Tag-assisted Liquid-phase Peptide Synthesis

Literature Seminar #2

2022.01.19

M1 Kazuki Oikawa

Contents

1. Introduction

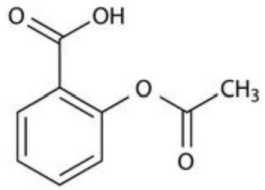
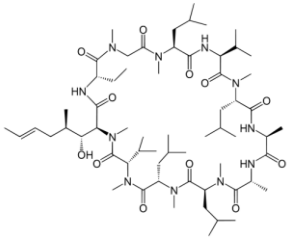
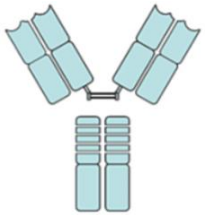
2. Benzyl-type tag

3. AJIPHASE®

4. Summary

Introduction

○ Peptide drugs

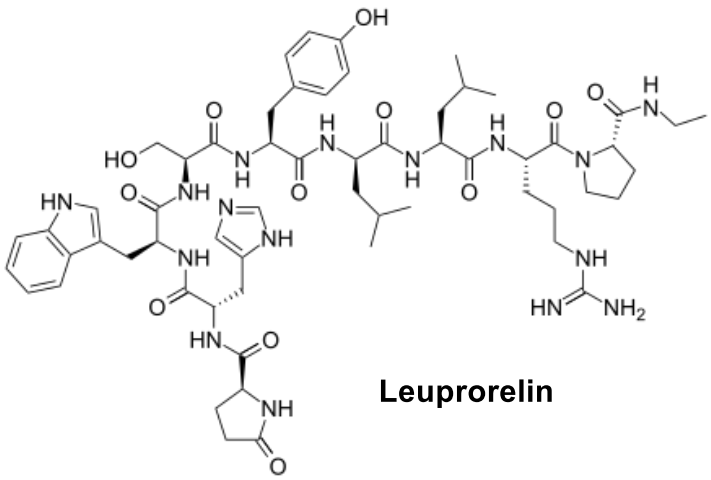
	Small molecule drugs	**Peptide drugs**	Macromolecular drugs
			
Molecular weight	300-500	**1000-10000**	50000-150000
Oral administration	○	○ **or ×**	×
Cell penetration	◎	○	×
Target selectivity	×	○	◎
Synthetic approach	Chemical	**Chemical or Biological**	Biological
Manufacturing cost	Low	**Relatively Low**	High
Possibility to launch	Low	**Relatively High**	Relatively High

Peptide drugs have the advantages of both small molecular drugs and macromolecular drugs!!

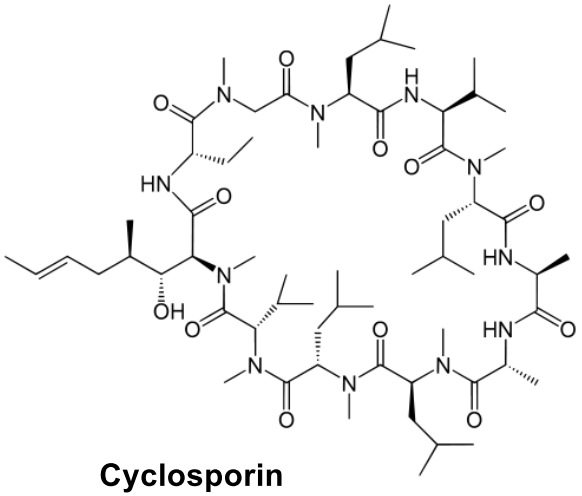
Lau, J. L.; Dunn, M. K. *Bioorg. Med. Chem.*, **2018**, *26*, 2700–2707.

Introduction

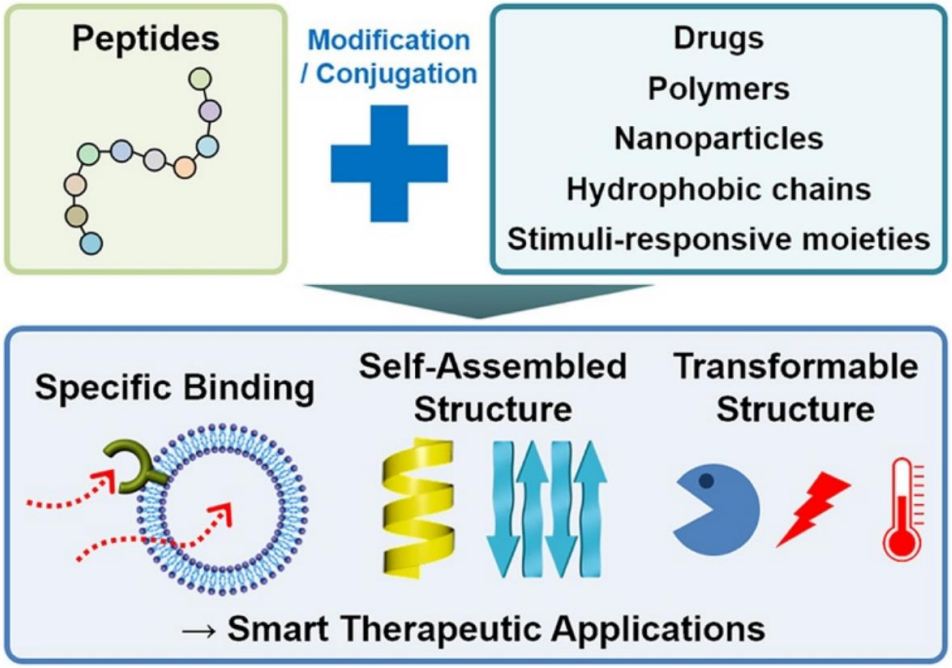
○ Peptide drugs



Leuprorelin



Cyclosporin



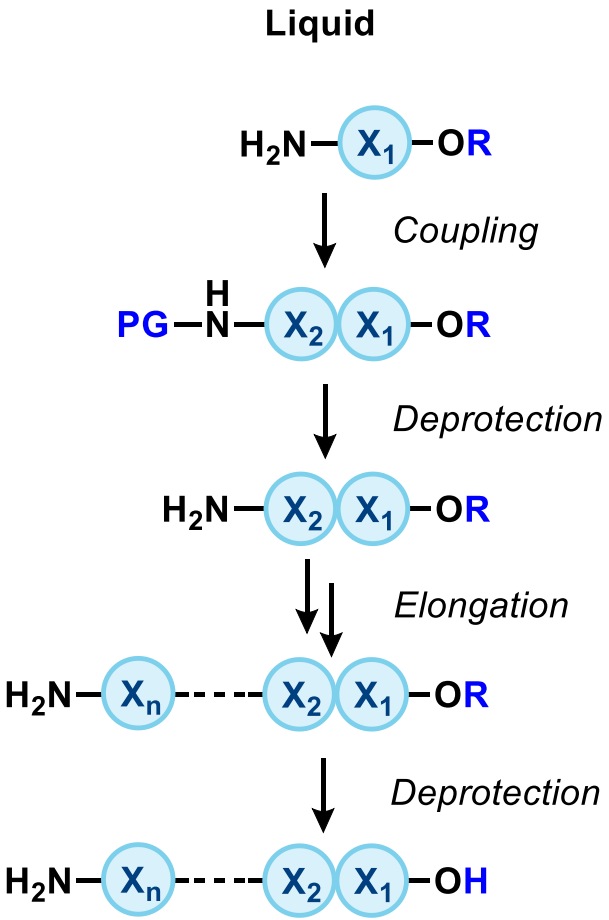
There is a great need for the development of peptide synthesis methods.

Lee, J.; Kim, C. *Macromol. Res.*, **2021**, *29(1)*, 2-14.

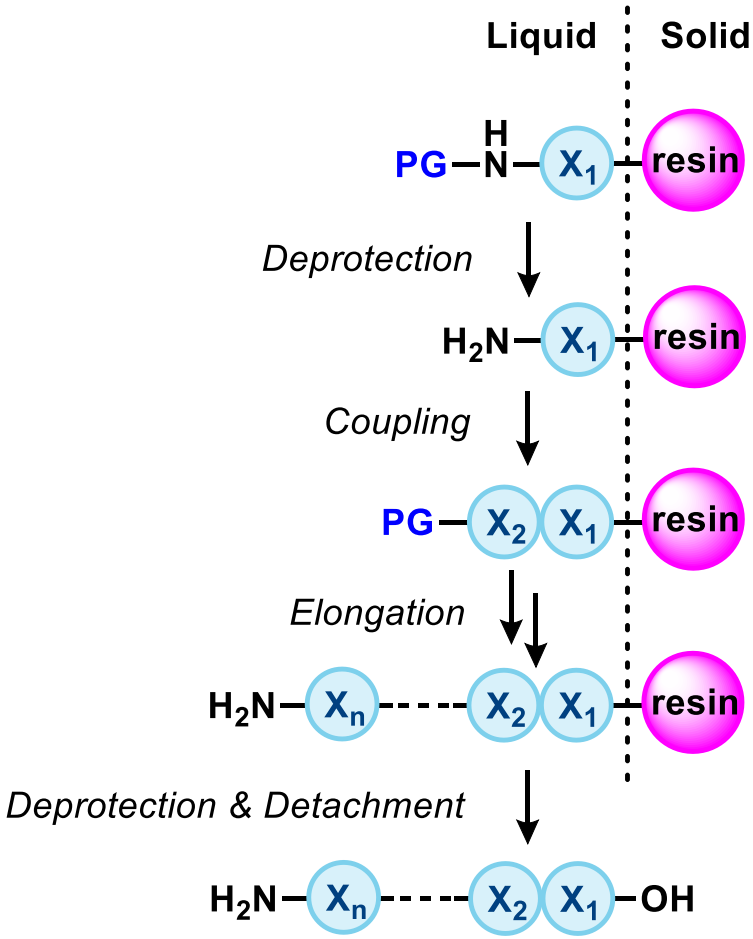
Introduction

○ Peptide synthesis

Liquid phase (LPPS)

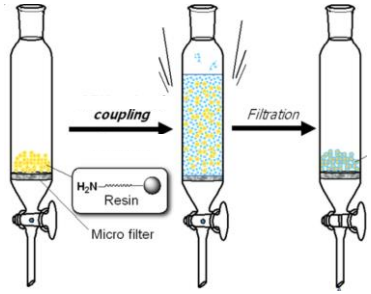
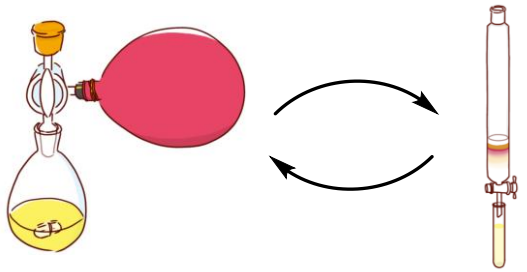


Solid phase (SPPS)



Introduction

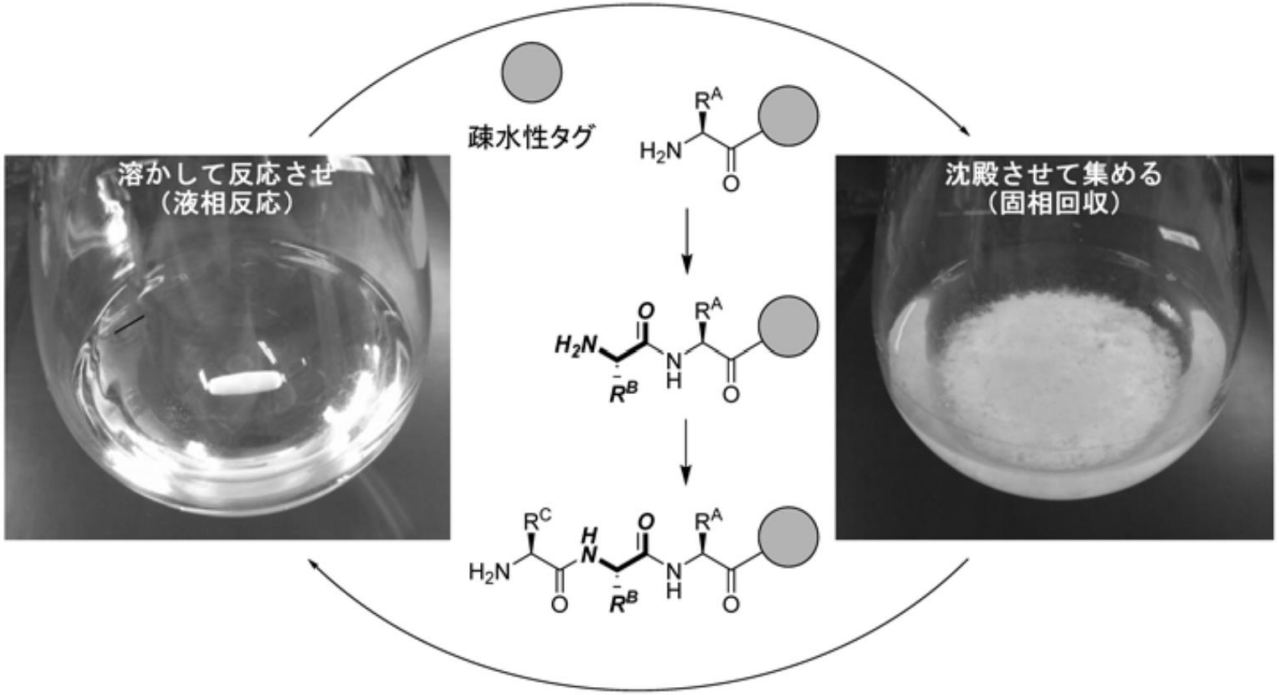
○ Peptide synthesis

	Solid phase (SPPS)	Liquid phase (LPPS)
		
Time	○	×
Solubility	○ (Biphasic)	× (As peptide chain grows)
Purification	○	×
Cost	×	○
Reagent	× (Excess)	○
Scale-up	△	○

New synthetic method of peptide that combines the advantages of SPPS and LPPS is needed.

