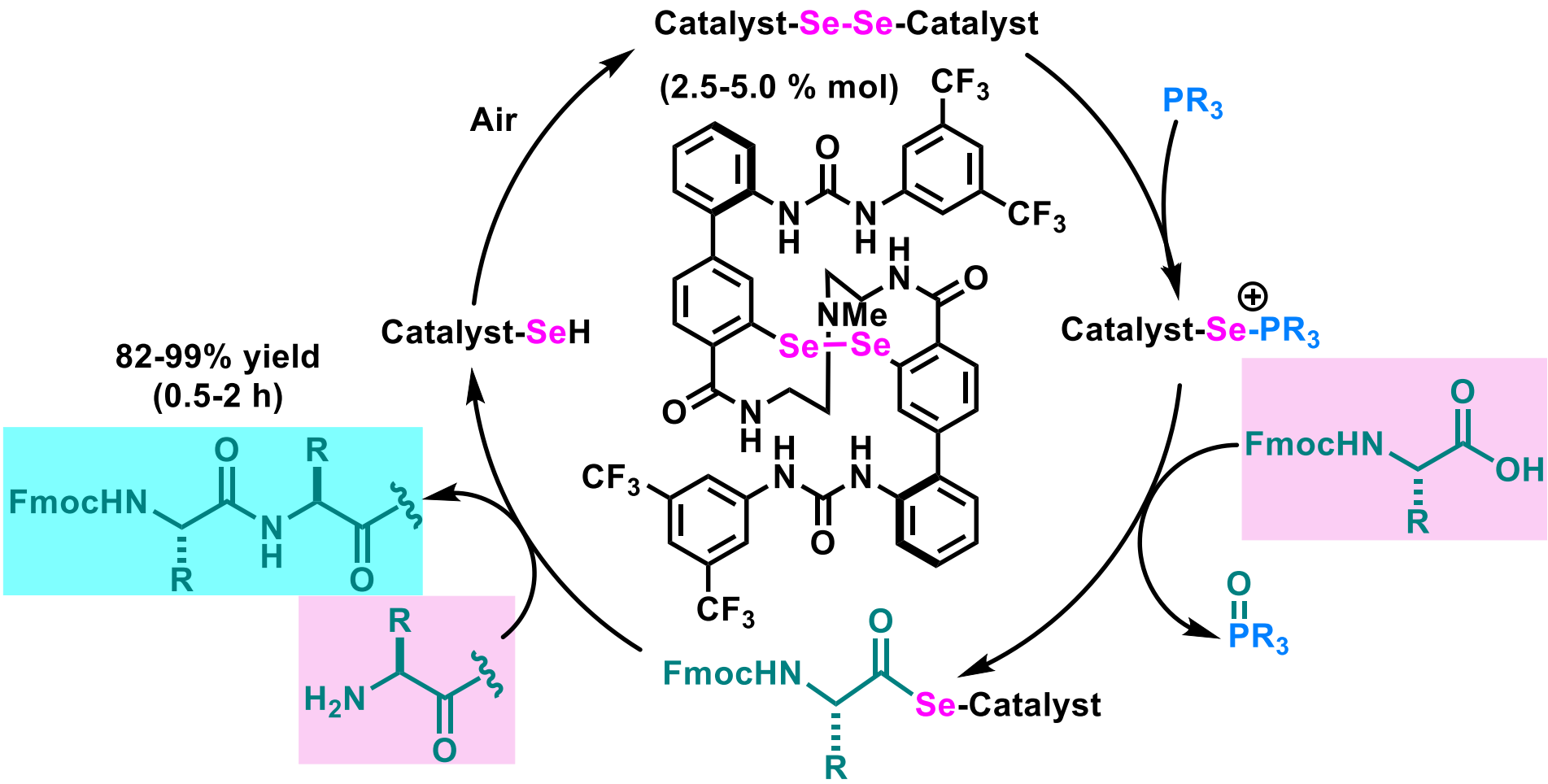
Designing an Organocatalyst-Driven Peptide Synthesis



