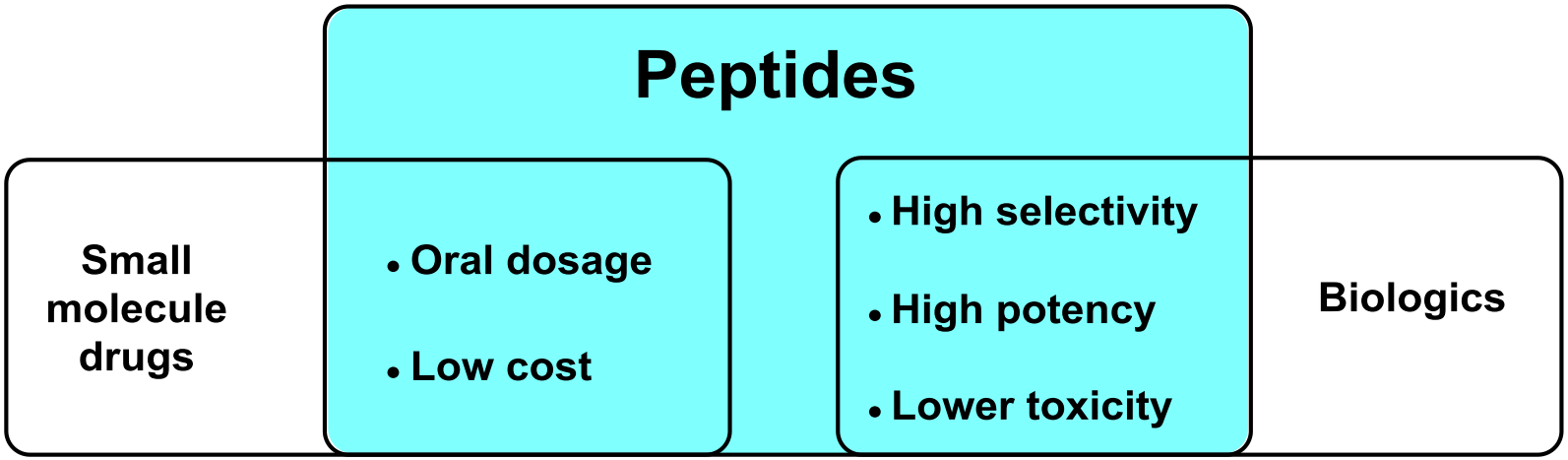
Epimerization of Peptide

2016/6/25 M1 Hirano Ryo

Introduction



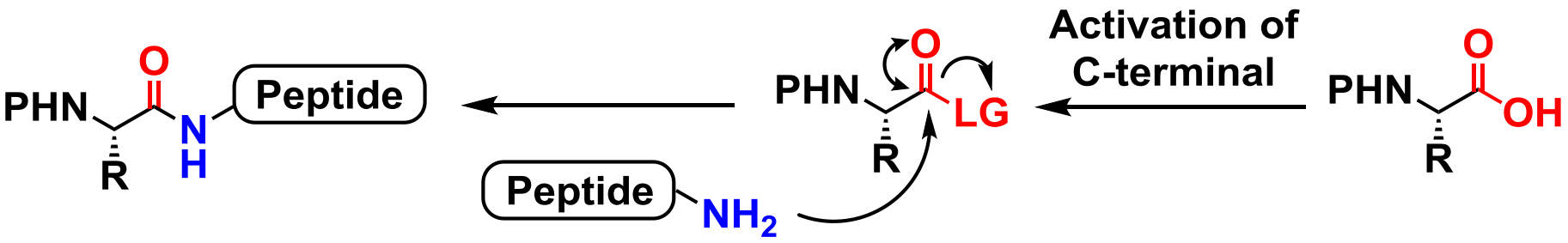
Disadvantage of peptide { **Poor metabolic stability**; **Poor membrane permeability**

Introduce unnatural amino acids by chemical synthesis

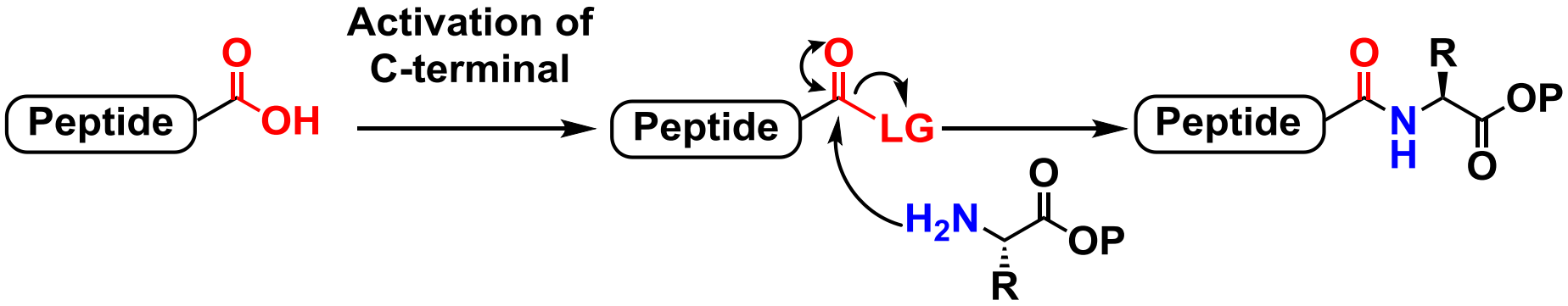
Fosgerau, K. Hoffman, T. *Drug Discovery Today* **2015**, *21*, 122

Introduction - chemical synthesis of peptide

C to N

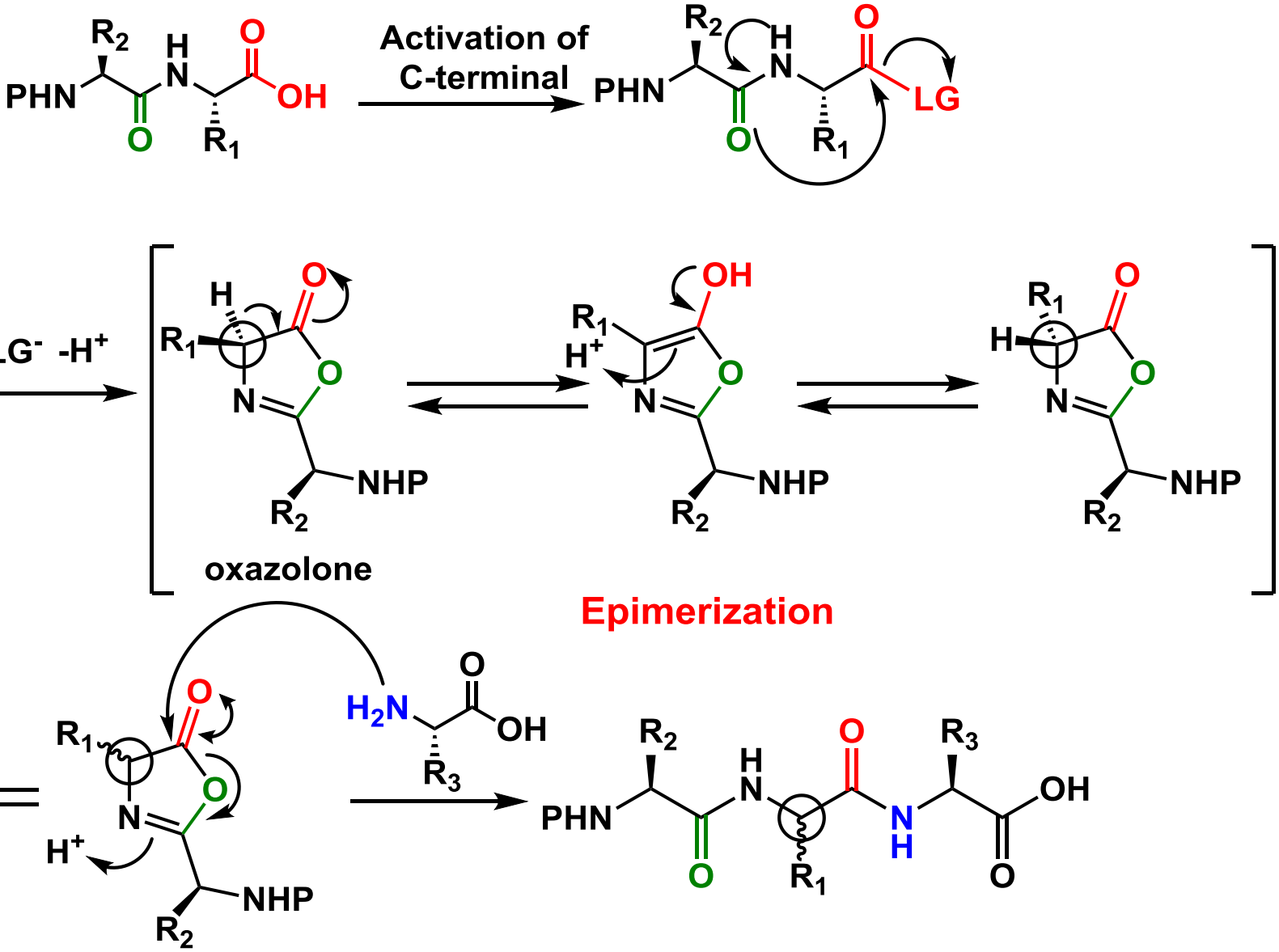


N to C



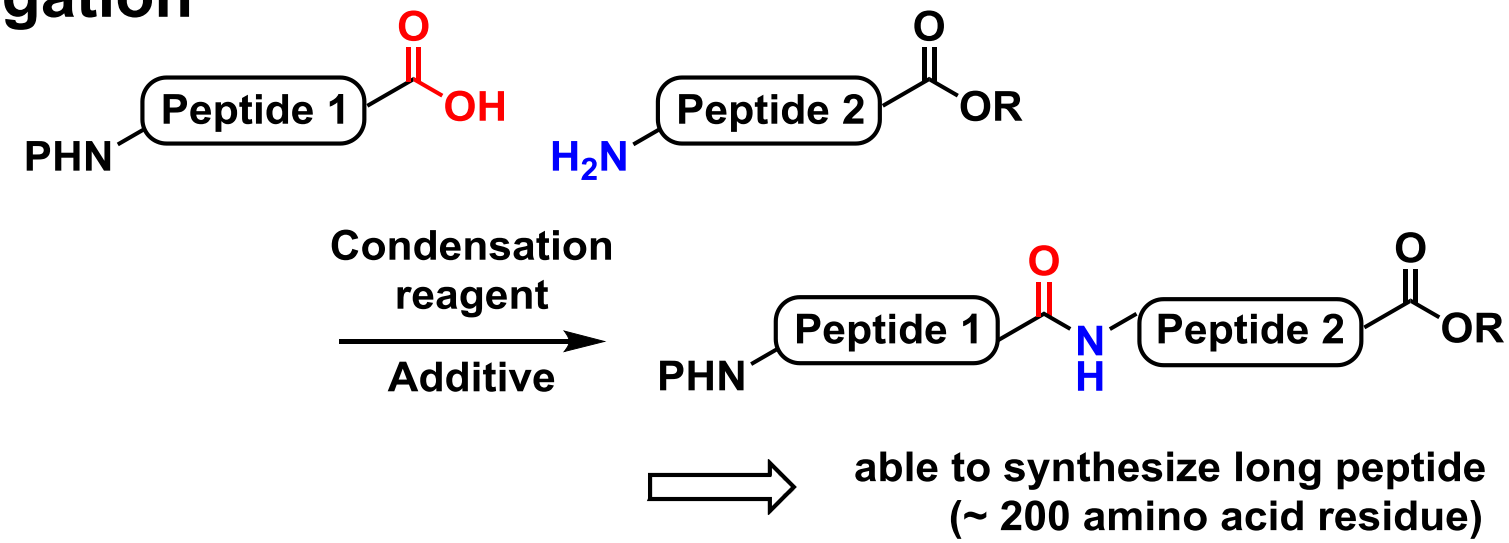
Introduction - N to C *synthesis*

But N to C synthesis causes epimerization



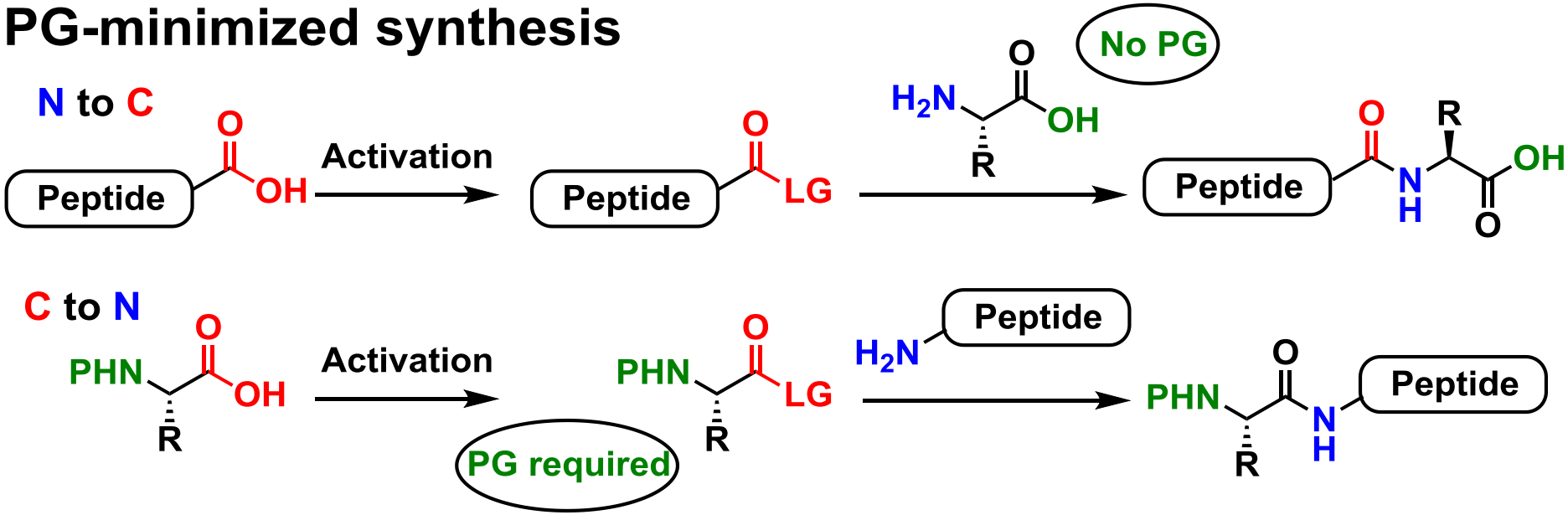
Introduction - importance of N to C synthesis