Introduction

○ Tag chemistry

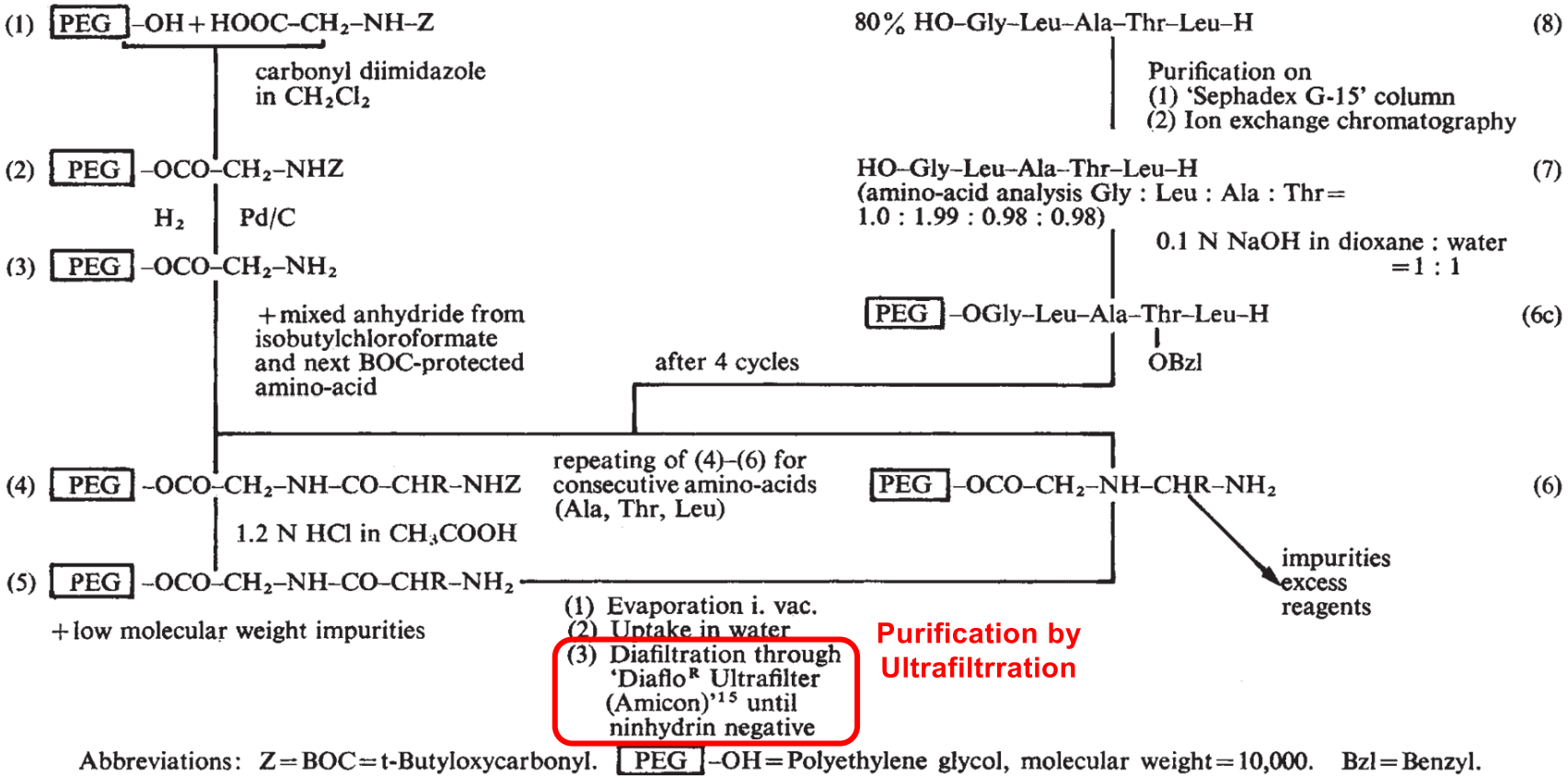


Requirements

- C-terminal protection (Orthogonal to N-terminal protecting group)
- Improve solubility in organic solvent
- Purification without complicated process

Introduction

○ Classical PEG tag



Bayer, E.; Mutter, M. *Nature*, **1972**, *237*, 2-14.

Introduction

○ Classical PEG tag

Problems

- Difficulty of reaction analysis
- Complexity of purification (want it even easier!)
- Loading efficiency of amino acid

Development of small-molecular-based hydrophobic tag

Contents

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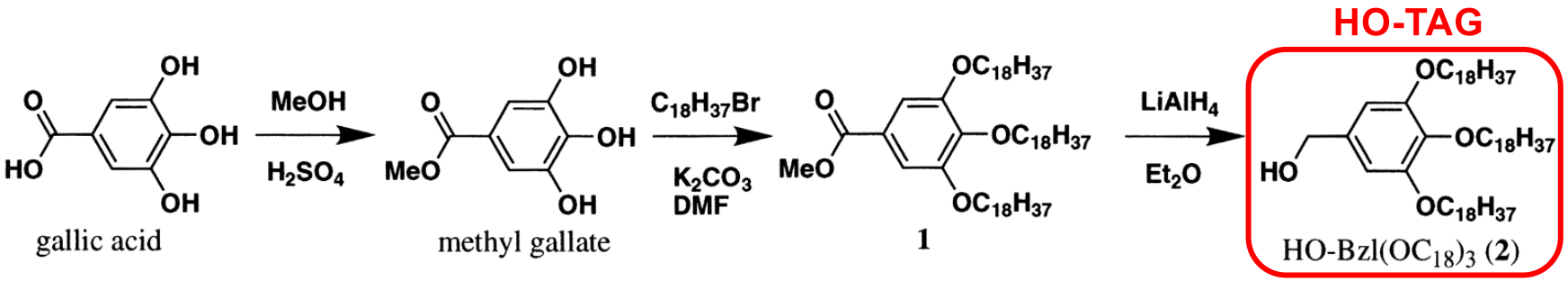
4. Summary

Benzyl-type tag

○ 3,4,5-Tris(octadecyloxy)benzyl alcohol

Concept

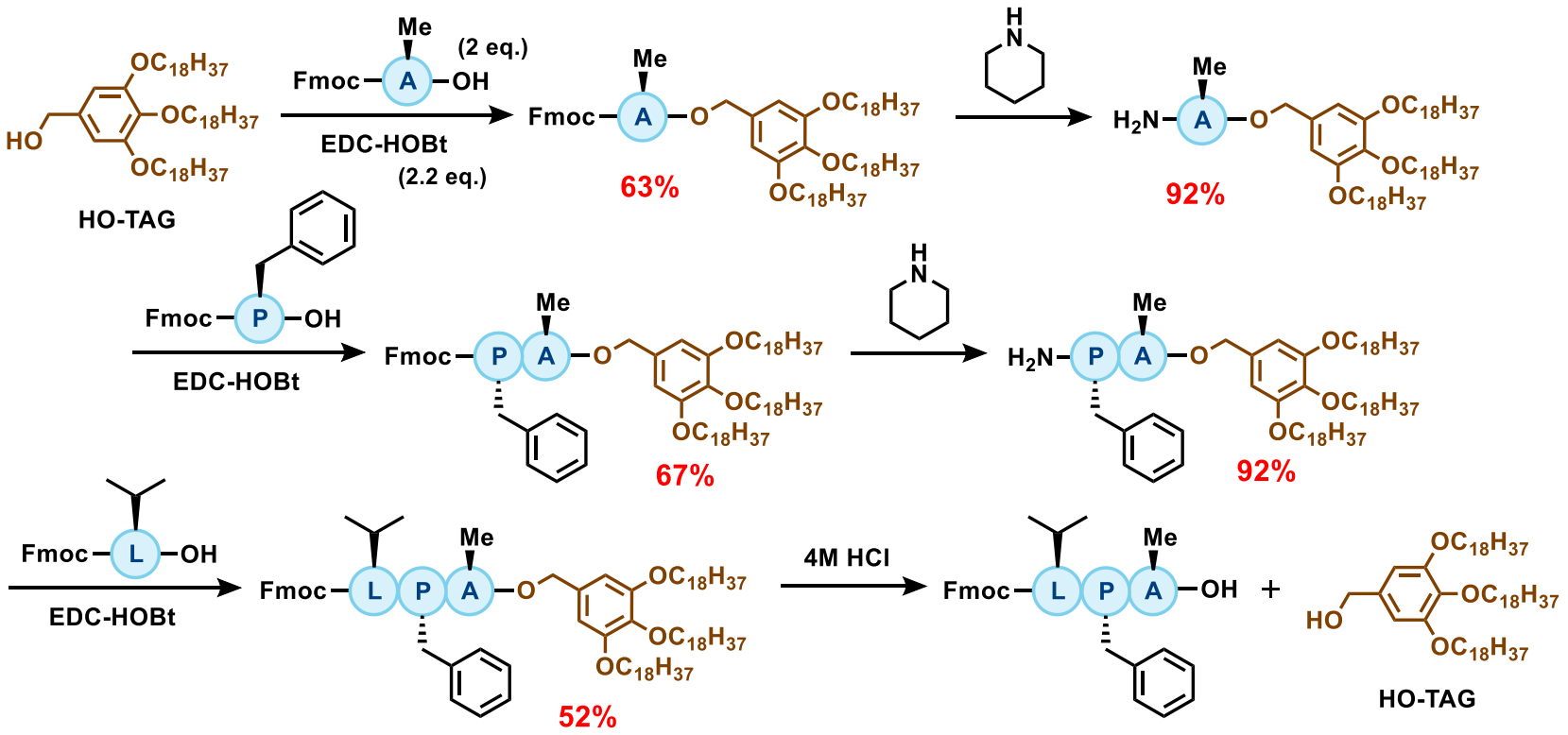
- Application to Fmoc strategy
- Well-defined molecular structure
- Easy purification (size-exclusion chromatography)



Tamiaki, H.; Obata, T.; Azefu, Y.; Toma, K. *Bull. Chem. Soc. Jpn.*, **2001**, *74*, 733-738.

Benzyl-type tag

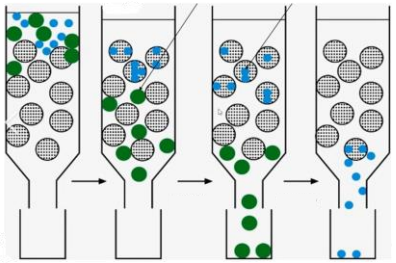
○ 3,4,5-Tris(octadecyloxy)benzyl alcohol



Purification

Coupling : Gel-filtration chromatography

Deprotection : Washing with methanol



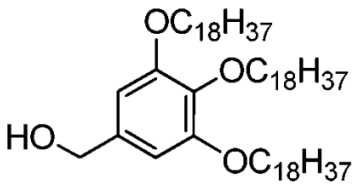
Tamiaki, H.; Obata, T.; Azefu, Y.; Toma, K. *Bull. Chem. Soc. Jpn.*, **2001**, *74*, 733-738.