2019.11.16. Literature Session
Takeuchi Aoi

Contents

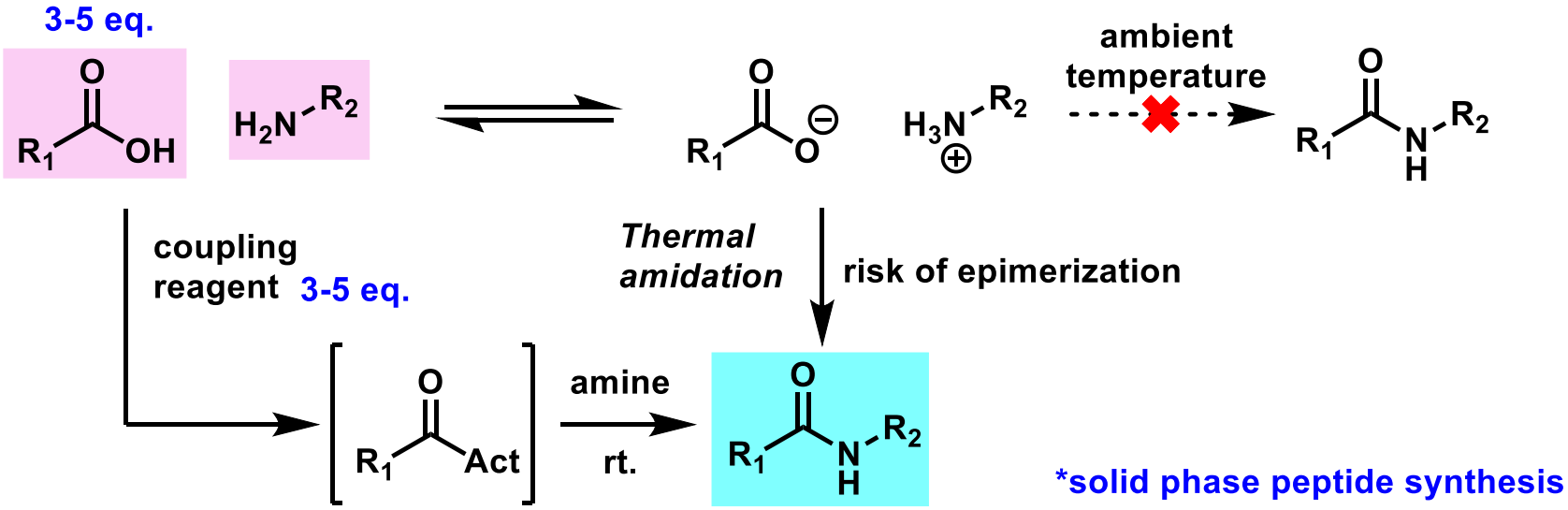
- **Introduction**

- **Main Paper**

“Rational Design of an Organocatalyst for Peptide Bond Formation”

(Handoko; Satishkumar, S.; Panigrahi, N. R.; Arora, P. S. *J. Am. Chem. Soc.* 2019, *141*, 15977.)

Classical Reagent-Driven Amidation[1)]



- applicable to various substrates
- low atom economy
- wasting much reagents and solvent

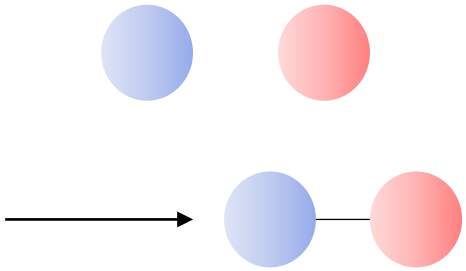
➢ "Non-classical" ways to construct amide bonds avoiding low atom economy has been pursued.

1) de Figueiredo, R. M.; Suppo, J.-S.; Campagne, J.-M. *Chem. Rev.* **2016**, *116*, 12029.

Concept of Atom Economy

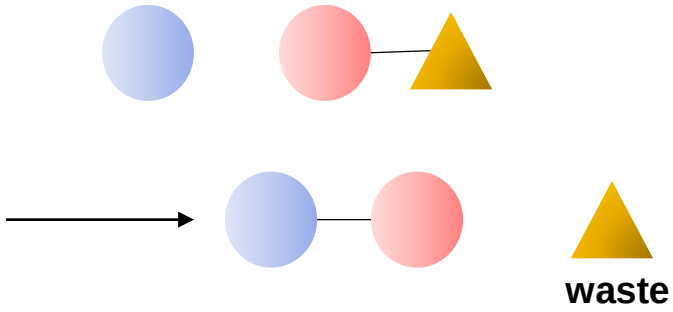
$$\text{atom economy}^{1)}\ (\%) = \frac{\text{molecular weight (desired product)}}{\text{molecular weight (all reactants)}} \times 100$$