Ligation



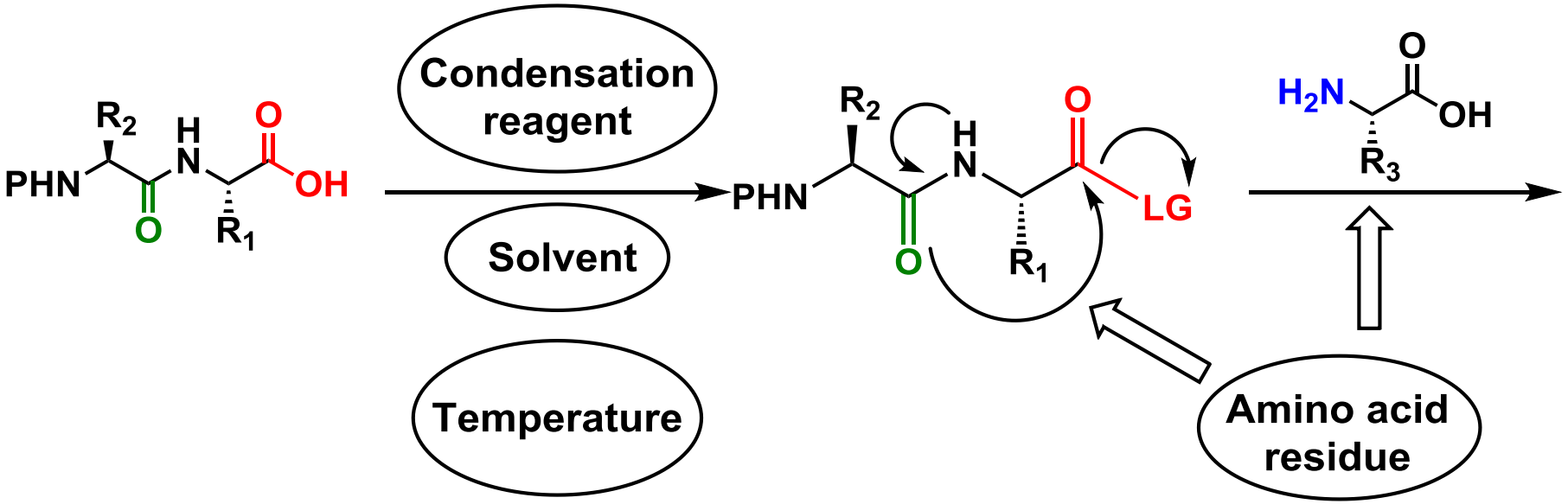
able to synthesize long peptide
(~ 200 amino acid residue)

PG-minimized synthesis



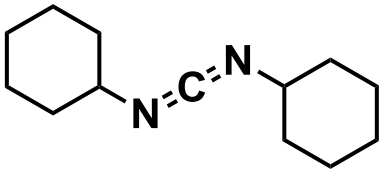
Contents

1. Factor of the epimerization

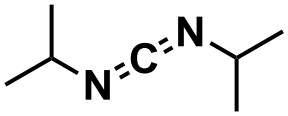


Factor of epimerization - condensation reagent

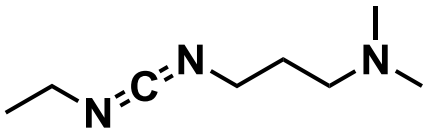
Condensation reagent



DCC

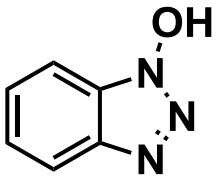


DIC

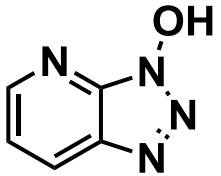


EDC

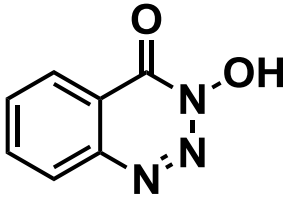
Additive



HOBt

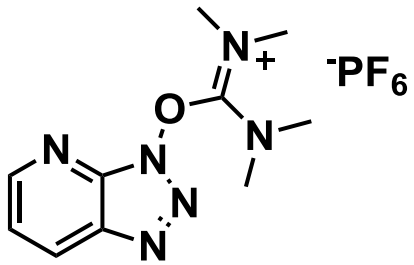


HOAt

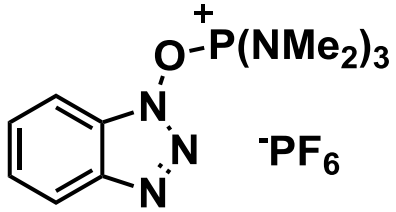


HOOBt

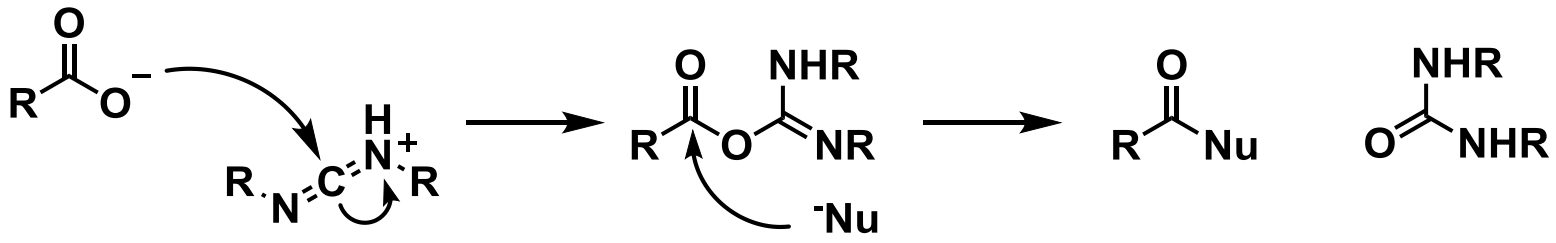
Condensation reagent + Additive



HATU

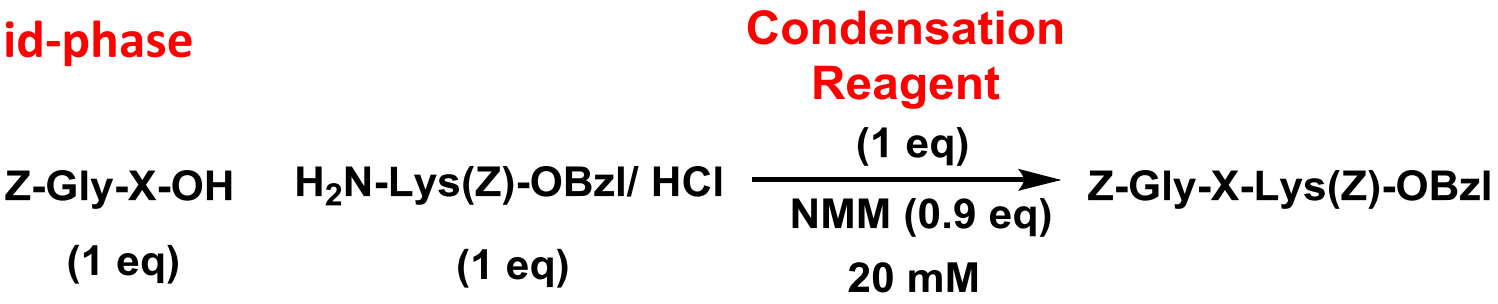


BOP



Factor of epimerization - condensation reagent

Liquid-phase



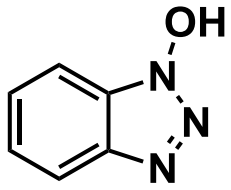
Epimerization rate

(X)	Ala	Leu	Phe	Val	Ile
DCC / DCM	10	14	18	5	9
EDC / DCM	25	25	21	22	29

(%)

(X)	Ala	Leu
DCC-HOBt / DMF	0.8	6.0
EDC-HOBt / DMF	2.0	9.0

(%)



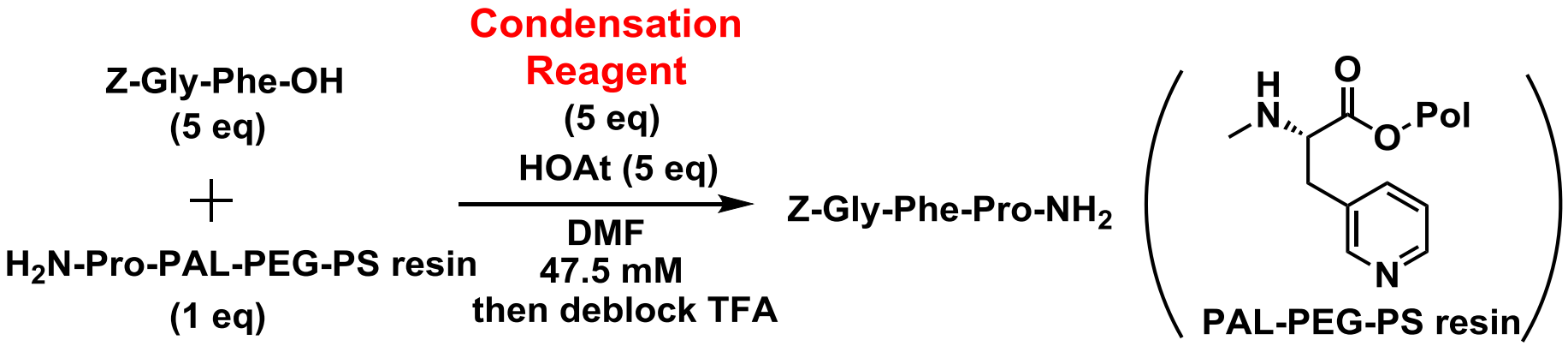
HOBt

N. L. Benoiton *et al*. *Int. J. Peptide Protein Res*. **1981**, *17*, 197.

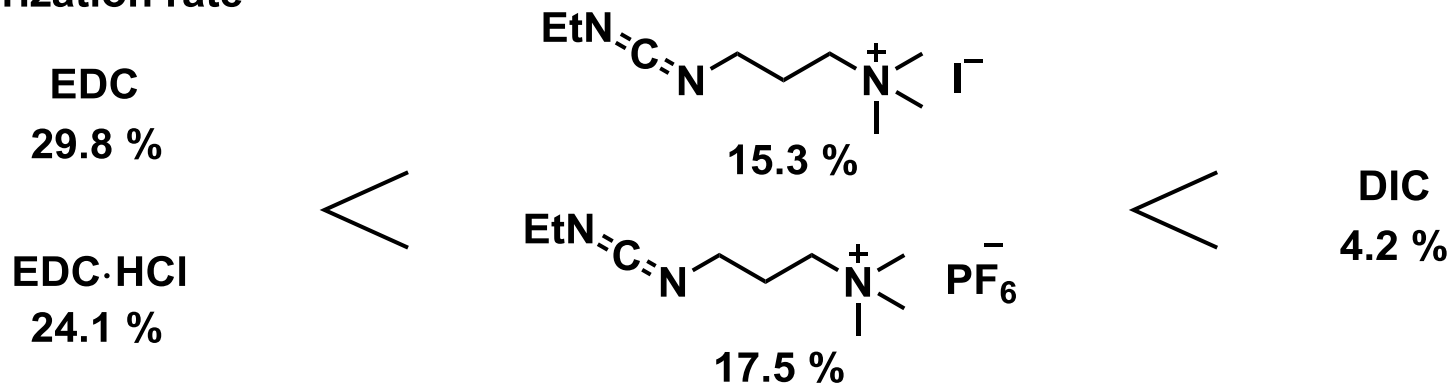
- **EDC tends to cause epimerization more than DCC.**

Factor of epimerization - condensation reagent

Solid-phase



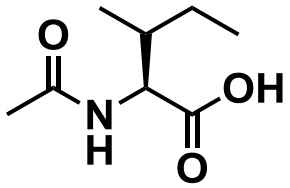
Epimerization rate



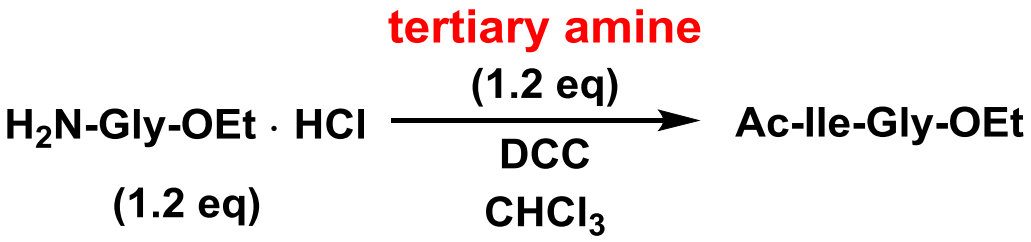
EDC and its derivative tends to cause more epimerization than DIC.

