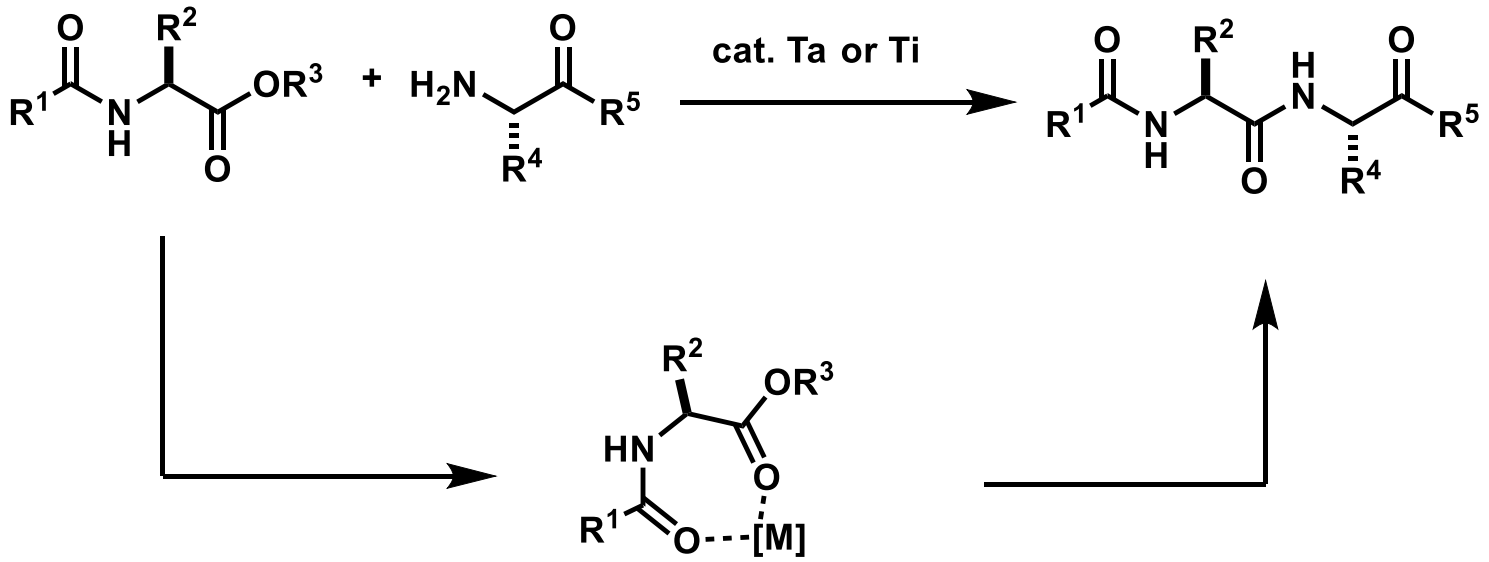
Substrate-Directed Peptide Synthesis with Lewis-Acid



2020.10.3. Literature Session
Nakata Yosuke

Contents

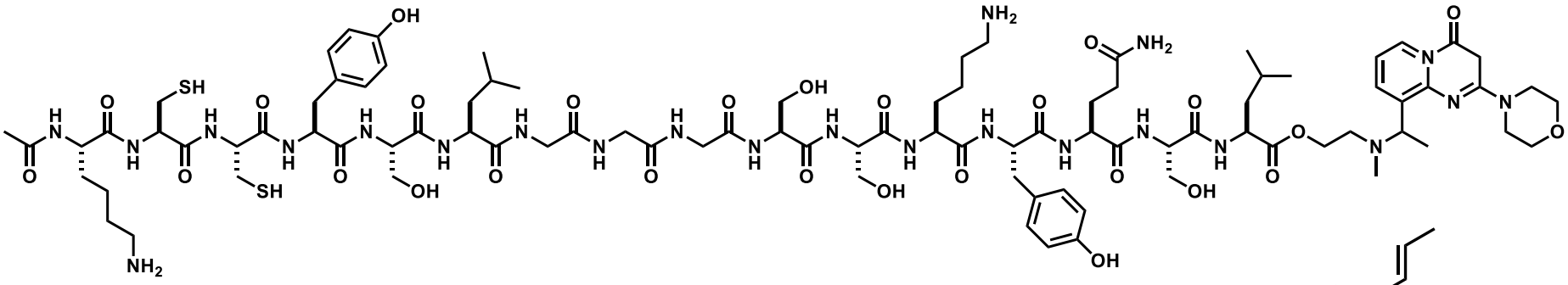
- **Introduction**
- **Substrate-Directed Synthesis of Dipeptide**
- **Substrate-Directed Synthesis of Oligopeptide**

(Muramatsu, W.; Hattori, T.; Yamamoto, H. *J. Am. Chem. Soc.* **2019**, *141*, 12288)

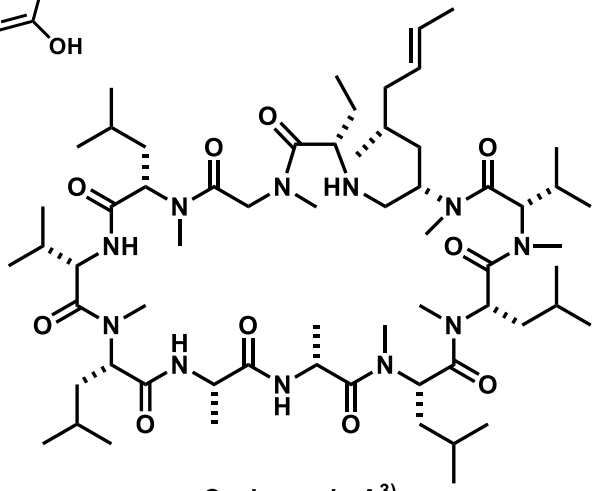
Contents

Demands for Peptide Synthesis

Peptides play an very important roll in pharmaceutical industry.[1)]



KCC-TGX[2)]
(anti cancer)



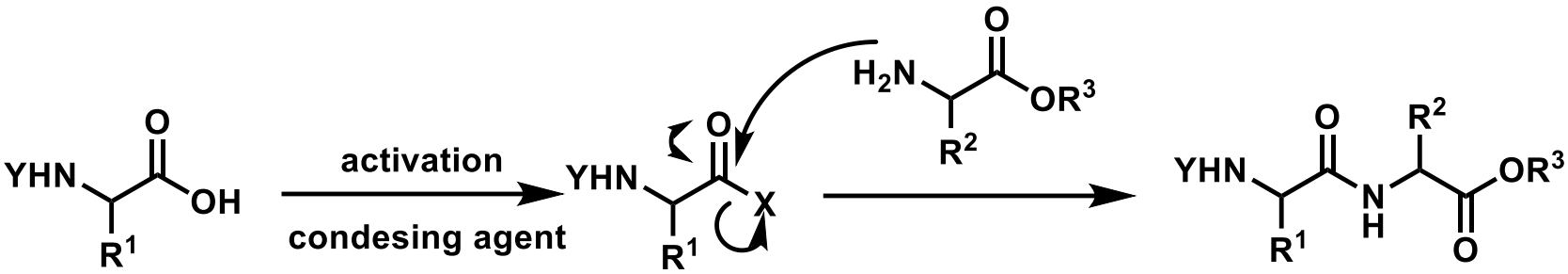
Cyclosporin A[3)]
(immunosuppressants)

Peptide-based drugs have been popular recently and occupy a certain ratio in the drug market.

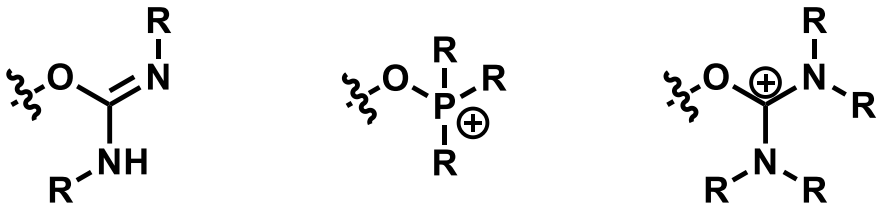
Methodology development for peptide synthesis is undoubtedly important.

1) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Comb. Chem*. **1999**, *1*, 55.
2) Wanyi, T.; Ravi, S. S.; Bin, Q.; Benyi, L.; Kun, C. *Anal. Chem.* **2019**, *91*, 6996.
3) Vinogradov, A. A.; Yin, Y.; Suga, H. *J. Am. Chem. Soc*. **2019**, *141*, 4167−4181.

Classical Condensing Agent-Mediated Amidation[1)]



X = halide, N_3,
OR (activated ester),
OCOR (anhydride),



O-acyl urea　　acyl phosphonium　　acyl uronium

Advantages

- wide substrate scope
- applicable to stepwise oligopeptide synthesis

Issues[2)]

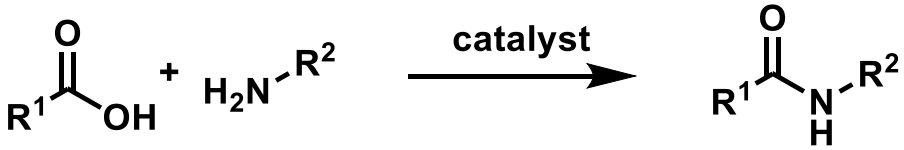
- excess amount of condensing agents and amino acids
- large volume of solvent

Catalytic amidation was supposed as a method avoiding the issue of amount of reagents.

1) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557.
2) Trost, B.M. *Science* **1991**, *254*, 1471.

Catalytic Amidation[1)]

Direct catalytic amidation of carboxylic acid with amine can avoid excess amount of condensing agents.