Benzyl-type tag

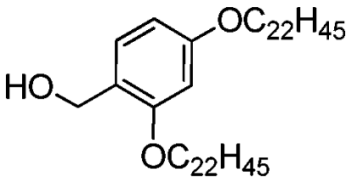
○ Didocosyloxybenzyl alcohol

Concept

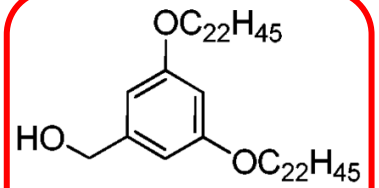
- Easier purification (precipitation)
- Tolerance of protecting group (Fmoc and Boc)



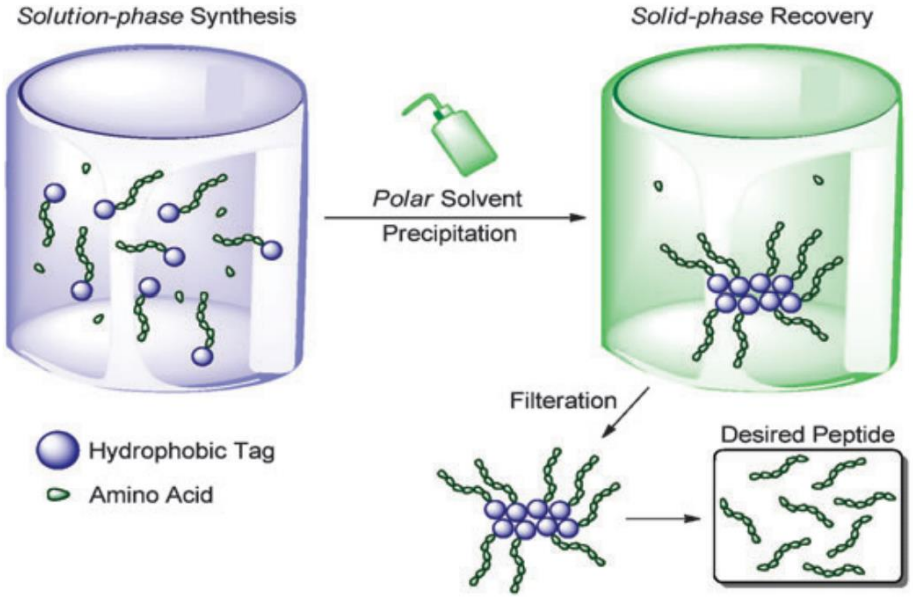
1 (HO-**TAGa**)



2 (HO-**TAGb**)



3 (HO-**TAGc**)

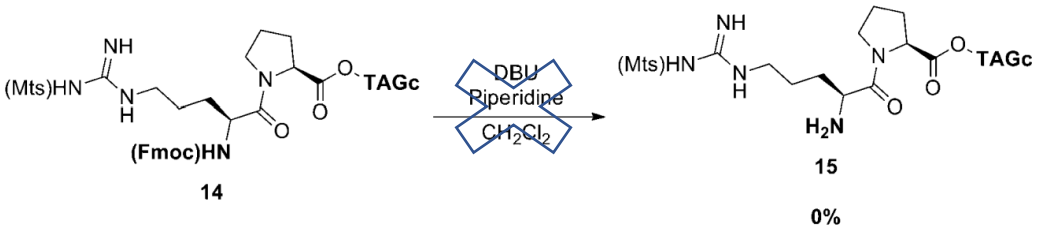


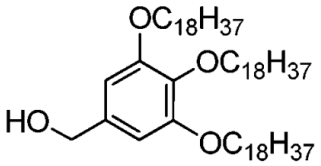
Tana, G.; Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. *Chem. Commun.*, **2002**, *237*, 2-14.

Benzyl-type tag

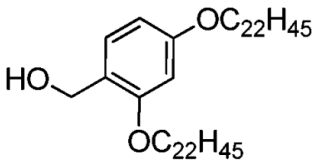
○ Didocosyloxybenzyl alcohol

C-terminal proline ⇨ Risk of diketopiperazine formation

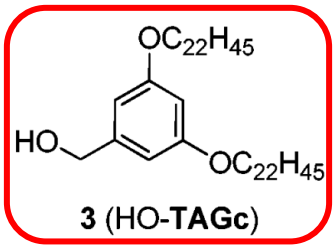




1 (HO-**TAGa**)



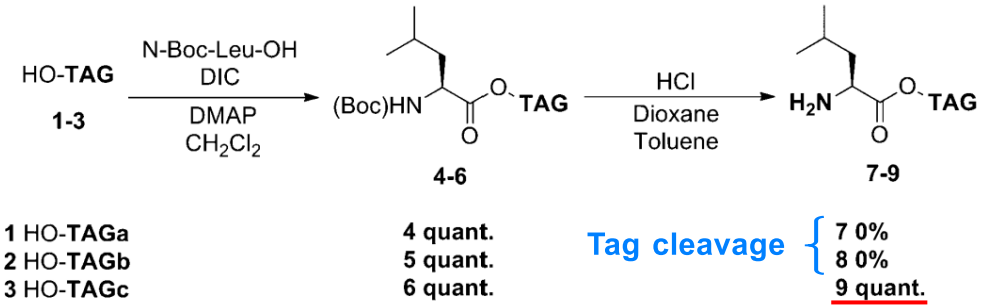
2 (HO-**TAGb**)



3 (HO-**TAGc**)

Benzyl type protection is weak to acid condition

⇒ Optimization of substituent group

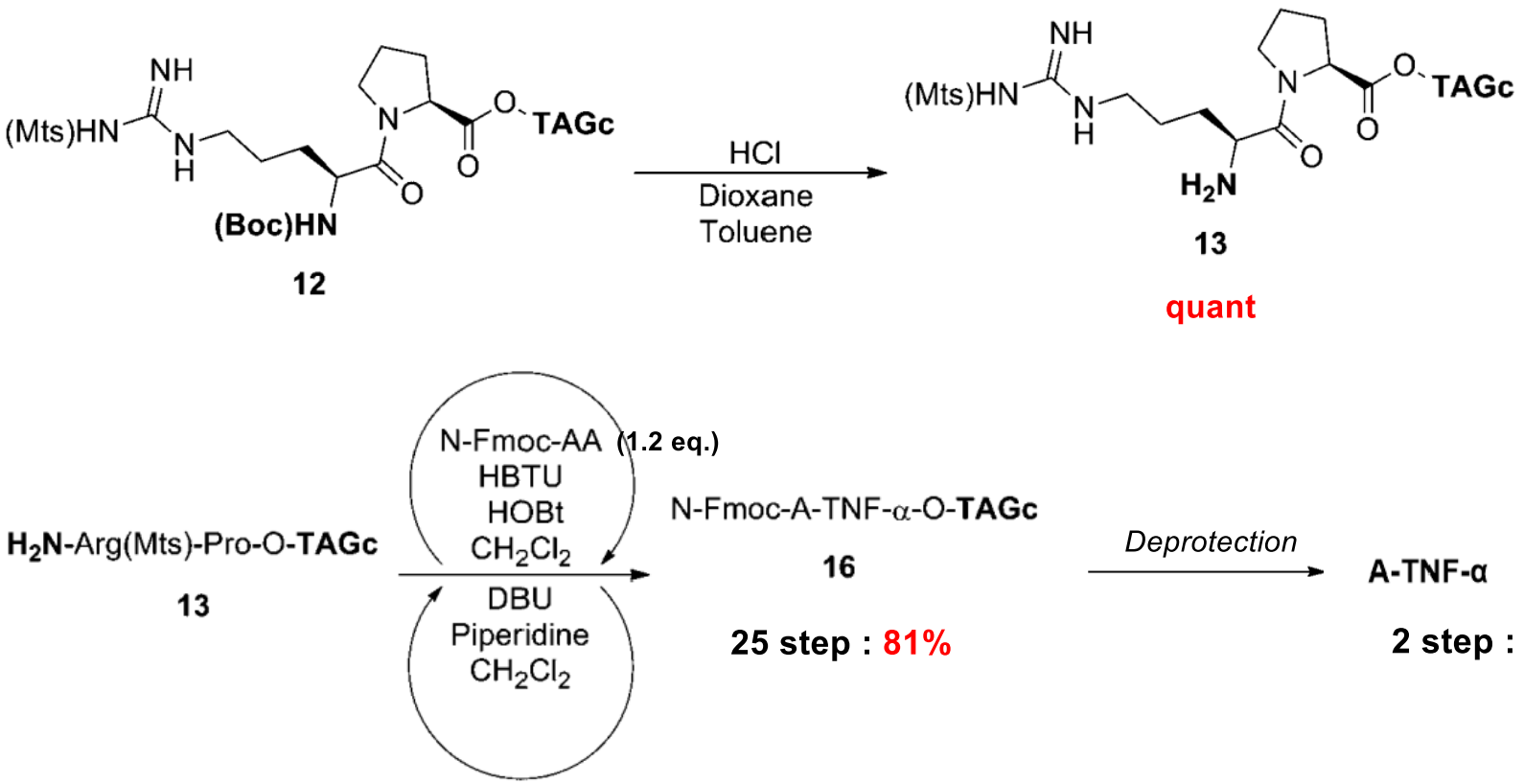


Tana, G.; Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. *Chem. Commun.*, **2002**, *237*, 2-14.

Benzyl-type tag

○ Didocosyloxybenzyl alcohol

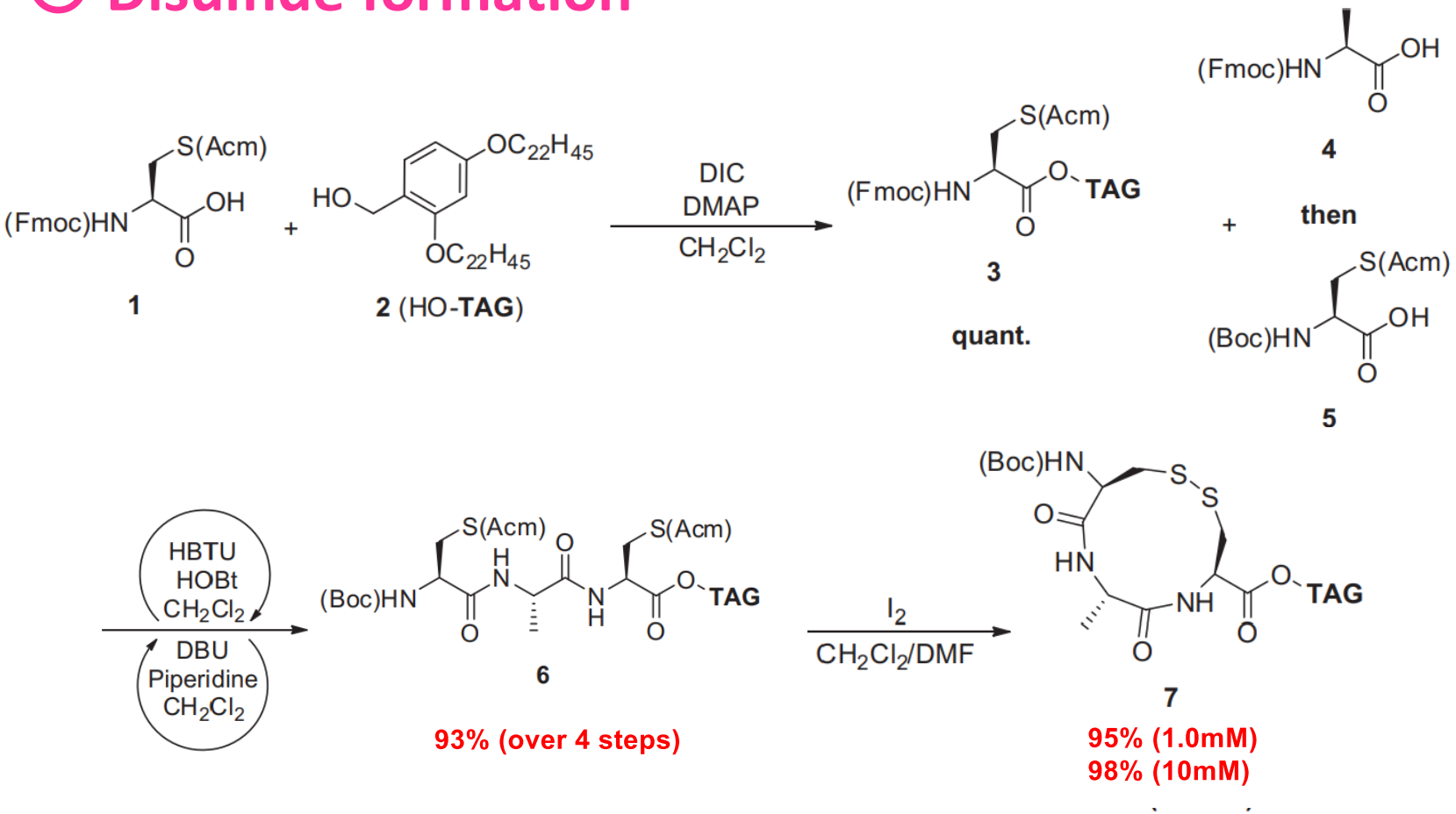
Synthesis of A-TNF-α (H-Asp-Phe-Leu-Pro-His-Tyr-Lys-Asn-Thr-Ser-Leu-Gly-His-Arg-Pro-OH)



Tana, G.; Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. *Chem. Commun.*, **2002**, *237*, 2-14.