L. A. Carpino, A. El-Faham *Tetrahedron* **1999**, *55*, 6813.

Factor of epimerization - condensation reagent



Ac-Ile (1 eq)

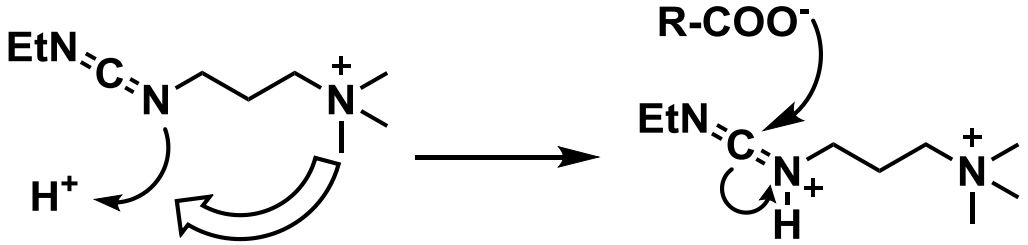


	NEt_3	DIPEA	None
Epimerization rate	9	10	4

(%)

L. E. Conklin et al. *Chem. Commun*. **1967**, 773.

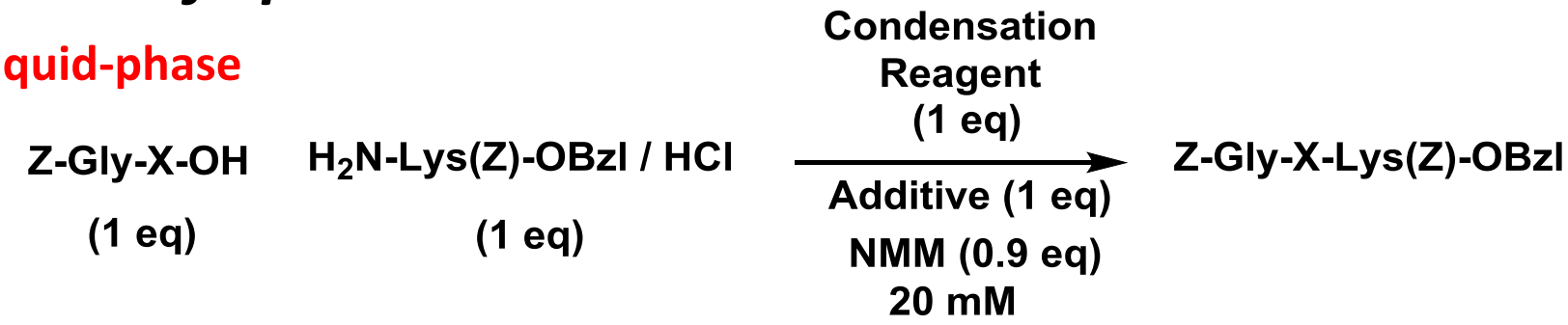
Basicity of amine correlates with epimerization?



Positive charge of amine
inhibits the protonation of carbodiimide?

Factor of epimerization - Solvent

Liquid-phase



Epimerization rate in **DCM** in **DMF**

(X)	Ala	Leu	Phe	Val	Ile
DCC-HOBt	0	0	0	0	0
DCC-HOOBt	-	0	-	-	0
DCC-HOSu	0	0	0	0	-
DCC	11	14	18	5	9

(%)

Ala	Leu	Phe	Val	Ile
-	0.7	1.7	5	6
-	0.2	-	-	0.3
1.4	0.6	2.8	12	13
15	11	15	30	44

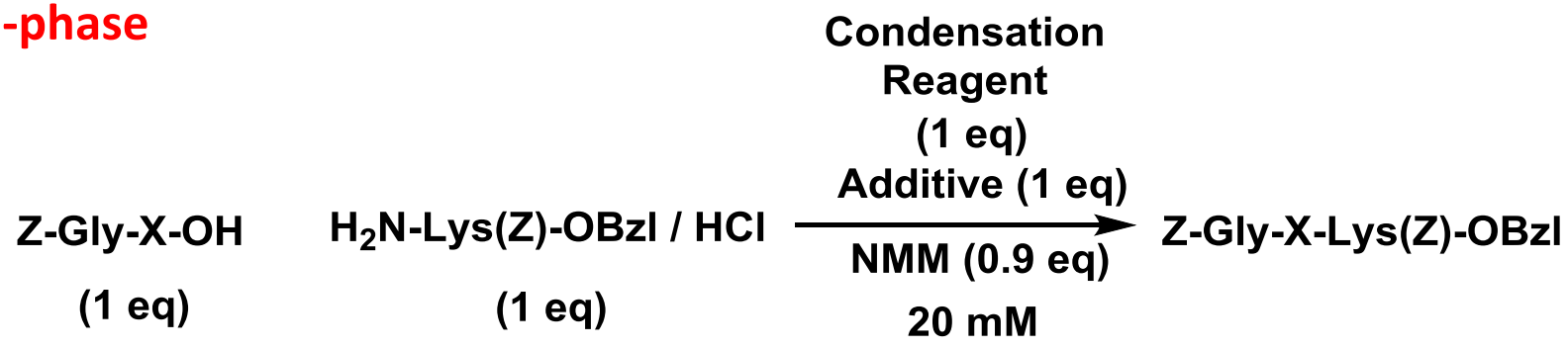
(%)

N. L. Benoiton *et al*. *Int. J. Peptide Protein Res*. **1981**, *17*, 197.

- **DMF increases epimerization rate.**

Factor of epimerization - Solvent

Liquid-phase



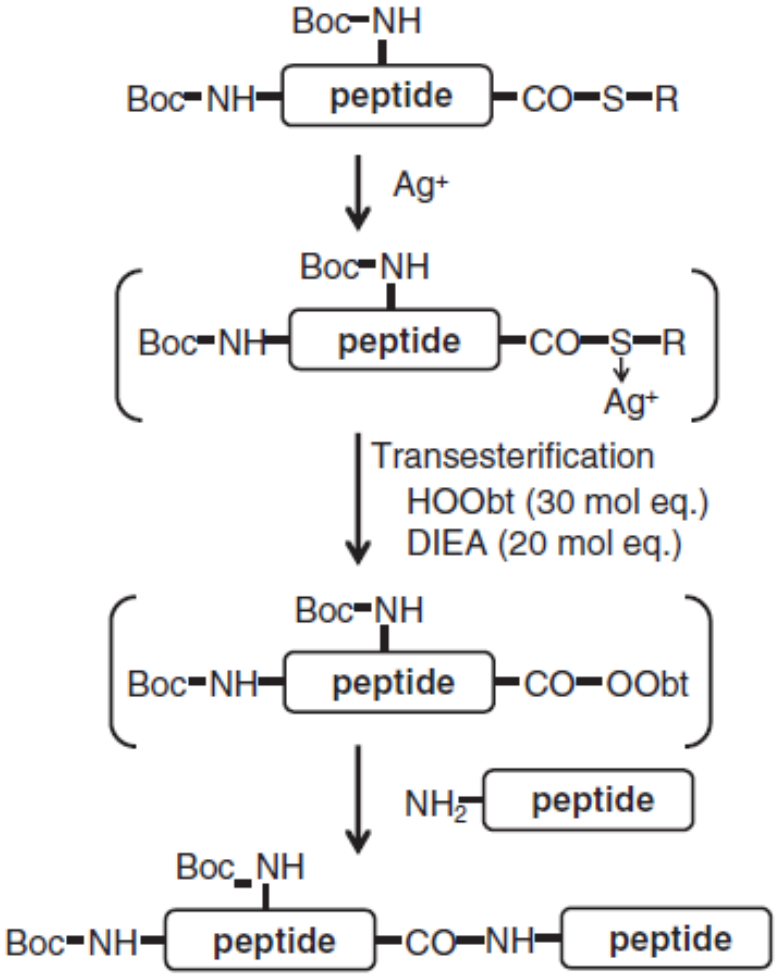
Epimerization rate (%)

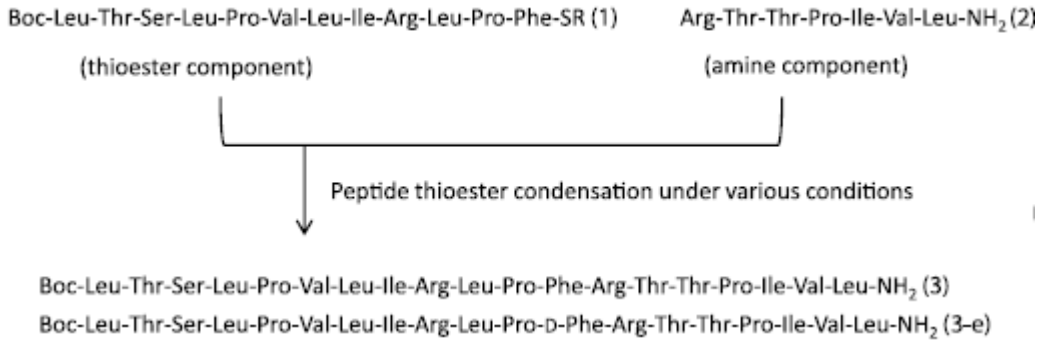
(X)	Ala	Leu	Phe	Val	Ile
DCC-HOBt / DCM	0	0	0	0	0
DCC-HOBt / DMF	-	0.7	1.4	5.5	0.3
DCC-HOBt / DMF-H_2O (2:1)	1.4	2.0	3.6	9.0	13

N. L. Benoiton *et al*. *Int. J. Peptide Protein Res*. **1981**, *17*, 197.

- **Polar solvent increases epimerization rate.**

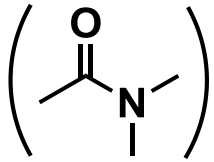
Factor of epimerization - Solvent





Liquid-phase

Solvent	epimerization rate
DMSO	23
DMAC	7
DMF	4



(%)

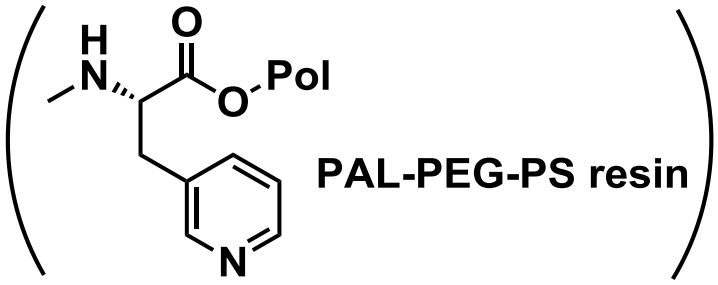
K. Teruya *et al*. *J. Pept. Sci*. **2012**, *18*, 669.

- **Polar solvent is likely to increase epimerization.**

Accelerates hydrogen abstraction from oxazolone?

Destabilizes intermediate?

Factor of epimerization - Solvent



Solid-phase

Fmoc-Asp(O-tBu)-Phe-OH

(5 eq)

+

H_2N-Lys(Boc)-PAL-PEG-PS resin

(1 eq)

Condensation Reagent (5 eq)
Additive (5 eq)

47.5 mM Solvent

then deblock TFA

Fmoc-Asp(O-tBu)-Phe-Lys(Boc)-NH_2

Coupling Reagent	Additive	Base	Solvent	Yield (%)	LDL- (%)
DIC	HOAt		DMF	90.0	23.1, 24.3
DIC	HOBt		DMF	89.0	30.2, 28.9
DIC [b]	HOAt		DMF	89.0	26.9 [b]
DIC [b]	HOBt		DMF	86.0	33.9 [b]
DIC	HOAt		DCM	85.0	0.1, 0.1
DIC	HOBt		DCM	86.0	0.9, 0.8

L. A. Carpino, A. El-Faham *Tetrahedron* **1999**, *55*, 6813.

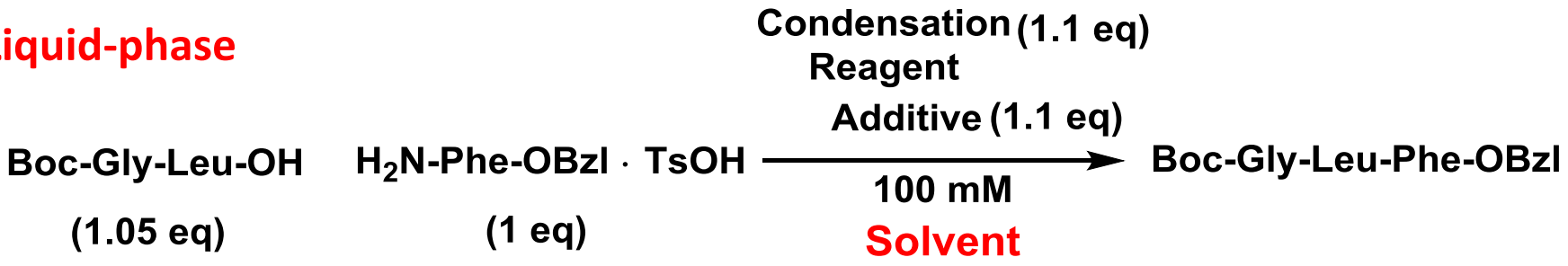
- **Same tendency is observed at solid-phase synthesis.**

Factor of epimerization - Solvent

So, apolar solvent is best? --- NO. Solubility problem occurs.

