K.ISHIHARA GROUP
green catalysis

Advances in Peptide synthesis

2023 / 09 / 02 (Sat.)
M2 Seminar
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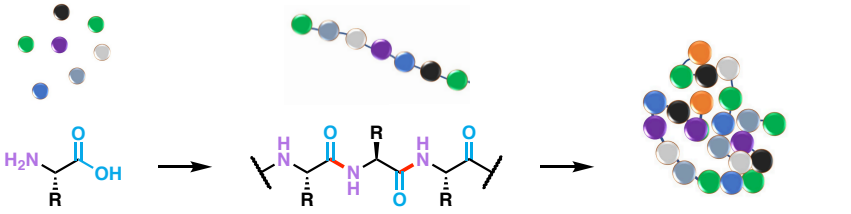
Contents

1. Introduction

1.1 Peptide

A peptide is a short chain of amino acids linked together by peptide bond, typically between two and 50 amino acids in length.

Peptide Bond



***L*-amino aicd**

common configuration in nature

Peptide

(2–50 amino acids)

Protein

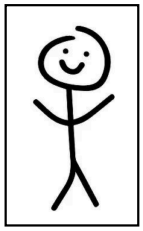
> 50 amino acids with secondary structure

1.2 Functions of Peptide

1. Hormones

e.g. Insulin (1922)

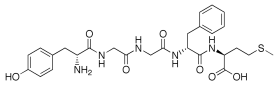
- **First peptide drug**
- **Regulate blood sugar lever**



2. Neurotransmitters

e.g. Enkephalin (1975)

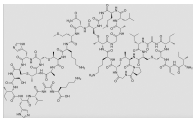
- **Regulate pain sensation in the body**



3. Antibiotics

e.g. Nisin (1930s)

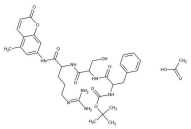
- **Used as a food preservative**
- **Inhibit the growth of bacteria**



4. Enzymes

e.g. Trypsin (1876)

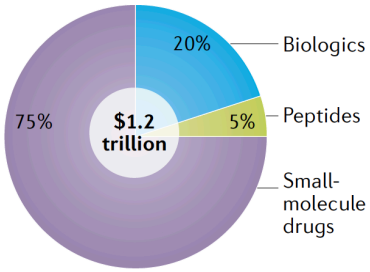
- **Break down proteins into smaller peptides**



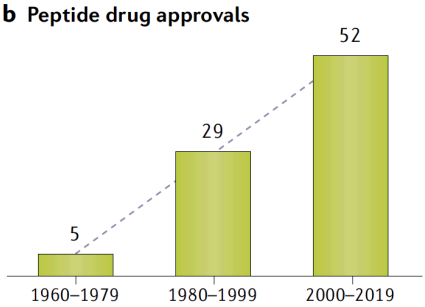
1.3 Current State of Peptide Drug Market[1,2]

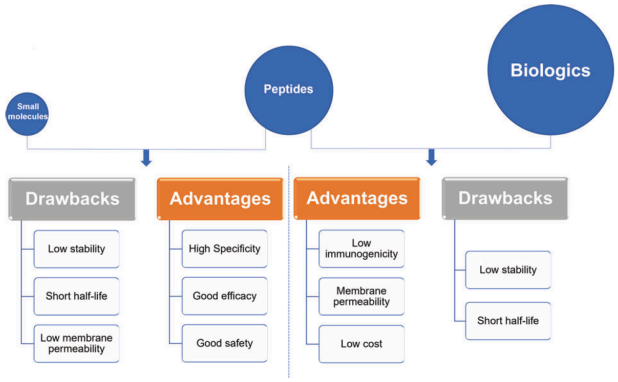
- **5% of the global pharmaceutical market**
- **US$50 billion in 2019**
- **average growth rate of 7.7%**

a Global pharmaceutical market (2019)



b Peptide drug approvals

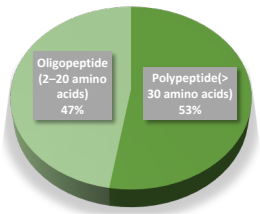




- **around 80 peptide drugs on the global market**
- **over 150 peptides in clinical development**
- **distribution of length in top 19 peptide drugs by sales[3]:**

9 oligopeptides

10 polypeptides



1) Muttenthaler, M.; King, G.F.; Adams, D.J. *et al. Nat Rev Drug Discov,* 2021, *20*, 309.
2) Wang, L.; Wang, N.; Zhang, W. *et al. Sig Transduct Target Ther,* 2022, *7*, 48.
3) Sources: 2019 company financial reports, https://www.fda.gov, and global sales analysis reports.

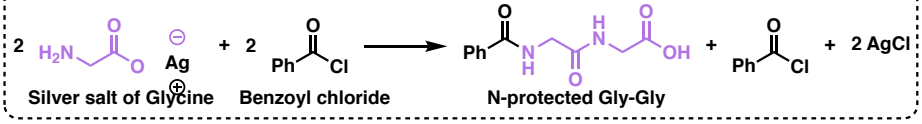
1. Introduction

1.4 Development of Approaches Accessing Peptide

1. Historical timeline of key milestones in peptide synthsis[4]

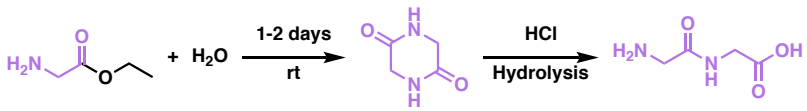
1880 — 1890 — 1900 — 1910 — 1920 — 1930 — 1940 — 1950 — 1960 — 1970 — 1980 — 1990 — 2000 — 2010 — 2020

1882 First *N*-protected dipeptide synthesis by T. Curtius[5]



Silver salt of Glycine — Benzoyl chloride — N-protected Gly-Gly

1901 First free dipeptide synthesis by E. Fischer[6]



Glycine ester — Piperazine-2,5-dione — Gly-Gly

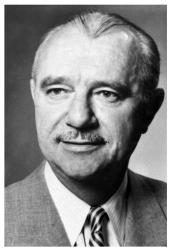


Photo from the Nobel Foundation archive.
Vincent du Vigneaud

1954 First chemical total synthesis of polypeptides by Du Vigneaud. V[7] (The Nobel Prize in Chemistry in 1955)

1963 Solid-phase peptide synthesis (SPPS) by R. B. Merrifield[8]

Photo from the Nobel Foundation archive.
Robert Bruce Merrifield

1984 The Nobel Prize in Chemistry 1984 was awarded to Robert Bruce Merrifield "for his development of methodology for chemical synthesis on a solid matrix"

Novel Methods:

1. Chemical ligation
2. Catalytic reaction (e.g. B, Ta, Se, etc.)
3. Flow chemistry

2. Sustainable Development Goals (SDGs)

Effective

- High yield
- High purity
- No epimerization

Practical

- Simple procedure
- Broad substrate scopes
- Easy purification

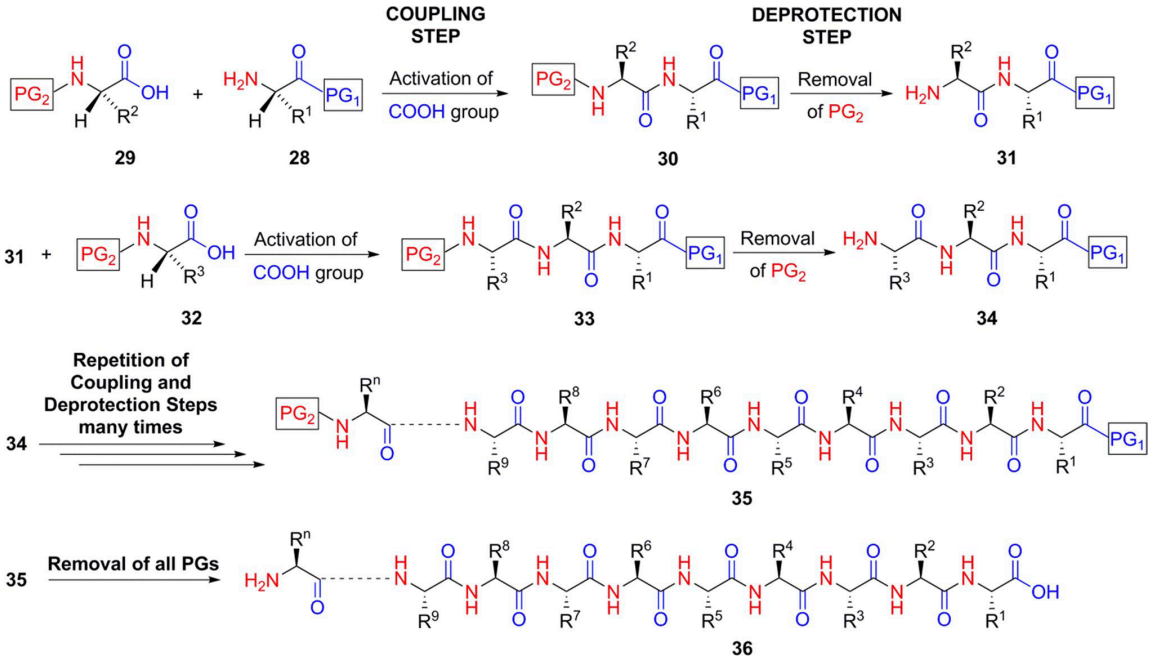
Green

- Mild conditions
- Less waste & low-cost
- Safe

4) Jaradat, D.M.M. *Amino Acids*. **2018**, *50*, 39.
5) Curtius T. *J Prakt Chemie.* **1882**, *26*, 145.
6) Fischer, E.; Fourneau, E. *European Journal of Inorganic Chemistry.* **1901**, *34,* 2868.
7) Du Vigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C. W.; Katsoyannis, P. G. *J. Am. Chem. Soc.* **1954**, *76*, 3115.
8) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

