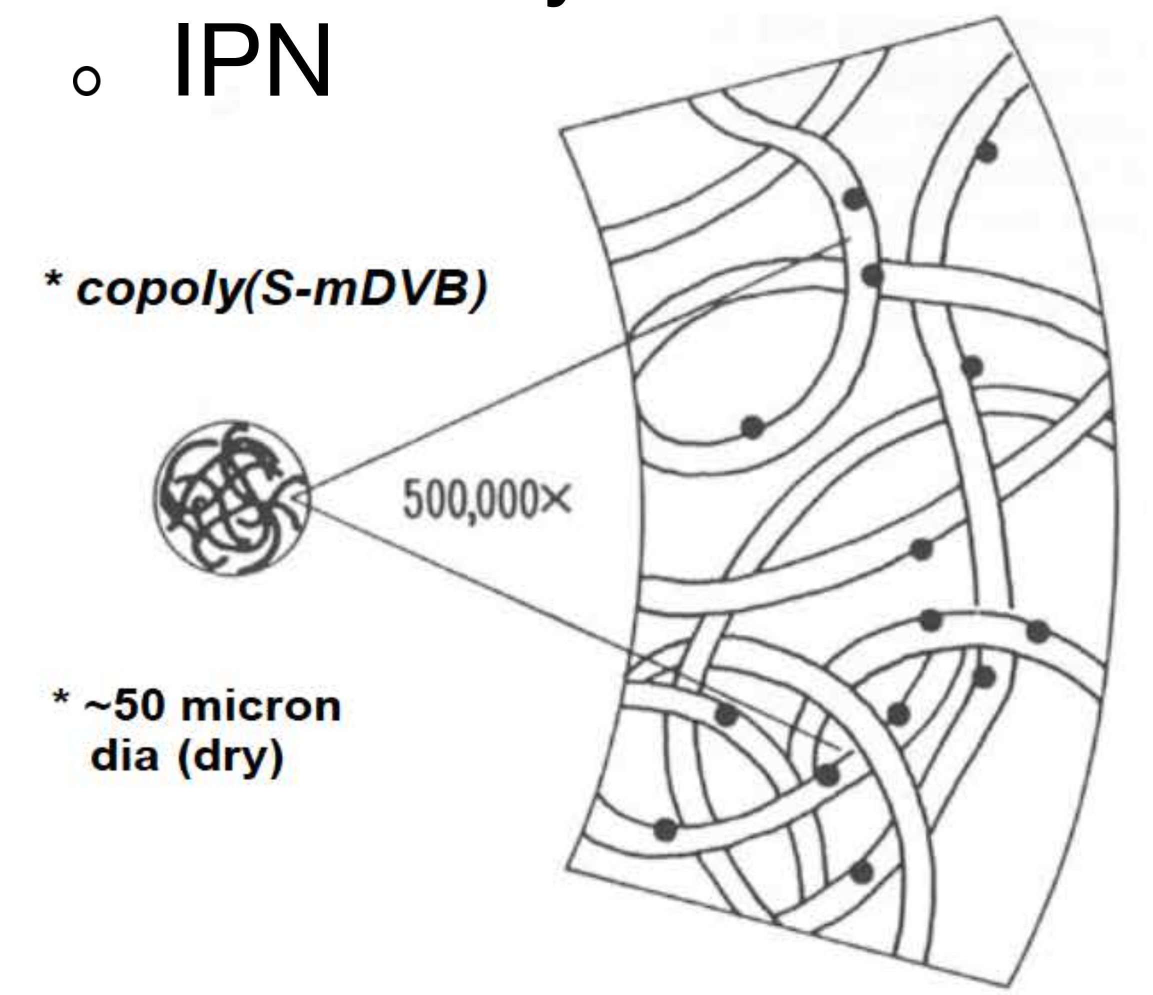
- ***enhanced solvation***
- stepwise synthesis
- purification by filtration
- quantitative recoveries
- general protocols
- automation

SPPS

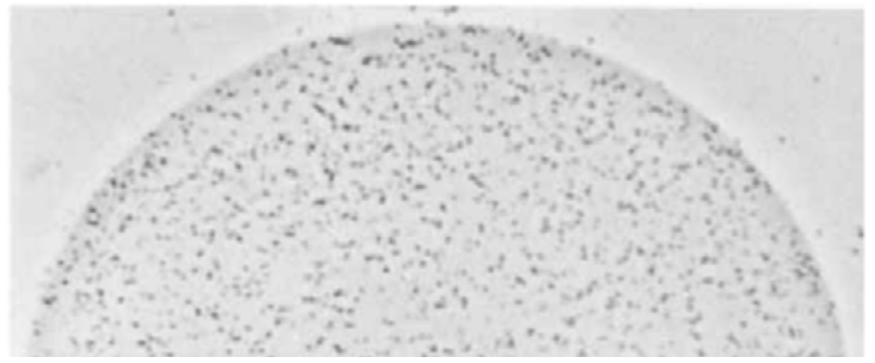
Principles of ^Polymer Chemistry

(Paul Flory)

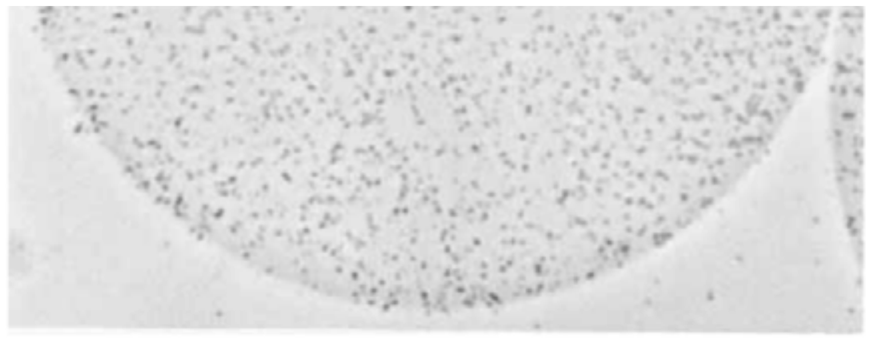
resin beads:
- randomly crosslinked
- IPN