Boc-Met-Val-Asn-Lys(ClZ)-Ile-Val-Gln-OPac
Z-Pyr-Phee-Thr(Bzl)-Asn-Val-Ser(Bzl)-OPac
} **insoluble even in DMSO (β-sheet aggregation occurs)**

Liquid-phase



Coupling reagent	Solvent	Yield (%): L + D	D-isomer (%)	Ester (%)
WSCI/HOBt	DMF	90	3.6	–
	TCM/TFE	80	0.5	2.1
	TCM/HFIP	25	1.2	9.1
WSCI/HOOBt	DMF	92	0.2	–
	TCM/TFE	92	0	<0.1
	TCM/HFIP	78	0	2.3

Chloroform : trifluoroethanol = 3 : 1
can dissolve peptide and suppress epimerization.

Sakakibara S. *et al*. *Int. J. Peptide Protein Res*. **1992**, 40, 294.

Factor of epimerization - C-terminal residue

GOOD

Free from epimerization: Gly, Pro, Hyp(Bn)

Suitable: Ala, Leu, Glu(OcHx), Gln, Asn

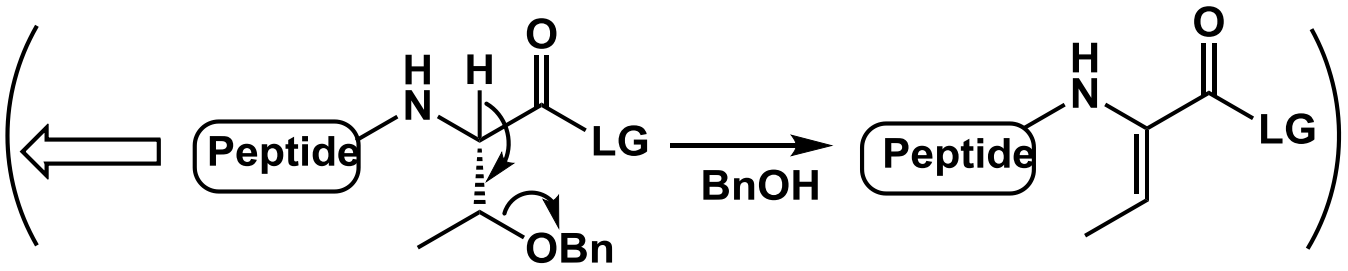
Acceptable: Lys(ClZ), Asp(OcHx), Met, Ser(Bn)

Not suitable: His(Bom), Cys(Acm), Arg(Ts), Ile, Val, Phe, Tyr(BrZ), Trp(Hoc), Thr(Bn)

BAD

Sterically Hindered amino acid
takes a long time for coupling reaction.

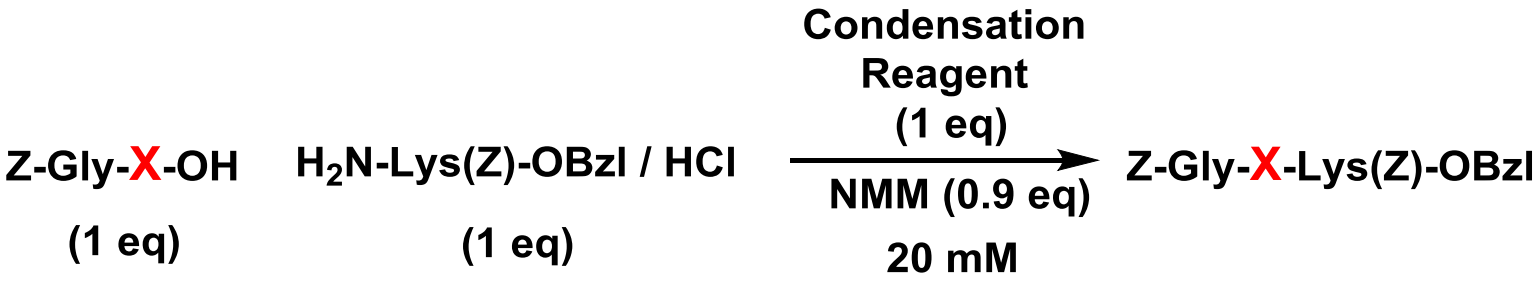
⇒ **Risk of epimerization increases.**



Thr(OBn) decomposes when activated.

Sakakibara S. *Biopolymers (Peptide Science)* **1999**, *51*, 279.

Factor of epimerization - C-terminal residue



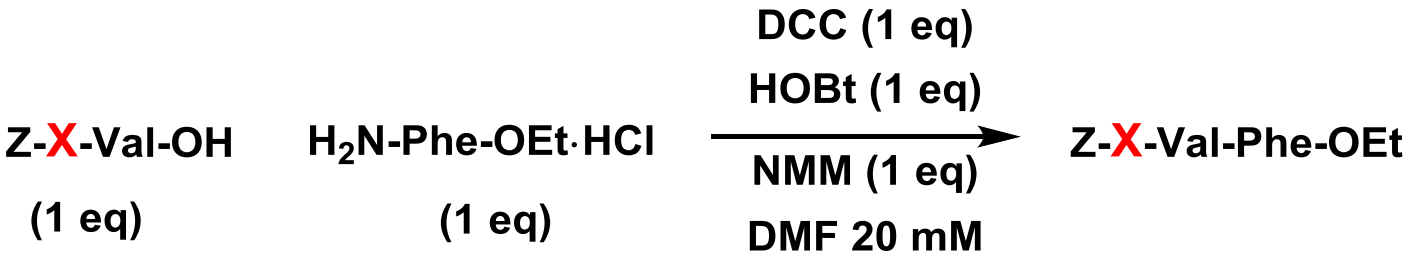
Epimerization rate (%)

(X)	Ala	Leu	Phe	Val	Ile
DCC / DCM	10	14	18	5.0	9.0
DCC / DMF	15	12	15	30	44
DCC-HOBt / DMF-H_2O (2:1)	1.4	2.0	3.6	9.0	18

N. L. Benoiton *et al*. *Int. J. Peptide Protein Res*. **1981**, *17*, 197.

- **Epimerization rate differs depending on C-terminal residue.**
- **Val or Ile tends to easily epimerize in polar solvents.**

Factor of epimerization - C-terminal residue



Epimerization rate

(X)	Leu	Ala	Gly
DCC	18.9	12.0	2.0
DCC-HOBt	5.9	3.6	2.5

(%)

- **Hindered residue increases epimerization.**

Thorpe-Ingold Effect?

N. L. Benoiton *et al*. *Int. J. Pept. Pro. Res*. **1992**, *40*, 559.

Factor of epimerization - N-terminal residue

GOOD

Suitable

Gly

Ala Leu Asn Lys

Glu Gln Asp Met

Ser His Cys Arg

Trp Phe Thr Tyr

Acceptable

Val Ile

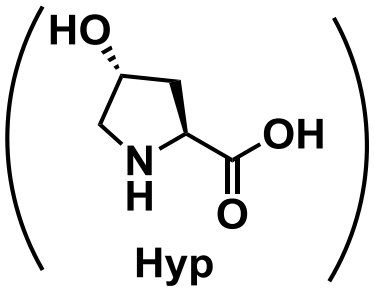
Not suitable

Pro Hyp

BAD

Sterically Hindered amino acid
takes a long time for coupling reaction

⇒ **Risk of epimerization increases**

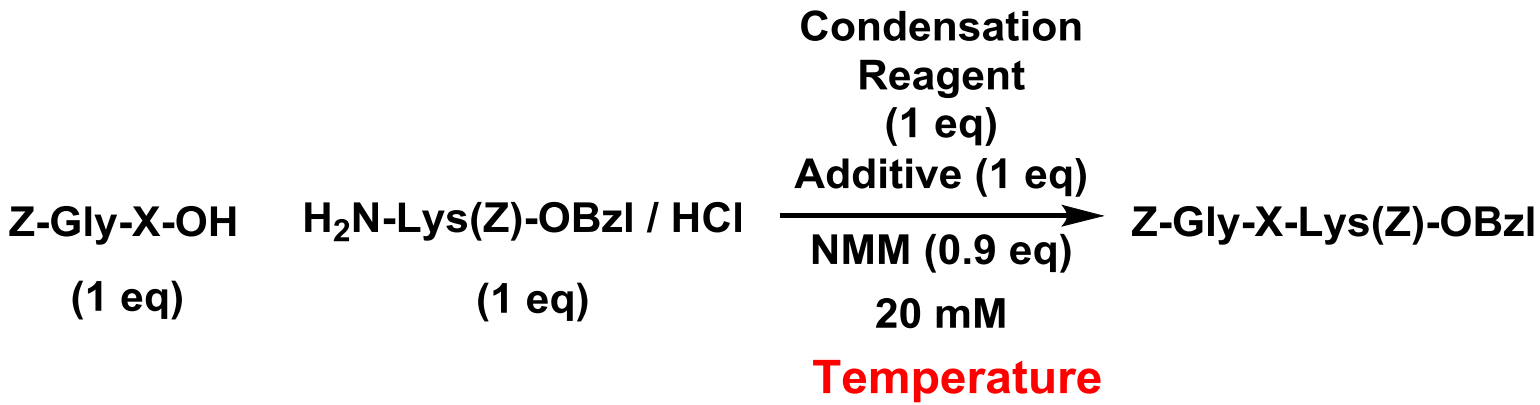


Sakakibara S. *Biopolymers (Peptide Science)* **1999**, 51, 279.

⇒ **Coupling at Hindered amino acid residue should be avoided.**

Factor of epimerization - temperature

Liquid-phase



in DMF (X)	Leu	Ile
DCC-HOBt / 5 °C	0.3	0.9
DCC-HOBt / 23 °C	0.8	6

(%)

N. L. Benoiton *et al. Int. J. Peptide Protein Res*. **1981**, *17*, 197.

- **Low temperature suppresses epimerization.**

Factor of epimerization - Summary

To supress epimerization・・・

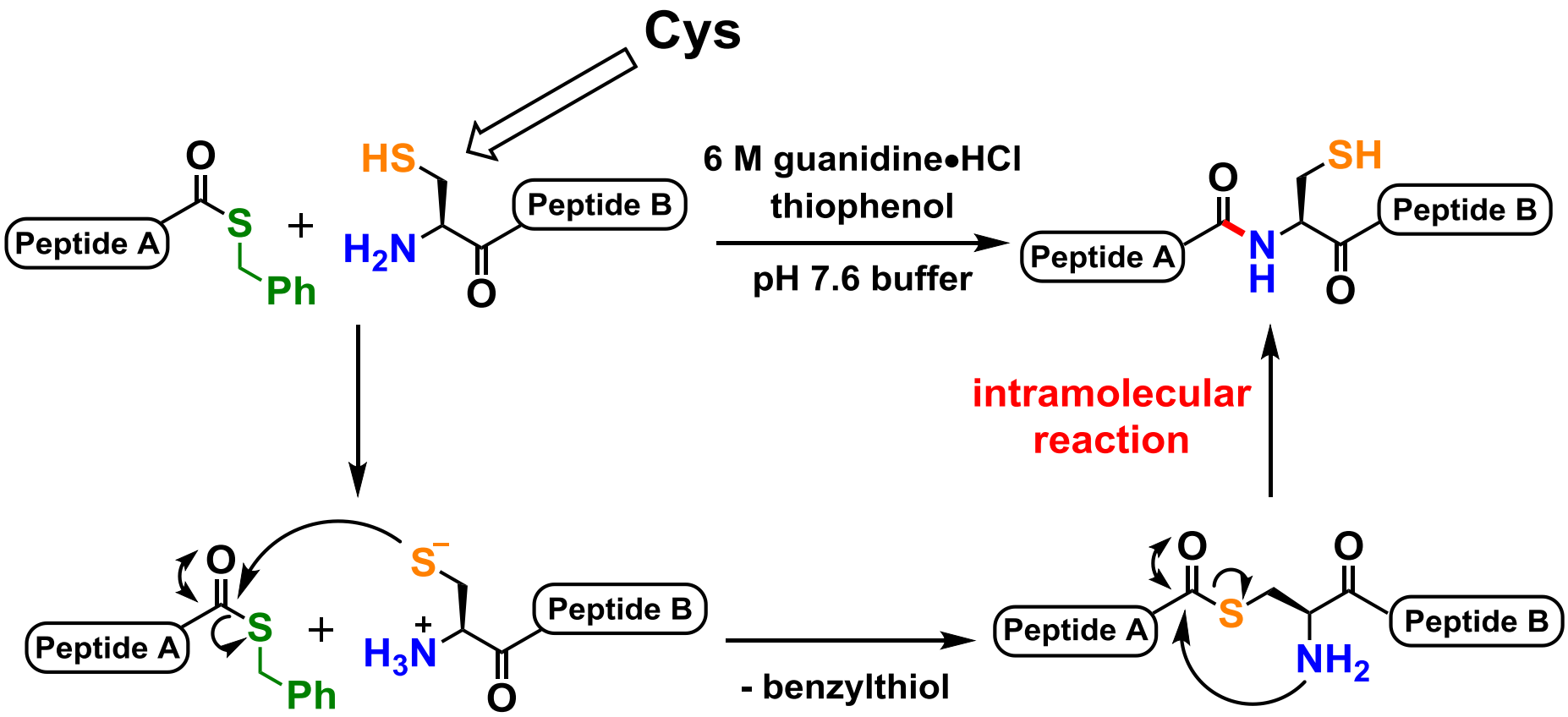
- **Conduct reaction at low temperature**
- **Use apolar solvent (but apolar solvent may cause solubility problem)**
- **Avoid coupling reaction using a peptide that conteins sterically hindered residue at C-Terminal or N-terminal.**
- **Aboid using a condensation reagent that contains amino group such as EDC.**
- **Epimerizagtion rate differs depend on the sequence of peptide.**