high atom economy



e.g.) pericyclic reaction, sigmatropic rearrangement

low atom economy

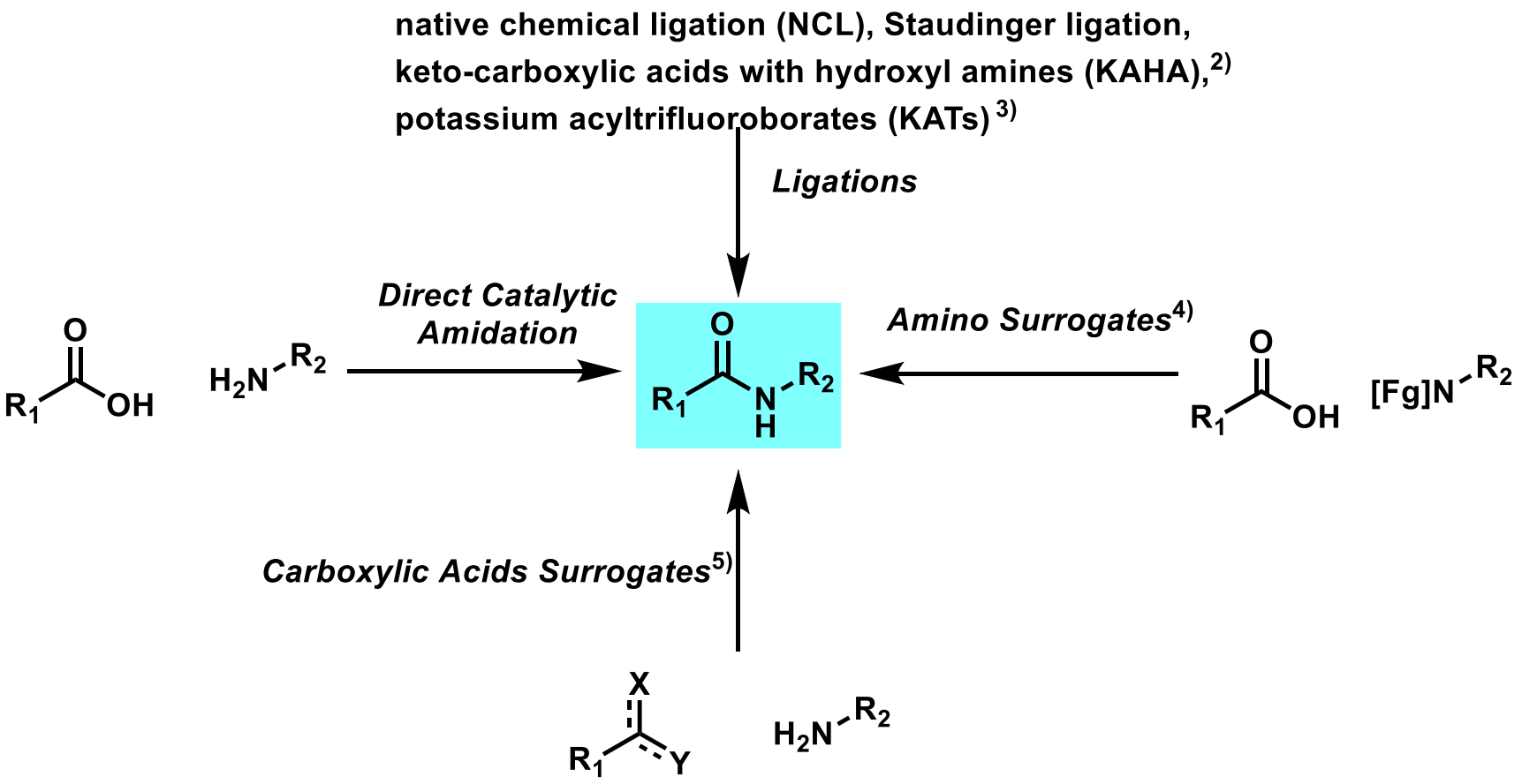


e.g.) elimination reaction, condensing reaction

Low atom economy of amide forming reaction has been recognized as one of the most important issue in green chemistry field.[2)]

1) Trost, B.M. *Science* **1991**, *254*, 1471.
2) Constable, D. J.; Dunn, P. J.; Hayler, J. D.; Humphrey, G. R.; Leazer Jr, J. L.; Linderman, R. J.; Lorenz, K.; Manley, J.; Pearlman, B. A.; Wells, A. *Green Chem.* **2007**, *9*, 411.