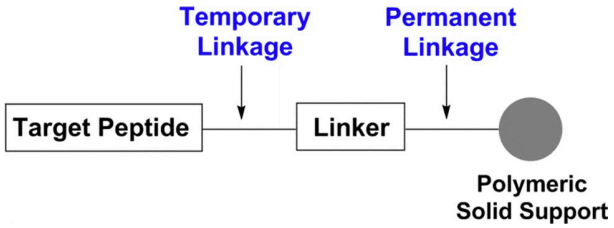
2. Classical Methods

Principle of Peptide Synthesis[4]



2.1 Solid-phase Peptide Synthesis (SPPS)

1.The Concept of SPPS[4]

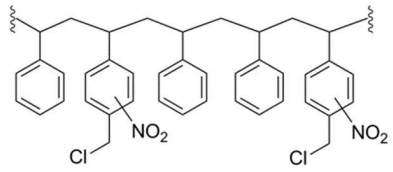


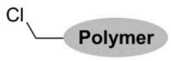
The concept of SPPS is based on attaching the first amino acid to a resin, then proceeding with peptide chain elongation to ultimately provide the target peptide.

Robert Bruce Merrifield

2. Merrifield's original SPPS[8] *(1963)*

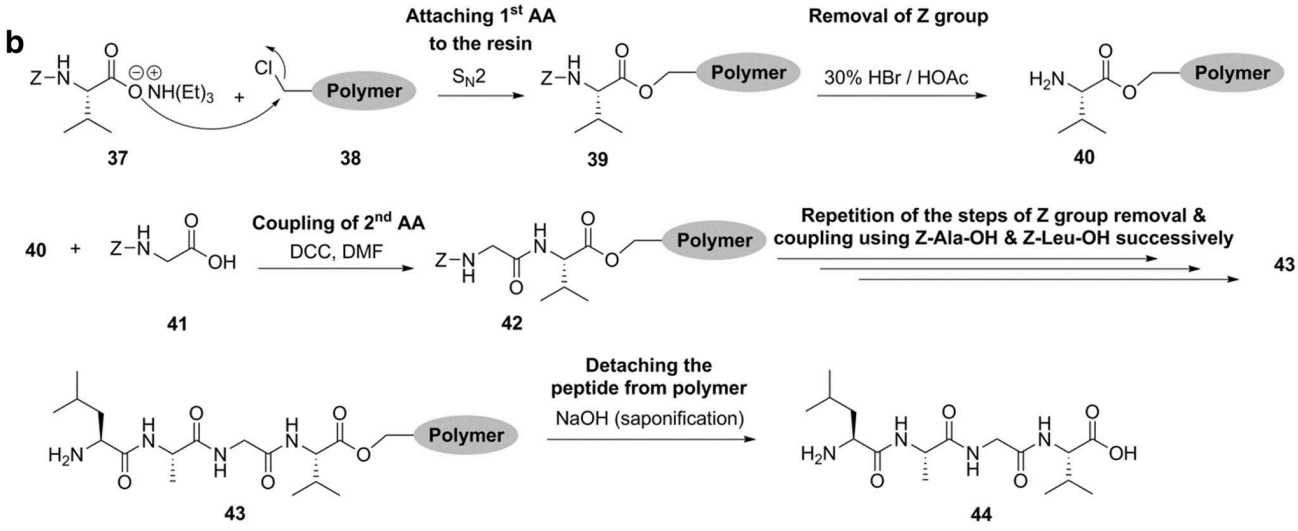
a Chloromethylated nitropolystyrene polymer — abbreviated as





38

b



2. Classical Methods

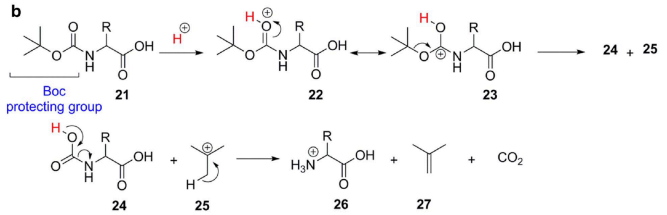
2.1 Solid-Phase Peptide Synthesis (SPPS)

3. Developments in SPPS[4]

1) Protecting strategy[9]

· Boc strategy by Albertson and McKay in 1957

Deprotection mechanism by acid:



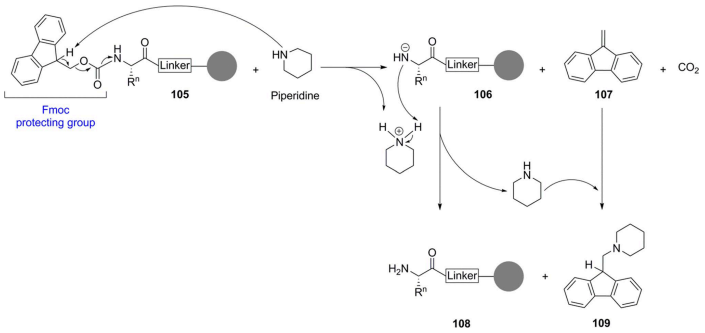
Drawbacks:

- Repetitive deprotection by TFA after each coupling steps could lead to side reactions and alteration of sensitive peptide bonds.

- Dangerous strong acid such as HF is required for the final cleavage of target peptide from resin and the removal of side-chain protecting groups.

· Fmoc strategy by Han and Carpino in 1970 (Common at present)

Deprotection mechanism by base:



Louis Carpino

Advantage:

- Mild deprotecting conditions

Disadvantage:

- More than 20 equiv of piperidine is required to remove Fmoc group.

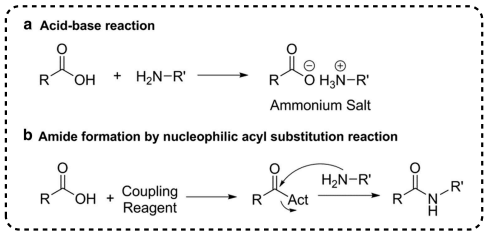
- Unsuitable for solution phase peptide synthesis due to some potential side reactions such as the reaction between the reactive dibenzofulvene and the liberated amine.

2) Coupling reagent

- Purpose:

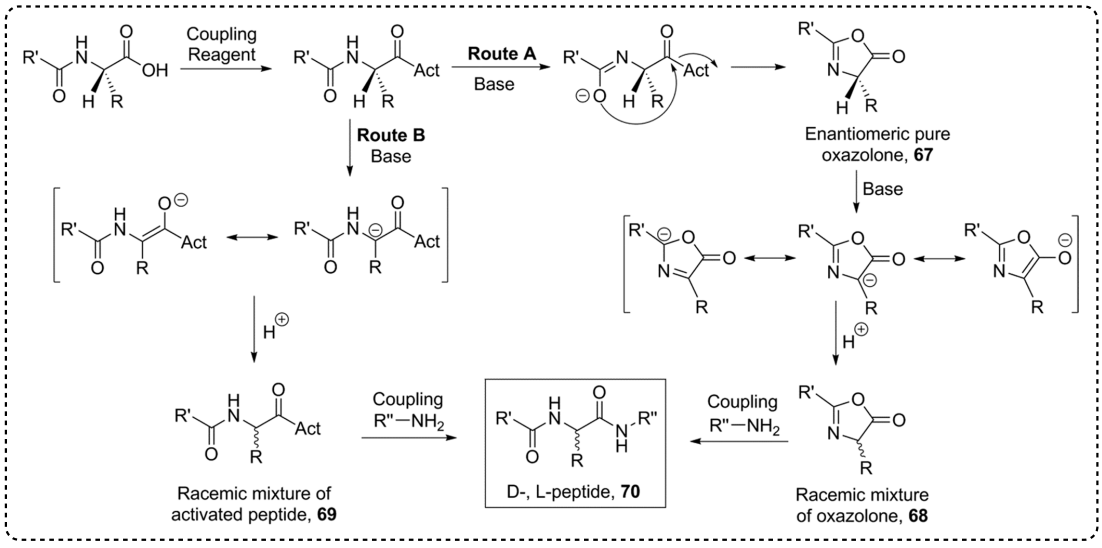
a) avoid forming inactive ammonium salt;

b) activate carboxy group



- Risk:

racemization



Route A proposed by Goodman and Stueben in 1962.[10]

Route B proposed by El-Faham and Albericio in 2011.[11]

9a) C. McKay, F.; F. Albertson, Noel. *J. Am. Chem. Soc.* 1957, *79*, 4686;
9b) A. Carpino, L.; Y. Han, G. *J. Am. Chem. Soc.* 1970, *92*, 5748.
10) Goodman, M.; C. Stueben, K. *J. Org. Chem.* 1962, *27*, 3409.
11) El-Faham, A.; Albericio, F. *Chem. Rev.* 2011, *111*, 6557.