2. To overcome epimerization

- NCL
- Flow reaction
- Additive

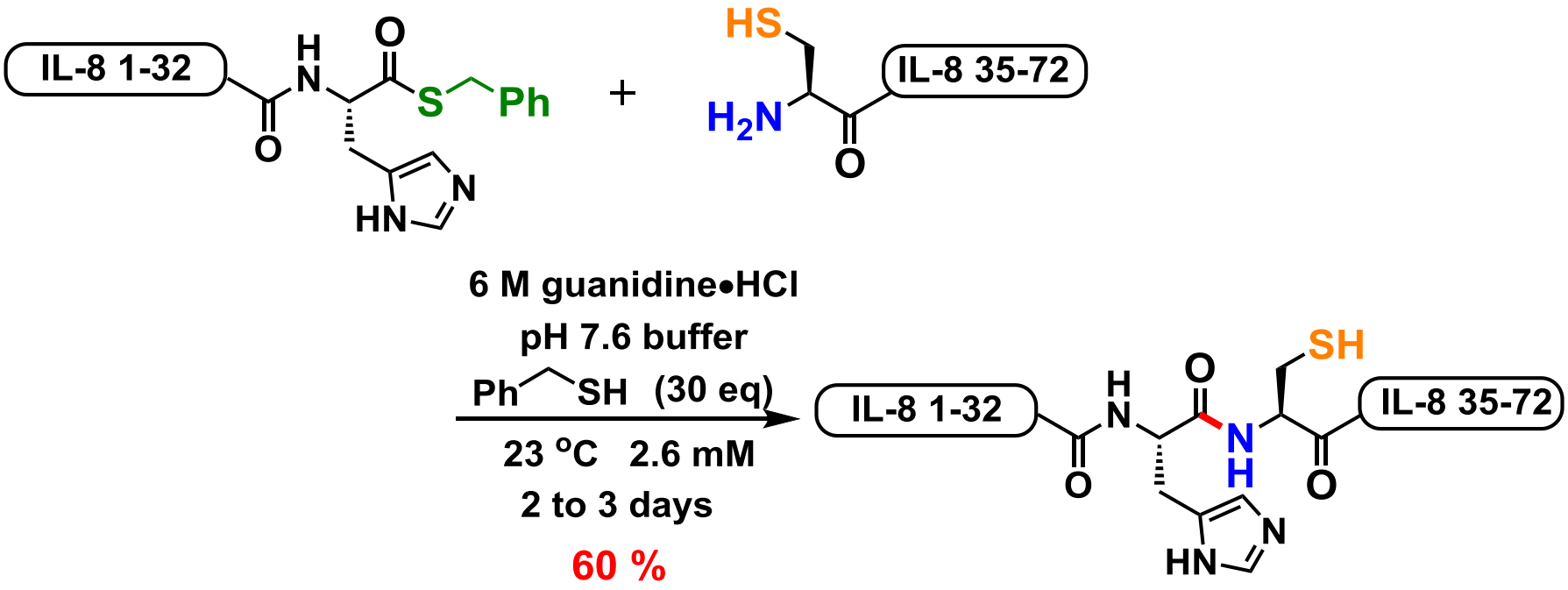
To overcome epimerization - NCL

NCL ··· Native Chemical Ligation



To overcome epimerization - NCL

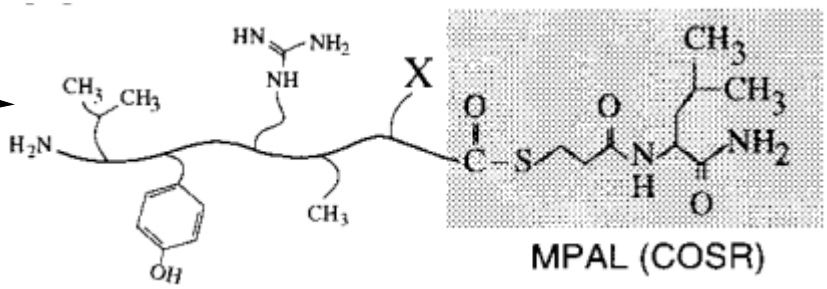
synthesis of IL-8



E. T. Baldwin *et al. Proc. Nati. Acad. Sci. USA* **1991**, *88*, 502.

Kent, S. B. H. *et al. Science* **1994**, *266*, 776.

To overcome epimerization - NCL



H_2N-Leu-Tyr-Arg-Ala-X-COSR
(1 eq)

H_2N-Cys-Arg-Ala-Asn-Lys-$CONH_2$
(2 eq)

6M guanidine
phosphate buffer (pH 8.5)
BnSH 4 %(v/v)
PhSH 4 %(v/v)
37 °C

H_2N-LYRAX-CRANK-$CONH_2$

Quant.

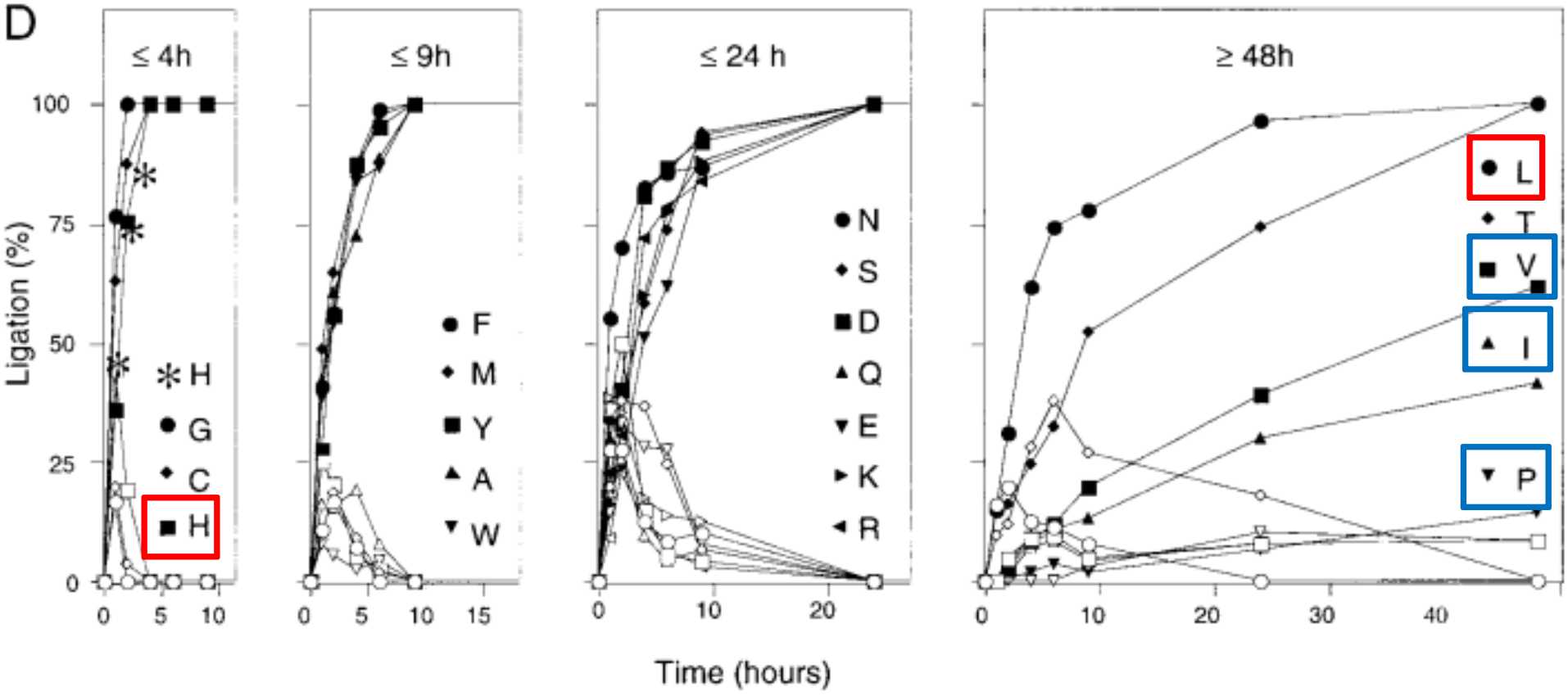
X = 20 kinds of amino acids

(X)	required time to complete the coupling	Epimerization rate
Leu	48 h	< 1 %
His	4 h	< 2 %

P. E. Dawson *et al*. *Proc. Nati. Acad. Sci. USA* **1999**, *96*, 10068

- **NCL enables epimerization-free coupling.**

To overcome epimerization - NCL



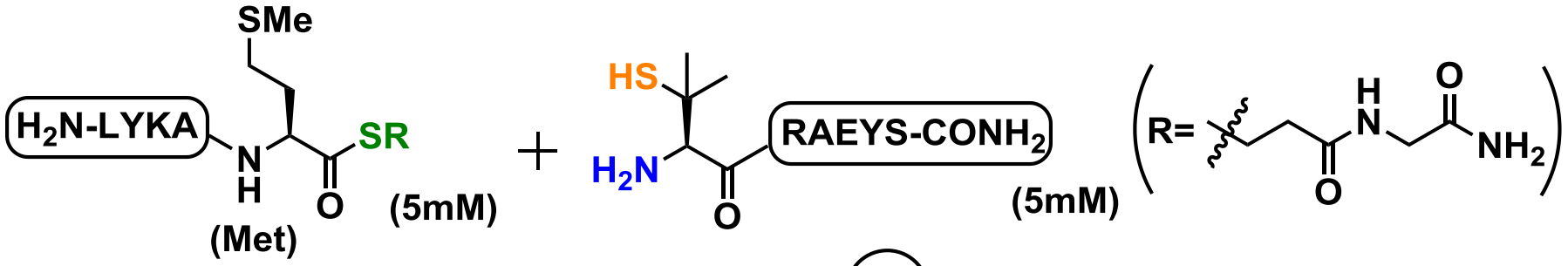
P. E. Dawson *et al*. *Proc. Nati. Acad. Sci. USA* **1999**, *96*, 10068.

- **If X is Val, Ile, or Pro, coupling reaction doesn't complete even in 48 hours.**

One have to consider where to connect peptides.

To overcome epimerization - NCL modification

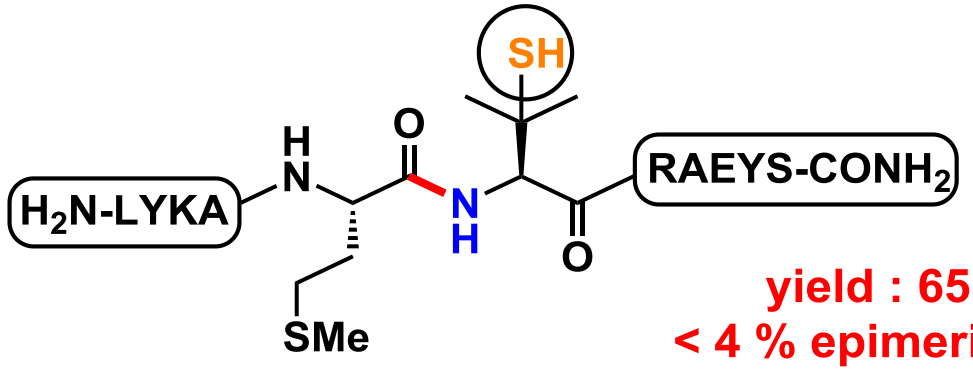
Connect peptide without using Cys



Ligation

6M guanidine hydrochloride
phosphate buffer (pH 8.5)

triscarboxyethylphosphine
(50 mM)
PhSH 5 %(v/v)
37 °C, 12 h

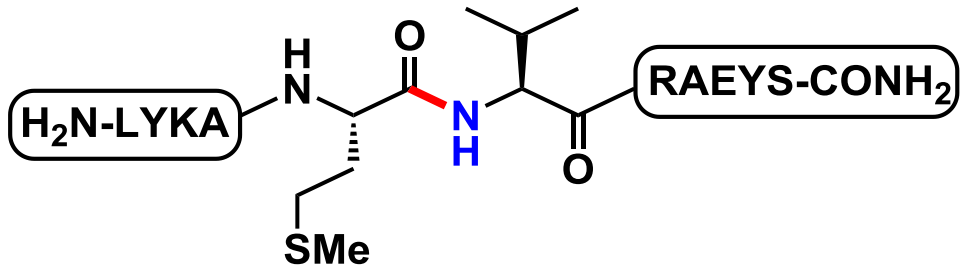


yield : 65 %
< 4 % epimerization

Desulfurization

3M guanidine hydrochloride
phosphate buffer (pH 6.5)

triscarboxyethylphosphine
(250 mM)
glutathione (40 mM)
VA-044 (200 mM)
65 °C, 2.5 h