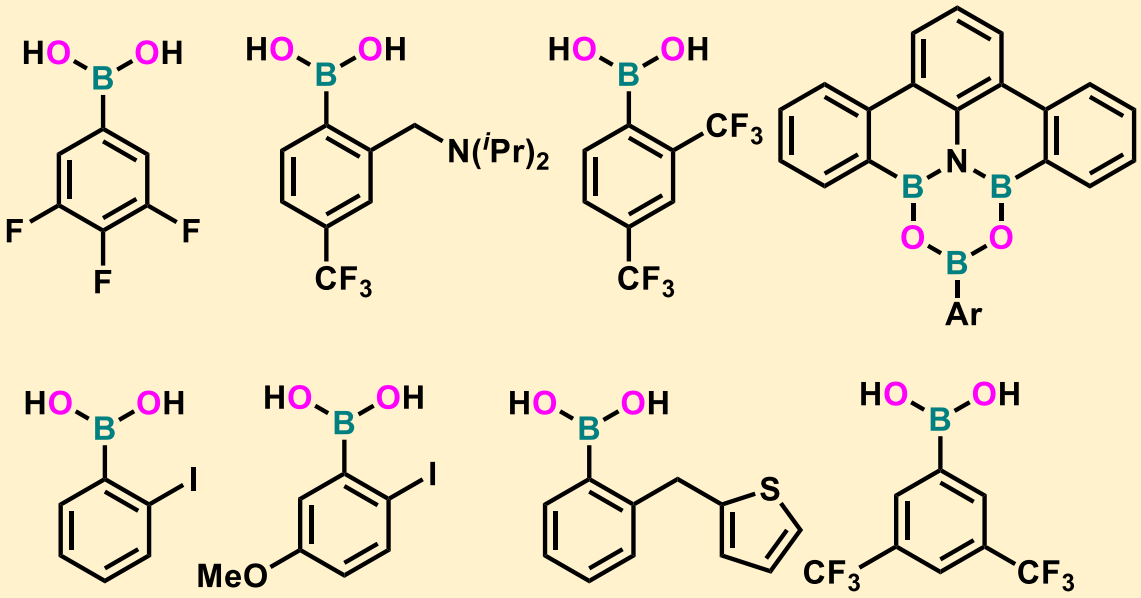
"Non-Classical" Amidation Approaches[1)]



1) de Figueiredo, R. M.; Suppo, J.-S.; Campagne, J.-M. *Chem. Rev*. **2016**, *116*, 12029.
2) Harmand, T. J. R.; Murar, C. E.; Bode, J. W. *Curr. Opin. Chem. Biol.* **2014**, *22*, 115.
3) Molander, G. A.; Raushel, J.; Ellis, N. M. *J. Org. Chem*. **2010**, *75*, 4304.
4) Ishihara, K.; Kuroki, Y.; Hanaki, N.; Ohara, S.; Yamamoto, H. *J. Am. Chem. Soc.* **1996**, *118*, 1569.
5) Blaise, E. E. *Compt. Rend.* **1901**, *132*, 38– 41

Direct Catalytic Amidation

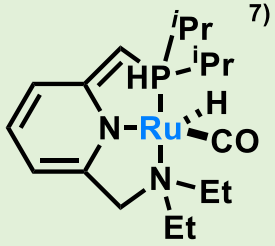
- **boronic acid derivatives[1)]**
  Yamamoto (1996)[2)]
- **metal calatyst**
  Williams (2012)[3)] Adolfsson (2012)[4)]
- **organocatalyst**
  Schmidt (2005)[5)] ,Awasthi (2015)[6)]