Catalyst

- **boronic acid derivatives[2), 3)]**
- **metal catalyst[4), 5)]**
- **organocatalyst[6), 7)]**

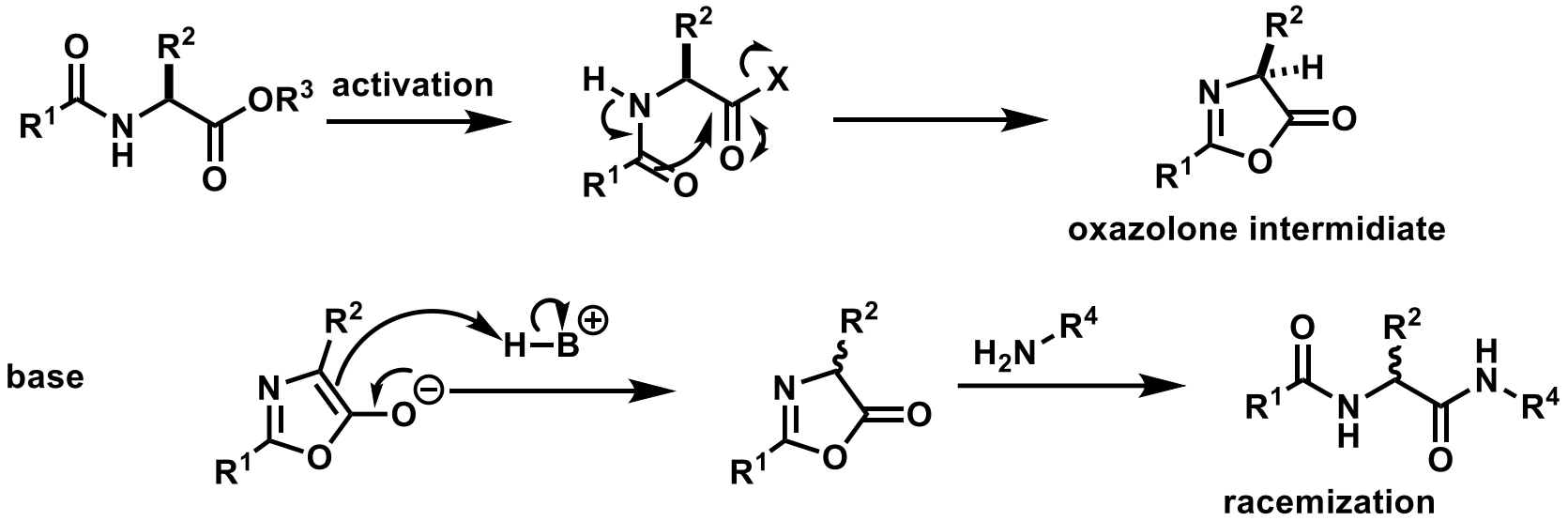
Problems

- **racemization occurs with some catalyst**
- **limited substrate scope**

Catalyst applicable to wide scope of amino acids without racemization has been pursued.

1) de Figueiredo, R. M.; Suppo, J.-S.; Campagne, J.-M. *Chem. Rev.* **2016**, *116*, 12029.
2) K. Wang, Y. Lu, K. Ishihara. *Chem. Commun*. **2018**, *54*, 5410.
3) El Dine, T. M.; Rouden, J.; Blanchet, J. *Chem. Commun*. **2015**, *51*, 16084.
4) Ghosh, S.; Bhaumik, A.; Mondal, J.; Mallik, A.; Sengupta.; Bandyopadhyay, S.; Mukhopadhyay, C. *Green Chem*. **2012**, *14*, 3220.
5) Nagarajan, S.; Ran, P.; Shanmugavelan, P.; Sathishkumar, M.;Ponnuswamy, A.; Nahm, K. S.; Kumar, G. G. *New J. Chem*. **2012**, *36*, 1312.
6) Mangawa, S. K.; Bagh, S. K.; Sharma, K.; Awasthi, S. K. *Tetrahedron Lett*. **2015**, *56*, 1960.
7) 191116_LS_Aoi_Takeuchi_Designing an Organocatalyst-Driven Peptide Synthesis

Racemization in Activation of Carboxylic Acid Moiety[1)]



Some of catalytic activation of carboxylic moiety of amino acid causes racemization via oxazolone intermediate.

→To avoid the problem, substrate-directed synthesis was envisioned as a solution.

1) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557.

Author's Profile

Prof. Hisashi Yamamoto

Research Career

1963 *B.S.* @Kyoto University advised by Prof. H. Nozaki

1971 *Ph.D.* @Harvard University advised by Prof. E. J. Corey

1980- *Associate Professor* @Nagoya University

1983- *Professor* @Nagoya University

2003- *Professor Emeritus* @Nagoya University

2012- Professor and Director @Molecular Catalyst Research Center, Chubu University

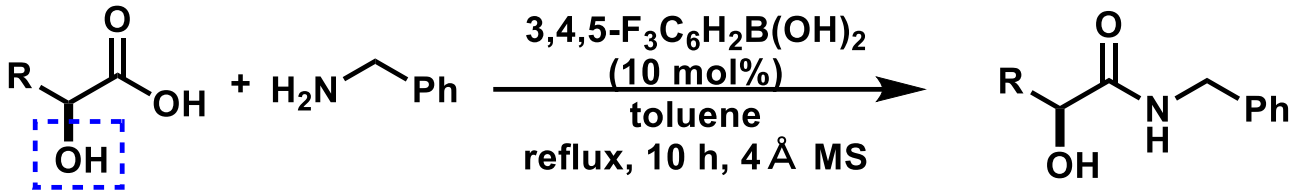
Research Interests

- **Lewis acid catalysis**
- **Peptide synthesis**

Contents

Previous Scheme by Author (1)

1. Catalytic Amidation by Boronic Acid[1)]



directing group

Authors firstly chose hydroxy-directed approach.

Since this report, hydroxy-directed anidation of α- and β- hydroxycarboxylic acids by boronic acid catalyst have been developed. [2), 3), 4), 5)]

Direct catalytic amidation by boronic acid

○ Amidation proceeded with high stereoselectivity.

△ A large quantity of molecular sieves were needed (waste problem).

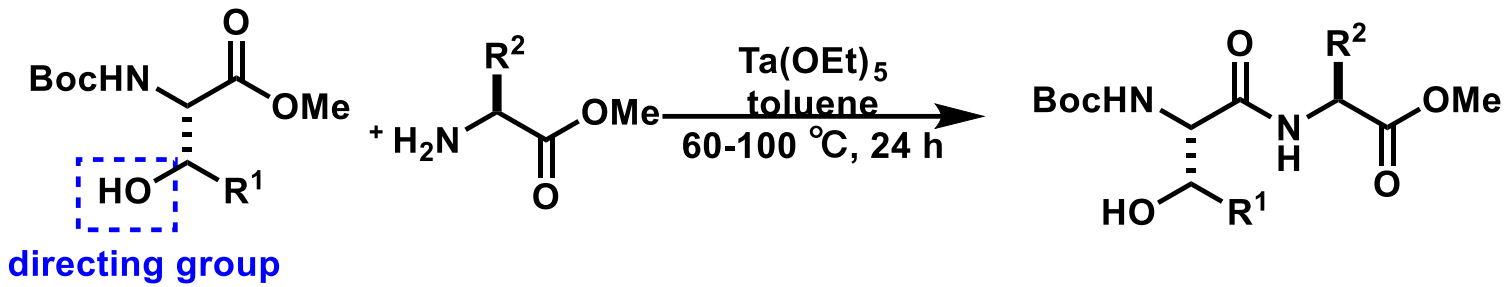
→Hydroxy-directed Lewis acid catalysis without generating water was selected for peptide synthesis.

1) K. Ishihara, S. Ohara, H. Yamamoto. *J. Org. Chem*. **1996**, *61*, 4196.
2) T. Maki, K. Ishihara, H. Yamamoto. *Tetrahedron* **2007**, *63*, 8645.
3) R. Yamashita, A. Sakakura, K. Ishihara. *Org. Lett.* **2013**, *15*, 3654.
4) K. Ishihara, Y. Lu. *Chem. Sci.* **2016**, *7*, 1276.
5) N. Shimada, M. Hirata, M. Koshizuka. N. Ohse, R. Kaito, K. Makino, *Org. Lett.* **2019**, *21*, 4303.

Previous Scheme by Author (2)

2. Hydroxy-Directed peptide synthesis by Lewis Acid

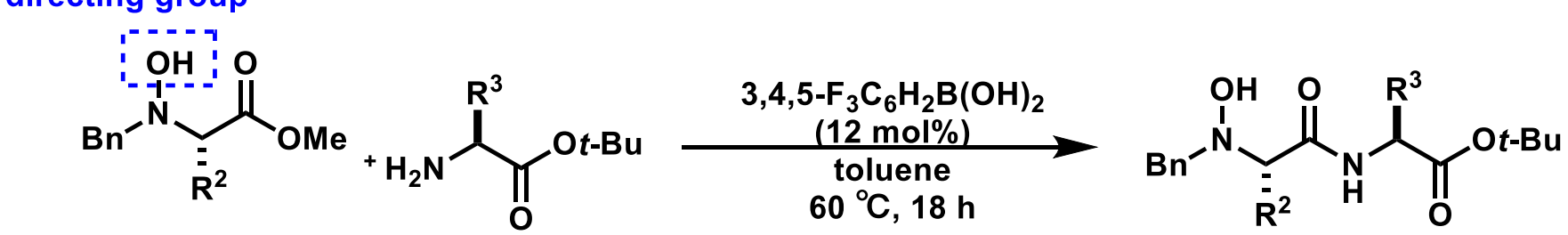
2-1. Tantalum-Catalyzed Amidation of Amino Acid methyl esters Possessing a Hydroxy Groups[1)]



○ **Amidation was applied to peptide synthesis without racemization.**

△ **Substrate scopes were limited to only Ser, Thr and their derivatives.**

2-2. Boronic-Acid-Catalyzed Amidation of N-Hydroxy Amino Acid methyl esters[2)]