Benzyl-type tag

○ Disulfide formation



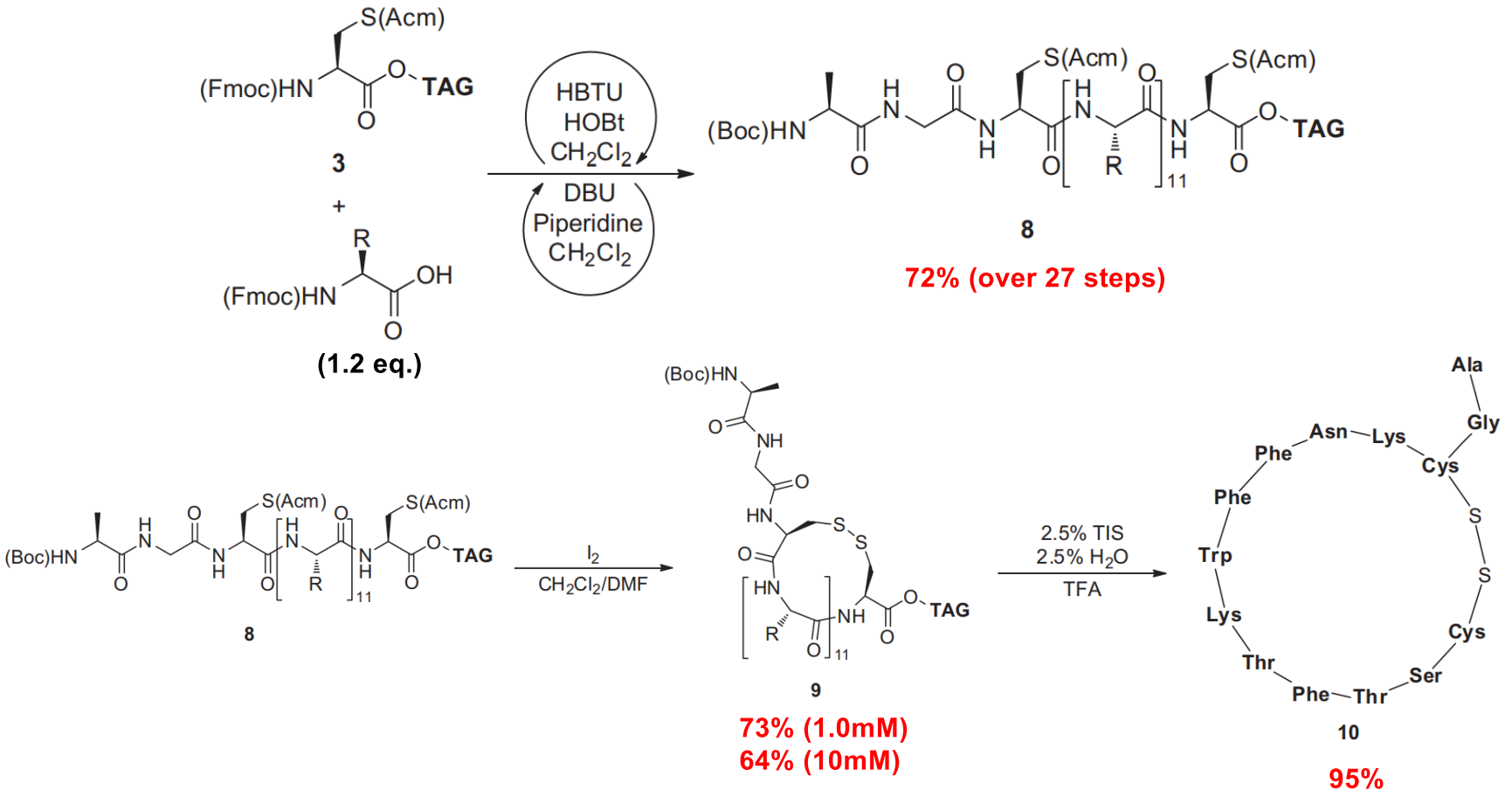
S-S bond was formed efficiently even at high concentrations!

Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 4476-4479.

Benzyl-type tag

○ Disulfide formation

Synthesis of Somatostatin

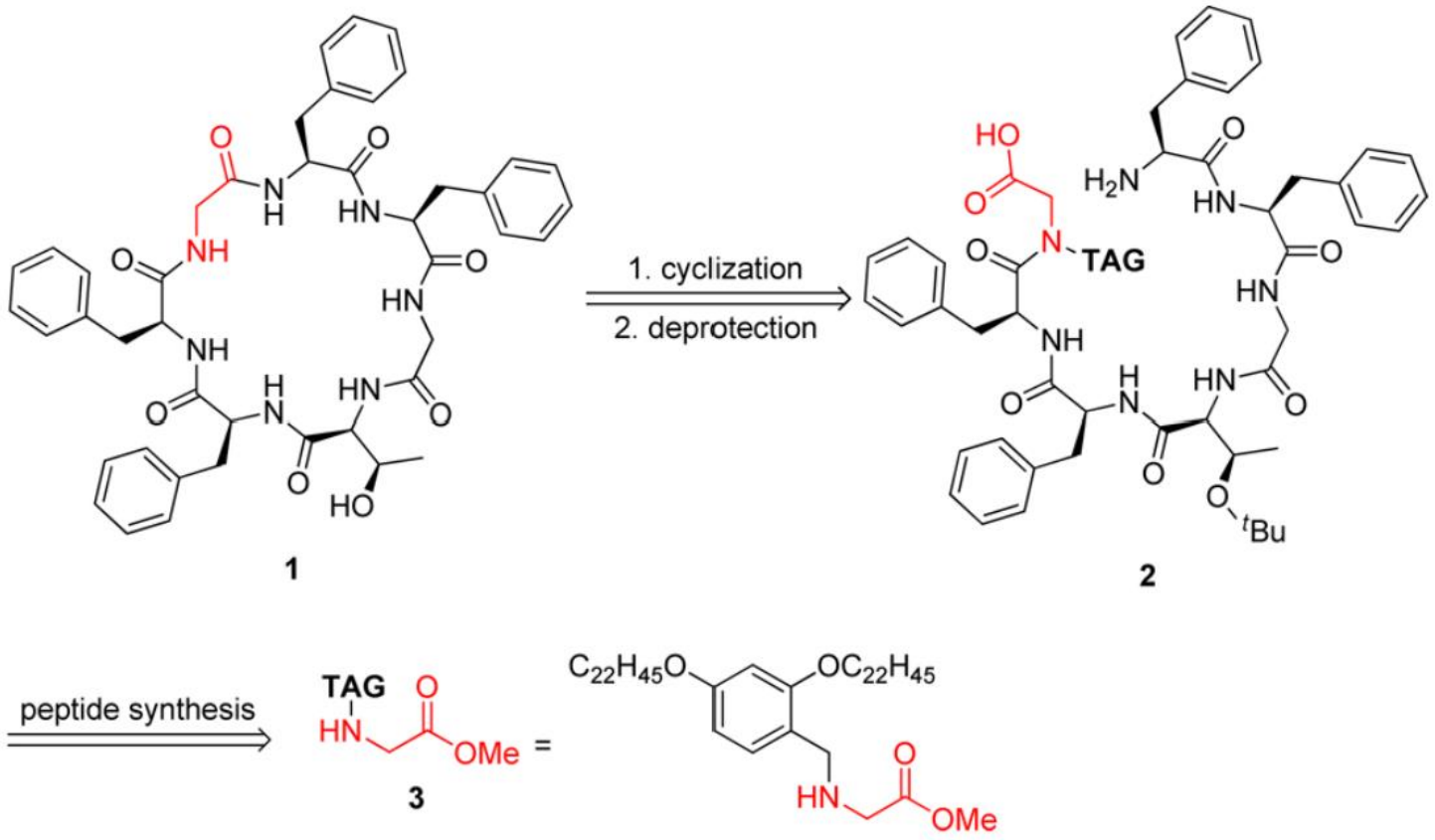


Kitada, S.; Fujita, S.; Okada, Y.; Kim, S.; Chiba, K. *Bioorg. Med. Chem. Lett.*, **2011**, *21*, 4476-4479.

Benzyl-type tag

○ Head-to-Tail cyclization

Retrosynthetic strategy for Mahafacyclin B

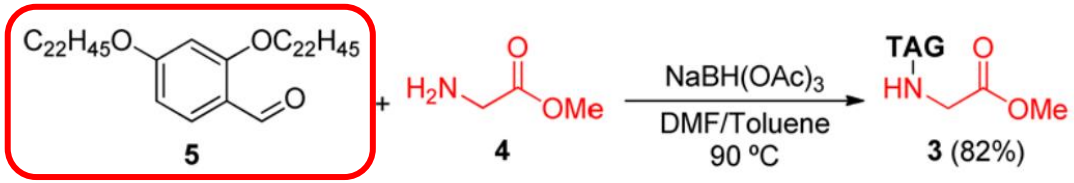


Fujita, Y.; Fujita, S.; Okada, Y.; Chiba, K. *Org. Lett.*, **2013**, *15(6)*, 1155-1157.