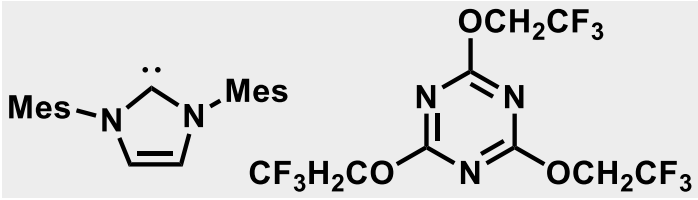


$ZrCl_4$ $ZrCl_2Cp_2$ $Zr(O^tBu)_4$ $Zr(OEt)_4$

$Ti(O^iPr)_4$ $Zn(O^tBu)_4$ $Hf(O^tBu)_4$ $HfCl_4$

$Ti(OBn)_4$ $Ta(OMe)_5$ $Nb(OEt)_5$





1) Hall, D, G. *Chem. Soc. Rev.* **2019**, *48*, 3475.
2) Ishihara, K.; Ohara, S.; Yamamoto, H. *J. Org. Chem.* **1996**, *61*, 4196.
3) Allen, C. L.; Chhatwal, A. R.; Williams, J. M. J. *Chem. Commun*. **2012**, *48*, 666.
4) Lundberg, H.; Tinnis, F.; Adolfsson, *Chem. Eur. J.* **2012**, *18*, 382.
5) Movassaghi, M.; Schmidt, M. A. *Org. Lett.* **2005**, *7*, 2453
6) Mangawa, S. K.; Bagh, S. K.; Sharma, K.; Awasthi, S. K. *Tetrahedron Lett.* **2015**, *56*, 1960.
7) Gnanaprakasam, B.; Milstein, D. *J. Am. Chem. Soc.* **2011**, *133*, 1682

Contents

Author's Profile

Prof. Paramjit Arora

C.V.

Born in New Delhi

B.S. Chemistry	**University of California at Berkeley**
Ph.D. Chemistry	**University of California at Irvine**
	(advised by Prof. James S. Nowick)
Postdoctoral Fellow	**California Institute of Technology**
	(advised by Prof. Peter B. Dervan)
Professor	**New York University**

Research Interests

- **Develop a systematic approach for targeting protein-protein interactions with synthetic ligands**
- **RNA binding ligands design**
- **Organocatalyst for amide bond synthesis**

Developing Catalytic-Driven Peptide Synthesis

- **Arora's strategy = organocatalyst**

(metal catalysts have risk of nonspecific coordination with amide bonds.)

- **It should have a significant impact to peptide-related industries to establish catalytic peptide synthesis which is waste-reducing and compatible with solid-phase.**

requirement

- **condensing agent-free reaction**
- **reduction the amount of amino acids**
- **no racemization**
- **compatible with Fmoc-based synthesis**
  (for application to solid-phase synthesis)

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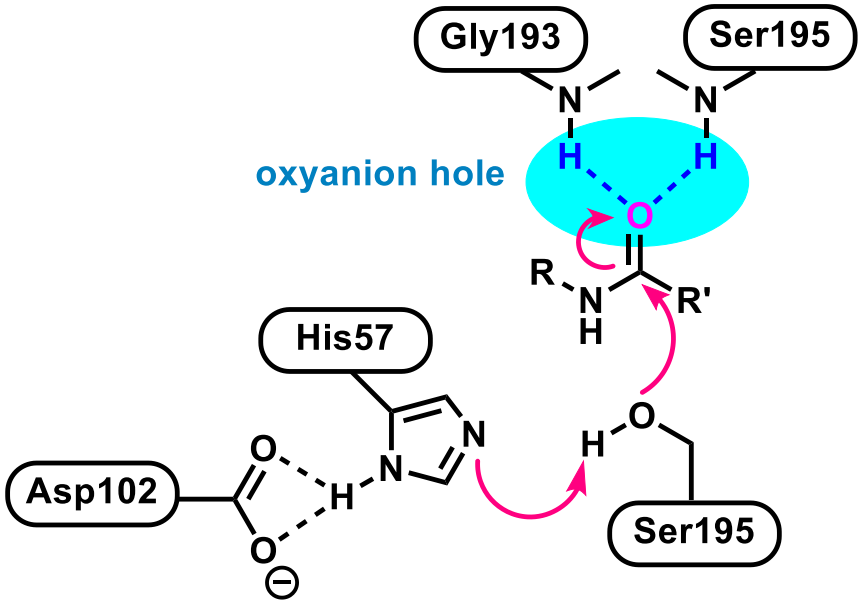
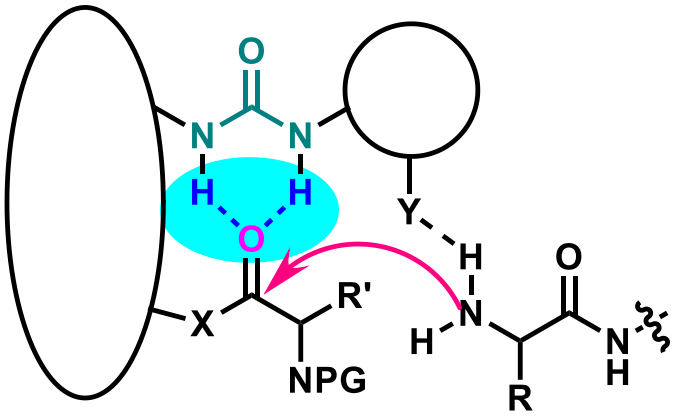
Biomimetic Catalyst Design Strategy

Catalytic design was inspired by two biosynthetic precedents.