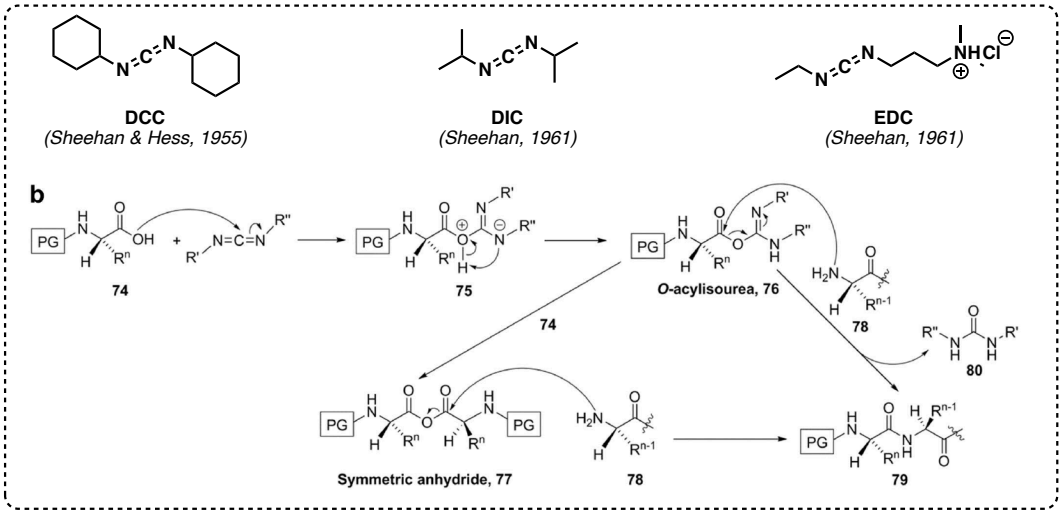
2. Classical Methods

2.1 Solid-Phase Peptide Synthesis (SPPS)

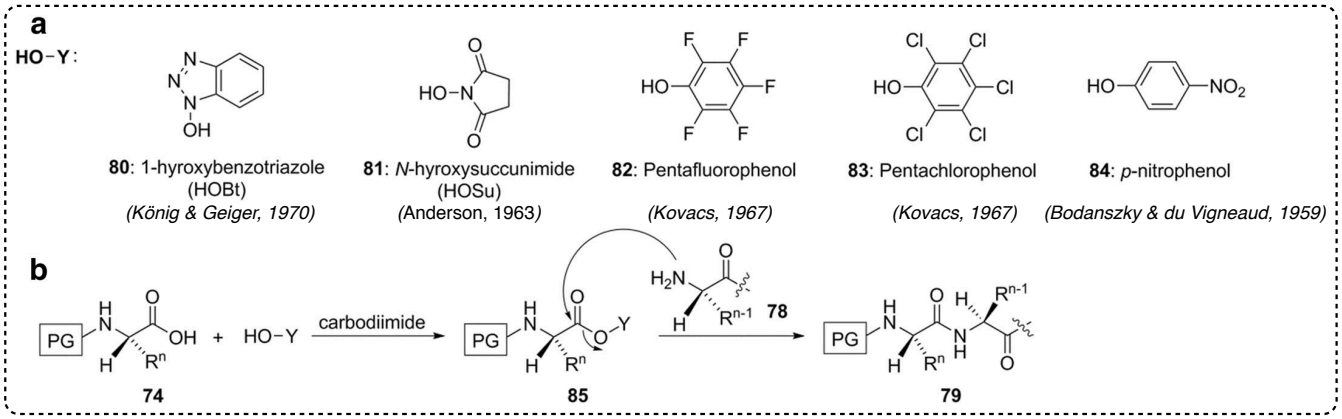
3. Developments in SPPS[4]

2) Coupling reagent

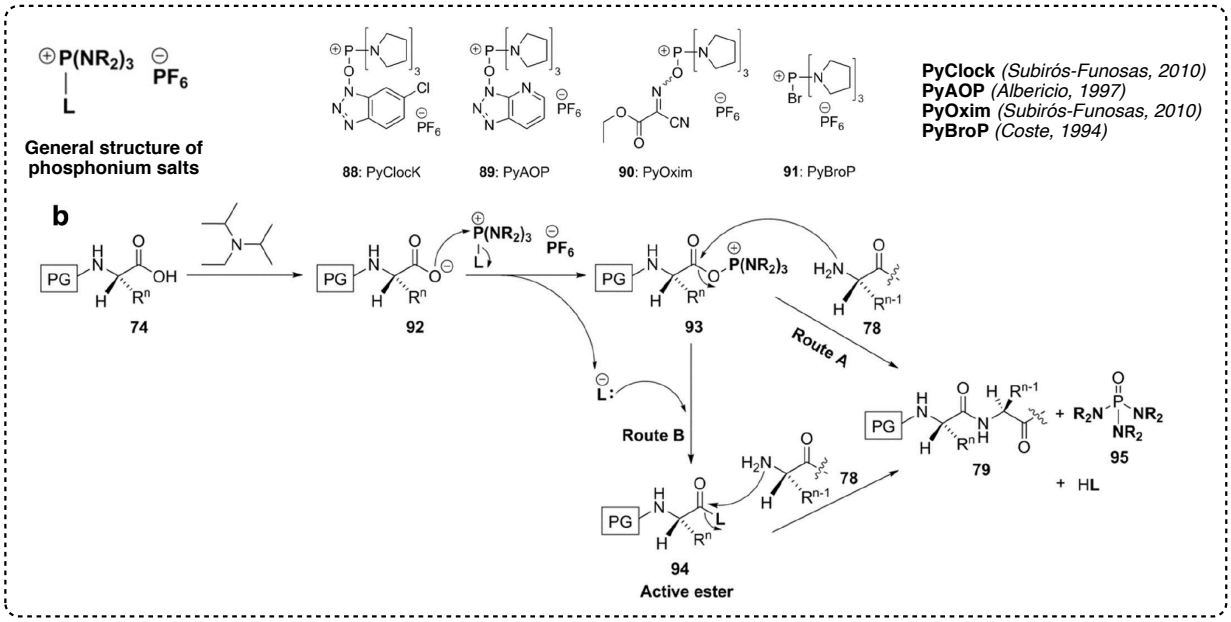
• **Carbodiimides**[12]



• **Active ester**[13]



• **Phosphonium salts**[14]



12a) *J. Am. Chem. Soc.* **1955**, *77*, 1067; 12b) *J. Org. Chem.* **1961**, *26*, 2525.
13a) *Chem. Ber.* **1970**, *103*, 788; 13b) *J. Am. Chem. Soc.* **1963**, *85*, 3039; 13c) *J. Org. Chem.* **1967**, *32*, 3696; 13d) *J. Am. Chem. Soc* **1967**, *89*, 183; 13e) *J. Am. Chem. Soc.* **1959**, *81*, 5688.
14a) *Org. Biomol. Chem*, **2010**, *8*, 3665; 14b) *Tetrahedron. Lett.* **1997**, *38*, 4853; 14c) *J. Org. Chem.* **1994**, *59*, 2437.

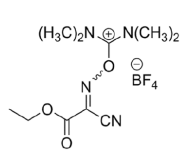
2. Classical Methods

2.1 Solid-Phase Peptide Synthesis (SPPS)

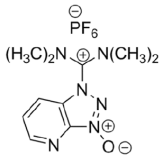
3. Developments in SPPS[4]

2) Coupling reagent

• Uronium / aminium salts[15]

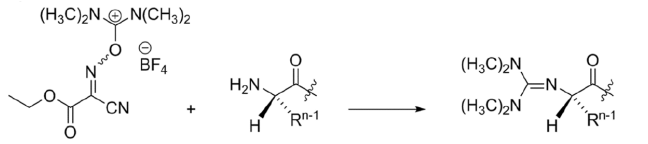


TOTU
(Breipohl and König, 1992)



HATU
(Carpino, 1993)

Side reaction with free *N*-amino group:



102 **78** **104**: guandine by-product

Common drawbacks of coupling reagent:

- Toxicity (reagent, solvent, by-product)

- Poor atom efficiency

4. Advantages and Disadvantages of SPPS[4, 16]

Advantages:

- Useful for providing large peptide (> 30-mer)[17]

- Easier purification than liquid-phase peptide synthesis

Popular in industry but ungreen

Disadvantages:

- Excess of amino acids, coupling reagents and base to maximize conversion of each step

- Excess of base (> 20 equiv) to remove Fmoc group

- Excess of solvent to wash resin

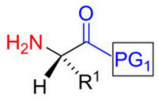
- Utilization of toxic solvent (e.g. DMF, NMP, DCM)

2.2 Liquid-Phase Peptide Synthesis (LPPS)

1. LPPS vs. SPPS

Same synthetic principle as SPPS

Main difference locates at C–PG_1 of the first amino acid[18]:



SPPS: PG_1 is an insoluble resin
LPPS: PG_1 is soluble in the reaction medium

2. Advantages and Disadvantages of LPPS

Advantages:

- Effective for providing short peptide (< 10-mer)

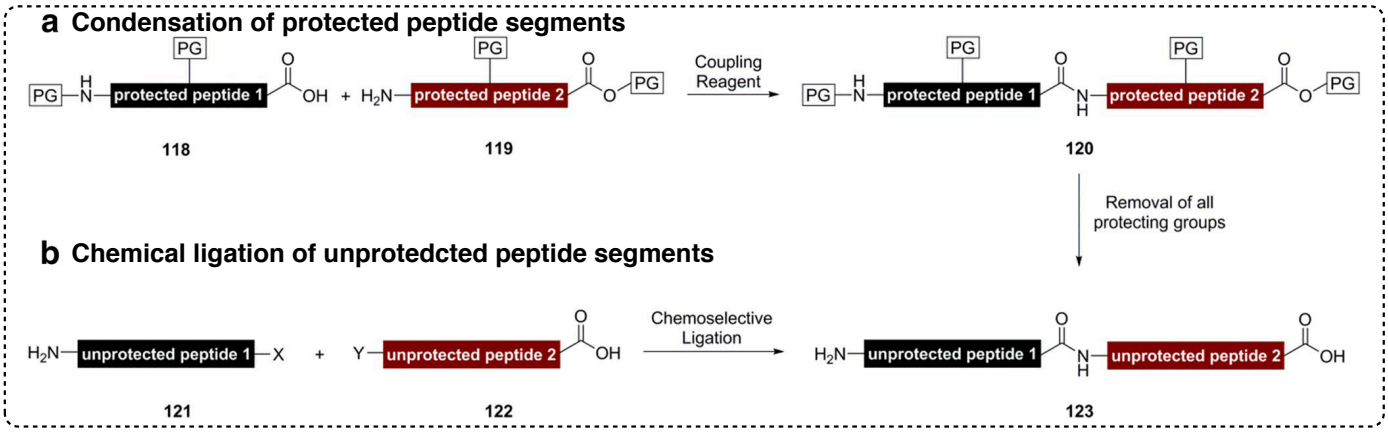
- Possible to minimize waste and price

Disadvantages:

- Repetitive isolation, purification and characterization after each coupling step.