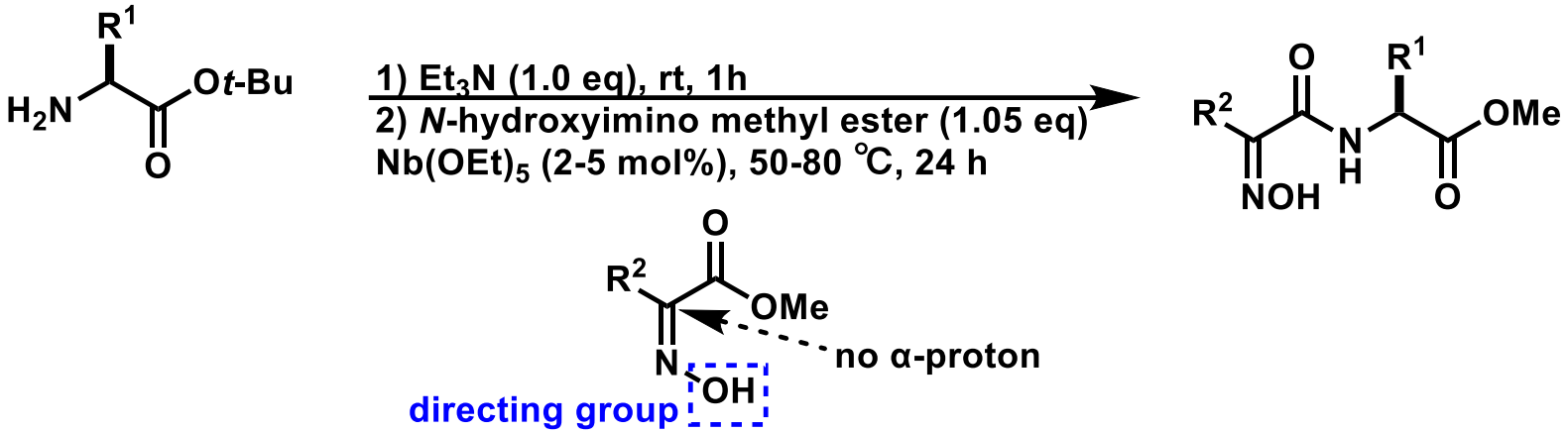
○ **Substrate scopes were extended to amino acids derivatives possessing 2° amine.**

△ **Longer synthetic steps to obtain targeted peptides**

1) H. Tsuji, H. Yamamoto. *J. Am. Chem. Soc.* **2016**, *138*, 14218.
2) H. Tsuji, H. Yamamoto. *Synlett* **2018**, *29*, 318.

Previous Scheme by Author (3)

2-3. Niobium-catalyzed Amidation of *N*-Hydroxyiminoesters[1)]



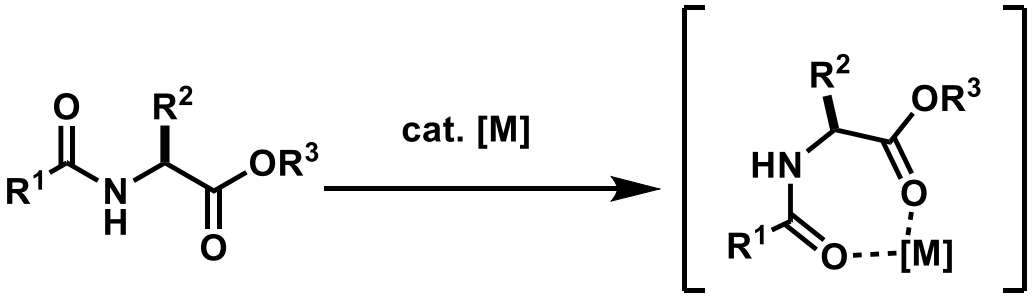
○ Racemization was not caused in an activation step due to absence of α-proton.

△ Poor selectivity of hydrogenation of iminoester

problems of hydroxy-directed Lewis acid catalysis

・・・limited substrate scope (2-1) and complicated steps to obtain targeted peptides (2-2, 2-3)

→To solve above two problems, carbonyl-directed catalysis was envisioned.



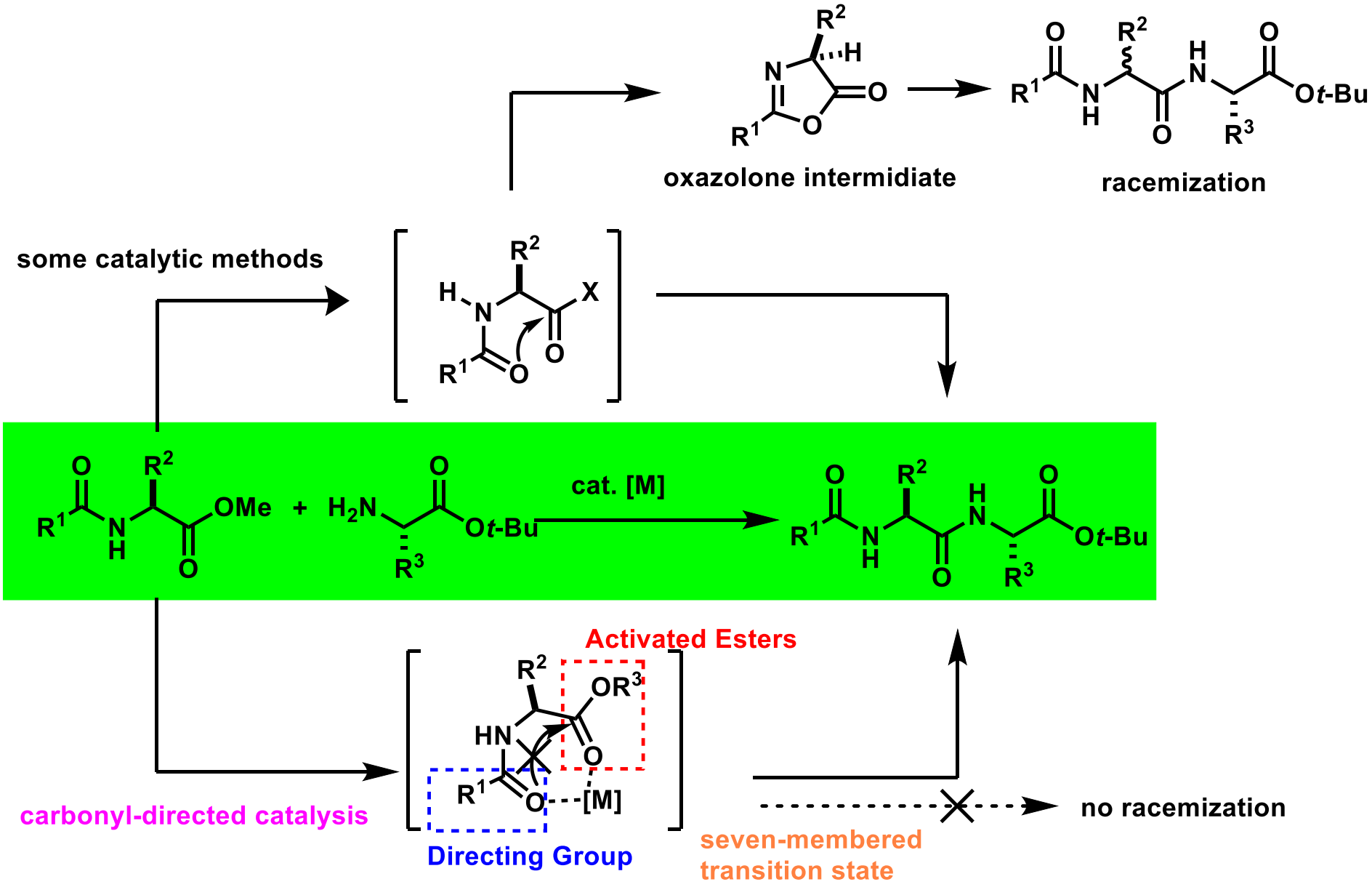
***N*-protection**

・applicable to most amino acids

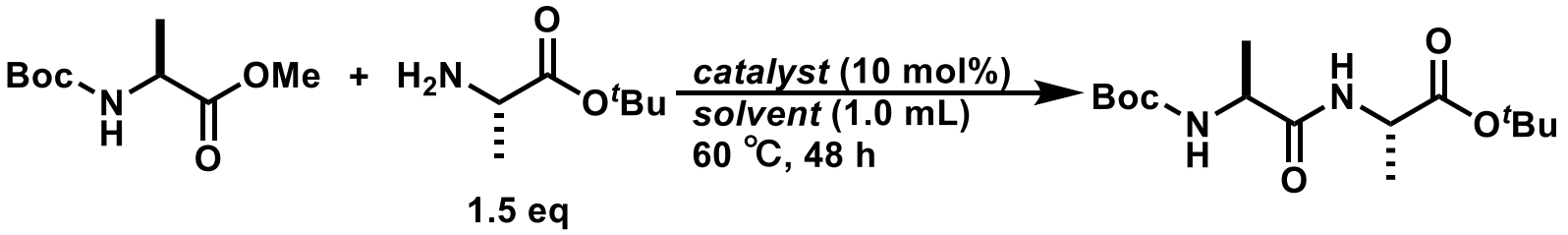
・easy protection/deprotection

1) W. Muramatsu, H. Tsuji, H. Yamamoto. *ACS Catal.* **2018**, *8*, 2181.

Concept of Carbonyl-Directed Amidation



Screening of solvent



solvent	*catalyst*	yield (%)	*solvent*	*catalyst*	yield (%)
DMSO	$Ta(OEt)_5$	<1	DCM	$Ta(OEt)_5$	4
CH_3CN	$Ta(OEt)_5$	3	Hexane	$Ta(OEt)_5$	44
Et_2O	$Ta(OEt)_5$	28	——	$Ta(OEt)_5$	70
AcOEt	$Ta(OEt)_5$	9	——	$Ta(OMe)_5$	56
Toluene	$Ta(OEt)_5$	18	——	$Ta(OMe)_5$	99[a]

[a] Temperature and time was changed to 45 ℃ and 72 h. 2.0 eq amino acid t-Bu ester was used.

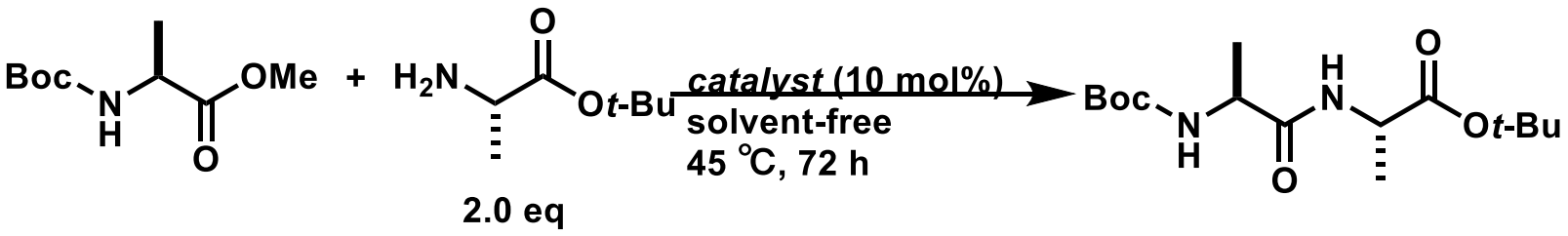
Aliphatic solvents conditions gave products in higher yields compared to polar solvents.