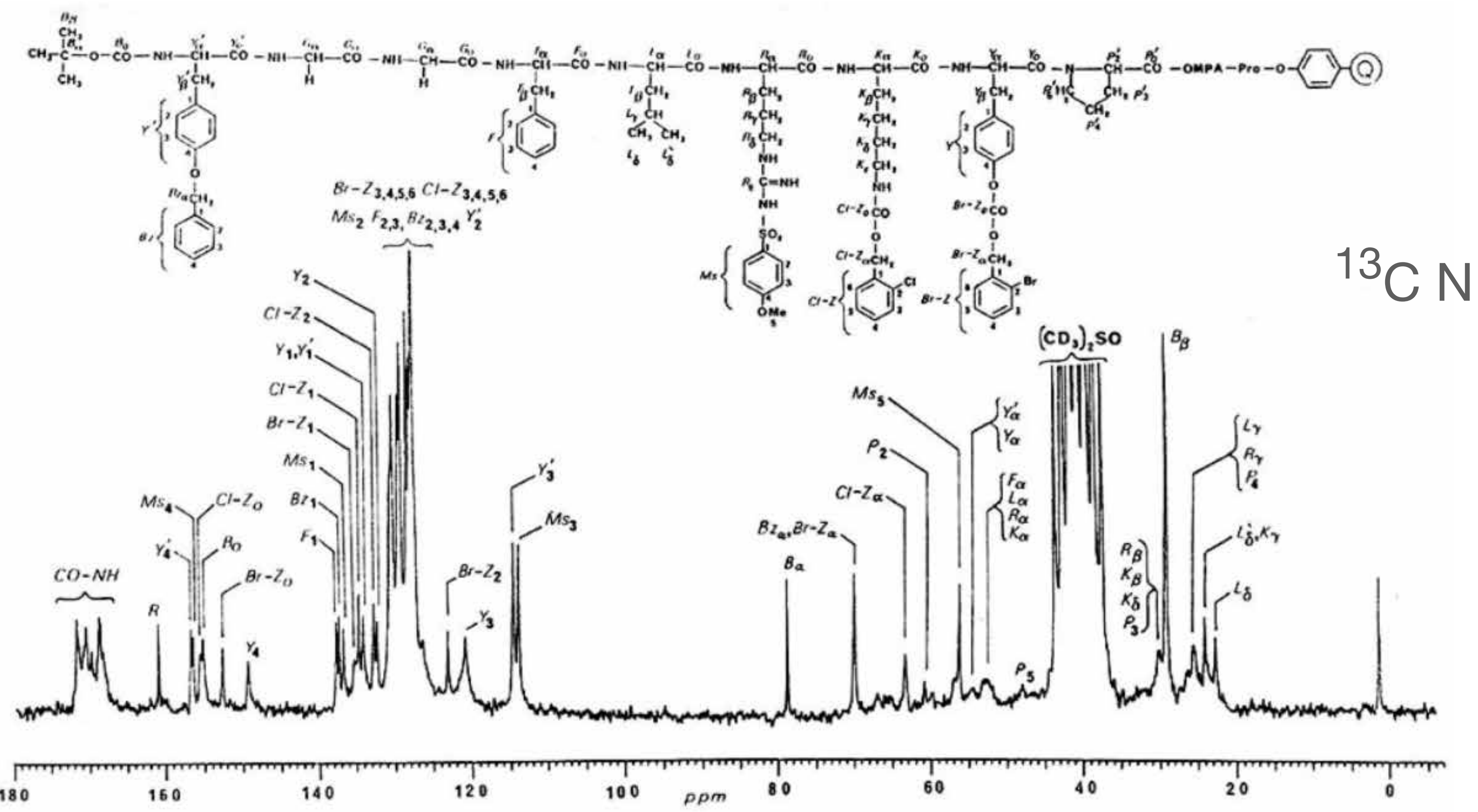
synthesis occurs ***within*** solvent-swollen beads:



~10^{14} peptides per bead

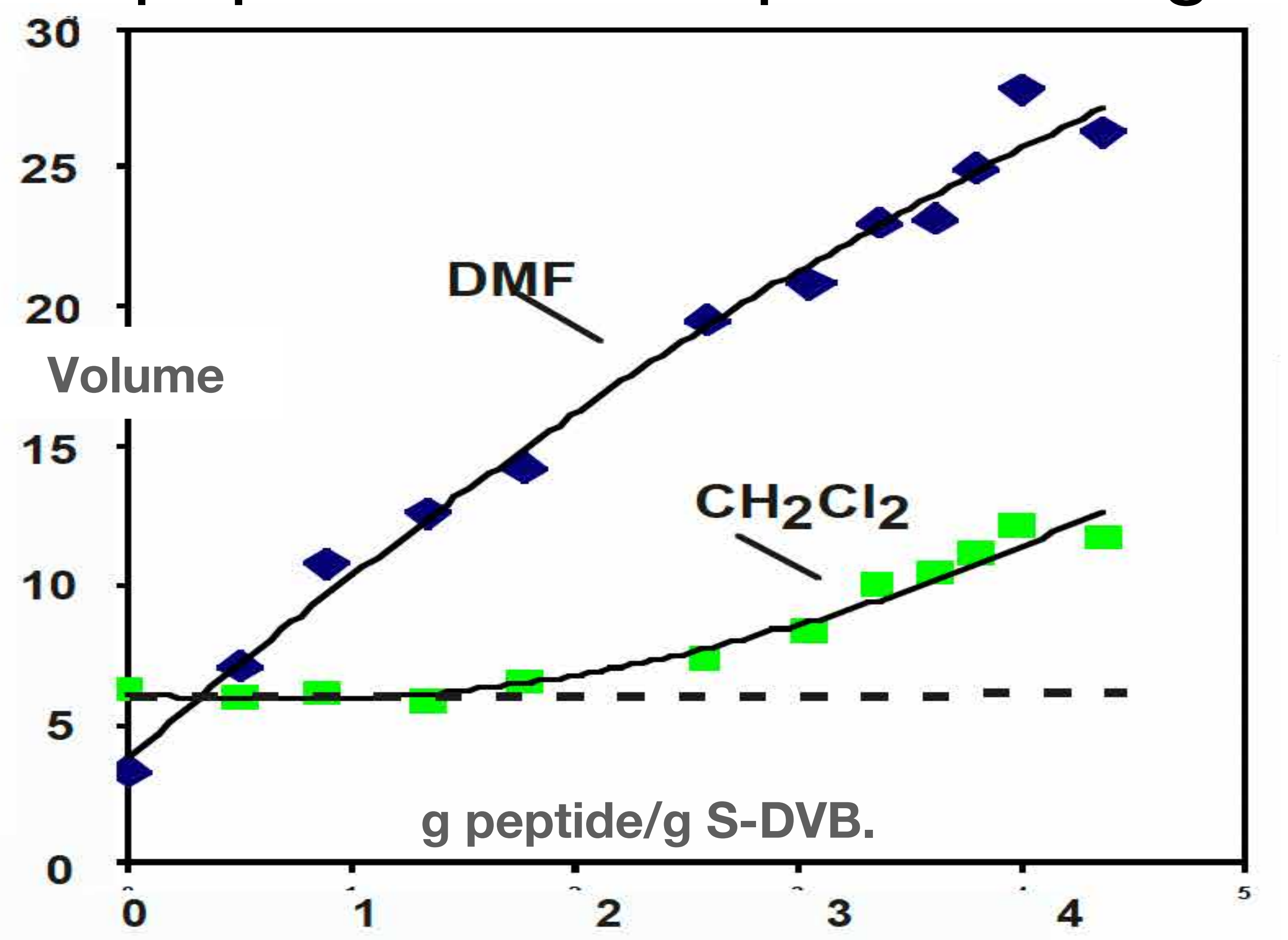


resin-bound peptides are effectively in solution - $\tau_c < 10^{-8}$ sec



^{13}C NMR peptide-resin

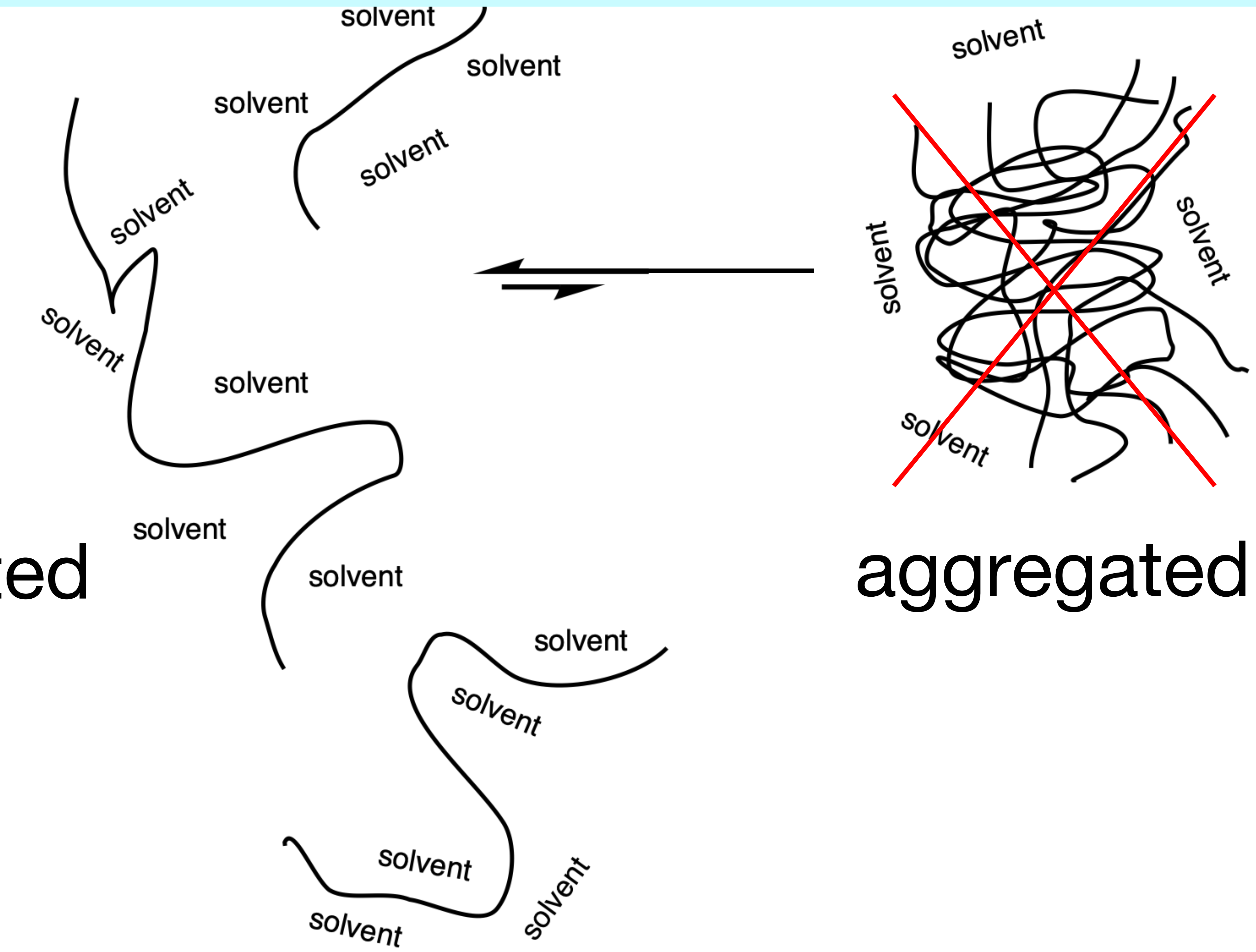
peptide-resin super swelling



Solvation of resin-bound peptides

Enhanced solvation of peptides within solvent-swollen resin beads is the *fundamental reason* for the *efficacy* and *versatility* of SPPS

solvated



aggregated

disfavored in peptide-resin

- *cross-links* prevent phase separation
- *dissimilar* properties of resin & peptide

the extent of solvation depends on the *relative free energies* of these two states