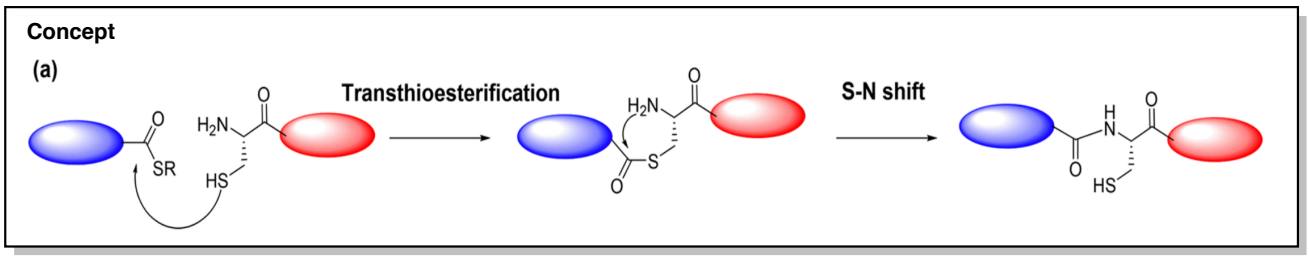
15a) Breipohl, G.; König, W. US patent, **1992**, 5166394 A. 15b) A.Carpino, L. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
16) Isidro-Llobet, A.; N. Kenworthy, M.; Mukherjee, S.; E. Kopach, M.; Wegner, K.; Gallou, F.; G. Smith, A.; Roschangar, F. *J. Org. Chem.* **2019**, *84*, 4615.
17) Winkler, D. F. H.; Tian, K. *Amino Acids.* **2015**, *47*, 787.
18) Tsuda, Y.; Okada, Y. Hughes AB (ed) Amino acids, peptides and proteins in organic chemistry, pp 203–251.(2011)

3. Novel methods

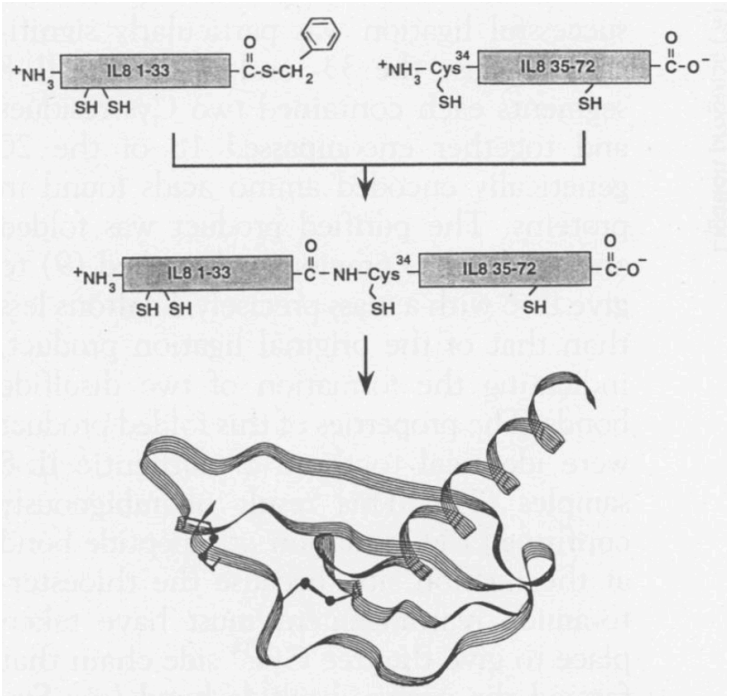


3.1 Chemical Ligation

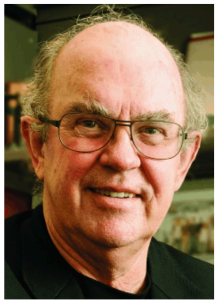
1. Native Cheical Ligation (NCL)



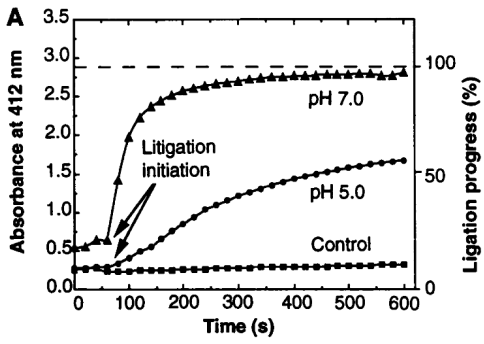
Synthesis of IL-8 by NCL[19] *(Kent, 1994)*



IL-8: Interleukin 8 (72 amino acids)



Stephen B. H. Kent



At pH 7.0, the reaction essentially complete within 5 min.

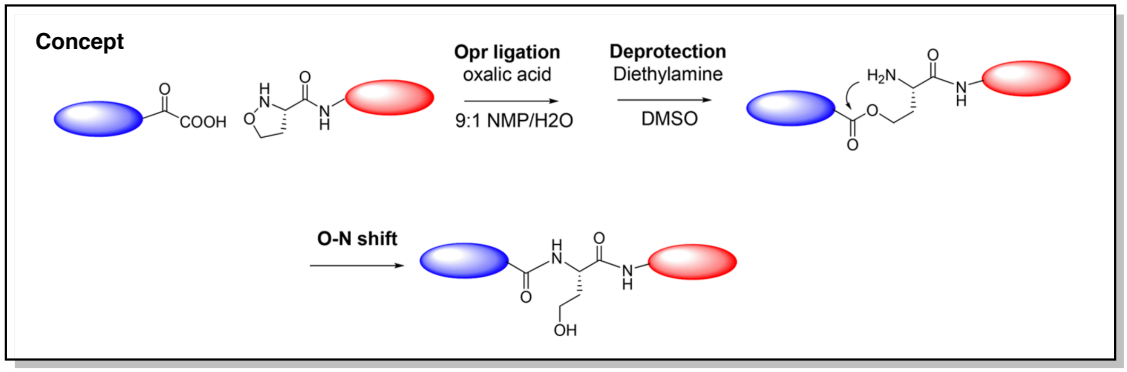
- **Direct formation of protein of moderate size**

19) E. Dawson, P.; W. Muir, T.; Clark-Lewis, L.; B. H. Kent, S. *Science*, **1994**, *266*, 776.