☆Solvent-free catalysis was found to be the best.

→Week C=O$^+$—Ta bond was more easily disrupted in more polar solvents.

→High concentration of substrate in solvent-free may also affect the yields.

Screening of Lewis Acid

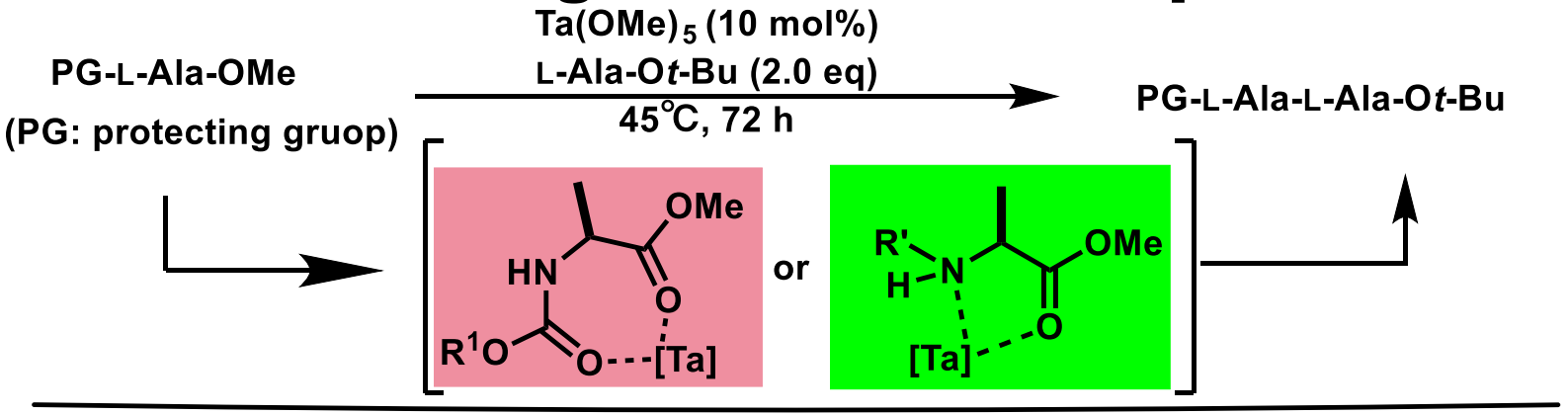


catalyst	yields (%)	*catalyst*	yields (%)
$Ta(OMe)_5$	99	$Nb(OEt)_5$	30
$Ta(OEt)_5$	78	$VO(OEt)_3$	<1
$Ta(OBu)_5$	57	$Fe(OMe)_2$	8
$Ta(acac)(OEt)_4$	8	$Hf(OEt)_4$	7
$Ta(NMe_2)_5$	69	$Nd(Oi\text{-}Pr)_3$	20
TaX_5 (X=F, Cl, Br)	2~10	Cp_2HfCl_2	12

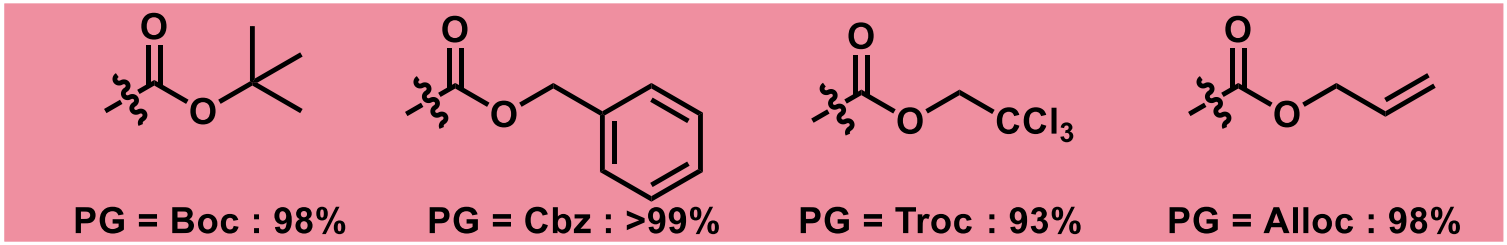
To coordinate to carbonyl oxygen, hard Lewis acid was selected.

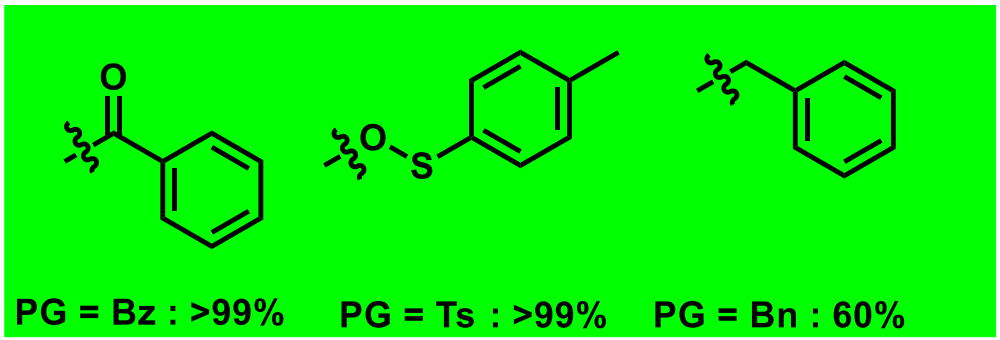
☆Lewis acid was optimized as $Ta(OMe)_5$.

Examining Protection Groups

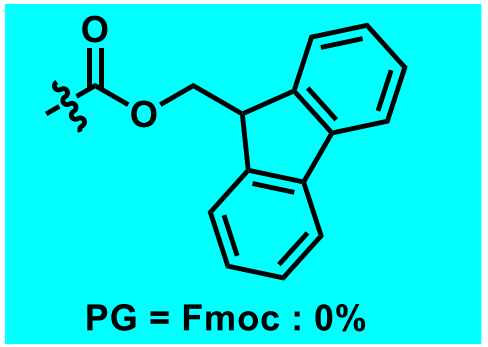


carbamate protecting groups





non-carbamate protecting groups



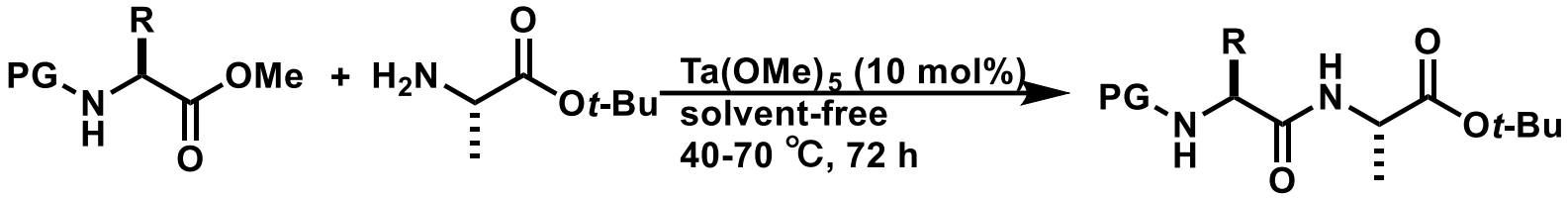
low yield

Commonly used carbamate protecting groups were torelated in this condition except Fmoc.

→Fmoc was deprotected due to free amines.[1)]

1) W. Muramatsu, H. Yamamoto. *J. Am. Chem. Soc.* **2019**, *141*, 18926.

Scope of Protected Amino Acid Methyl Esters



14 of *N*-protected 20 natural α-amino acid methyl esters were converted to desired dipeptides (45% ー >99%) without racemization.

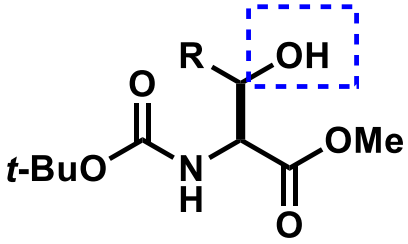
Conversion of Boc-L-Ser-OMe, Boc-L-Thr-OMe, and Boc-L-Asp-OMe was completed in a shorter time with smaller amount of catalyst.

→Secondary directing effect of hydroxy group (Ser, Thr) or *t*-Bu ester group at β-position (Asp)

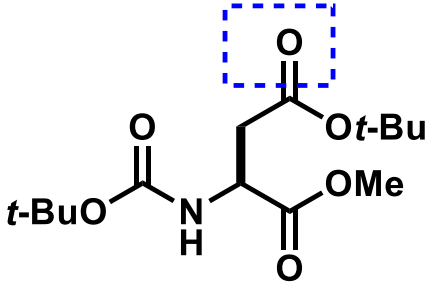
Boc-L-Val-OMe, Boc-L-Ile-OMe, and Boc-L-Tle were slightly converted.