1. mimicking oxyanion holes in enzymes by urea catalysts



generally accepted mechanism for serine proteases[1)]



idealized depiction of amide bond formation catalyst

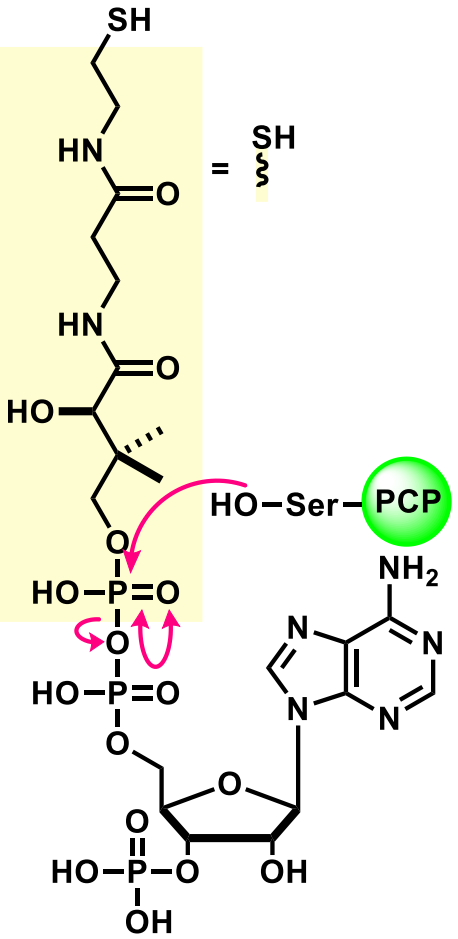
1) Zhang, Z.; Schreiner, P. R. *Chem. Soc. Rev.* **2009**, *38*, 1187.

Biomimetic Catalyst Design Strategy

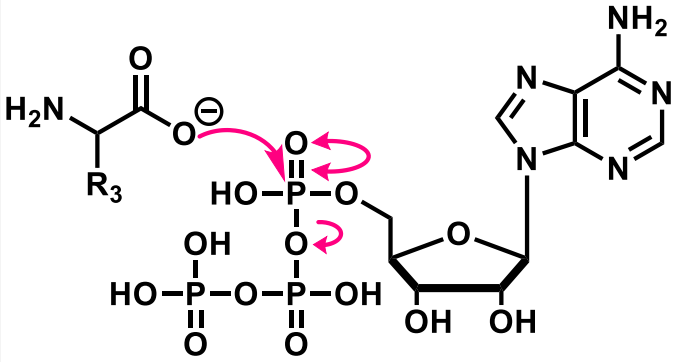
2. utilizing amino acid thioesters to access peptide

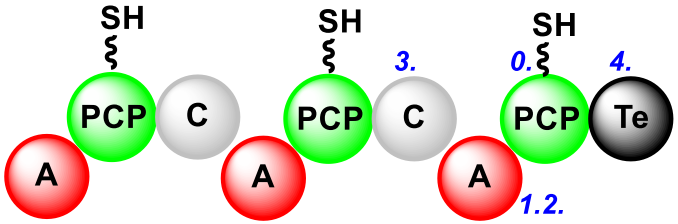
biosynthesis of non-ribosomal peptides synthetases (NRPS)[1)]