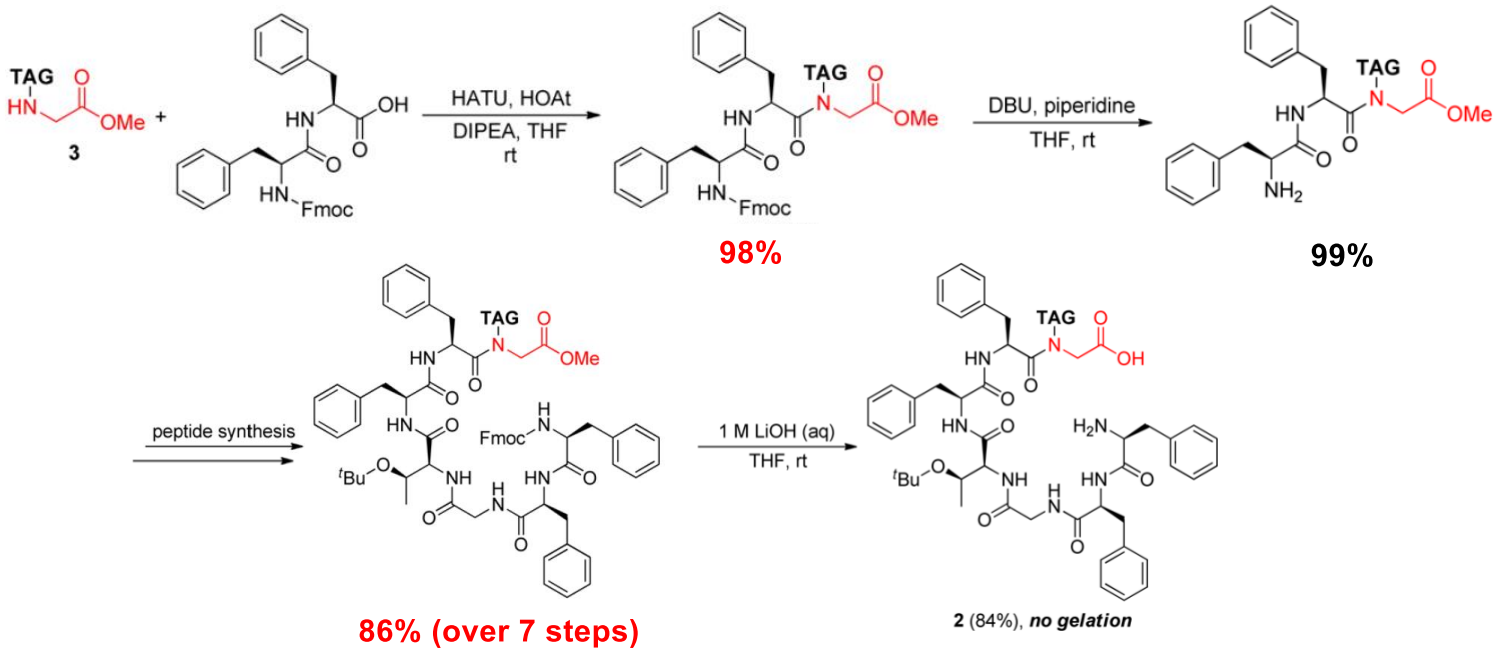
Benzyl-type tag

○ Head-to-Tail cyclization

Synthesis of tagged amino acid



Synthesis of linear peptide

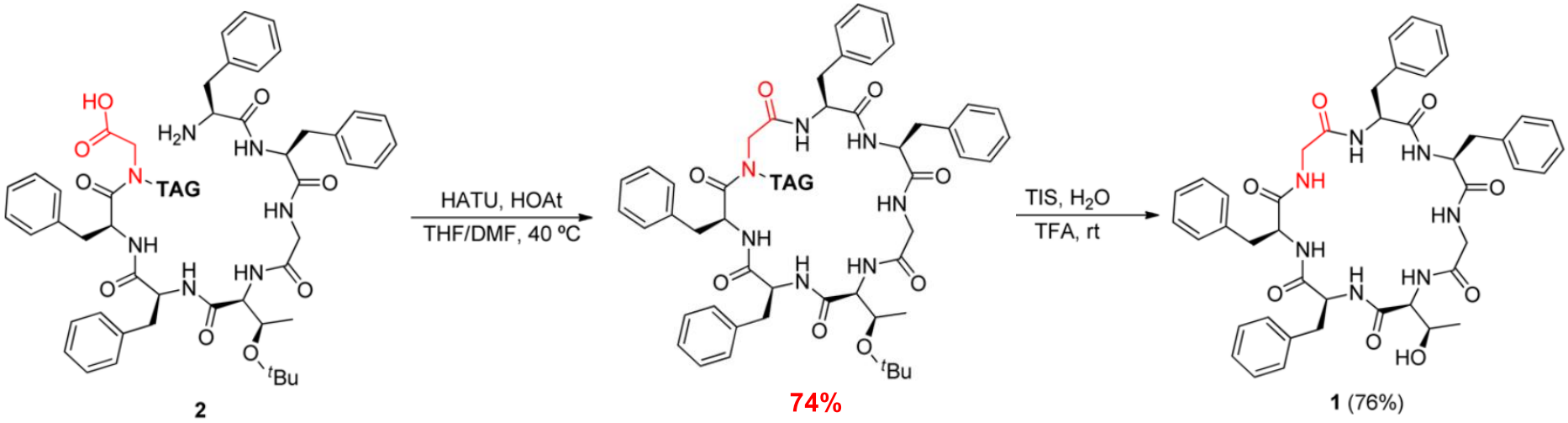


Fujita, Y.; Fujita, S.; Okada, Y.; Chiba, K. *Org. Lett.*, **2013**, *15(6)*, 1155-1157.

Benzyl-type tag

○ Head-to-Tail cyclization

Cyclization



2

74%

1 (76%)

No cyclodimerization

No gelation

Various peptides can be synthesized with high efficiency, purity, and yield by controlling solubility and reactivity with hydrophobic tags.

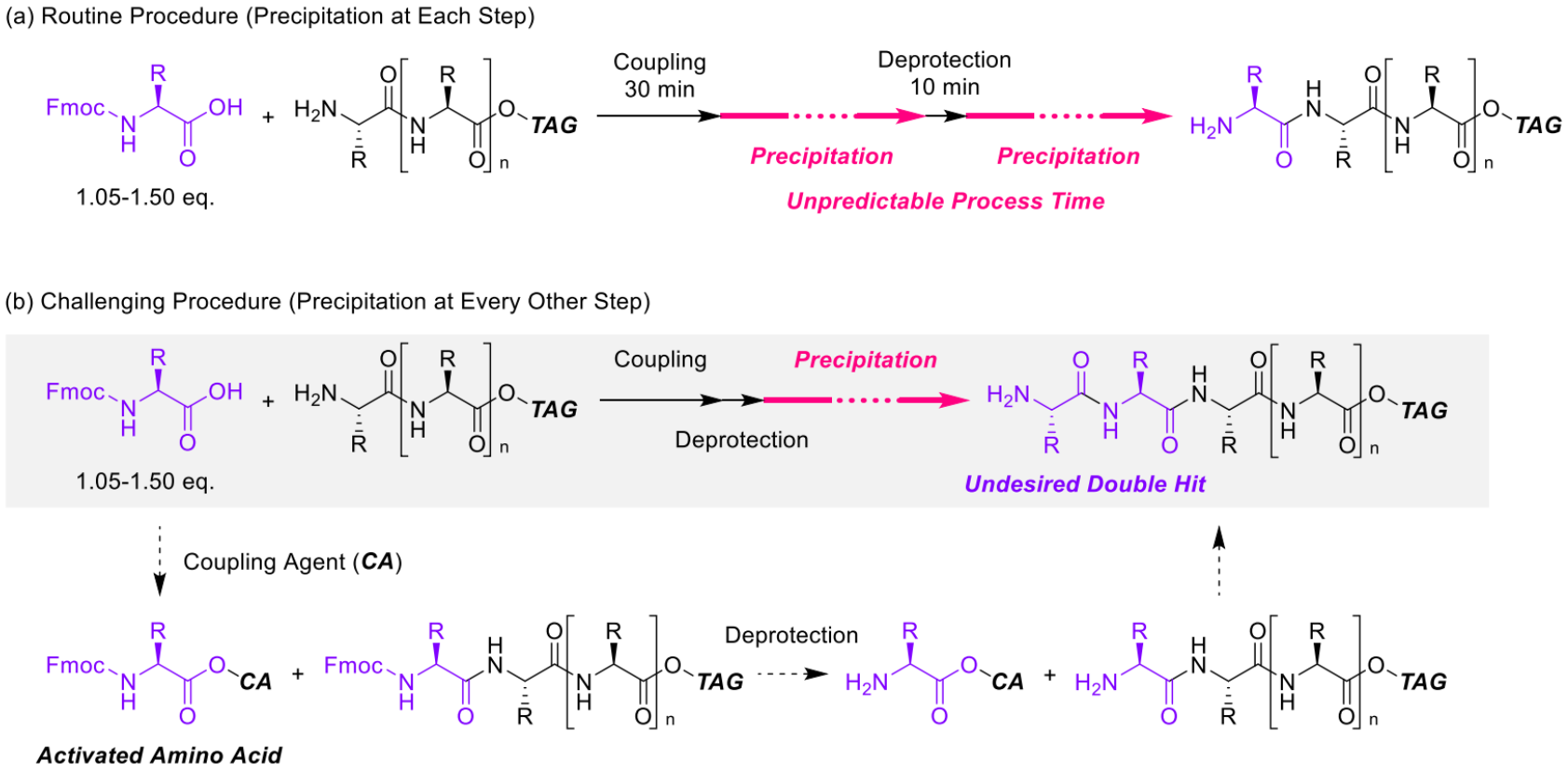
Fujita, Y.; Fujita, S.; Okada, Y.; Chiba, K. *Org. Lett.*, **2013**, *15(6)*, 1155-1157.

Benzyl-type tag

○ Improved process

Precipitation at every step ⇨ ○ Prevent "Double Hit"

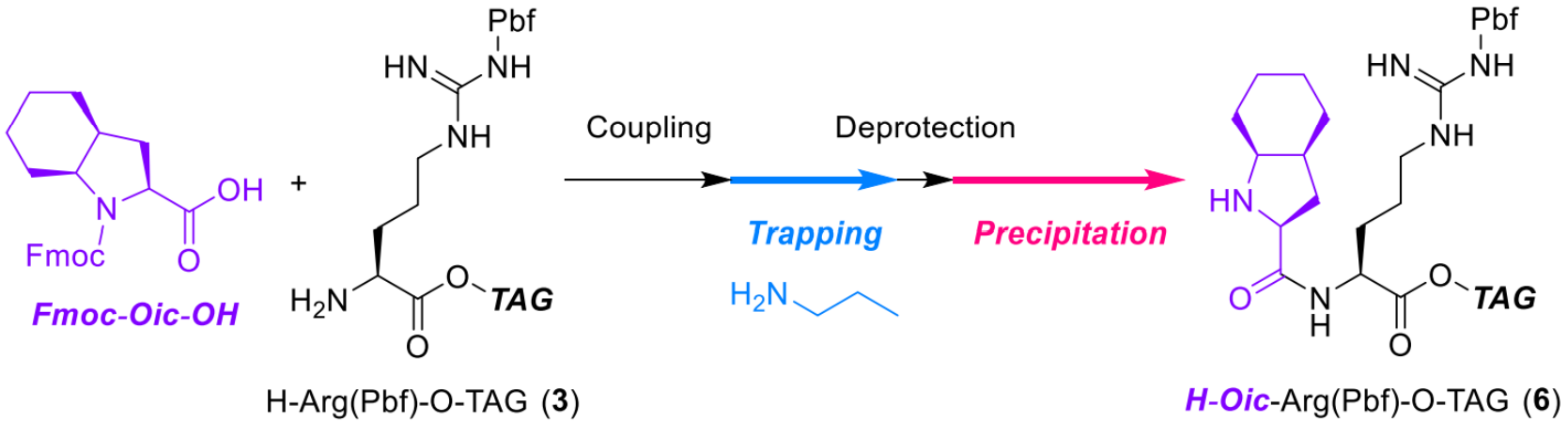
× Excessive use of solvents



Okada, Y. *et al. Org. Process. Res. Dev.* **2019**, *23*, 2576-2581.

Benzyl-type tag

○ Improved process



Trapping remaining Fmoc-AA-OH using sacrificial primary amine

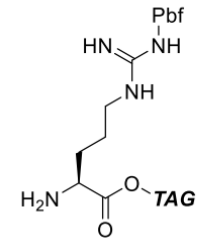
⇒ Reduce the number of precipitation steps

Okada, Y. *et al. Org. Process. Res. Dev.* **2019**, *23*, 2576-2581.

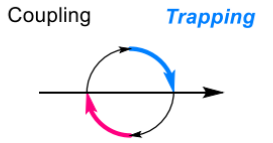
Benzyl-type tag

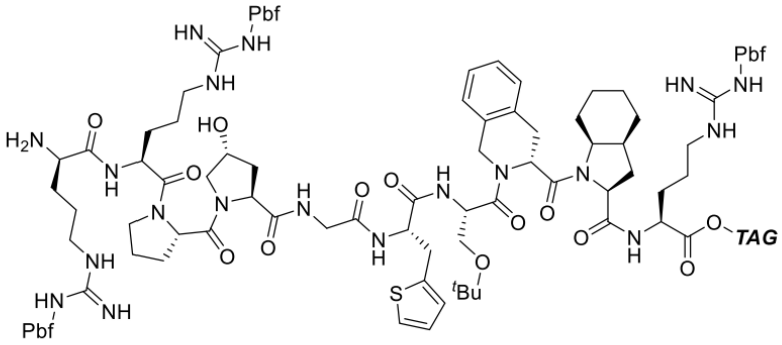
○ Improved process

Large scale synthesis of Icatibant acetate



H-Arg(Pbf)-O-TAG (**3**)

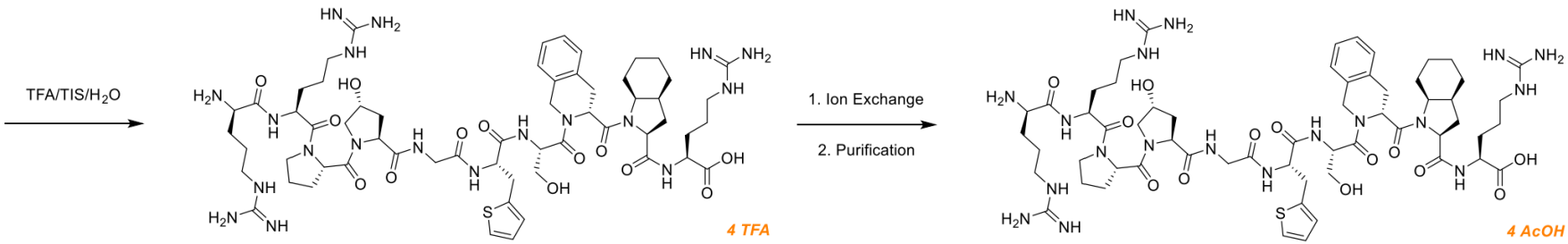




H-D-Arg(Pbf)-Arg(Pbf)-Pro-Hyp-Gly-Thi-Ser(^{t}Bu)-D-Tic-Oic-Arg(Pbf)-O-TAG (**7**)

71% (over 18 steps)

20 L Flask (for 100 gram scale)



H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH (**8**)
82% (*purity: 86%*)

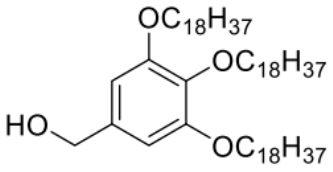
H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH (**2**)
65% (*purity: >99%*)

169 g , 109 mmol

Okada, Y. *et al. Org. Process. Res. Dev.* **2019**, *23*, 2576-2581.