→The bulky side chains at the α-position sterically hindered formation of the seven-membered transition state.

another directing groups

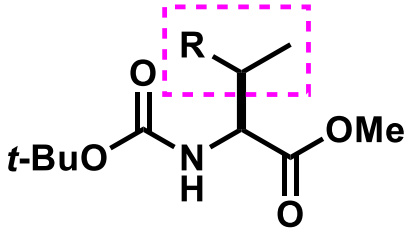


Boc-L-Ser-OMe (R = H)
Boc-L-Thr-OMe (R = Me)

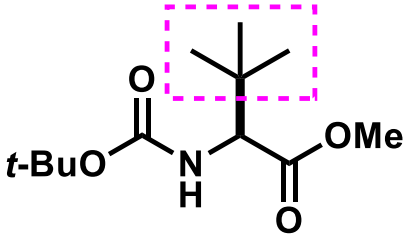


Boc-L-Asp-OMe

bulky side chains

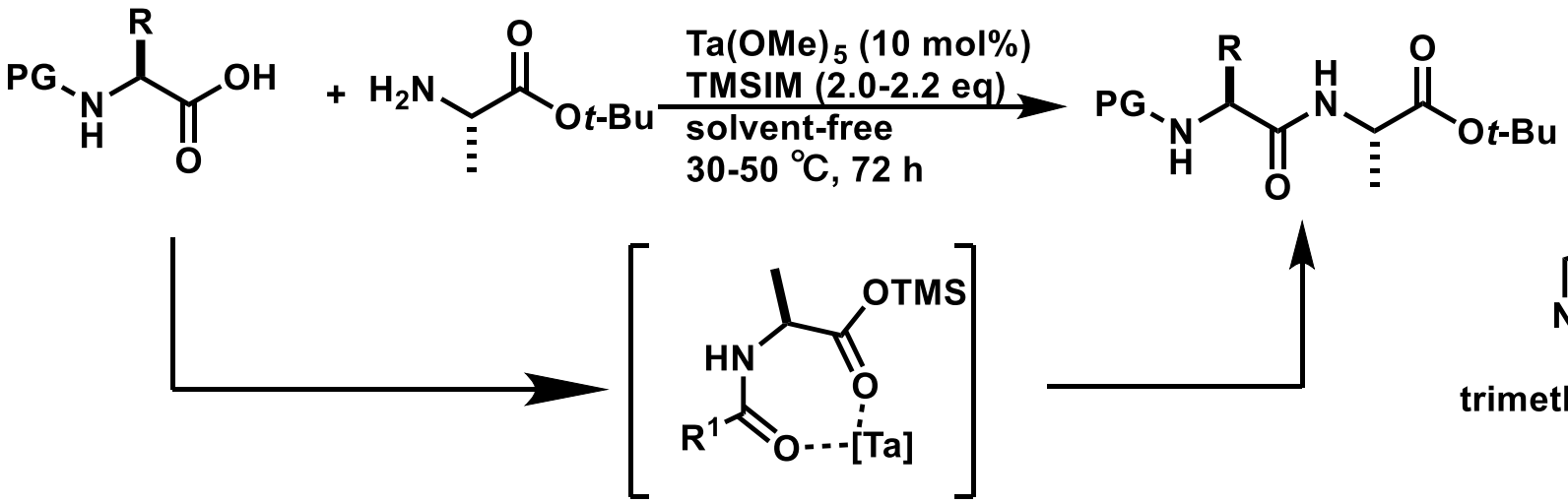


Boc-L-Val-OMe (R = Me)
Boc-L-Ile-OMe (R = Et)



Boc-L-Tle-OMe

Alteration to Silyl Esters



trimethylsilylimidazole
(TMSIM)

⁻OTMS is a better leaving group than ⁻OMe. (pK_a (TMSOH) = 11, pK_a (MeOH) = 16)

Boc-L-Val-OMe, Boc-L-Ile-OMe, and Boc-L-Tle were converted to desired peptides in 97, 74, 37% in yield respectively.

Yields of conversion about 6 of Boc- protected 20 natural α-amino acids were improved from using methyl esters (45% ⁻ 77% → 64% ⁻ 97%) .

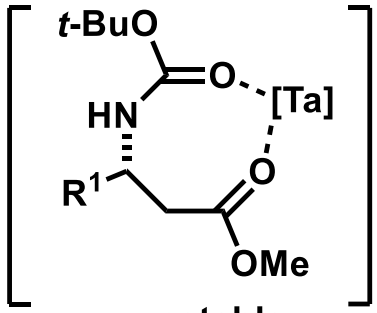
Fmoc-L-Ala-OH was converted to a desired peptide in a good yield.

(0% (previous method) → 85% (in this method))

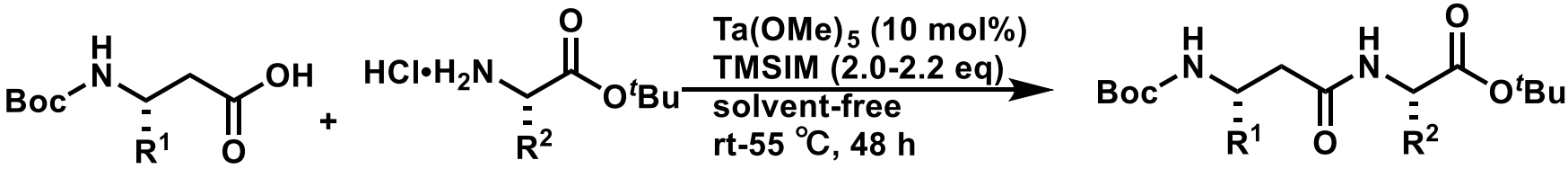
Extended Scope (Amino Acid Homologues)[1)]

Boc- protected β-amino acid methyl esters did not converted to dipeptides by the $Ta(OMe)_5$ catalyst because of unstable eight-membered transition state.

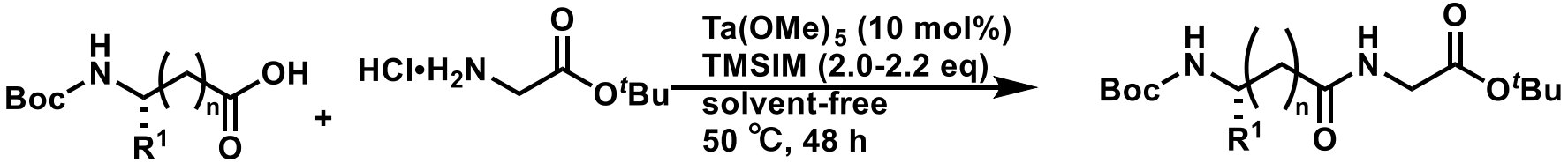
So, Carbonyl-directing $Ta(OMe)_5$ catalysis with trimethylsilylimdazole (TMSIM) was applied to amidation of β- or longer chain amino acids.



unstable



90% - >99%



n = 2 Boc-γ-HoGly-Gly-O*t*-Bu : 80%
n = 3 Boc-δ-HoGly-Gly-O*t*-Bu : 98%
n = 4 Boc-ε-HoGly-Gly-O*t*-Bu : 92%
n = 10 Boc-λ-HoGly-Gly-O*t*-Bu : 94%

Not only β-amino acids but also longer chain amino acids converted to dipeptides in good yields

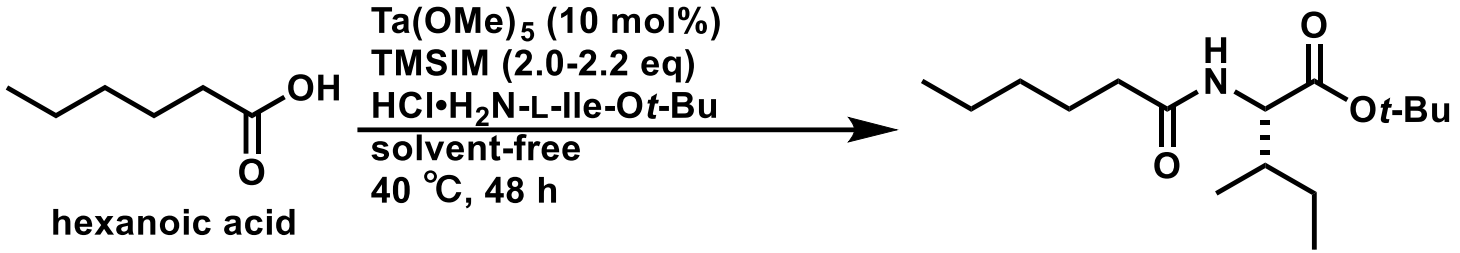
Substrate scope was extended.

1) W. Muramatsu, H. Yamamoto, *J. Am. Chem. Soc.* **2019**, *141*, 18926.

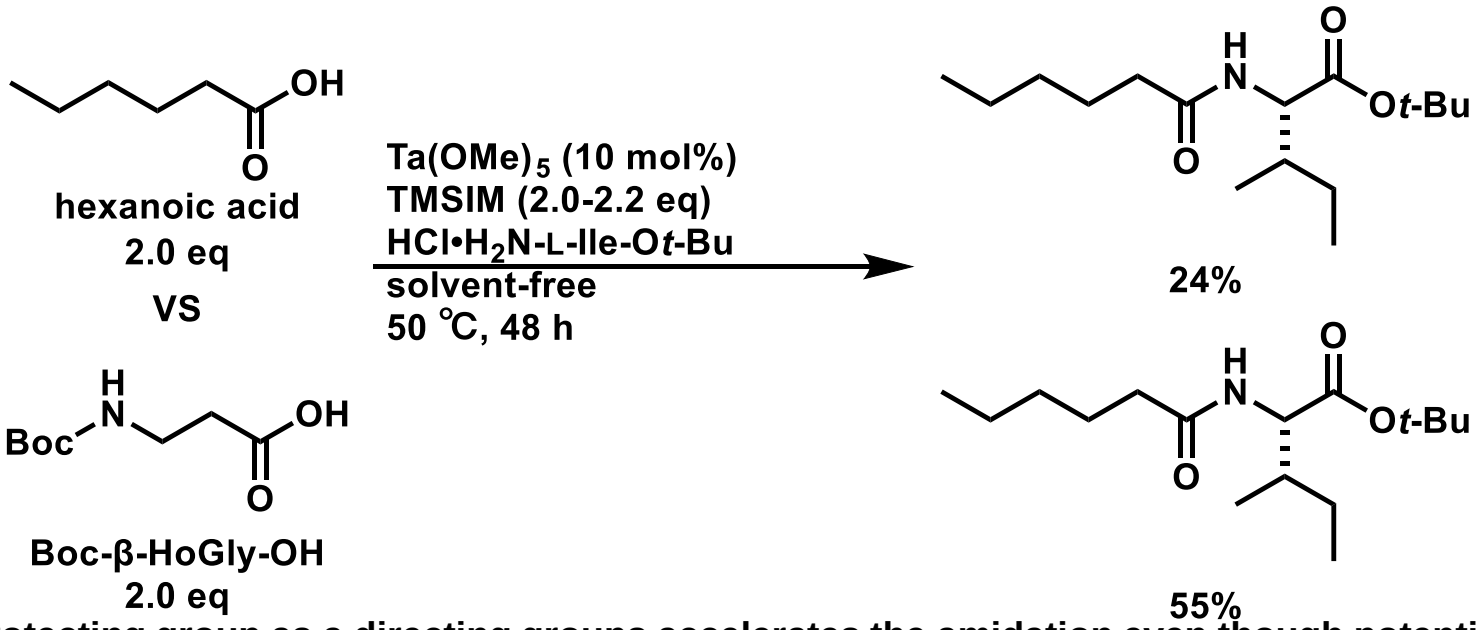
Control Experiments

To probe the mechanism of amidation by $Ta(OMe)_5$ and silyl ester system, experiments below were conducted as a control.