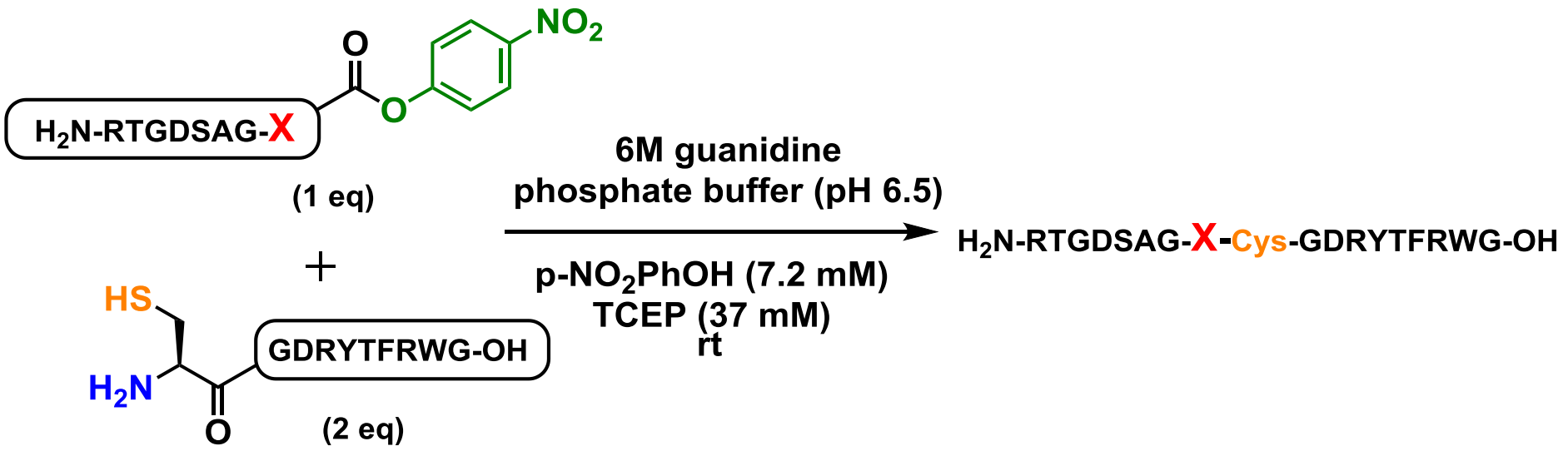


yield : 93 %

O. Seitz *et al*. *Angew. Chem. Int. Ed*. **2008**, *47*, 6807.

To overcome epimerization - NCL modification

Connect peptides at sterically hindered amino acid

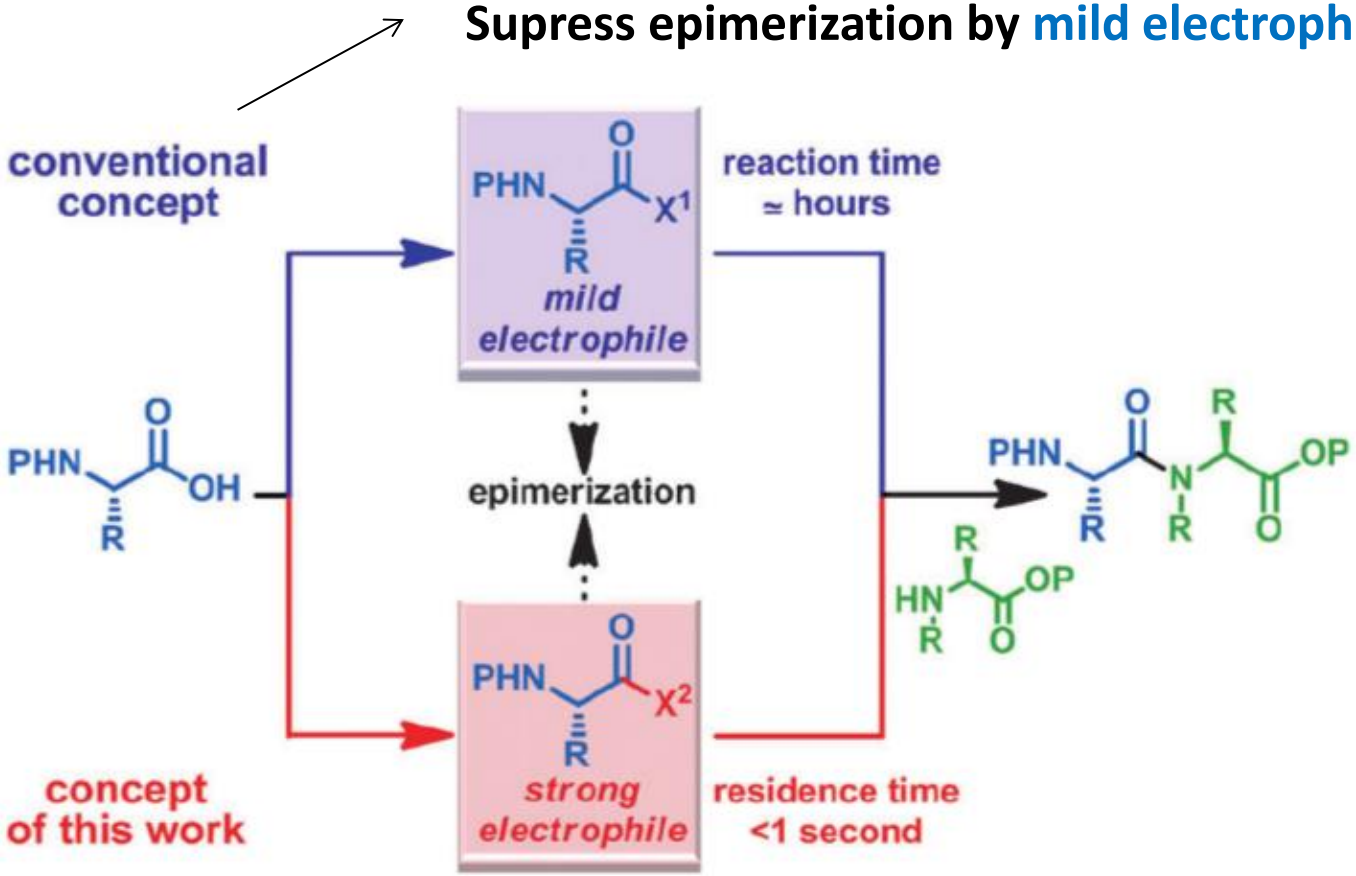


(X)	required time to complete the coupling	Yield
Thr	2 h	79 %
Val	6 h	69 %
Ile	7 h	70 %

epimerization rate
5 ~ 6 %

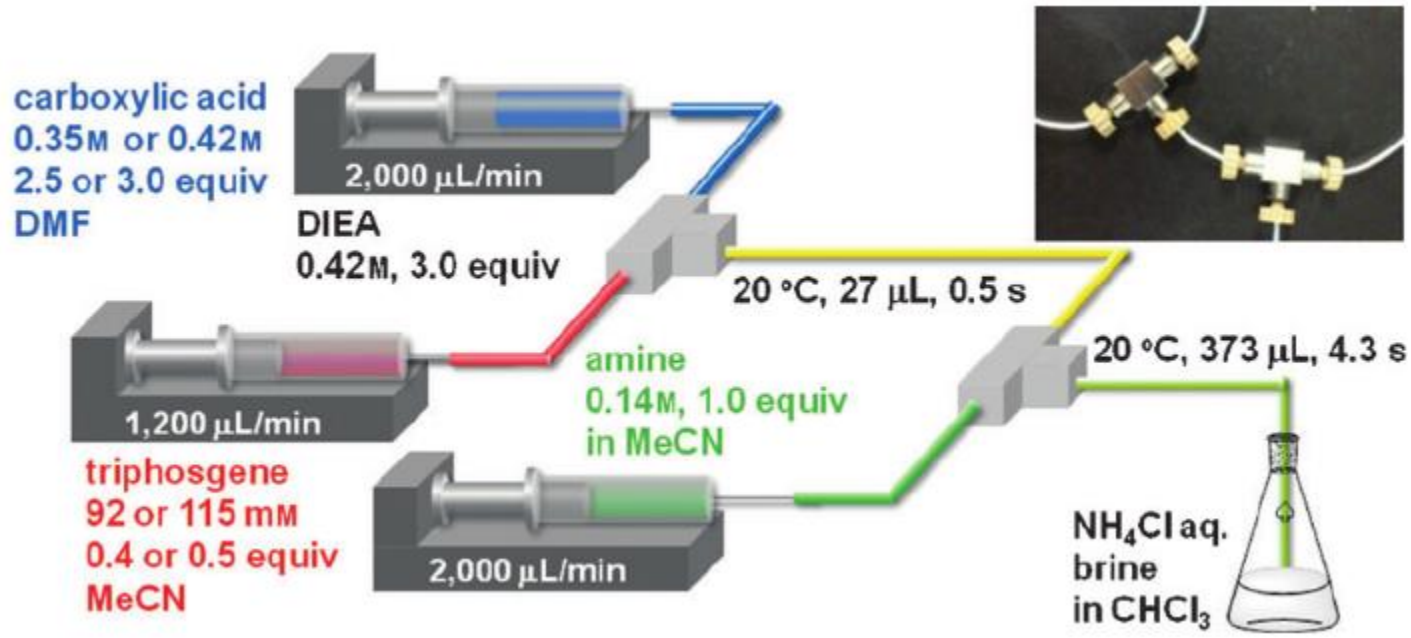
S. J. Danishefsky *et al. J. Am. Chem. Soc*. **2008**, *130*, 15814.

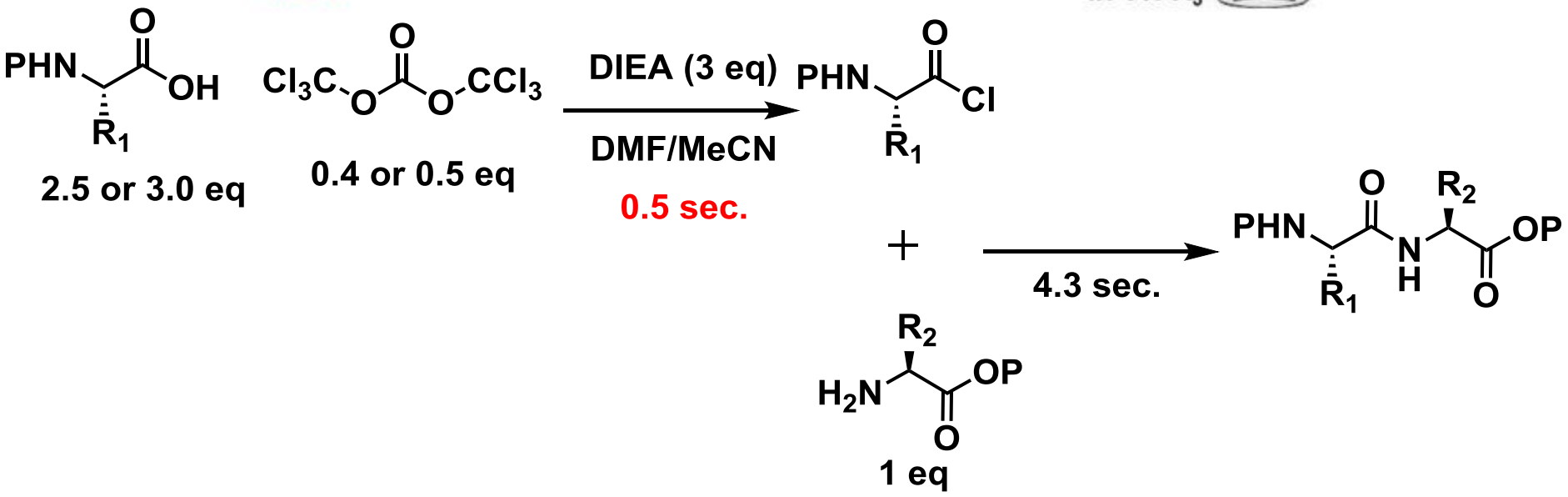
To overcome epimerization - Flow reaction



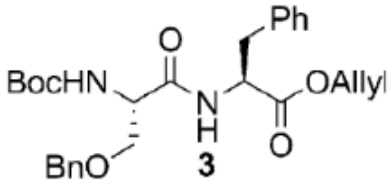
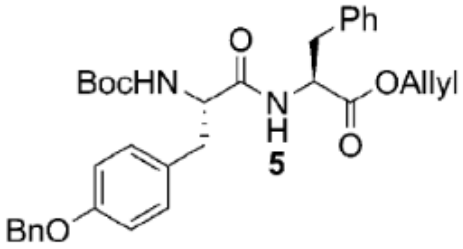
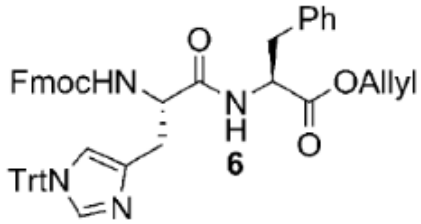
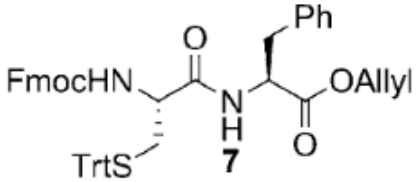
T. Takahashi *et al*. *Angew. Chem. Int. Ed*. **2014**, *53*, 851.