Benzyl-type tag

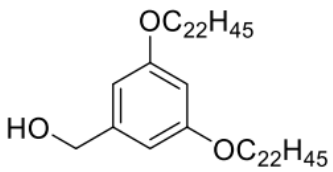
○ Short summary



3,4,5-Tri-Substituted (**1**)[8]

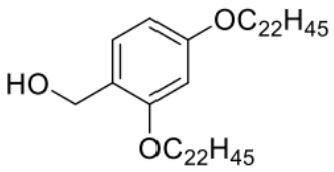
"Standard"

HO–*TAG*



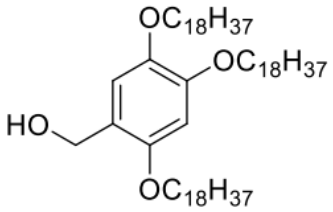
3,5-Di-Substituted[13a]

Acid Resistant
Not Colorimetric
Boc-chem. Compatible



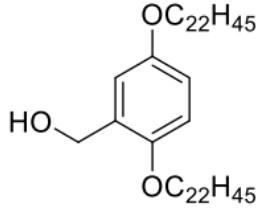
2,4-Di-Substituted[13b]

Readily Cleavable
Colorimetric (Purple)
Fmoc-chem. Only



2,4,5-Tri-Substituted[13c]

Readily Cleavable
Colorimetric (Blue)
Fmoc-chem. Only



2,5-Di-Substituted[13d]

Acid Resistant
Fluorometric
Boc-chem. Compatible

A variety of benzyl-type tags with different properties have greatly expanded the scope of peptide compound synthesis.

Okada, Y. *et al. Org. Process. Res. Dev.* **2019**, *23*, 2576-2581.

Contents

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2. Benzyl-type tag

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AJIPHASE®

○ Fluorene-derived tag

Concept

- Fmoc strategy
- Prevent diketopiperazine formation
- Productization

Benzyl type tag

Risk of diketopiperazine formation

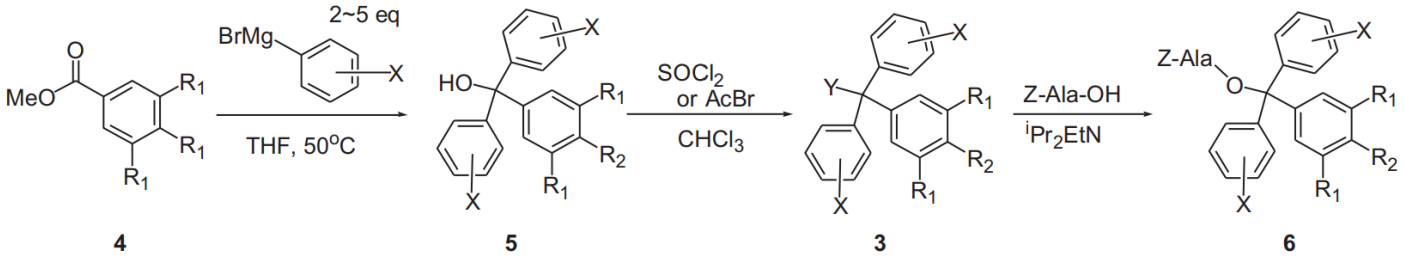
Trityl structure (SPPS type)

Takahashi, D.; Yamamoto, T. *Tetrahedron. Lett.* **2012**, *53*, 1936-1939.

AJIPHASE®

○ Fluorene-derived tag

Development of trityl type tag



Anchor support compound	R1	R2	R3	X	Y	Loading yield[a] (%) (Z-Ala esterification)		Degradation of **6**[b] (%)	
								MeOH (40 °C)	10% AcOH/$CHCl_3$
3a[c]	$OC_{18}H_{37}$	$OC_{18}H_{37}$	$OC_{18}H_{37}$	4-Cl	Cl	97	Degradation	69	100
3b[c]	$OC_{22}H_{45}$	H	$OC_{22}H_{45}$	4-Cl	Cl	89	Degradation	19	74
3c[d]	$OC_{18}H_{37}$	$OC_{18}H_{37}$	$OC_{18}H_{37}$	3,5-F	Cl	95	Degradation	4	63
3d[d]	$OC_{22}H_{45}$	H	$OC_{22}H_{45}$	3,5-F	Cl	0[e]	Loading failure	Not tested	Not tested
3e[d]	$OC_{22}H_{45}$	H	$OC_{22}H_{45}$	3,5-CF_3	Br	0[e]	Loading failure	Not tested	Not tested

Achieving a balance between the stability of the ester bond and a high loading yield using the trityl-type tag was difficult…

Takahashi, D.; Yamamoto, T. *Tetrahedron. Lett.* **2012**, *53*, 1936-1939.

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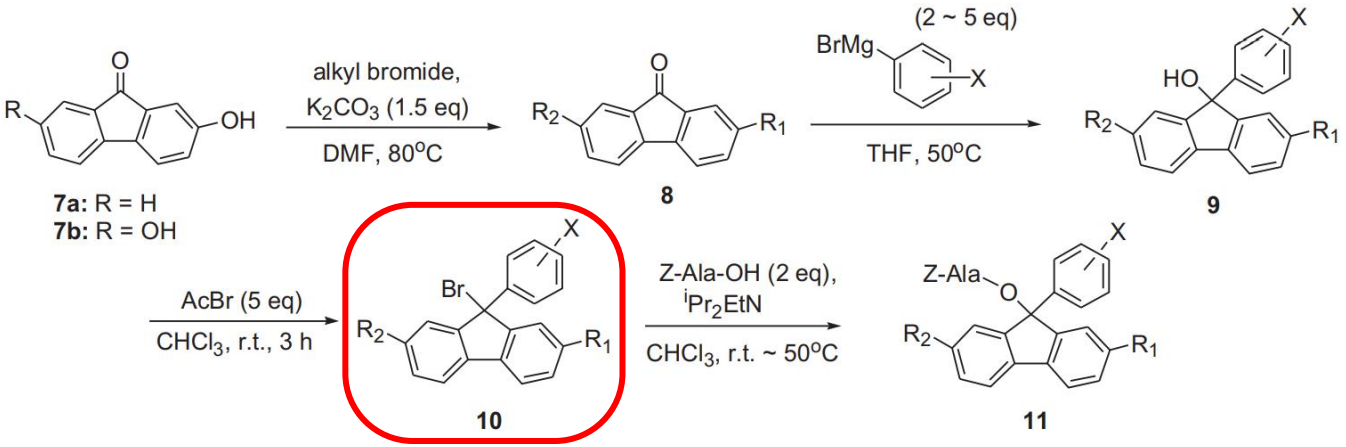
○ Fluorene-derived tag

Development of fluorene-type tag



Anchor support compound	R_1	R_2	X	Loading yield[a] (%) (Z-Ala esterification)	Degradation of **11**[b] (%)	
					MeOH (40 °C)	10% AcOH/$CHCl_3$
10a	$OC_{22}H_{45}$	$OC_{22}H_{45}$	H	96	1.8	3.2
10b	$OC_{22}H_{45}$	$OC_{22}H_{45}$	4-Cl	98	1.0	3.0
10c	$OC_{22}H_{45}$	$OC_{22}H_{45}$	3-F	93	0.3	0.3
10d	$OC_{22}H_{45}$	$OC_{22}H_{45}$	3-CF_3	88	Not detected	Not detected
10e	$OC_{12}H_{15}$ $OC_{22}H_{45}$	H	4-Cl	92	0.4	0.8
10f	$OC_{12}H_{15}$ $OC_{22}H_{45}$	H	3-F	95	Not detected	Not detected
10g	$OC_{12}H_{15}$ $OC_{22}H_{45}$	H	3-CF_3	85	Not tested	Not tested

Fluorene-derived tag (10f) could have the performance for use as a tag!

Takahashi, D.; Yamamoto, T. *Tetrahedron. Lett.* **2012**, *53*, 1936-1939.

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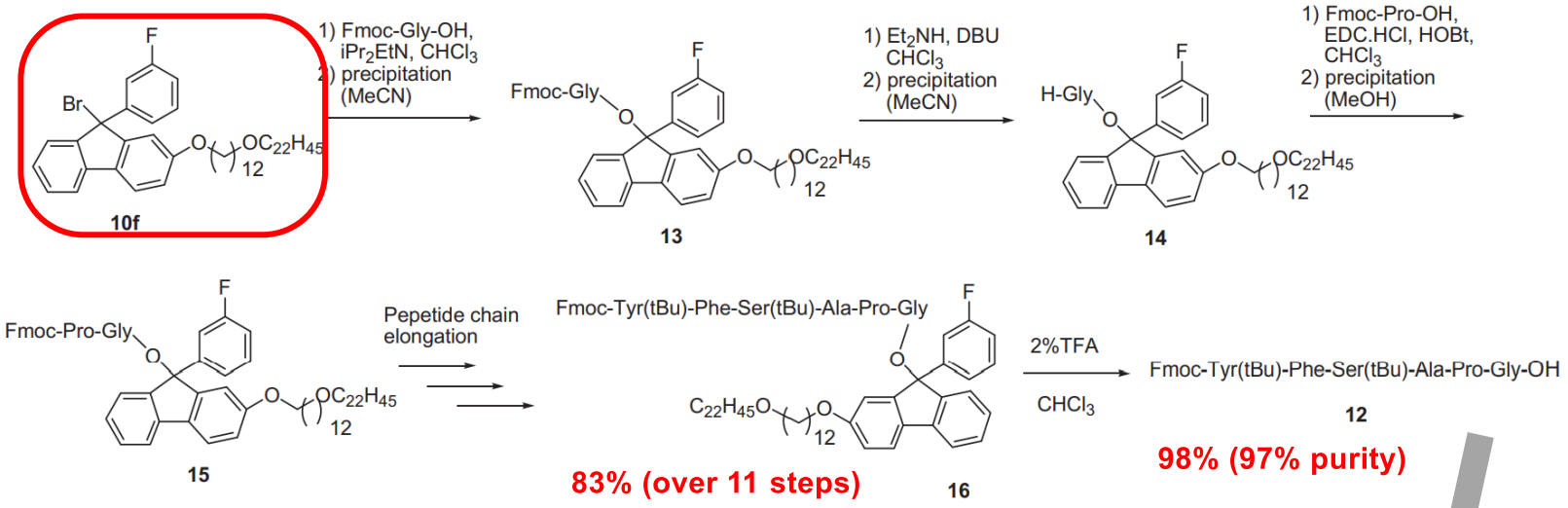
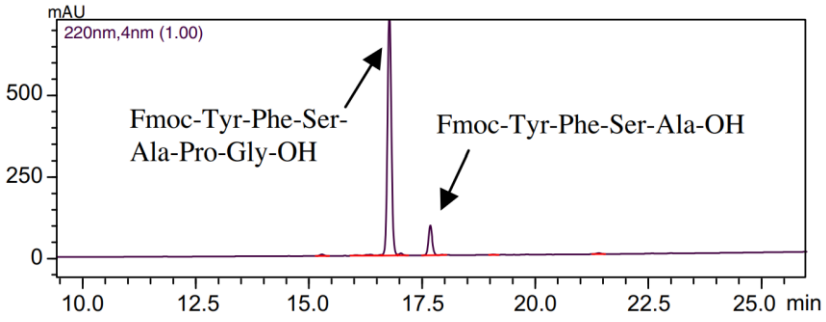
○ Fluorene-derived tag

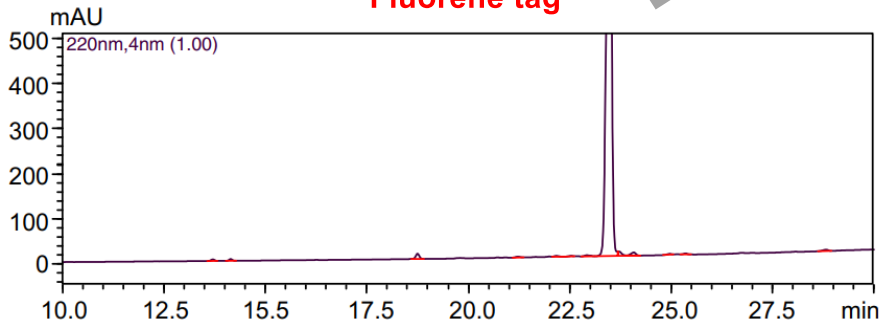
Synthesis of peptide without diketopiperazine formation



Conventional benzyl tag



Fluorene tag



Takahashi, D.; Yamamoto, T. *Tetrahedron. Lett.* **2012**, *53*, 1936-1939.