Generation of silyl esters was confirmed by ^{1}H and ^{29}Si NMR spectrum.

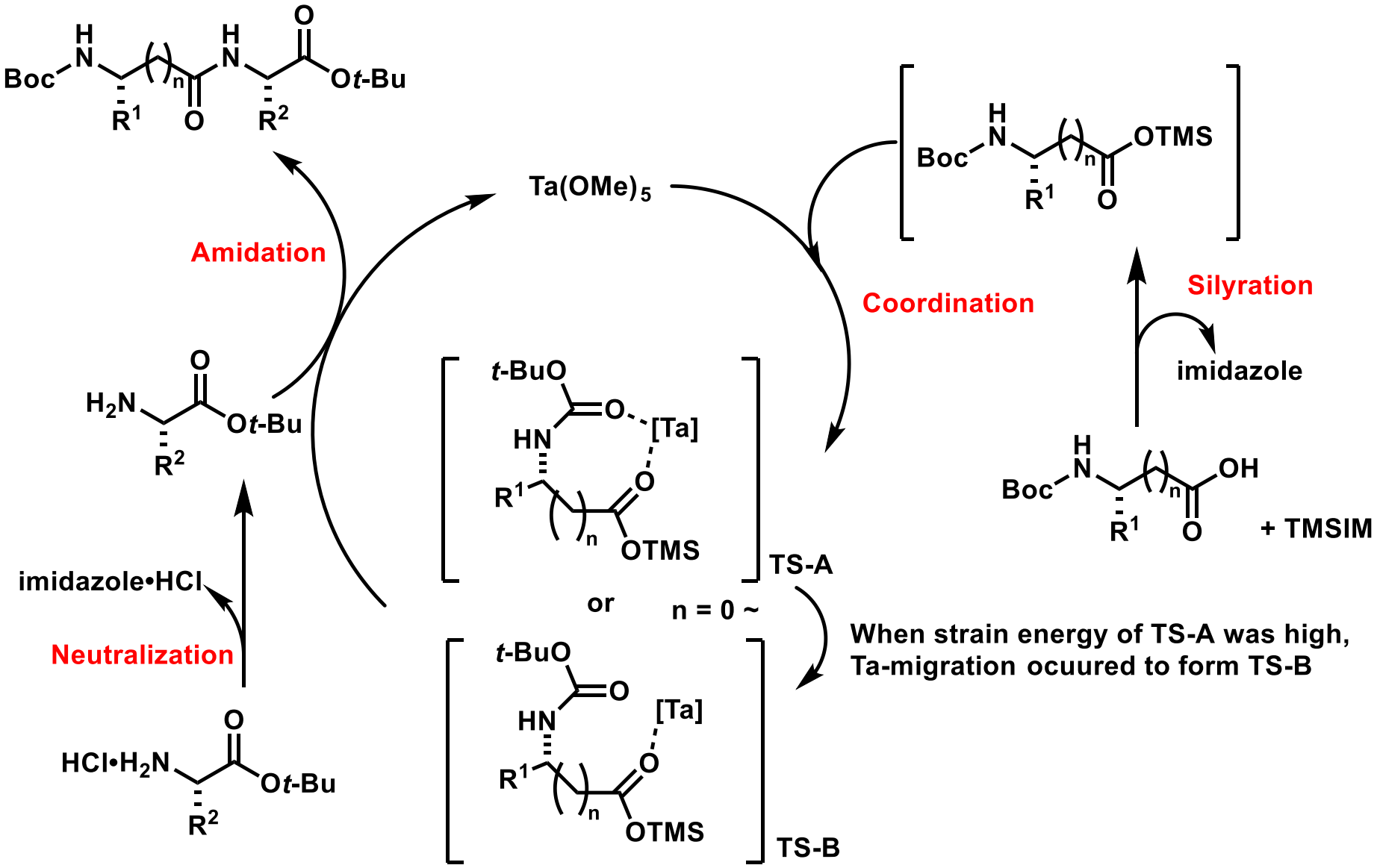


Amidation proceeds without *N*-protecting groups as directing groups in this system.



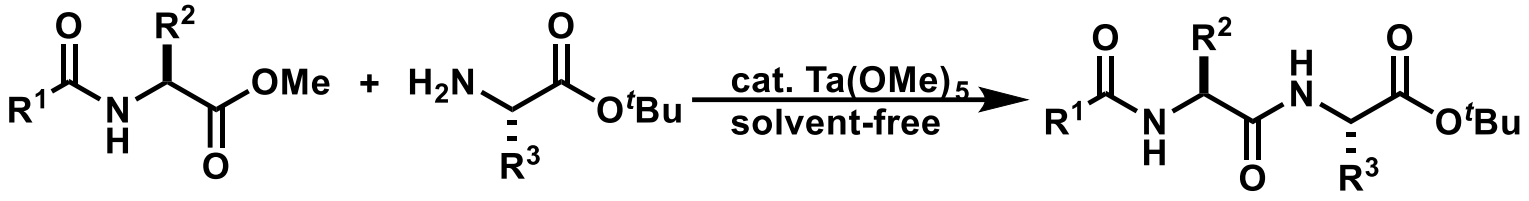
***N*-protecting group as a directing groups accelerates the amidation even though potentially generating 8-membered transition state**

Plausible Mechanistic Pathway



Short Summary

- **$Ta(OMe)_5$ enabled solvent-free carbonyl-directed catalysis of dipeptide without racemization.**



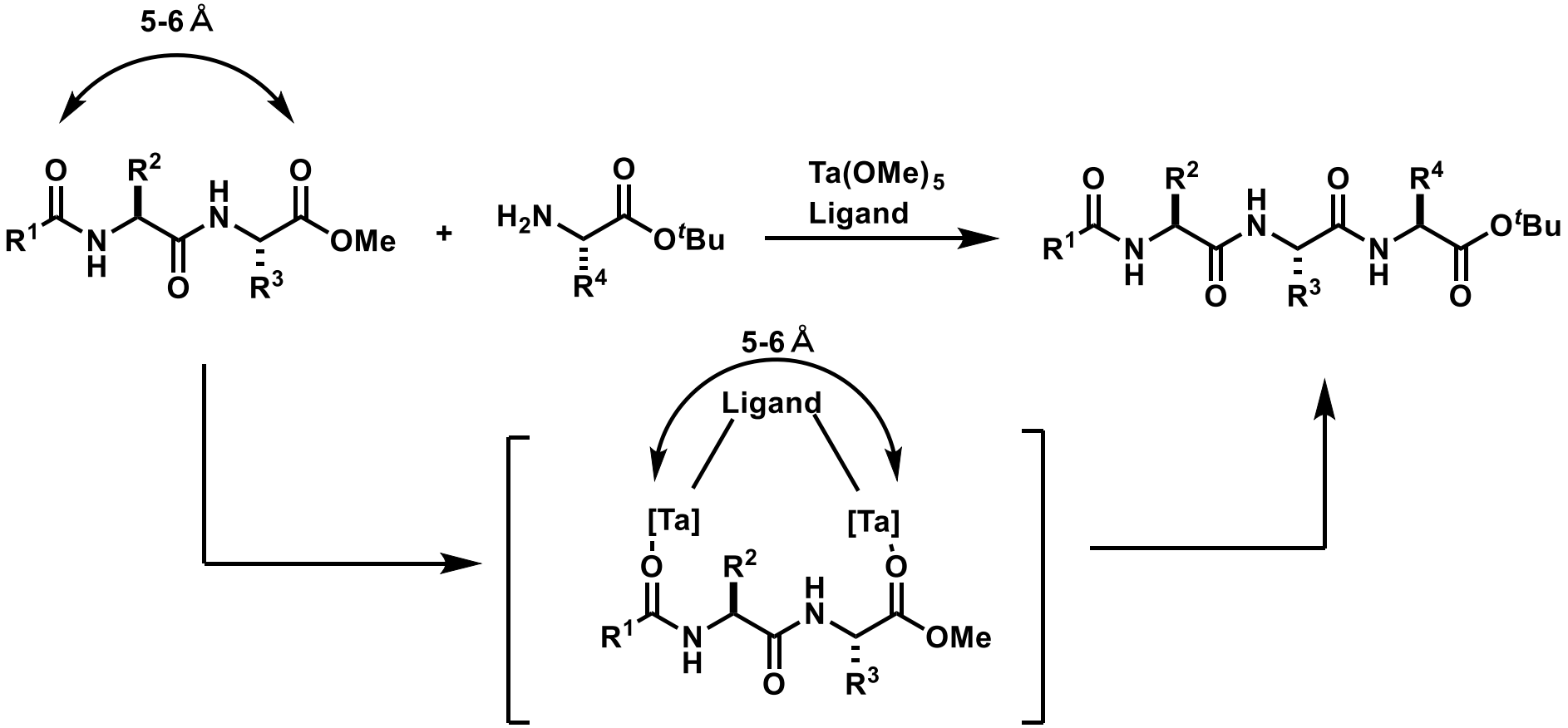
- **Utilizing silyl ester extended substrate scope not only to α-amino acids possessing bulky side chains but also to β- or longer chain amino acids.**

Contents

Substrate-Directed "Remote" Peptide Bond-Formation

According to author's previous report[1),2)], tantalum catalysts can activate the carbonyl oxygen at least six atoms away from directing group.

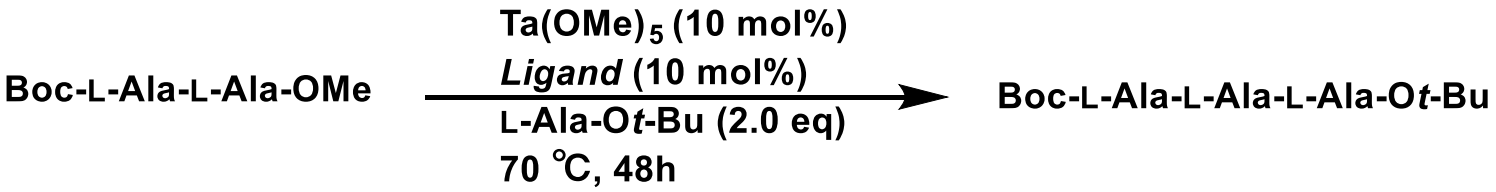
→This carbonyl-directed catalytic system can activate remote C-terminal carbonyl oxygen from N-terminal protecting group of dipeptides.