To overcome epimerization - Flow reaction



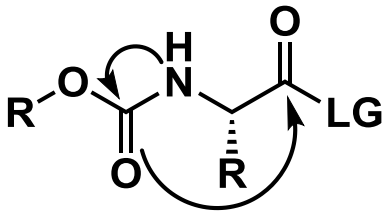


To overcome epimerization - Flow reaction

Entry	Structure of desired product	Cond.	Yield [%] (desired)	Yield [%] (epimer)
1		flow A[a]	92	1
		batch[d]	57	2
2		flow B[b]	quant.	<1
		batch[d]	40	<1
3		flow B[b]	92	2
		batch[d]	75	17
4		flow A[a]	94	<1
		batch[d]	71	1

[a] Carboxylic acid: 2.5 equiv, triphosgene: 0.4 equiv. [b] Carboxylic acid: 3.0 equiv, triphosgene: 0.5 equiv. [c] Carboxylic acid: 2.5 equiv, triphosgene: 0.4 equiv, DIEA: 2.5 equiv, solvent A: MeCN, reaction temperature: 10 °C. [d] Reaction time for the activation of carboxylic acid and the amidation: 30 s. Fmoc = 9-fluorenylmethyloxycarbonyl.

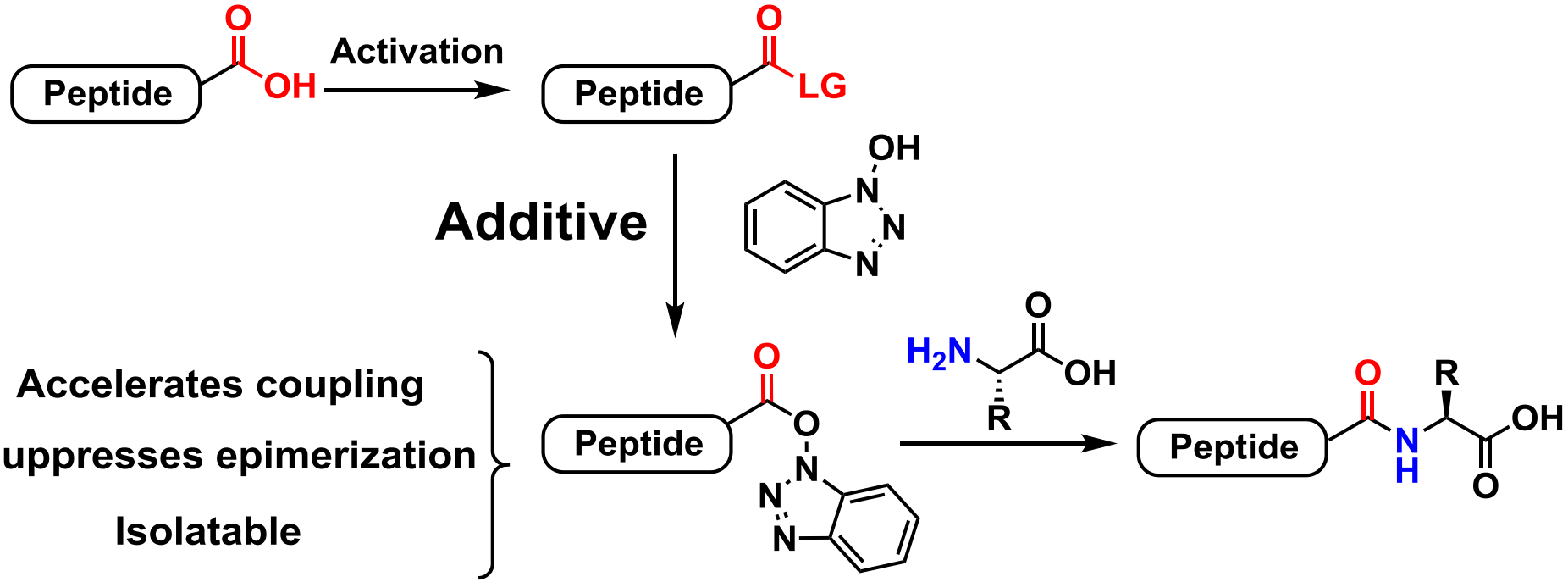
Desired peptide is gained in good yield by flow reaction.

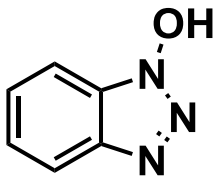


But carbamate is less epimerizable than amide・・・.

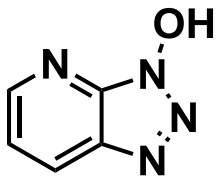
It's still uncertain flow reaction can inhibit epimerization of peptide.

To overcome epimerization - Additive

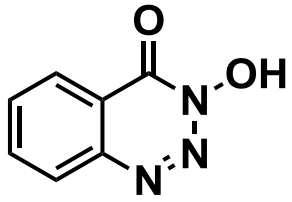




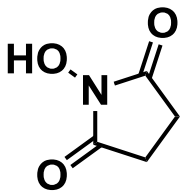
HOBt



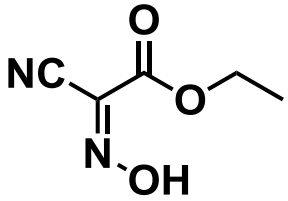
HOAt



HOOBt



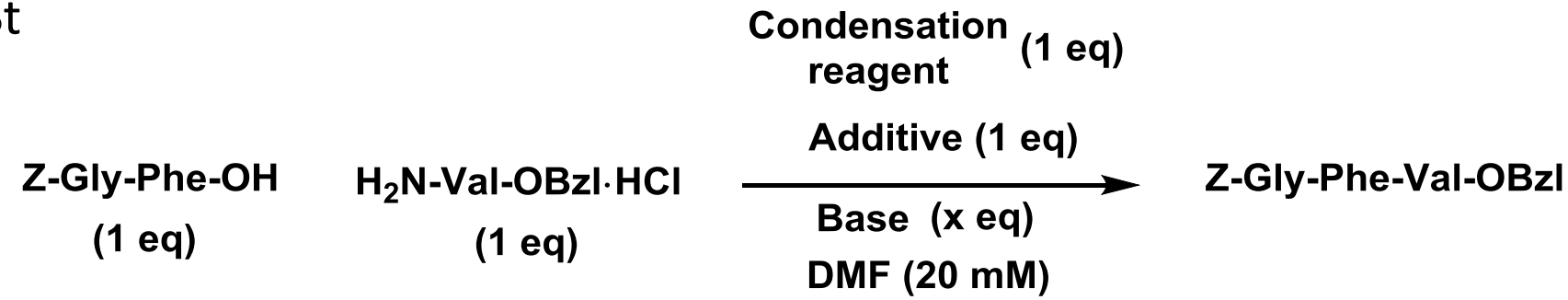
HOSu



Oxyma

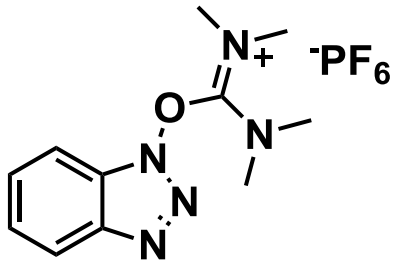
To overcome epimerization - Additive

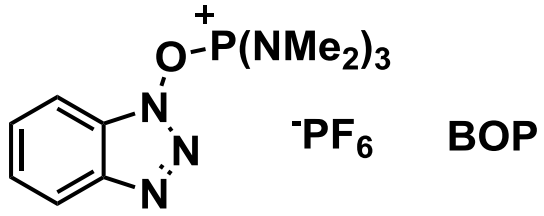
HOBt



	Base (x eq)	Epimerization rate
DCC	NMM (1eq)	20.5 %
DCC / HOBt	NMM (1eq)	0.7 %
BOP / HOBt	DIPEA (2 eq)	0 %
HBTU / HOBt	DIPEA (2 eq)	1.5 %

(%)



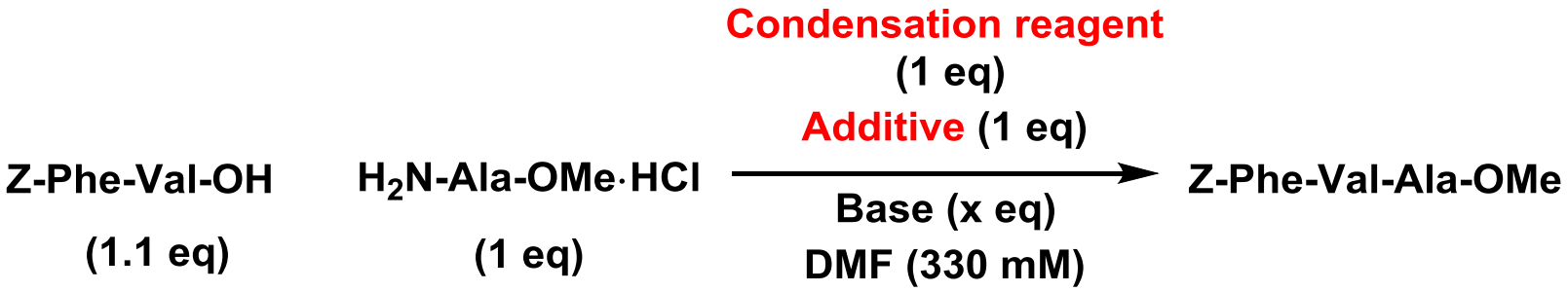


- **HOBt suppresses epimerization.**

N. L. Benoiton *et al*. *Int. J. Pept. Pro. Res*. **1992**, *40*, 559.

To overcome epimerization - Additive

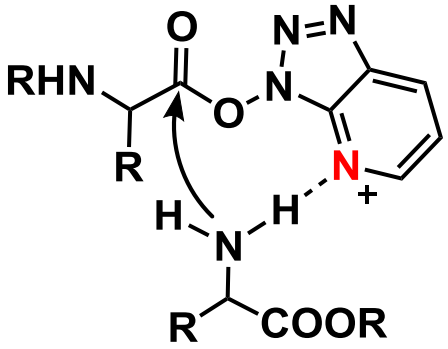
HOAt



	Base (x eq)	Time	Epimerization rate
EDC / HOAt	NMM (1eq)	1.25 h	< 1~2 %
EDC / HOBt	NMM (1eq)	2.25 h	4.1 %
HATU	DIPEA (2 eq)	3.5 h	< 1~2 %
HBTU	DIPEA (2 eq)	4 h	4.1 %

(%)

HOAt accelerates coupling and suppresses epimerization better than HOBt.

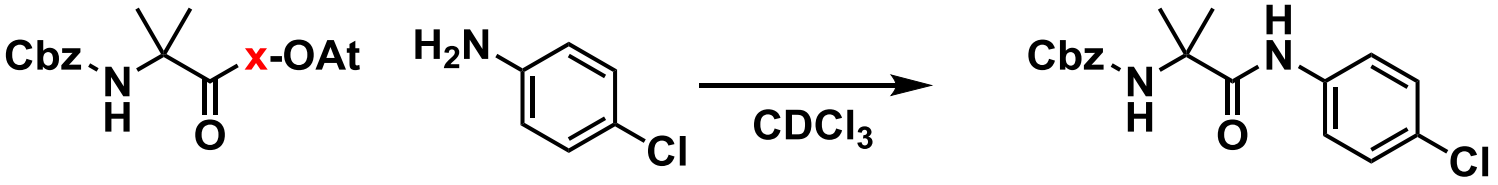


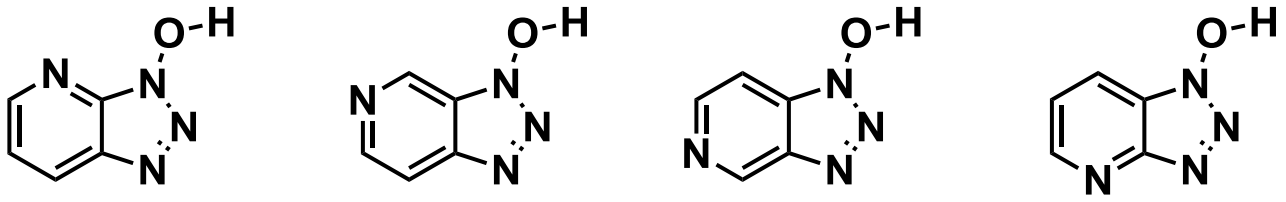
N atom draws amino group?

Louis A. Carpino *J. Am. Chem. Soc*. **1993**, *115*, 4397.