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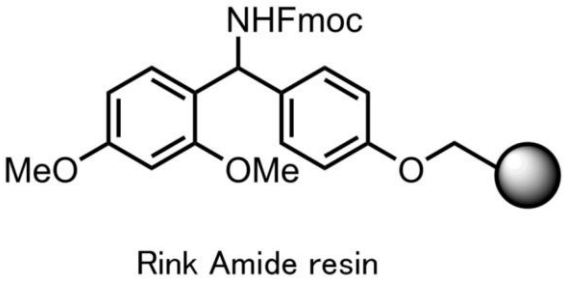
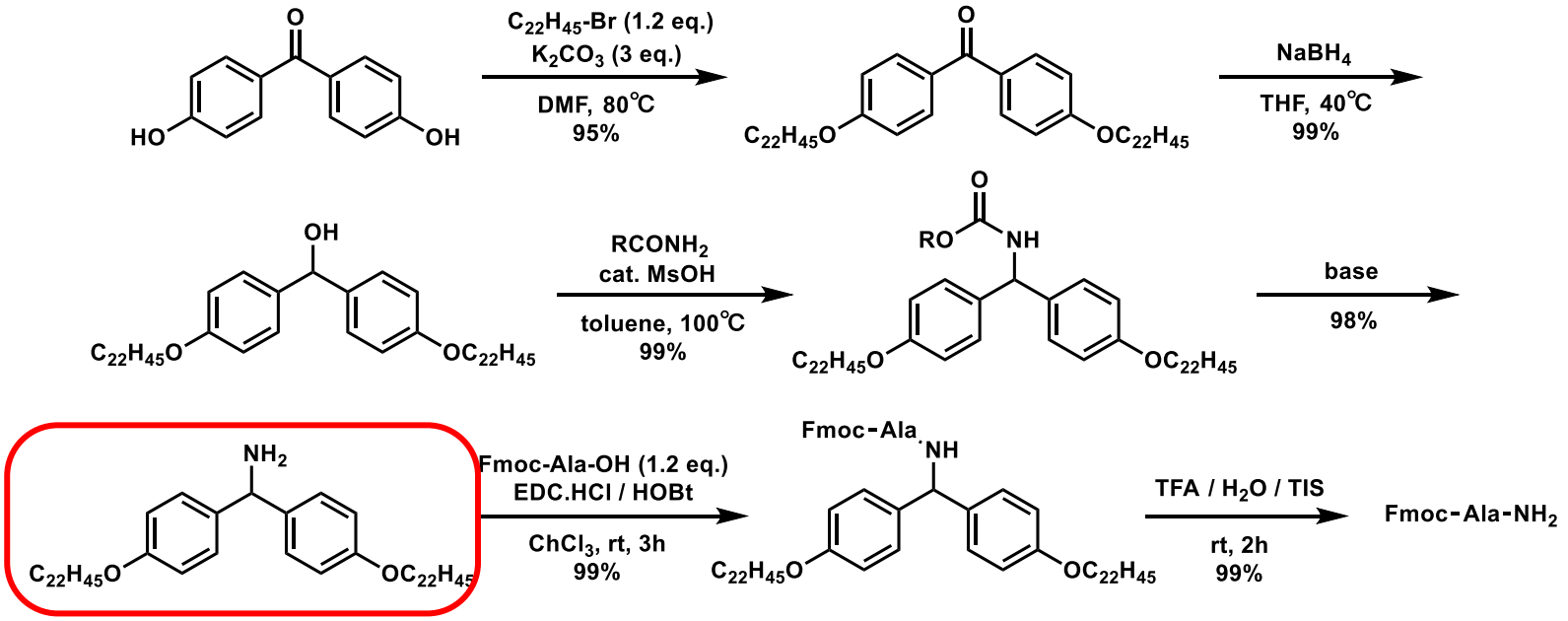
○ Diphenylmethyl-derived tag

Concept

- Fmoc strategy
- Synthesis of C-terminal amide peptide



Rink Amide resin



Takahashi, D.; Yamamoto, T. *Org. Lett.* **2012**, *14(17)*, 4514-4517.

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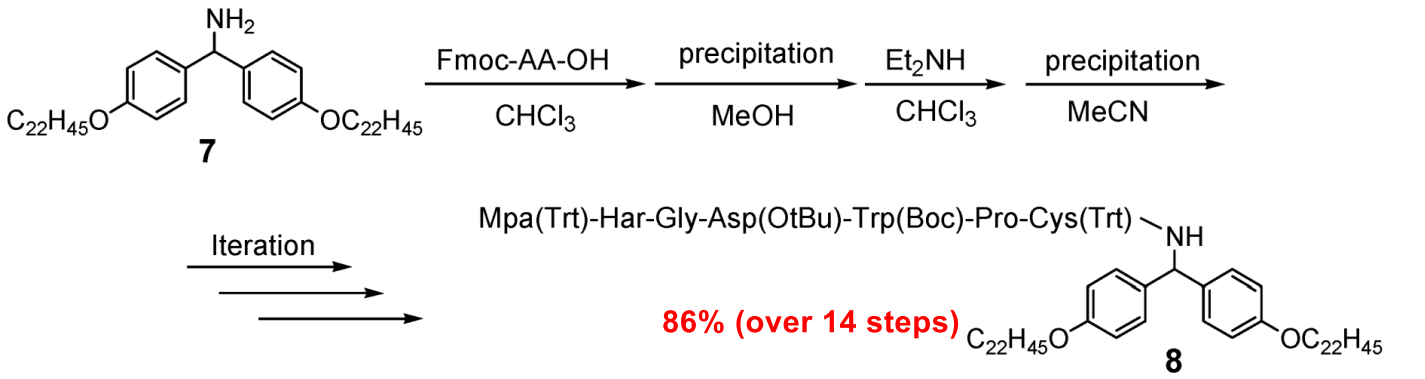
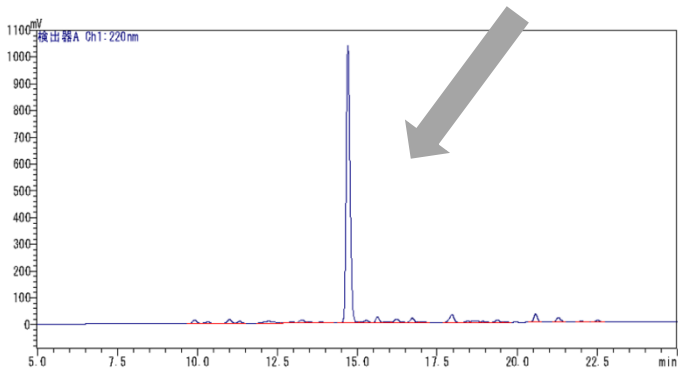
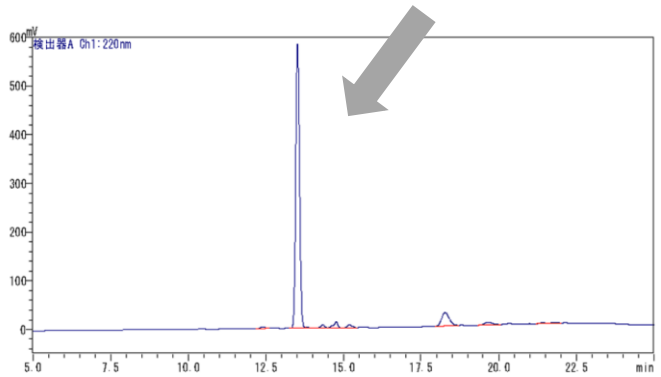
○ Diphenylmethyl-derived tag

Synthesis of Eptifibatide







Takahashi, D.; Yamamoto, T. *Org. Lett.* **2012**, *14(17)*, 4514-4517.

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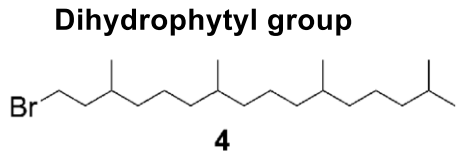
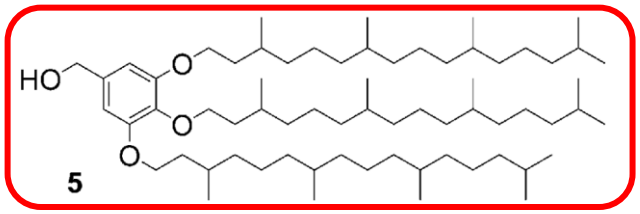
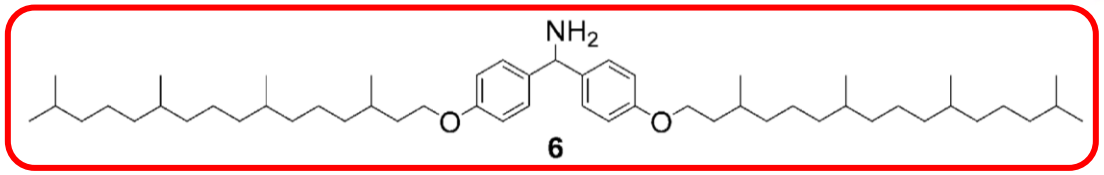
○ Further development

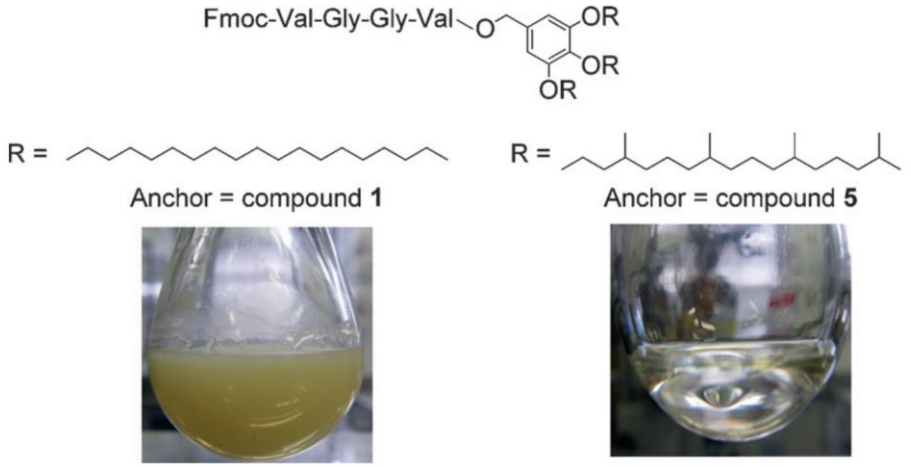
Concept

- Higher solubility in organic solvents
- More efficient washing process (Extraction)
- Longer peptide and hydrophobic peptide synthesis









Takahashi, D.; Inomata, T.; Fukui, T. *Angew. Chem. Int. Ed.* **2017**, *56*, 7803-7807.

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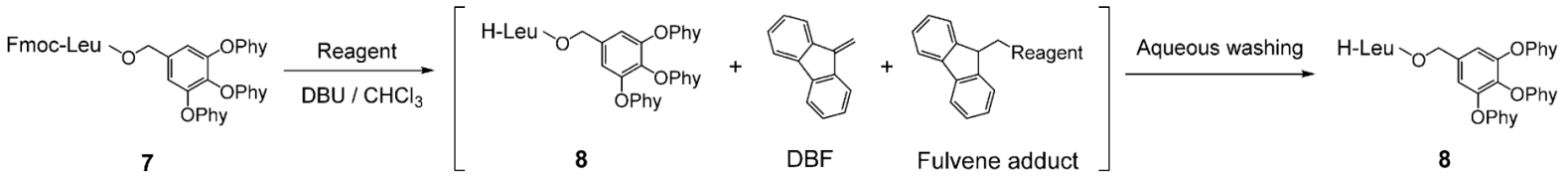
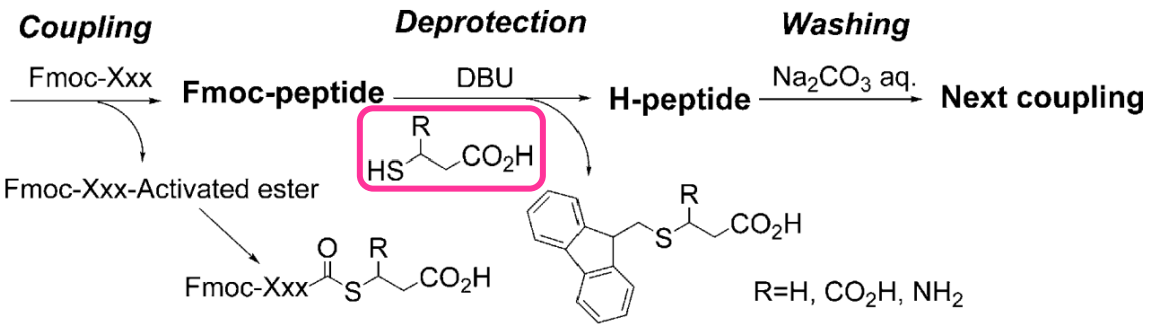
○ Further development



Reagent (equiv)	DBU [equiv]	Solvent	DBF/fulvene adduct	Washing solvent	Layer separability[a]	Removal rate [%]
diethylenetriamine (30)	0	$CHCl_3$	35:65	HCl aq.	–	48
piperidine (5)	3	$CHCl_3$	31:69	HCl aq.	–	2
piperidine (5)	3	CPME	8:92	HCl aq.	–	0
Mpa (3)	6	CPME	3:97	Na_2CO_3 aq.	+	100
Mpa (3)	6	$CHCl_3$	1:99	Na_2CO_3 aq.	+	30
thiomalic acid (3)	9	$CHCl_3$	1:99	Na_2CO_3 aq.	+	100
cysteine (3)	6	$CHCl_3$	2:98	Na_2CO_3 aq.	+	8

[a] "–": Insufficient phase separation; "+": sufficient phase separation. See the Supporting Information for details. Mpa = mercaptopropionic acid.