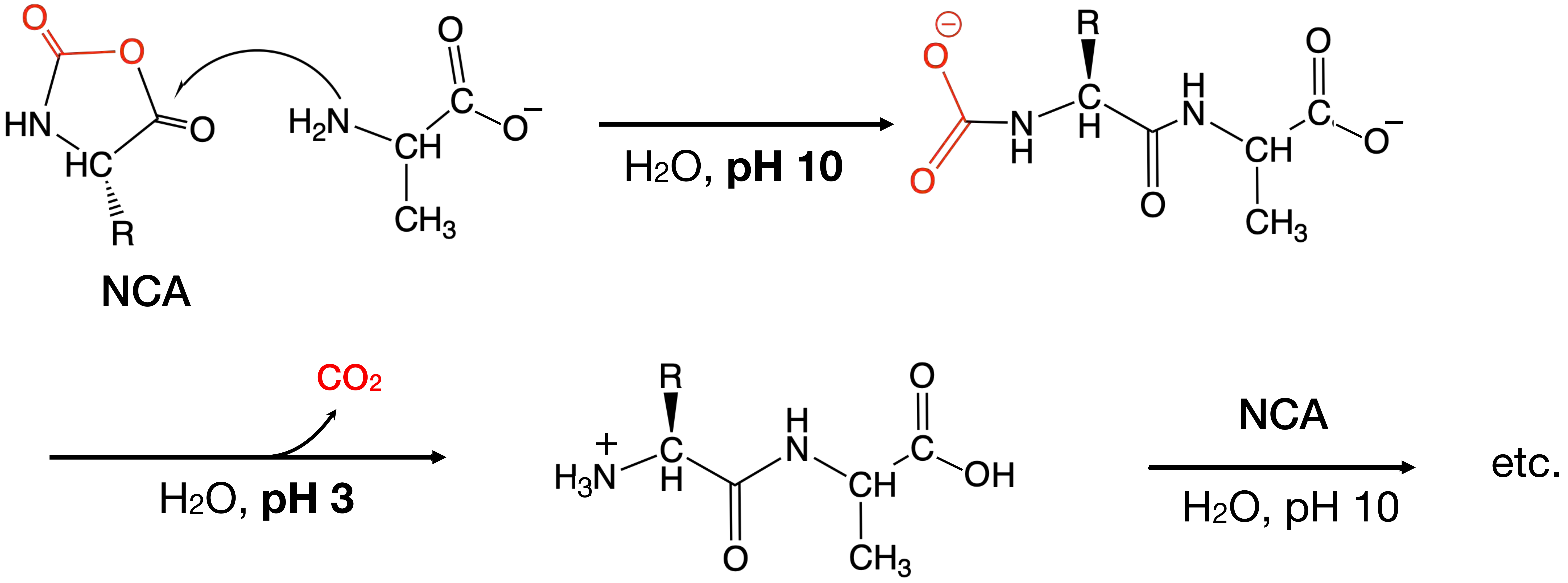
Dang, Dhayalan, & Kent
Org. Lett. 2015, **17**, 3521–3523

Features of a Green SPPS

- **atom economy**
  - minimal protecting groups
  - minimal excess reactants
- **benign reactants & reagents**
- **benign solvent**
- **efficient purification (PMI)**

“Controlled Synthesis of Peptides in Aqueous Solution.

1. The Use of α-Amino Acid N-Carboxyanhydrides.” (NCAs)



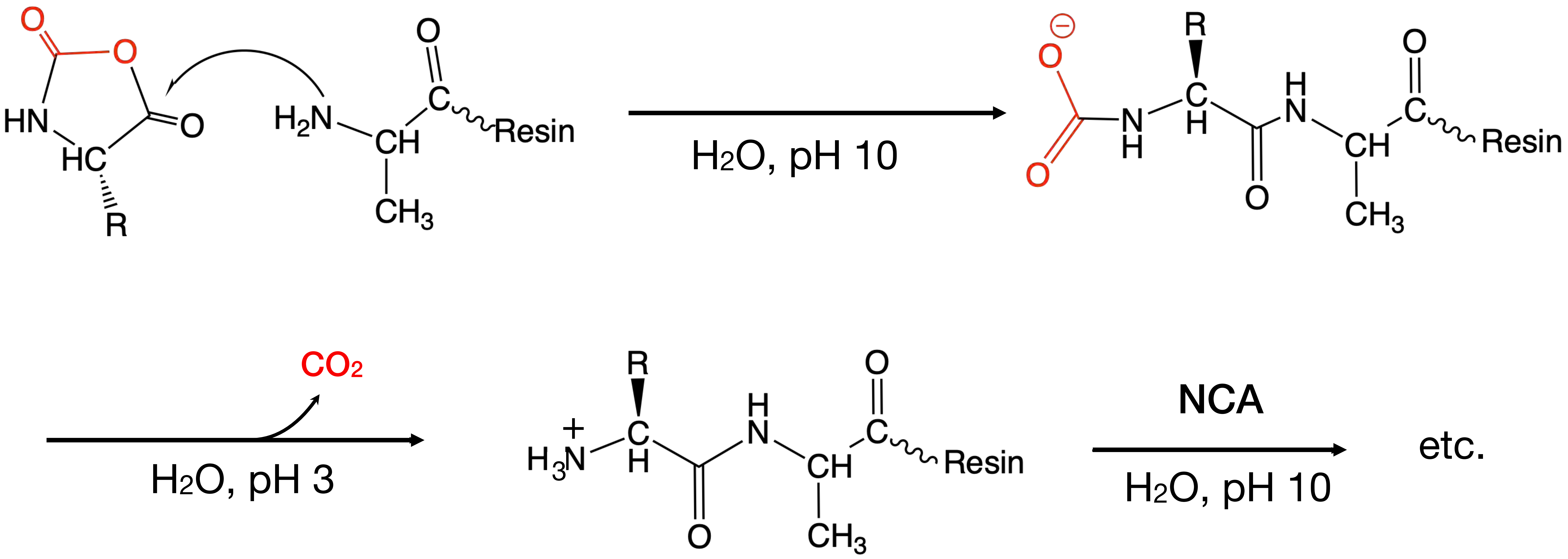
- **atom economy**
  - minimal protecting groups
  - minimal excess reactants
- **benign reagents**
- **benign solvent**

Denkewalter, et al.
J.Am.Chem.Soc.
88, 3163-3164 (1966)

SPPS using α-Amino Acid N-Carboxyanhydrides

on ChemMatrix resin

NCA



De Marco, Gentilucci, et al., *ACS Sustainable Chem. Eng.* **1,** 566–569 (2013)

Green Solid Phase Peptide Synthesis

Atom economy

- N-carboxyanhydrides
  - minimal side-chain protection
    - Lys
    - Ser/Thr(?)
- high resin loading
  - >1 millimol/g resin
- maximum ***concentration*** of NCA
- minimal ***excess*** of NCA