3. Novel methods

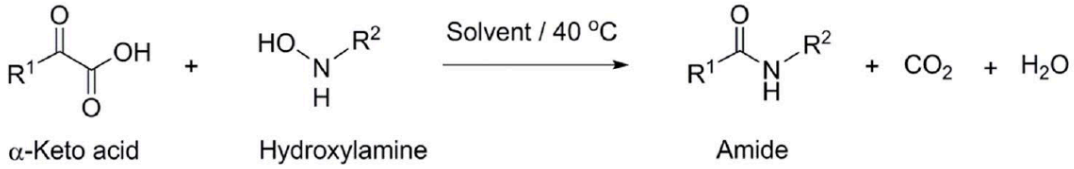
3.1 Chemical Ligation

2. α-Ketoacid Hydroxylamine (KAHA) Ligation

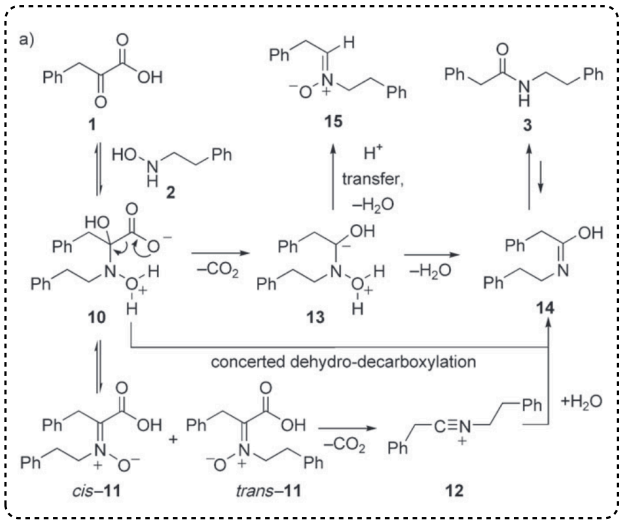


Jeffrey W. Bode

General Reaction decarboxylative amide synthesis



Possible reaction pathway



Bode's KAHA Ligation[20] *(2006)*

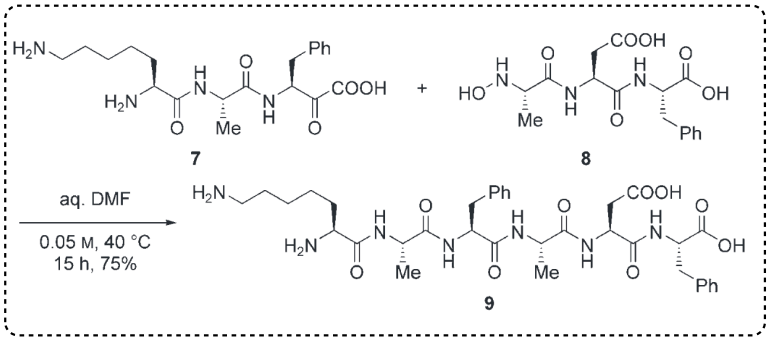
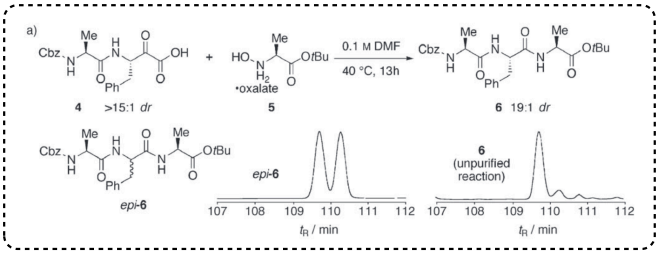


Table 2: Ketoacid–hydroxylamine peptide ligations of selected protected- and unprotected-peptide substrates.

Entry	Ketoacid	Hydroxylamine	Product[a]	Yield[b] [%]
1	FmocAlaPro	AlaO*t*Bu	Fmoc-AlaProAla-O*t*Bu	72
2	FmocAlaVal	GlyOEt	Fmoc-AlaValGly-OEt	58
3	FmocLys(Boc)-Glu(*t*Bu)PheAla	AlaO*t*Bu	Fmoc-Lys(Boc)Glu(*t*Bu)Phe-AlaAla-O*t*Bu[c]	80
4	H_2N-LysAlaPhe	AlaAsp(*t*Bu)PheO*t*Bu	H_2N-LysAlaPhe-AlaAsp(*t*Bu)Phe-O*t*Bu	74
5	FmocAspAlaPhe	AlaAsp(*t*Bu)PheO*t*Bu	Fmoc-AspAlaPhe-AlaAsp(*t*Bu)PheO*t*Bu	74

[a] All reaction performed at 0.02–0.1 M in DMF or DMSO containing ca. 5% H_2O at 40 °C for 10–24 h using 1 equiv ketoacid and 1.2–2 equiv hydroxylamine oxalates; [b] Yields of pure products following preparative TLC or RP-HPLC. The reported yields include the preparation of the ketoacids by oxidation of the appropriate cyanoylide followed by coupling with the hydroxylamine; [c] 0.01 M, 48 h.



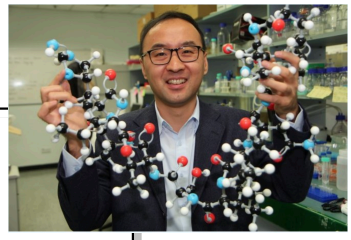
Demonstration of preservation of stereochemistry during the reaction:

Epimerizationof the ketoacid does not occur duringthe ligation reaction.

20) W. Bode, J.;* M. Fox, R.; D. Baucom, K. *Angew. Chem. Int. Ed.* **2006**, *45*, 1248.

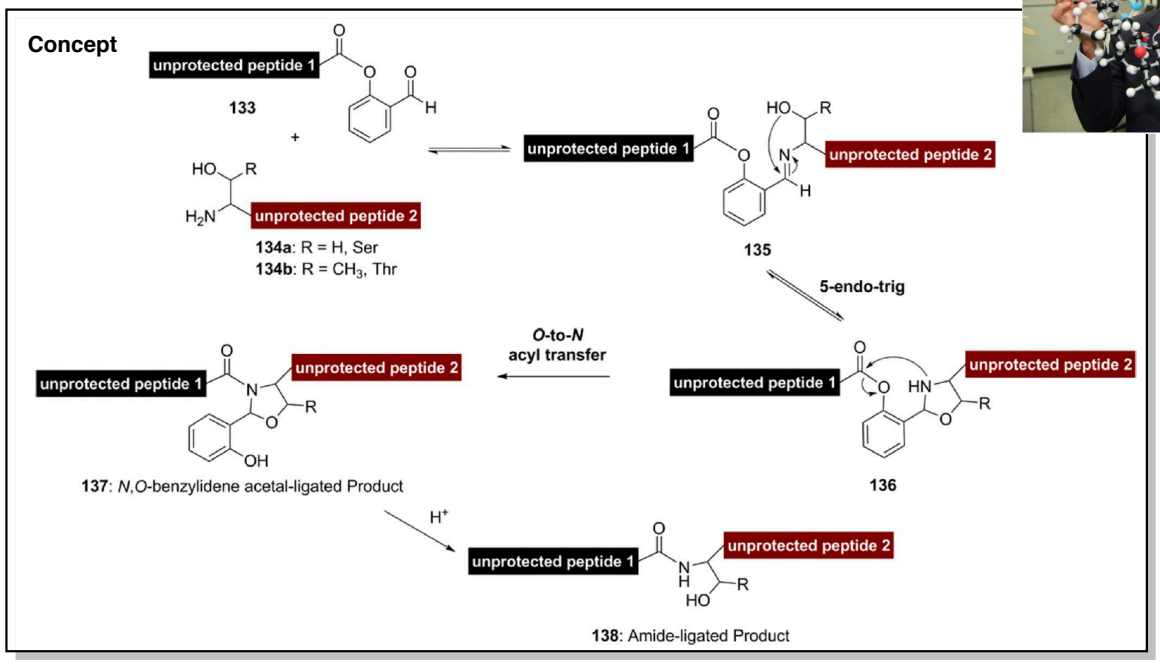
3. Novel methods

3.1 Chemical Ligation

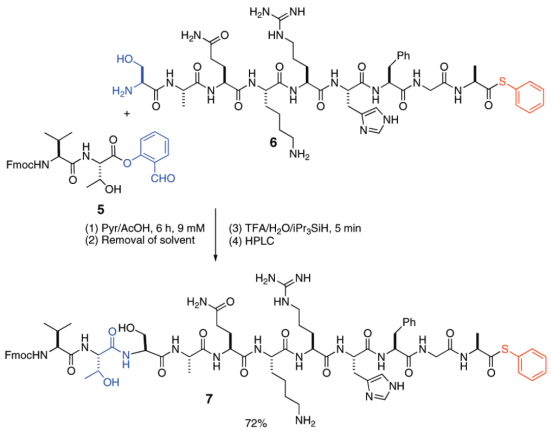


Xuechen LI

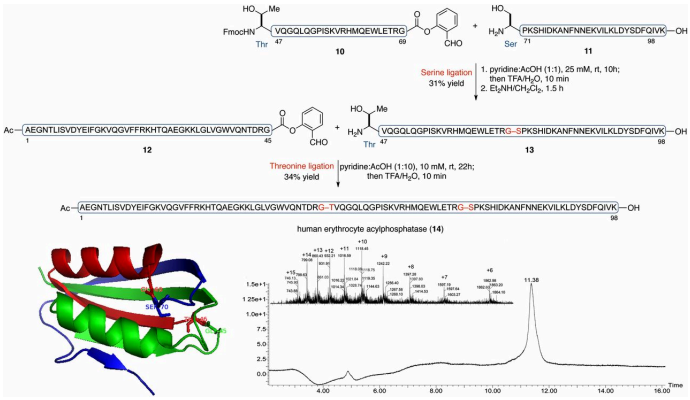
3. Ligation Serine / Threonine Ligation (STL)



Li' s STL[21] *(2010)*



Total chemical synthesis of human erythrocyte phosphatase protein (98-mer)[22] *(Li, 2013)*



Summary of chemical ligation methods:

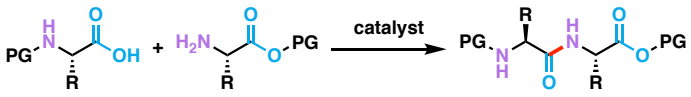
- Common steps: 1) a variable chemoselective capture step
2) invariable rearrangement

- Suitable for synthesis of large peptide

21) Li, X.; Lam, H.; Zhang, Y.; Chan, C. *Org. Lett.* **2010**, *12*, 1724.
22) Zhang, Y.; Xu, C.; Lam, H.; Li, X. *Proc. Natl. Acad. Sci. USA.* **2013**, *110*, 6657.