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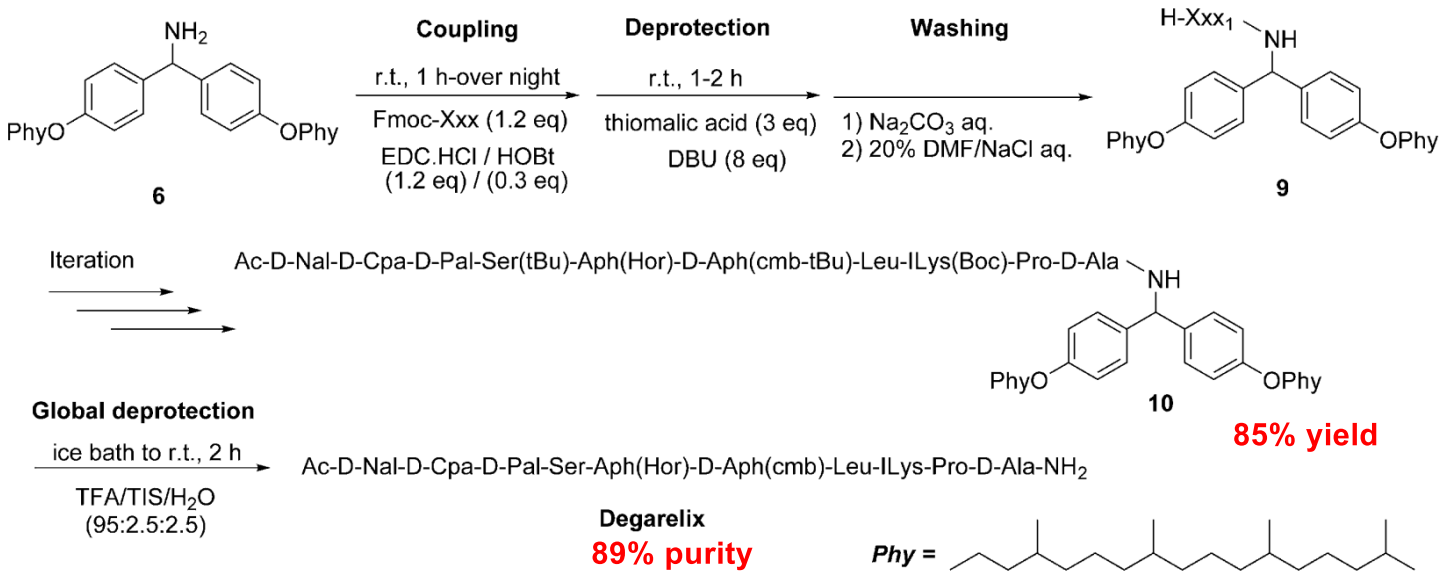
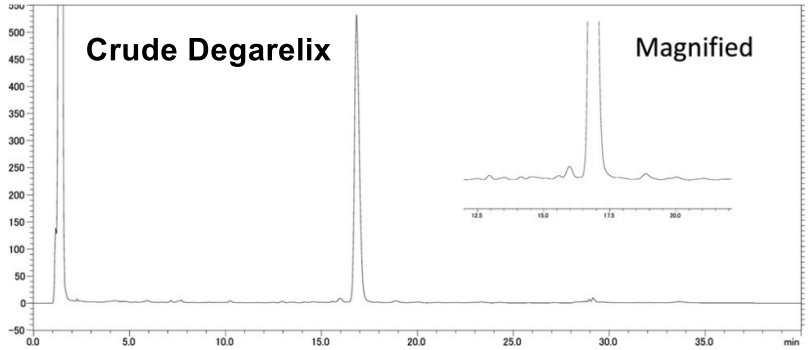
○ Further development

Synthesis of Degarelix





Takahashi, D.; Inomata, T.; Fukui, T. *Angew. Chem. Int. Ed.* **2017**, *56*, 7803-7807.

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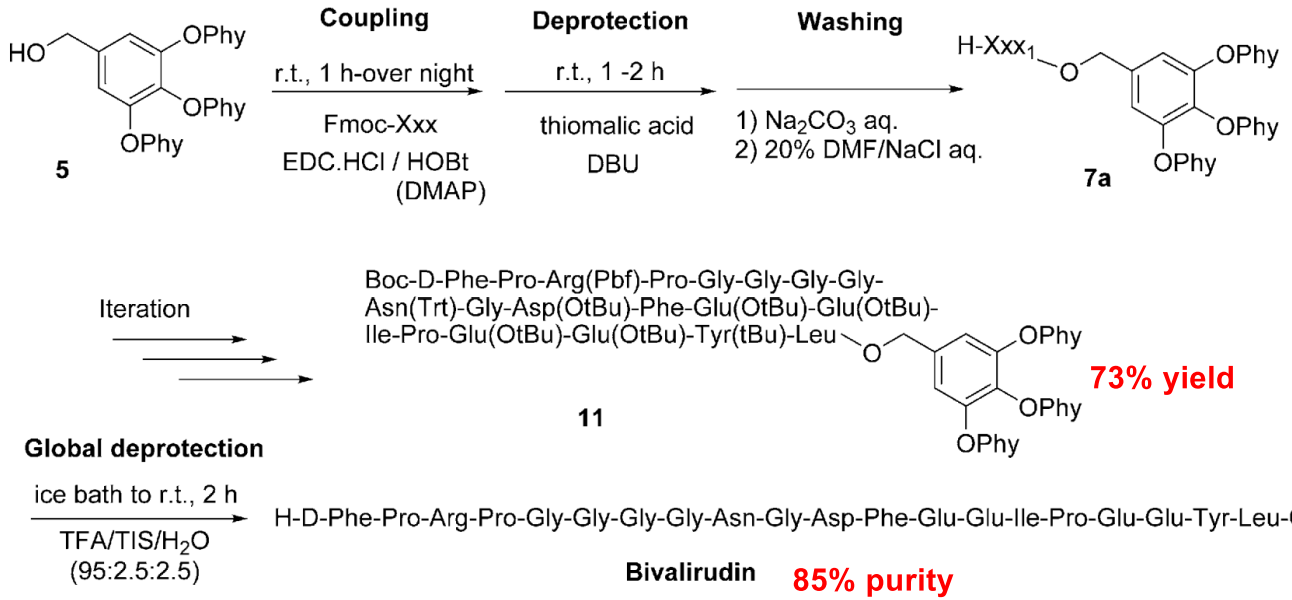
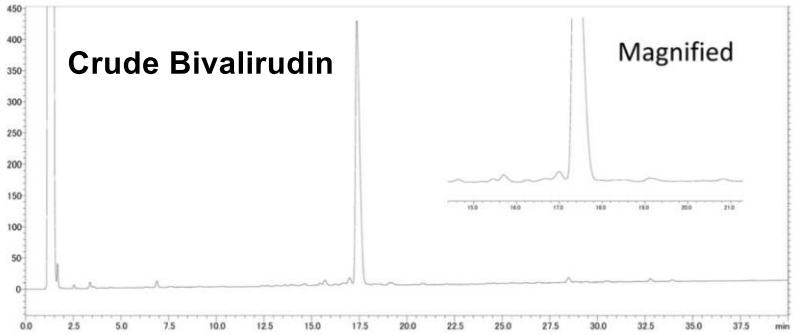
○ Further development

Synthesis of Bivalirudin





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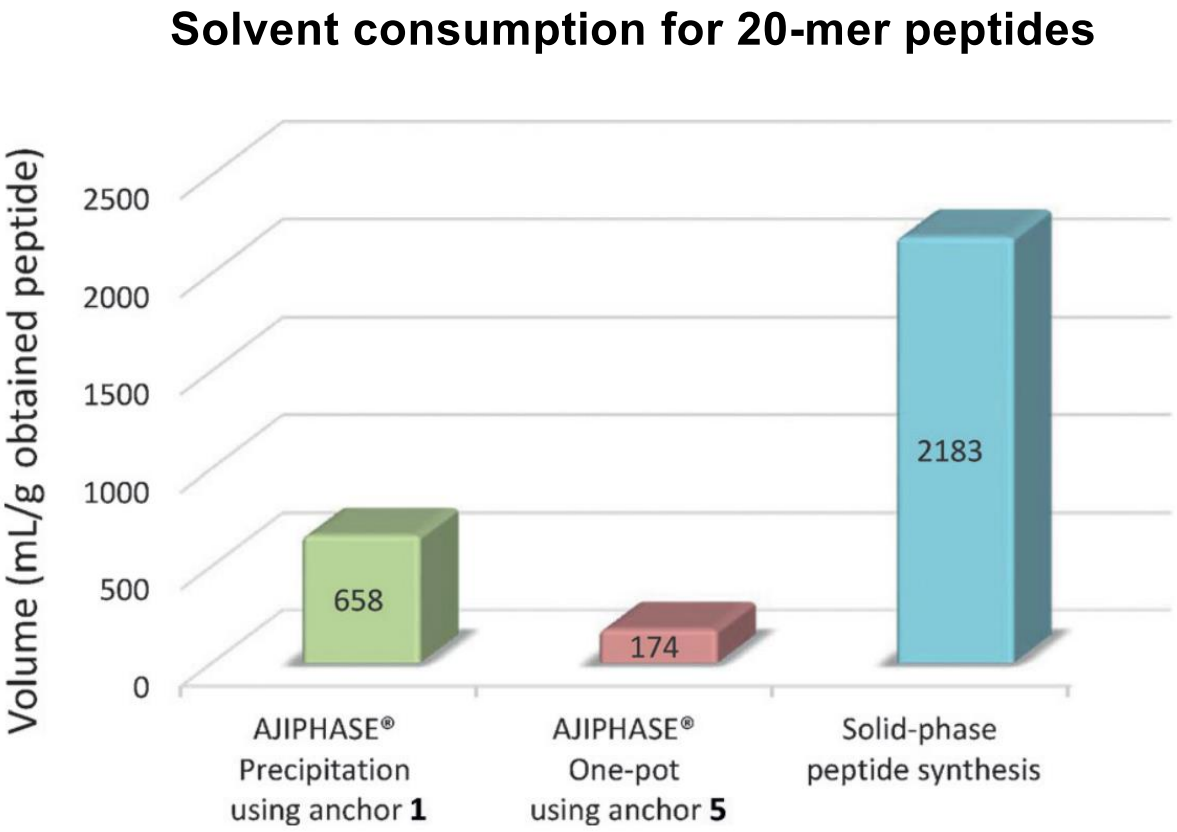

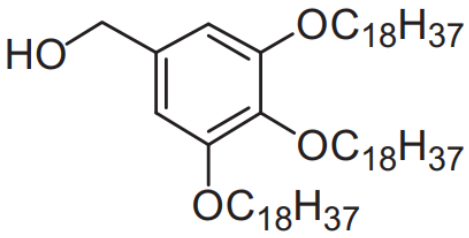

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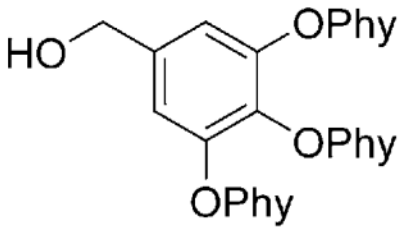

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2. Benzyl-type tag

3. AJIPHASE®

4. Summary

Summary

- **Peptide drugs have been attracting attention in recent years and more efficient synthetic method is needed.**
- **Hydrophobic tagging can streamline the synthetic process of peptide with a simpler purification method.**
- **Hydrophobic tags can also control the solubility and reactivity of peptide compounds.**
- **Large-scale peptide synthesis and commercialization of tags have been achieved through structural optimization of tags.**