Solvent

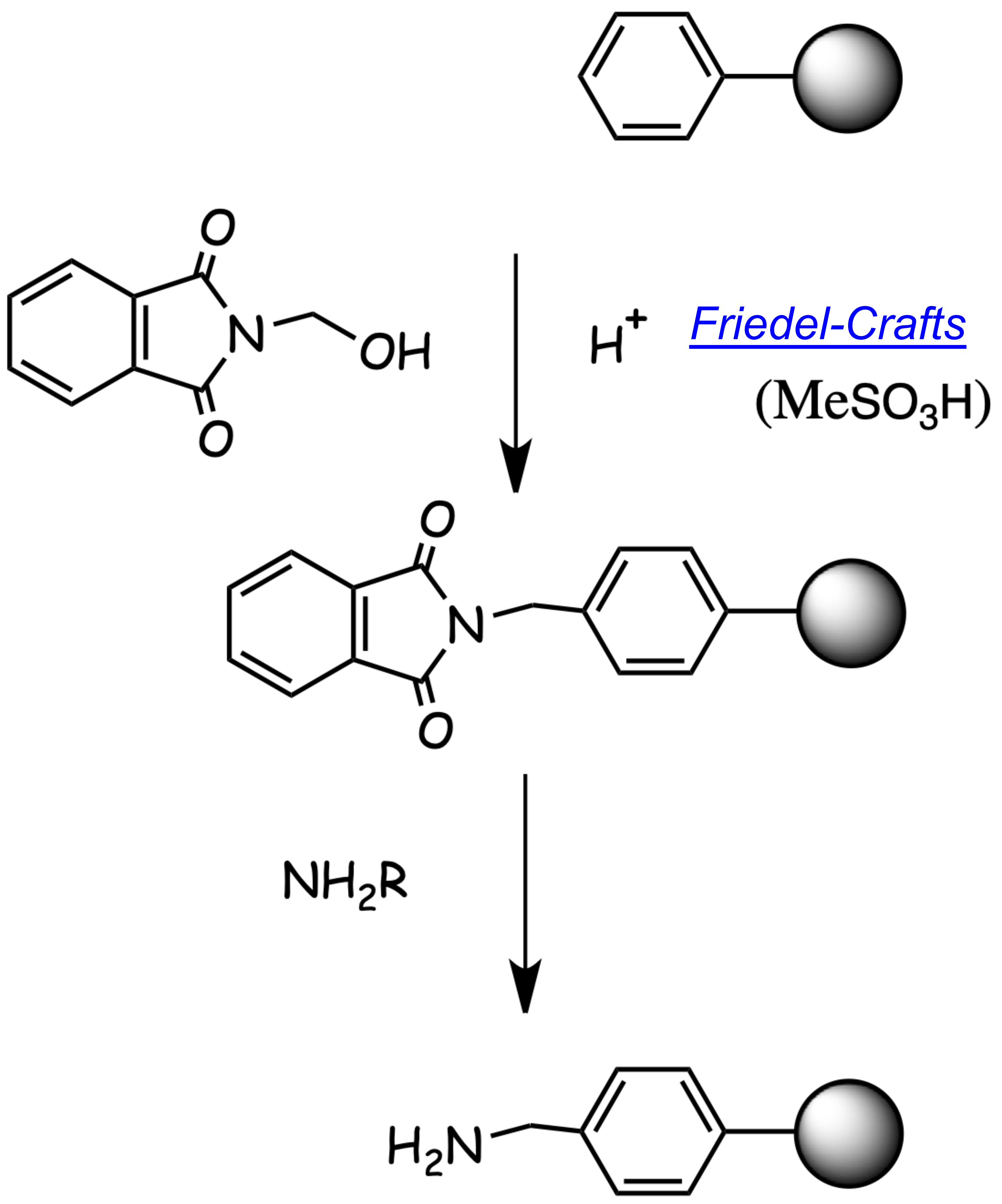
- water

Resin

- aqueous solvent-compatible

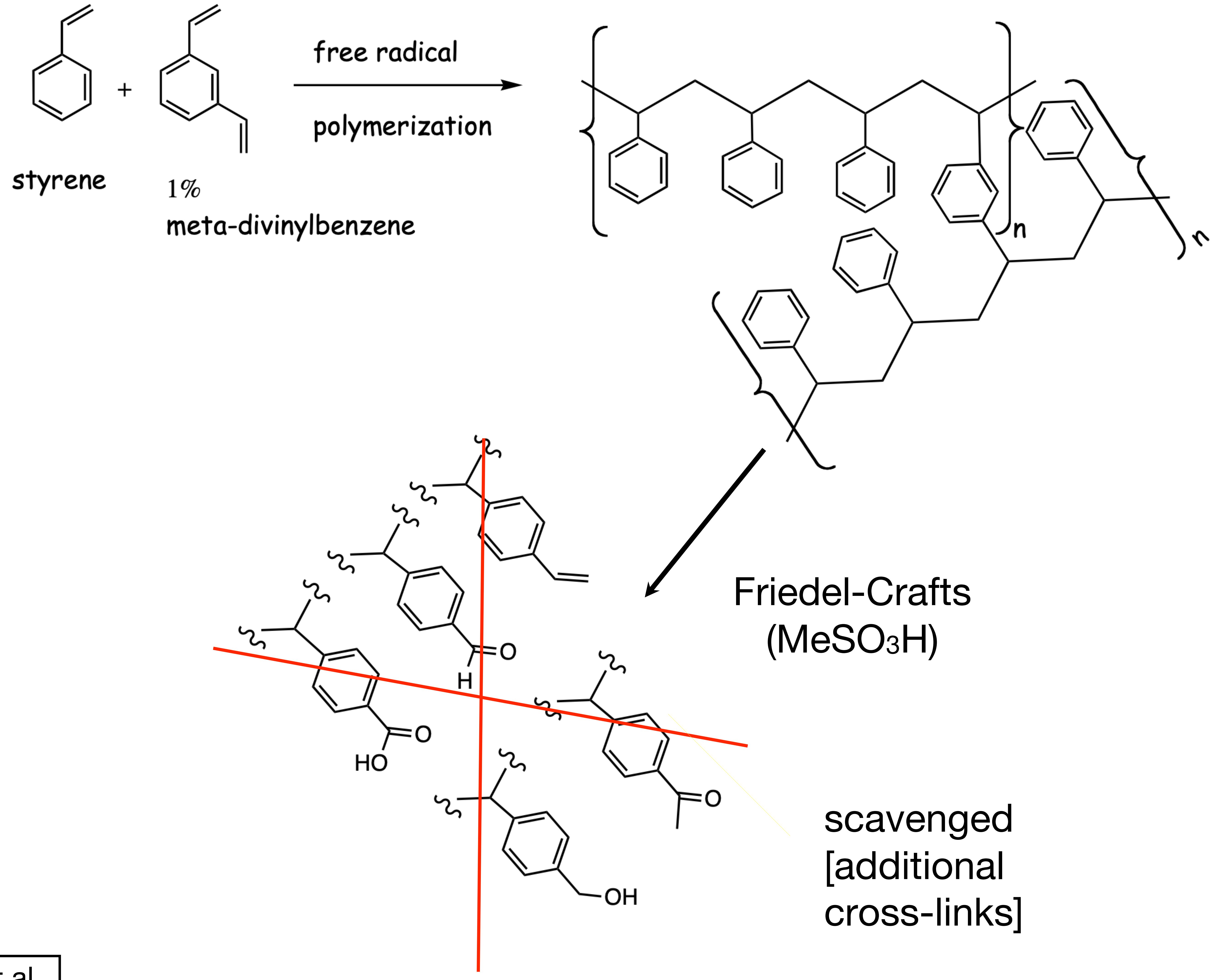
Preparation of chemically defined resins for SPPS