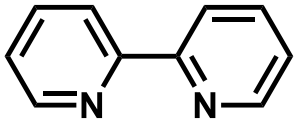


To achieve the "remote" activation, ligands which satisfy above conditions were screened.

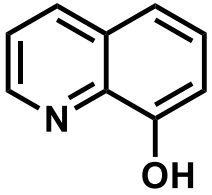
1) H. Tsuji, H. Yamamoto. *J. Am. Chem. Soc.* **2016**, *138*, 14218.
2) W. Muramatsu, H. Tsuji, H. Yamamoto. *ACS Catal.* **2018**, *8*, 2181.

Ligand Screening

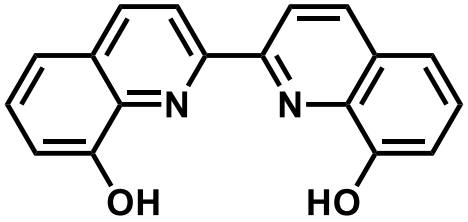




L1
66%

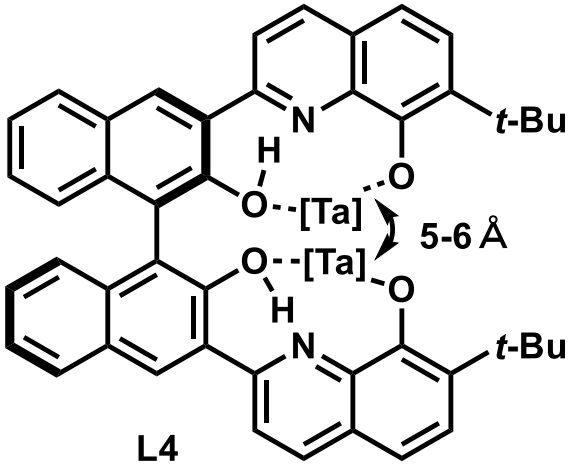


L2
68%

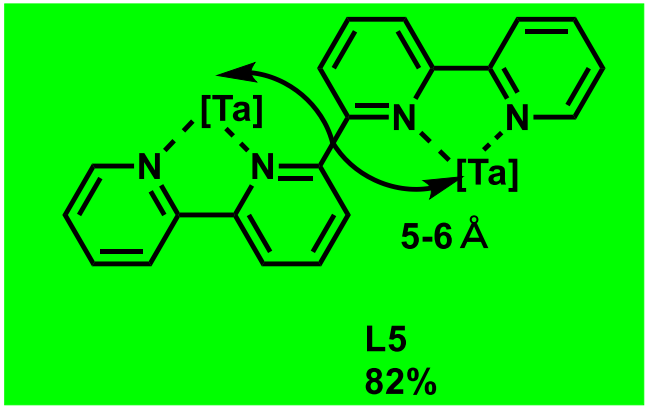


L3
74%

Firstly, L1~L3 gave good yields, then Ligands were designed to carry two tantalum atoms in 5~6 Å distance.



L4
71%



L5
82%

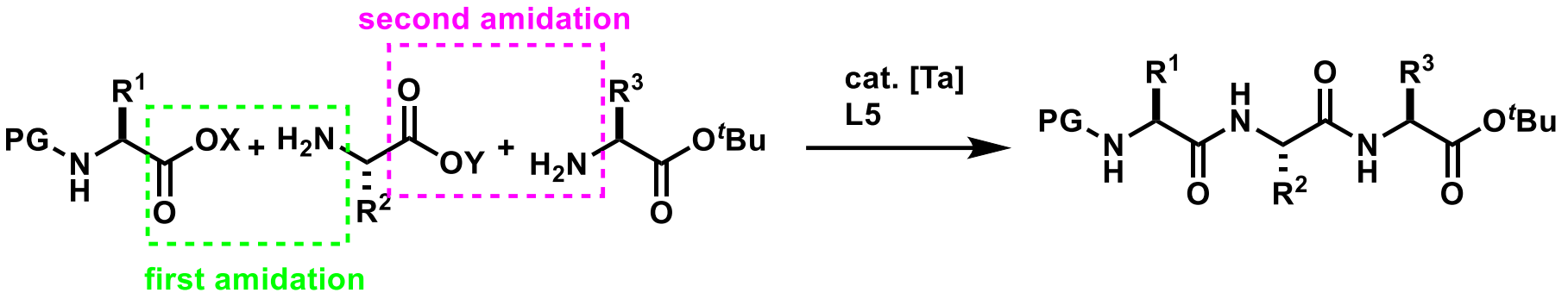
Ligand was optimized as L5.

Solvent conditions were optimized as solvent-free.

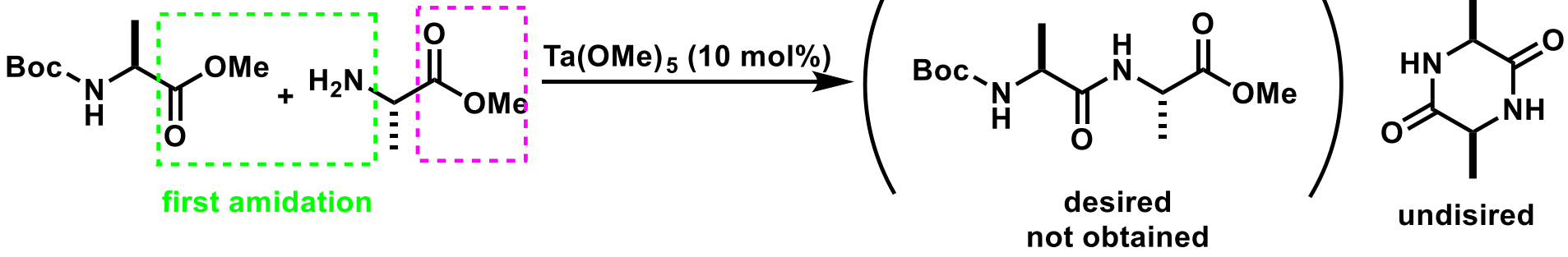
1) H. Tsuji, H. Yamamoto. *J. Am. Chem. Soc.* **2016**, *138*, 14218.
2) H. Tsuji, H. Yamamoto. *Synlett* **2018**, *29*, 318.

Application to Triply Convergent Synthesis (1)

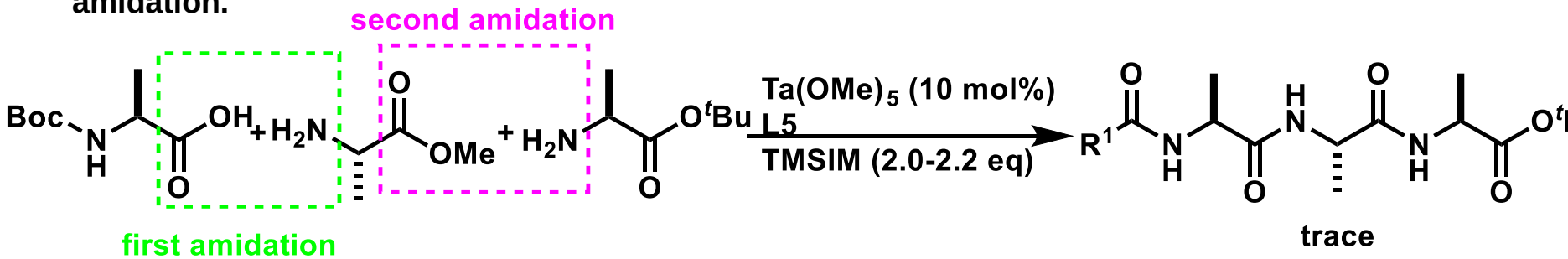
Three-component peptide-forming reaction using $Ta(OMe)_5$ and L5 was investigated.



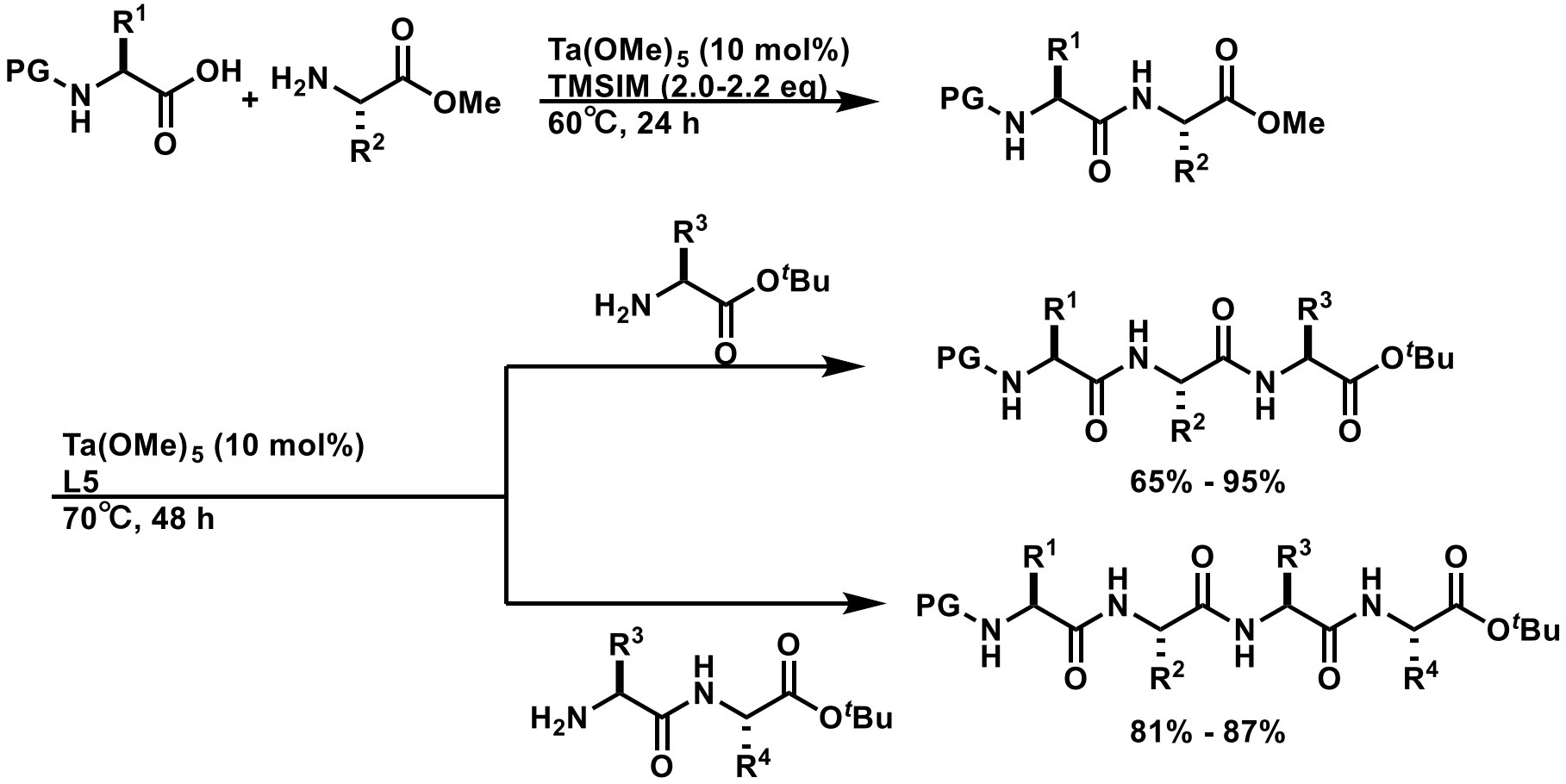
Using Amino acid methyl ester as the nucleophile in the first amidation caused generation of the cyclic L-Ala-L-Ala.