3. Novel methods

3.2 Catalytic condensation of Protected Amino Acids

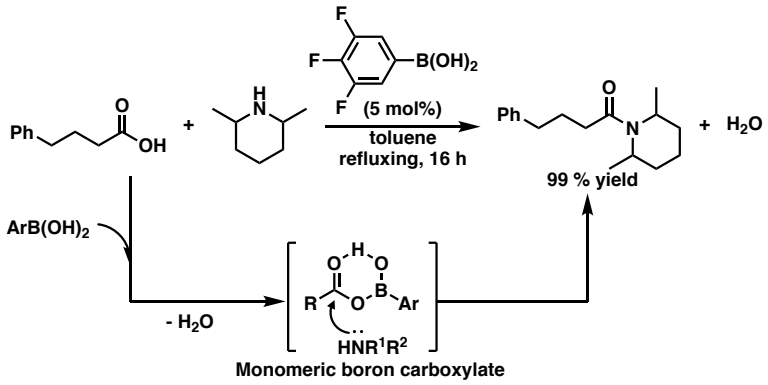


Typical catalyst: B, Metal, Se

1. Boron-catalyzed condensation

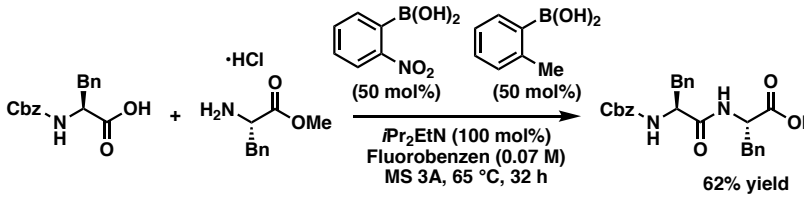
- **Basis: arylboronic acid catalyzed amidation by Yamamoto and Ishihara in 1996**[23]

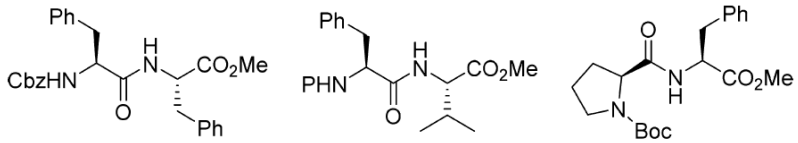
Yamamoto, H



- **First boron-catalyzed peptide synthesis by Whiting in 2013**[24]

Whiting, A



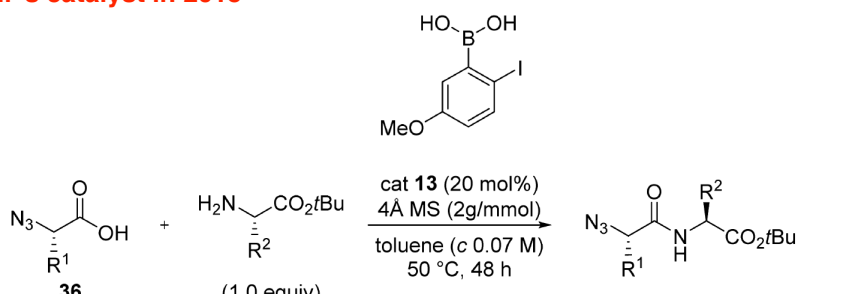


total 100 mol% catalyst
narrow substrate scope
low yield

no racemization

- **Hall' s catalyst in 2015**[25]

Hall, D. G.



narrow substrate scope
low yield

no racemization

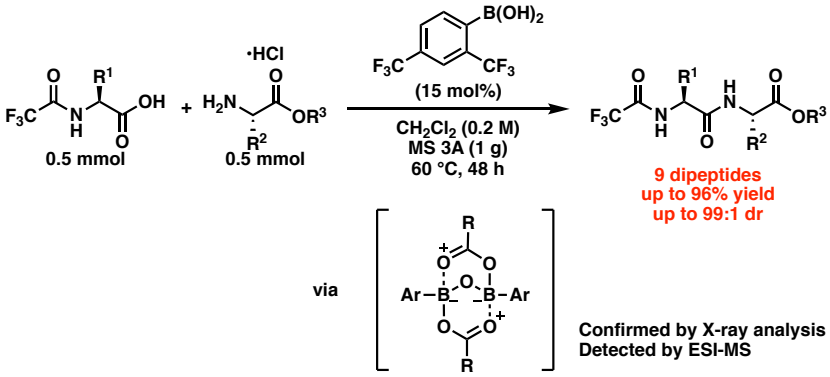
23) Ishihara, K.; Ohara, S.; Yamamoto, H. *J. Org. Chem.* **1996**, *61*, 4196.
24) Liu, S.; Yang, Y.; Liu, X.; Ferdousi, F. K.; Batsanov, A. S.; Whiting, A. *Eur. J. Org. Chem.* **2013**, *2013*, 5692.
25) Fatemi, S.; Gernigon, N.; Hall, D. G. *Green Chem.* **2015**, *17*, 4016.

3. Novel methods

3.2 Catalytic condensation of Protected Amino Acids

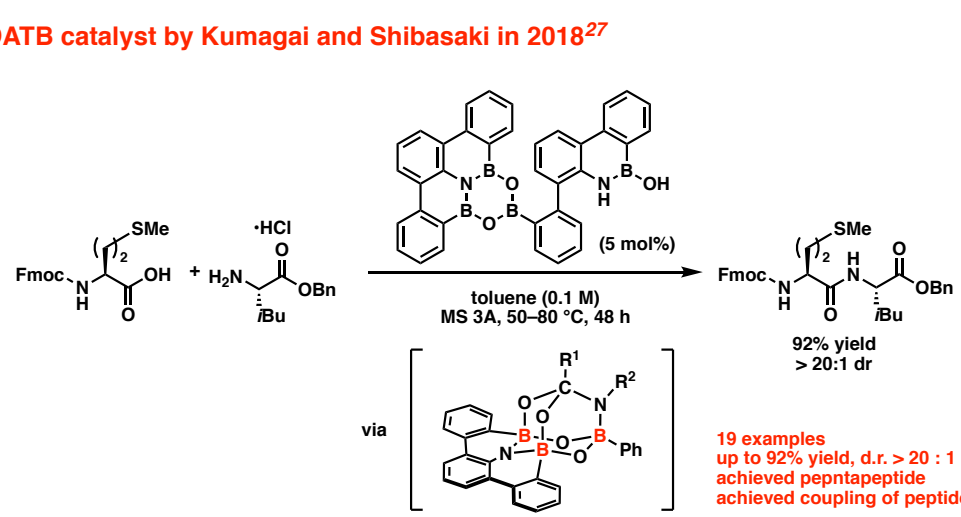
1. Boron-catalyzed condensation

- **Ishihara' s catalyst in 2018**[26]



Ishihara, K

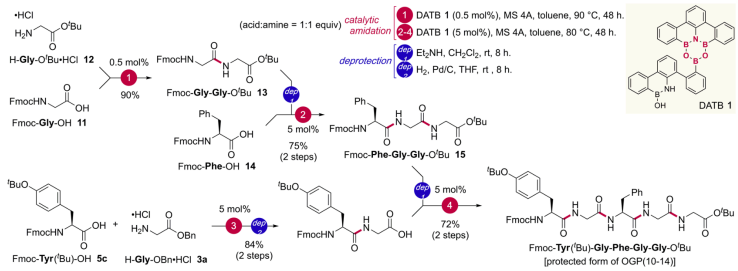
- **DATB catalyst by Kumagai and Shibasaki in 2018**[27]



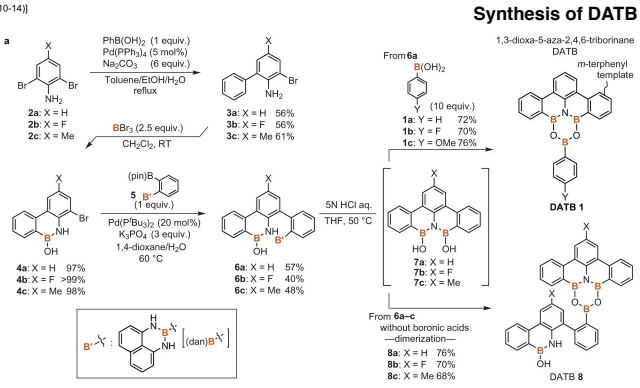
Shibasaki, M

Kumagai, N

Scheme 5. Catalytic Assembly of Five α-Amino Acids To Afford Pentapeptide Fmoc-Tyr(ᵗBu)-Gly-Phe-Gly-Gly-OᵗBu



Synthesis of pentapeptide by DATB



26) Wang, K.; Lu, Y.; Ishihara, K. *Chem. Commun.* **2018**, *54*, 5410.
27a) Liu, Z.; Noda, H.; Shibasaki, M.; Kumagai, N. *Org. Lett.* **2018**, *20*, 612. 17b) Noda, H.; Furutachi, M.; Asada, Y.; Shibasaki, M.; Kumgai, N. *Nat. Chem.* **2017**, *9*, 571.