BioBeads Sx1 (Biorad)



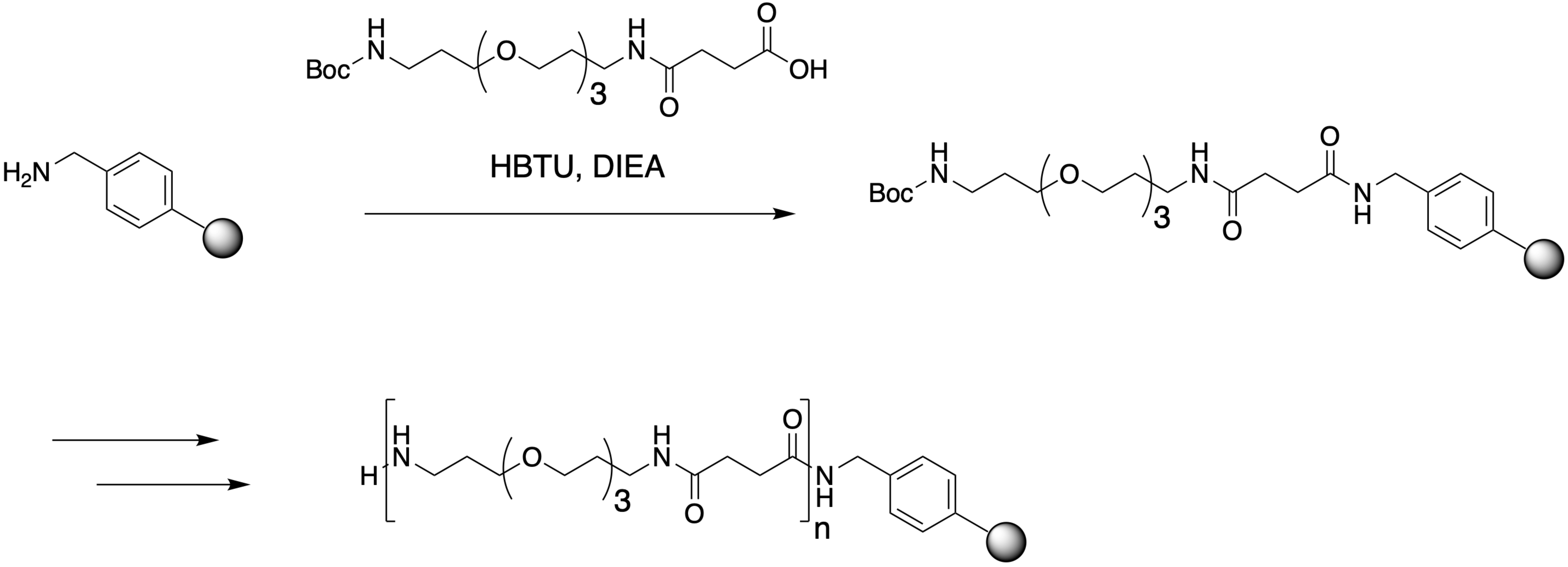
aminomethyl–resin

Paul W. R. Harris et al. ,
Tetrahedron Letters
52 (2011) 6024–6026



Mitchell A.R., Kent S.B.H., Engelhard M., Merrifield R.B.
J. Organic Chem **43**, 2845-2852 (1978).

Aqueous solvent-compatible resin for SPPS



NH_2–(TTD-Succ)$_n$–Resin

Zachary P. Gates, Balamurugan Dhayalan, Stephen B. H. Kent, *Chem.Communications,* 2016,**52**,13979—13982.

Green Solid Phase Peptide Synthesis

Atom economy

- N-carboxyanhydrides
  - minimal side-chain protection
    - Lys(Boc)
    - Ser/Thr?
- high resin loading
  - ~1 millimol/g resin
- maximum ***concentration*** of NCA
- minimum ***excess*** of NCA