In one-pot reaction, the second amidation was affected by imidazole generated in the first amidation.



Application to triply Convergent synthesis(2)



After the first amidation, crude product was washed by water to remove imidazole.

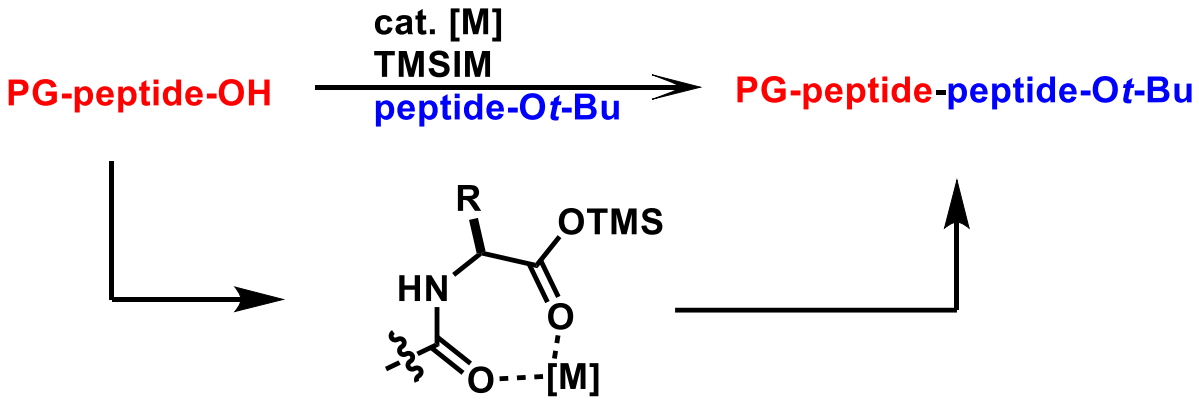
Without purification of the crude product, the second amidation was proceeded.

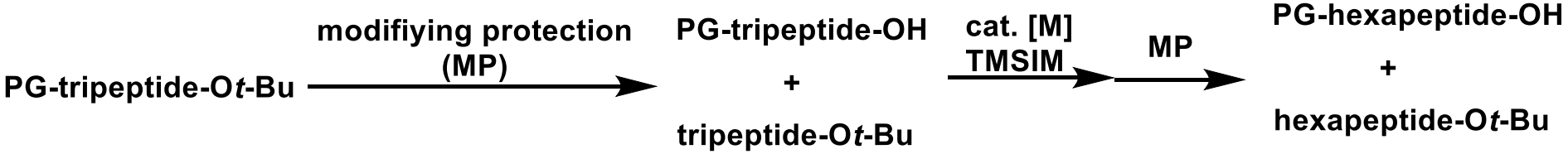
Dipeptides consisting of natural α-amino acid were converted to tri- or tetrapeptide in good yields without any stereochemical problems.

Peptide Coupling Reaction

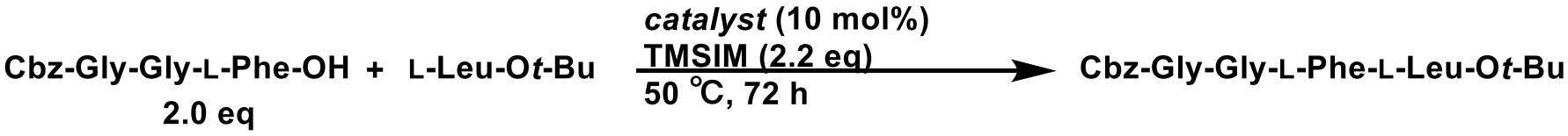
Tripeptides can be generated by $Ta(OMe)_5$ and L5 system.

→Realizing carbonyl-directed chemical ligation of tripeptides theoretically enables synthesis of any oligopeptide possessing 3×2^n residues in very few steps.





Lewis Acid Screening



catalyst	yield (%)	dr	*catalyst*	yield (%)	dr
$Mg(ClO_4)_2$	trace		$Ti(OMe)_4$	75	90:10
$Al(OEt)_3$	trace		$Ti(OEt)_4$	64	90:10
$Pd(phen)_2^{2+}\bullet 2PF_6^-$	trace		$Ti(Oi\text{-}Pr)_4$	72	91:9
$Zr(OBu)_4$	34	85:15	$Ti(OBu)_4$•monomer	68	91:9
Cp_2NbCl_2	trace		$Ti(Ot\text{-}Bu)_4$	trace	
$CpHfCl_2$	22	88:12	$Ti(OCH_2CH(Et)Bu)_4$	5	
$Ta(OMe)_5$	0		$CpTiCl_3$	trace	
Cp_2WCl_2	trace		Cp_2TiCl_2	44	75:25

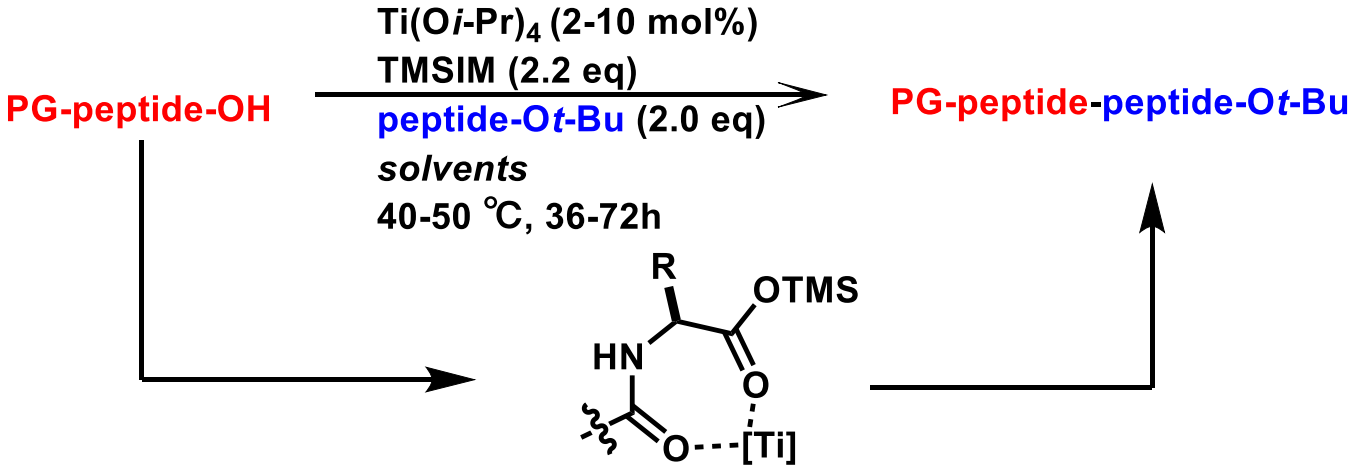
Lewis acid was optimized as $Ti(Oi\text{-}Pr)_4$.

→$Ti(Oi\text{-}Pr)_4$ is more Lewis acidic than $Ta(OMe)_5$.

Carbonyl oxygen of normal amide bond is less Lewis basic than carbonyl oxygen of carbamate protecting group.

→Catalyst needs more Lewis acidity.

Peptide Coupling Reaction



oligopeptide	yield	dr	*solvents*
Cbz-Gly-Gly-L-Phe-L-Leu-O*t*-Bu	72	94:6	DMSO
Cbz-Gly-Gly-L-Leu-L-Met-O*t*-Bu	99	97:3	——
Cbz-L-Pro-L-Leu-Gly-L-Val-O*t*-Bu	98	>99:1	——
Boc-Gly-Gly-Gly-L-Ala-L-Ala-O*t*-Bu	>99	>99:1	$CHCl_3$
Cbz-Gly-Gly-Gly-Gly-L-Ile-O*t*-Bu	>99	>99:1	DMSO
Cbz-Gly-L-Pro-L-Leu-Gly-L-Asp(*t*-Bu)-O*t*-Bu	>99	>99:1	$CHCl_3$
Boc-L-Ala-L-Me-L-Ala-Gly-L-Ala-L-Ala-O*t*-Bu	98	>99:1	$CHCl_3$

Peptide elongation proceeded well even in the synthesis of hexapeptide from two tripeptides with high stereoselectivity.

This result indicates that synthesis of oligopeptides of any length is theoretically feasible in this method.

Summary

- **Carbonyl-directed catalytic amidation solves problems of racemization and extended substrate scope compared with previous catalytic methods.**

- **Carbonyl-directed catalysis also shows high stereoselectivity in synthesis of tripeptide and hexapeptide, which indicates possibility of oligopeptide synthesis in a few steps.**

- **This solvent-free catalysis is useful as a method avoiding the issue of amount of solvent.**