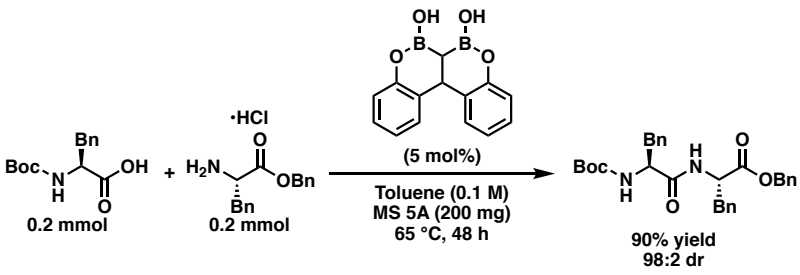
3. Novel methods

3.2 Catalytic condensation of Protected Amino Acids

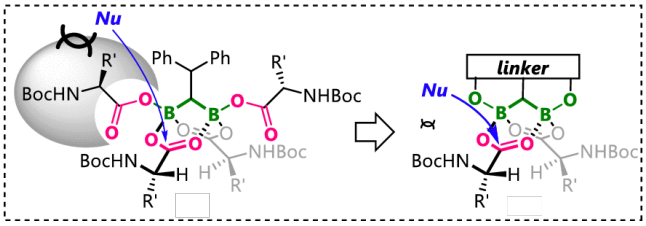
1. Boron-catalyzed condensation

• **Gem-DBA by Takemoto in 2020**[28]

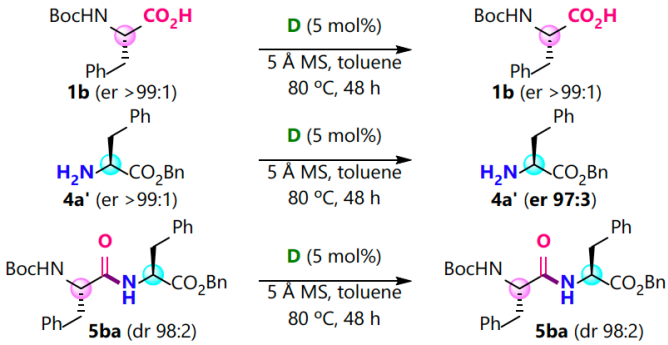
Takemoto, Y



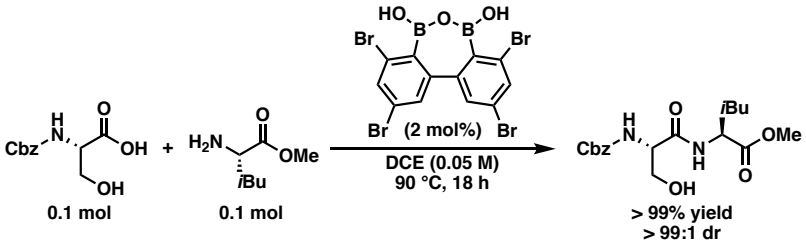
24 examples
up to 97% yield, d.r. > 99 : 1
achieved tetrapeptide
achieved coupling of peptides



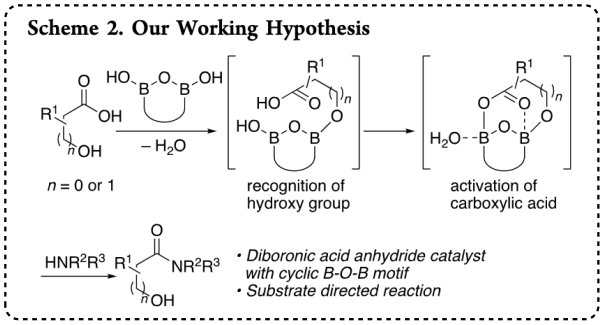
B) Epimerization Tests



• **DBAA by Shimada in 2020**[29]



35 examples
up to 99% yield, d.r. > 99 : 1
achieved tripeptide



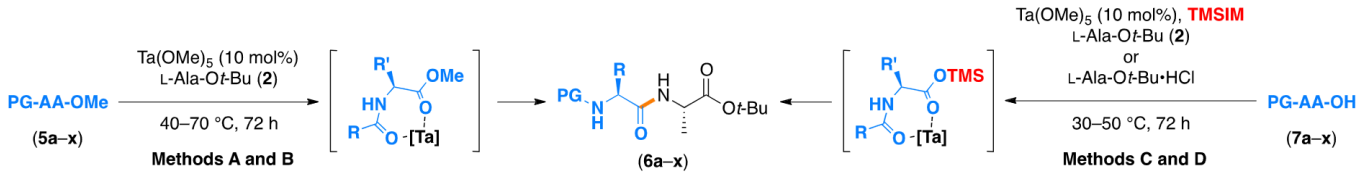
Shimada, N

28) Michigami, K.; Sakaguchi, T.; Takemoto, Y. *ACS Catal.* **2020**, *10*, 683.
29) Koshizuka, M.; Makino, K.; Shimada, N. *Org. Lett.* **2020**, *22*, 8658

3. Novel methods

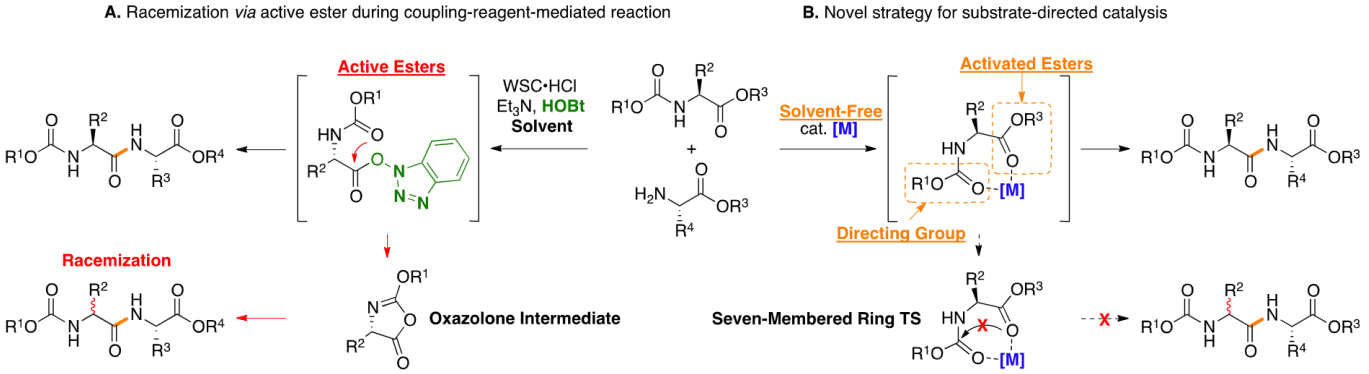
3.2 Catalytic condensation of Protected Amino Acids

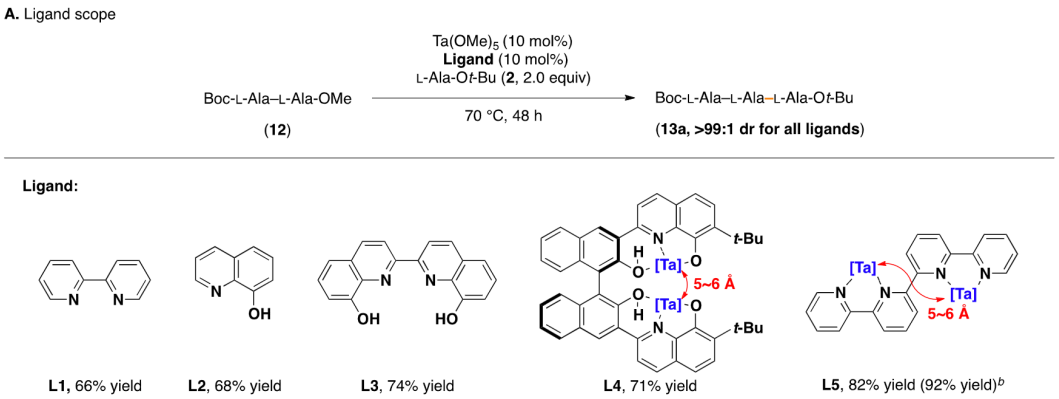
2. Ta-catalyzed condensation by Yamamoto in 2019[30]

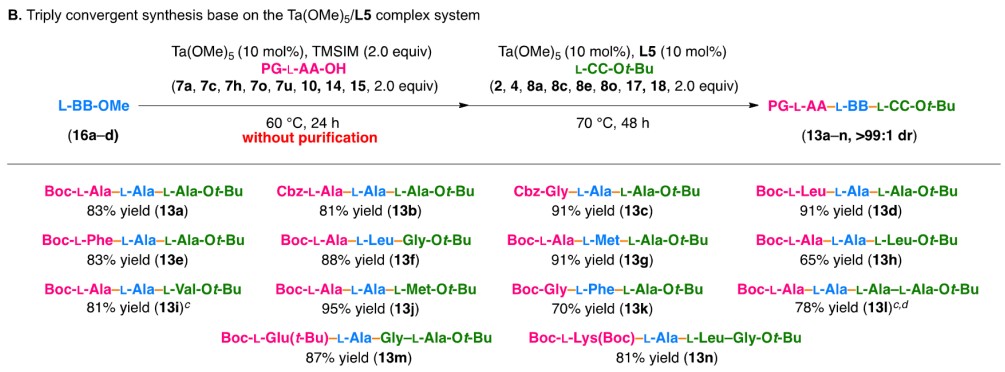


59 examples
up tp >99% yield, d.r. > 99 :1
achieved hexapeptide
achieved coupling of peptides

Adcantage:







Yamamoto, H

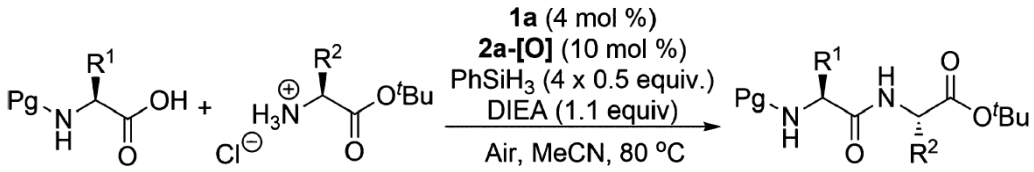
30) Yamamoto, H. J. Am. Chem. Soc. 2019, 141, 12288.