Solvent

- water

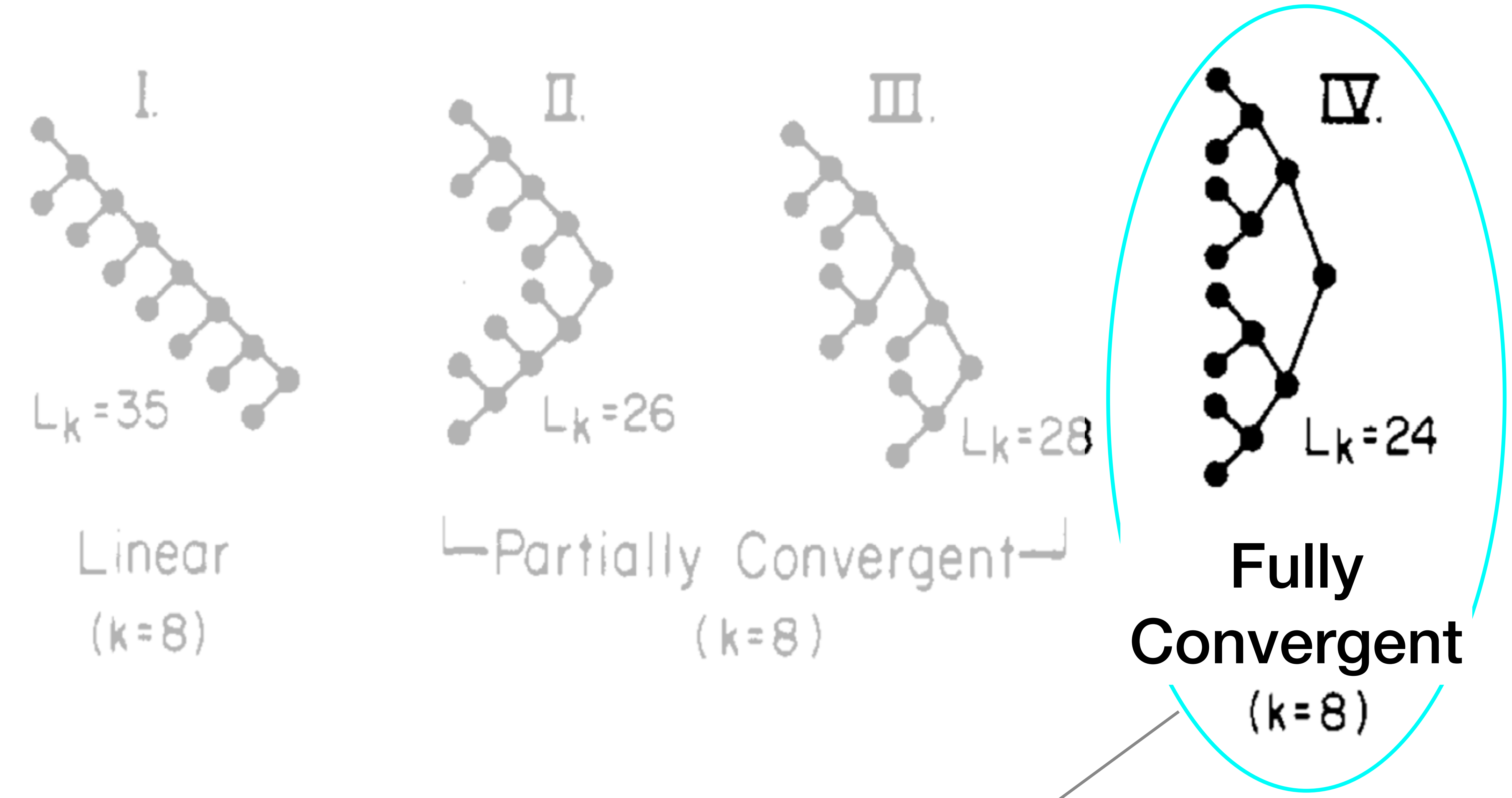
Resin

- aqueous solvent-compatible

Deprotection/cleavage/purification

- *without* TFA
- efficient purification (PMI)
  - tag-assisted
  - displacement mode prep HPLC

Convergent chemical synthesis



Systematic Synthesis Design. 6. Yield Analysis and Convergency

James B. Hendrickson

Journal of the American Chemical Society / 99: 5439 (1977)

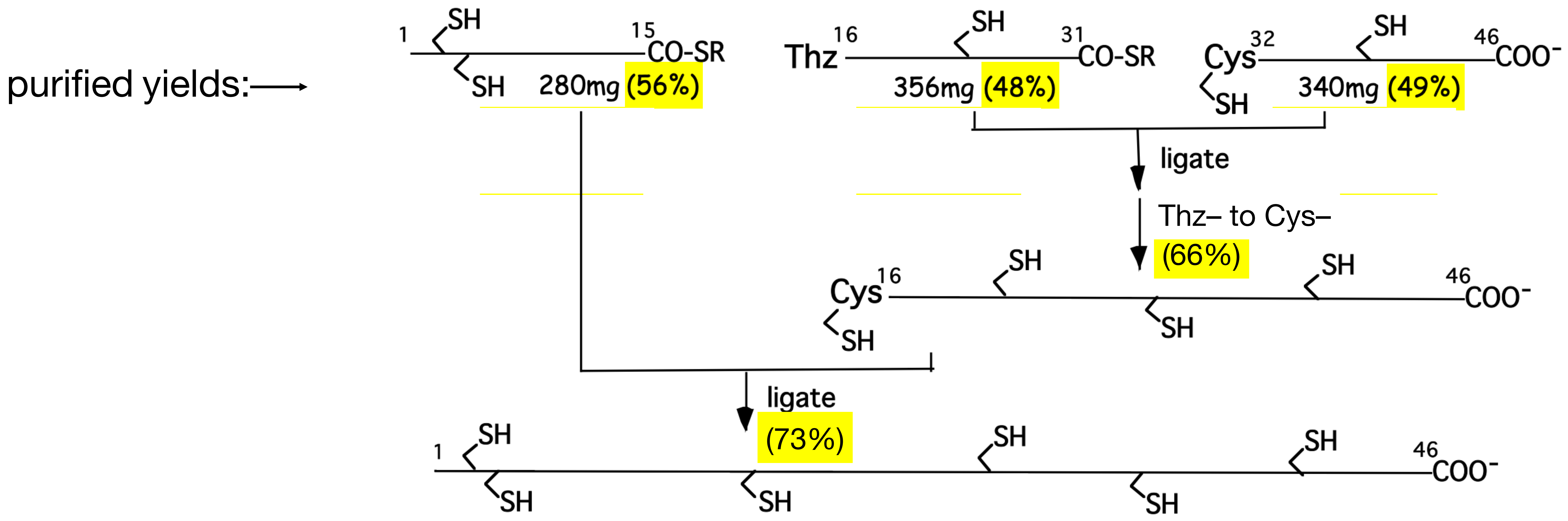
- most efficient use of starting materials
- minimum exposure to reaction conditions
- versatile analogue synthesis
- ***isolation of intermediate products***
- ***enhanced yields & purity***

Convergent segment ligation *vs.* stepwise synthesis

SEGMENT LIGATION

0.4 millimole scale SPPS

46 residue peptide chain of Crambin



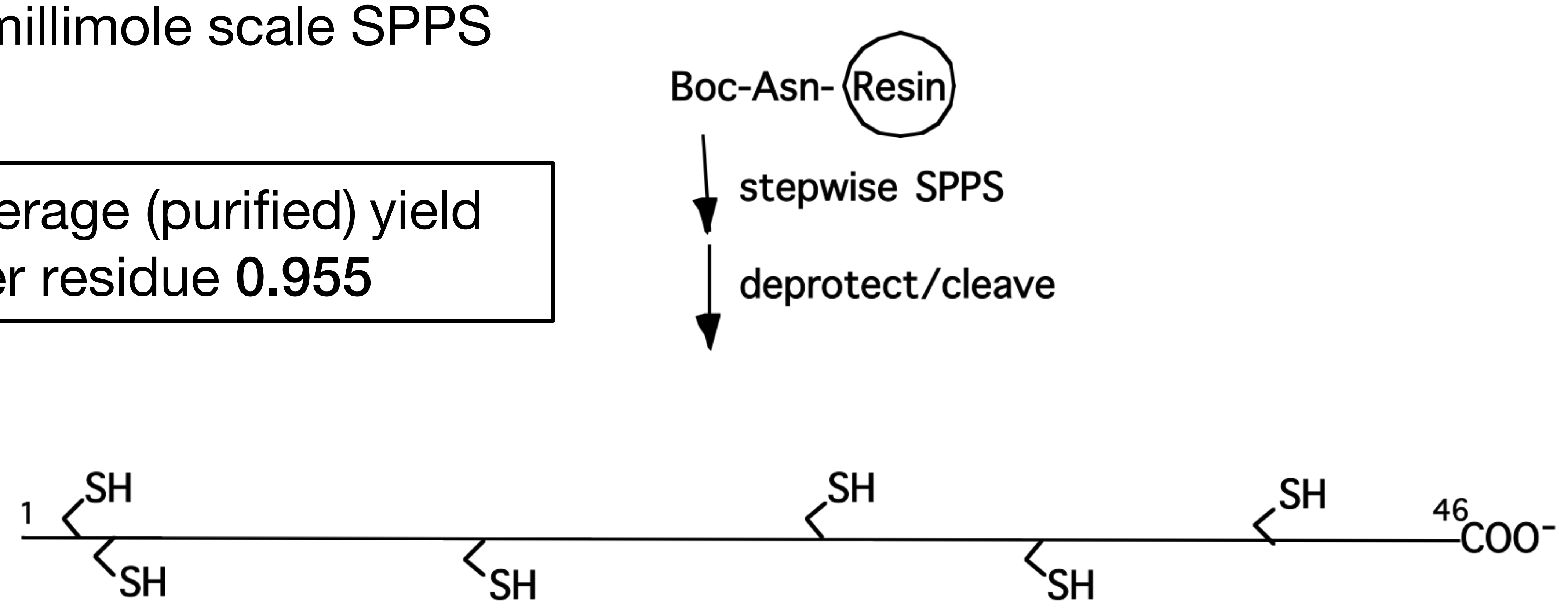
average (purified) yield
per residue **0.955**