To overcome epimerization - Additive

HOAt





7-HOAt **6-HOAt** **5-HOAt** **4-HOAt**

ester	A
Z-Aib-7-OAt	7–8
Z-Aib-6-OAt	25
Z-Aib-5-OAt	95[a]
Z-Aib-4-OAt	95
Z-Aib-OBt	210

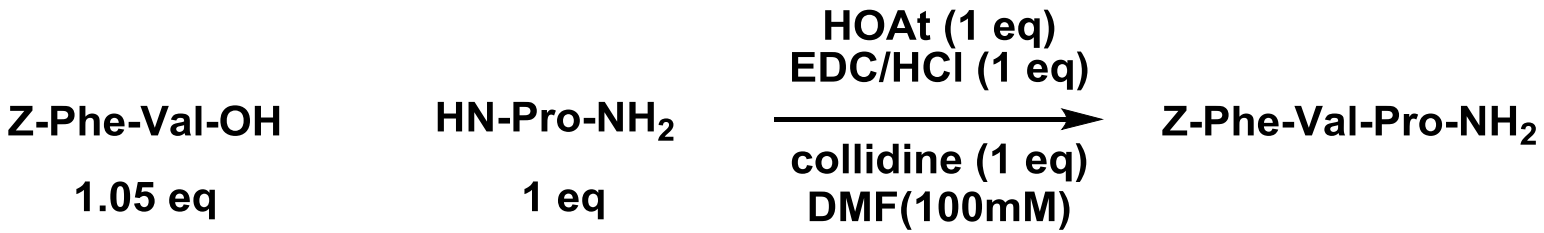
Half-times for reaction completion (min)

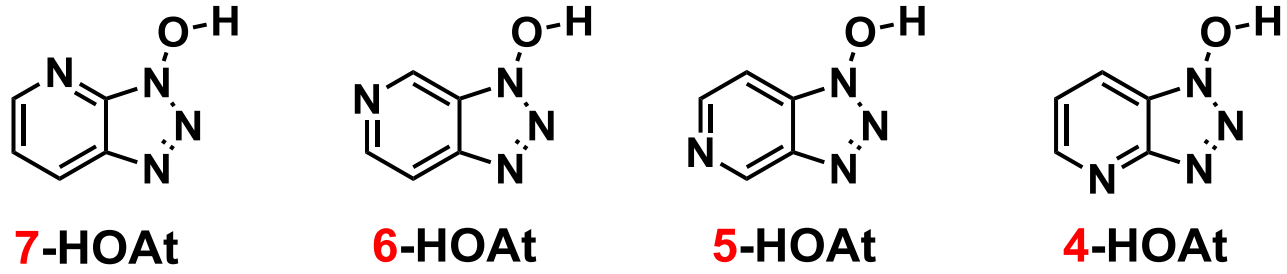
Louis A. Carpino et al. *Org. Lett.*, **2000**, *2*, 2253.

7-HOAt accelerates the reaction best.

To overcome epimerization - Additive

HOAt





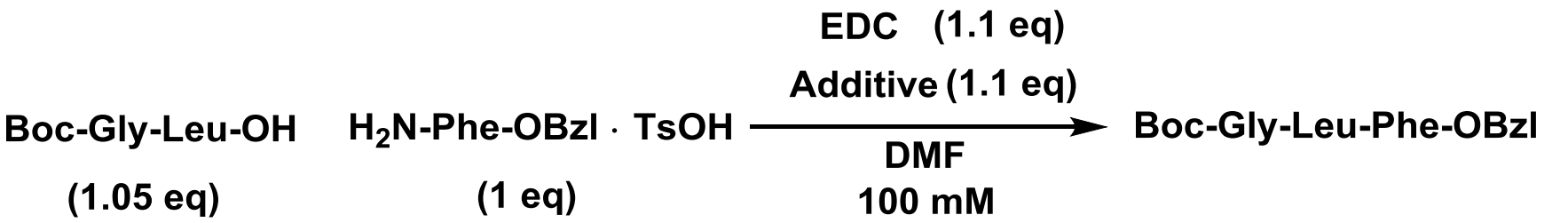
additive	Z-Phe-Val-Pro-NH_2 % LDL (yield, %)
7-HOAt	6.2 (95)
6-HOAt	11.0 (95)
5-HOAt	12.0 (95)
4-HOAt	13.2 (95)
HOBt	19.8

Louis A. Carpino *et al*. *Org. Lett*., **2000**, *2*, 2253.

7-HOAt inhibits epimerization best.

Drawing of amino group by N atom seems to exist.

To overcome epimerization - Additive



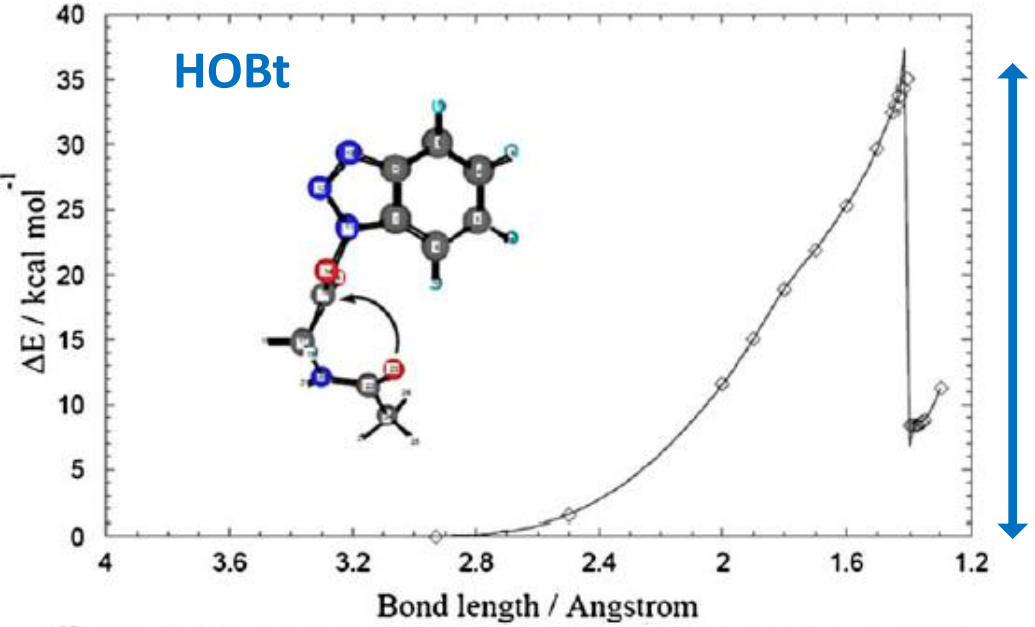
	Yield	epimerization rate
EDC-HOBt	90	3.6
EDC-HOOBt	92	0.2

(%)

HOOBt suppresses epimerization better than HOBt.

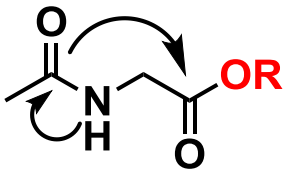
Sakakibara S. *et al. Int. J. Peptide Protein Res*. **1992**, 40, 294.

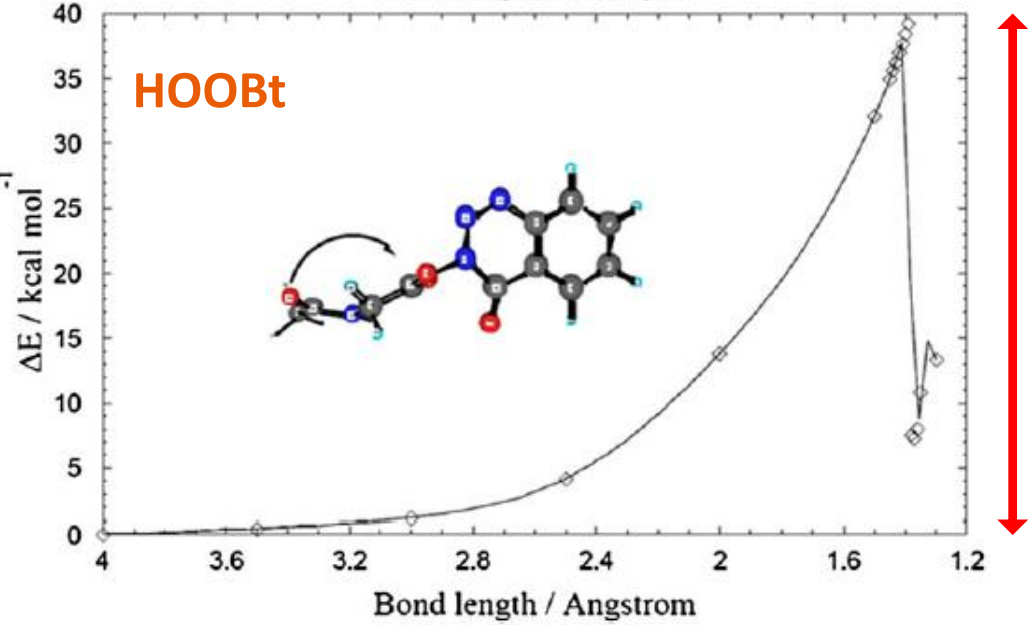
To overcome epimerization - Additive



35 kcal/mol

Potential energy surface scans for carbonyl oxygen





40 kcal/mol

Energy barrier for cyclization

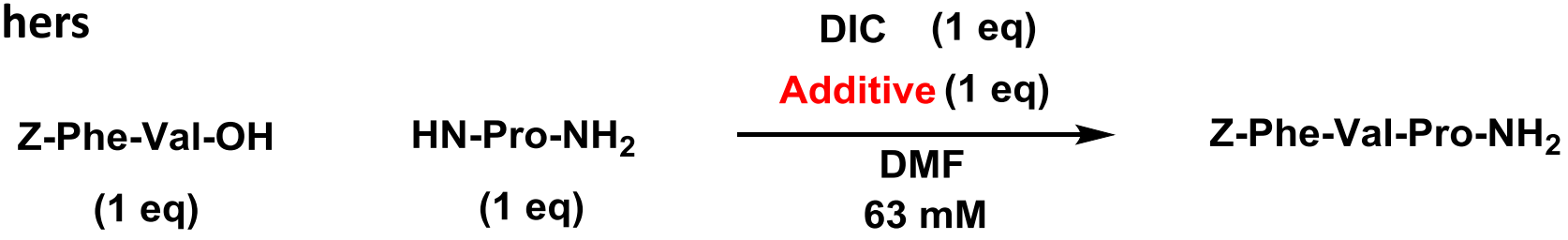
HOBt < HOOBt

HOOBt suppresses epimerization better.

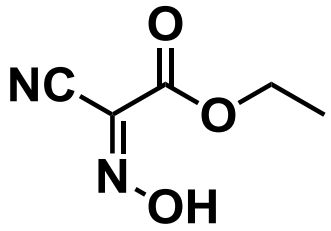
K. Teruya *et al*. *J. Pept. Sci*. **2012**, *18*, 669.

To overcome epimerization - Additive

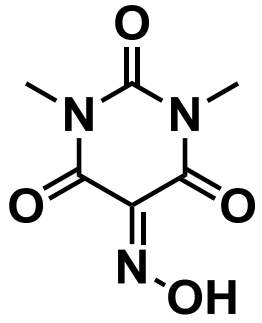
Others



Entry	Coupling reagent	Yield[b] (%)	LDL/LLL[c] (%)
1	DIC/HOBt (**1**)	96.3	14.8
2	DIC/HOAt (**2**)	97.6	5.9
3	DIC/OxymaPure (**7**)	91.9	7.7
4	DIC/Oxyma-B (**14**)	90.7	5.1



OxymaPure

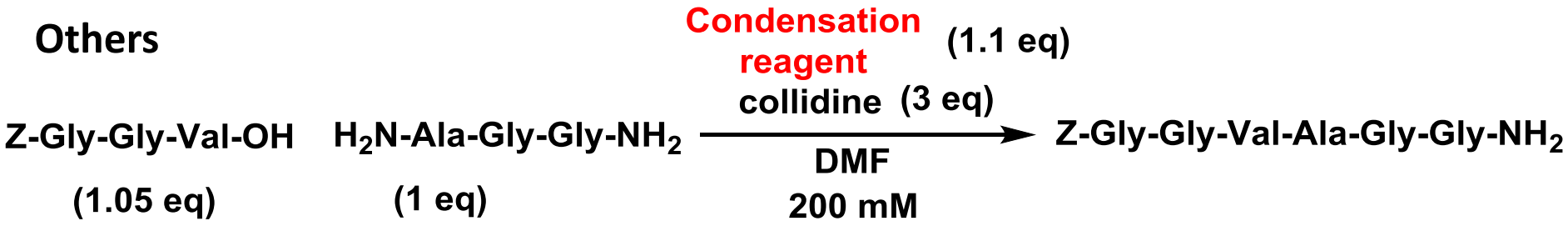


Oxyma-B

F. Albericio *et al*. *Org. Biomol. Chem*., **2014**, *12*, 8379.

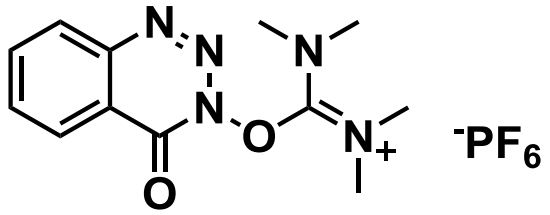
To overcome epimerization - Additive

Others



coupling reagent	base	solvent	yield (%)	LDL (%)
HDATU, **12**	TMP (3)	DMF	98.4	0.8
HDTU, **3**	TMP (3)	DMF	95.0	3.3
HATU[a]	TMP (3)	DMF	96.6	2.4
HBTU[a]	TMP (3)	DMF	85.6	8.2

HDATU



HDTU

L. A. Carpino, A. El-Faham *et al*. *J. Org. Chem*. **2004**, *69*, 54.

It is uncertain What makes additive effective.

To overcome epimerization - Summary

- **NCL realizes epimerization-suppressed fragment coupling by enabling intramolecular coupling reaction.**

- **Flow reaction may enable epimerization-suppressed coupling by shortening preactivation time.**

- **Additive can inhibit epimerization but its reason is remained to be solved.**

Summary

- **Epimerization rate is strongly effected by coupling condition.**

- **Epimerization rate depends on the sequence of amino acids, so where to connect peptide is important to conduct ligation.**

- **Shortening the existing time of intermediate can suppress the epimerization.**