3. Novel methods

3.2 Catalytic condensation of Protected Amino Acids

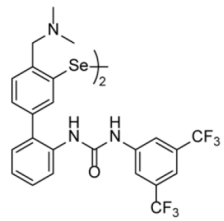
Paramjit S. Arora

3. Se-catalyzed condensation by S. Arora in 2022[31]

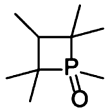


15 dipeptides
up to 94% yield, >99:1 dr

catalyst

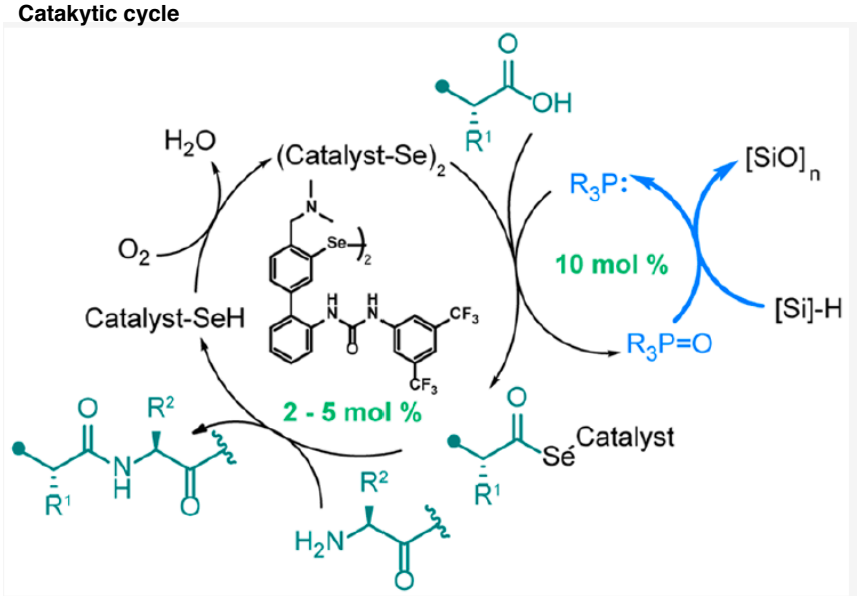


1a

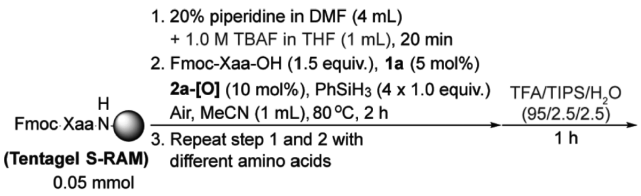


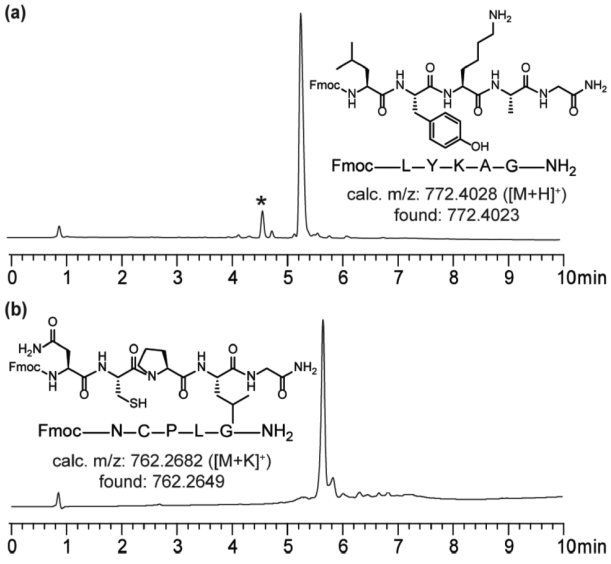
2a-[O]

Catakytic cycle



Catalytic solid-phase peptide synthesis



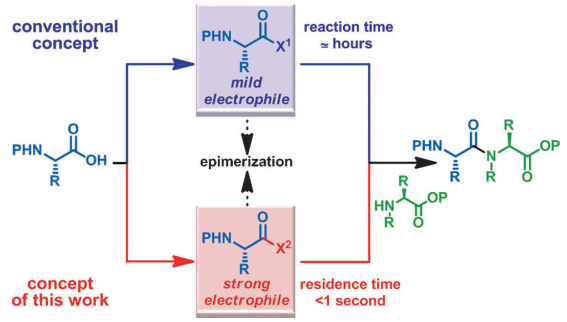


31) Handoko.; Nihar R. Panigrahi, N.; S. Arora, P. *J. Am. Chem. Soc.* **2022**, *144*, 3637.

3. Novel methods

3.3 Peptide Synthesis in Flow (Fuse, S)[32]

1. Activating agent: triphosgene (2014)



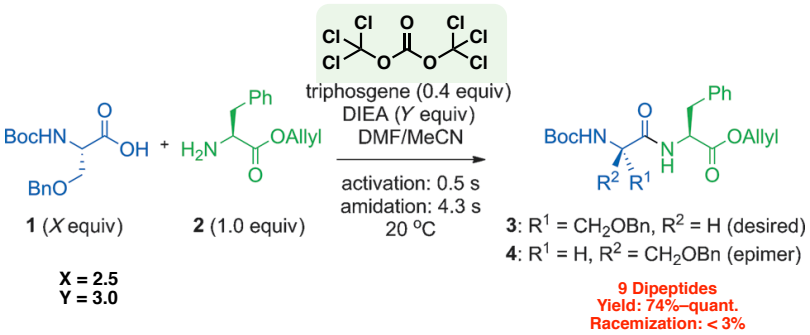
Ideas:

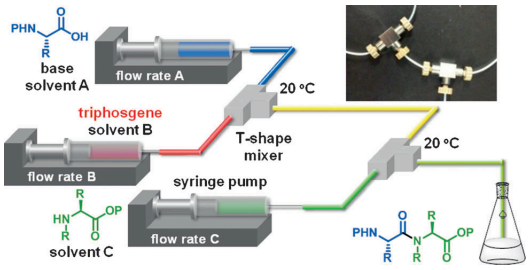
- Rapid and strong activation increased efficiency.
- Short residence time suppressed racemization.

Achievements:

- Fast, efficient and low waste synthesis of small peptide
- 1 Tetrapeptide (60% yield, < 1% epimer)

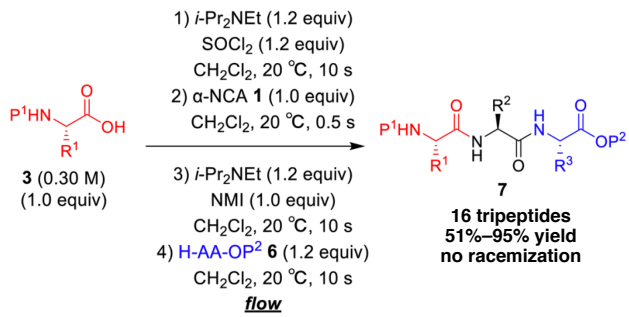
Shinichiro Fuse

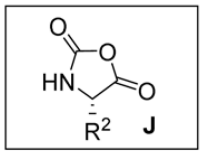




Microflow synthetic reactor

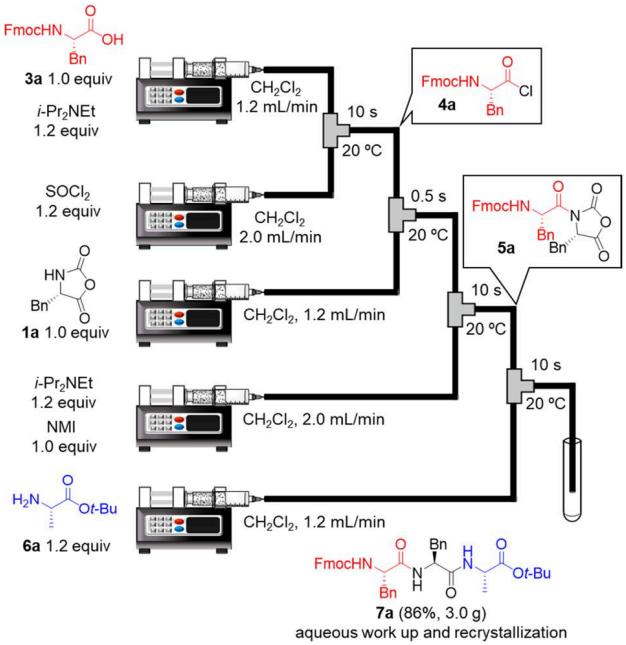
2. Activating agent: *N*-carboxyanhydrides (NCAs) (2023)





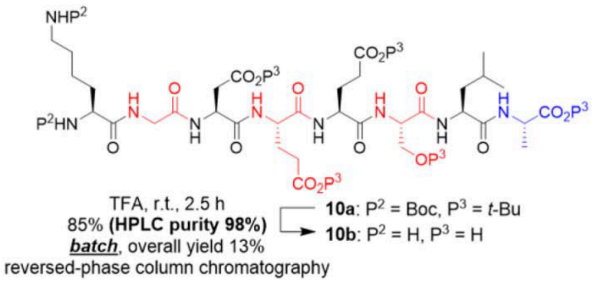
NCAs

☑ one-flow ☑ short reaction time (<minutes) ☑ no column chromatography



Application:

Total synthesis of beefy meaty peptide (overall yield 13%)



32a) Fuse, S.; Mifune, Y.; Takahashi, T. *Angew. Chem. Int. Ed.* **2014**, *53*, 851.
32b) Sugisawa, N.; Ando, A.; Fuse, S. *Chem. Sci.* **2023**, *14*, 6986.