overall yield (SPPS-NCL1-NCL2): 450mg (0.5x0.66x0.73) = **24%**

STEPWISE SPPS

0.4 millimole scale SPPS

average (purified) yield
per residue **0.955**



overall yield: 0.955^{46} = **12%**

Chemical ligation condensation of unprotected peptides is inherently 'Green'!

- Protecting groups - minimal/none
- Reaction conditions - benign
- Solvent - water (w or w/o 'solubility modifiers')
- Energy - reactions at/near room temperature
- Efficiency - minimal excesses of reactants
- Atom economy - complete/very good
- Products - degradable

*****commercial availability*** of β-SH Xaa's**

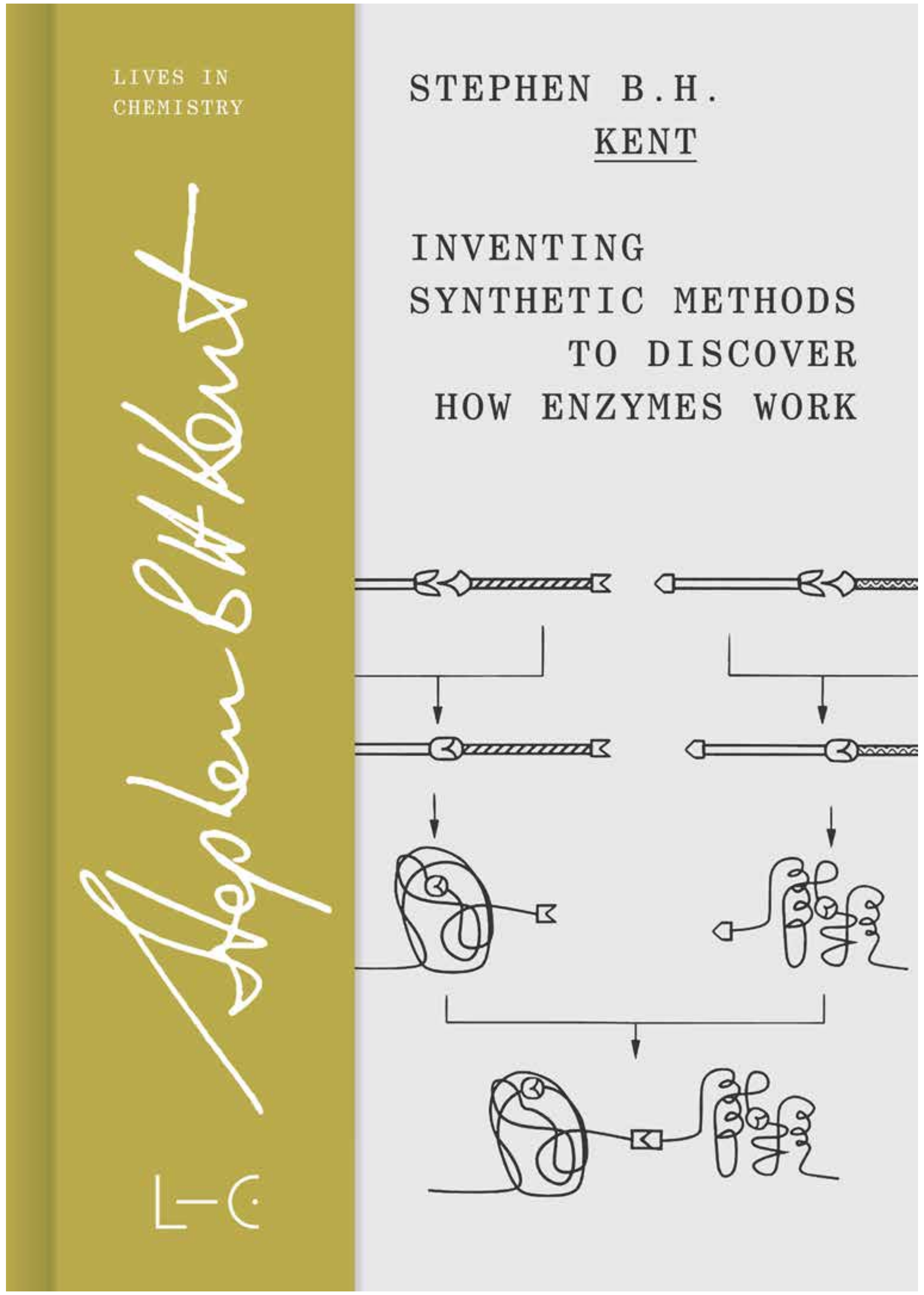
– please! –

Green peptide synthesis

stepwise SPPS using N-carboxyanhydrides

convergent segment condensation by NCL

- enhanced yields
- enhanced purity



LIVES
IN
CHEMISTRY

GDCh HISTORY OF
CHEMISTRY
DIVISION

Outstanding chemists unveil

- how they started they career
- how their ideas evolved
- how they balanced work & life
- how they built a team
- how they overcame hurdles
- how they survived harsh conditions

GDCh

GERMAN CHEMICAL SOCIETY