0. phosphopantetheinylation



1. adenylation

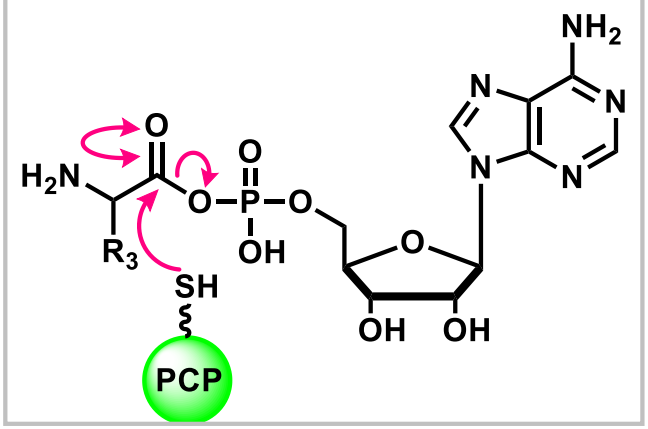




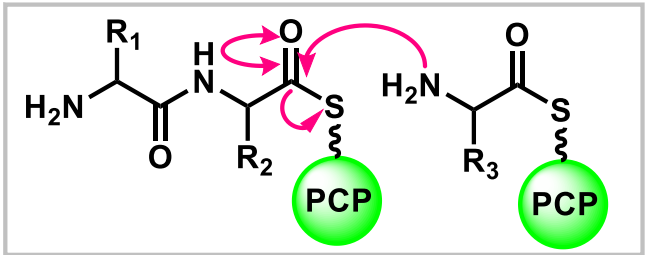
Initiation module	*Elongation module*	*Termination module*

*PCP = Peptydil Carrier Protein

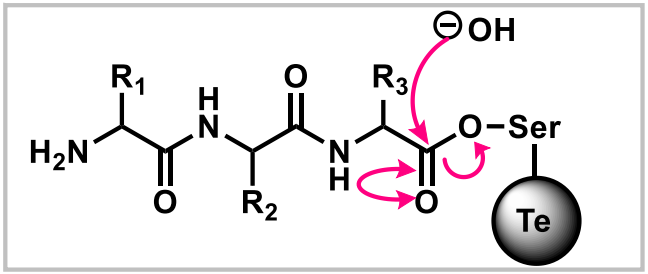
2. thiolation



3. condensation



4. release

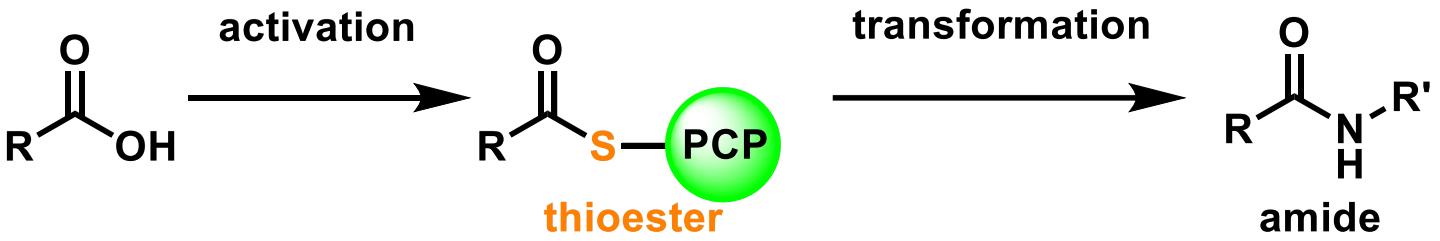


1) Süssmuth, D. R.; Mainz, A. *Angew Chem., Int. Ed.* **2017**, *56*, 3770.

Biomimetic Catalyst Design Strategy

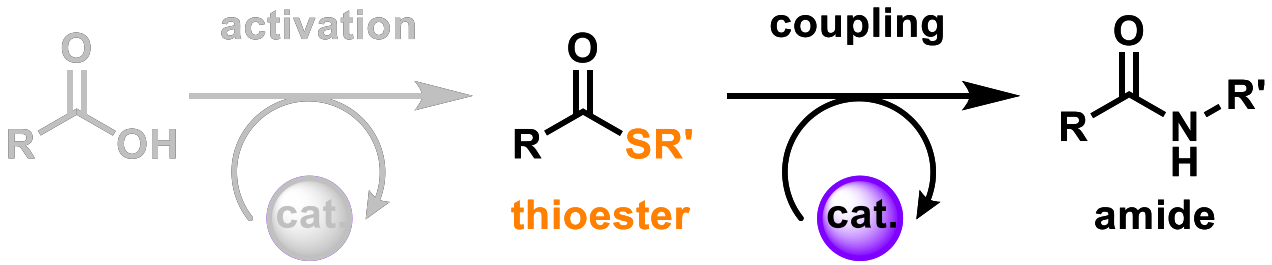
utilizing amino acid thioesters to access peptide

biosynthesis of non-ribosomal peptides synthetases (NRPS)[1)]



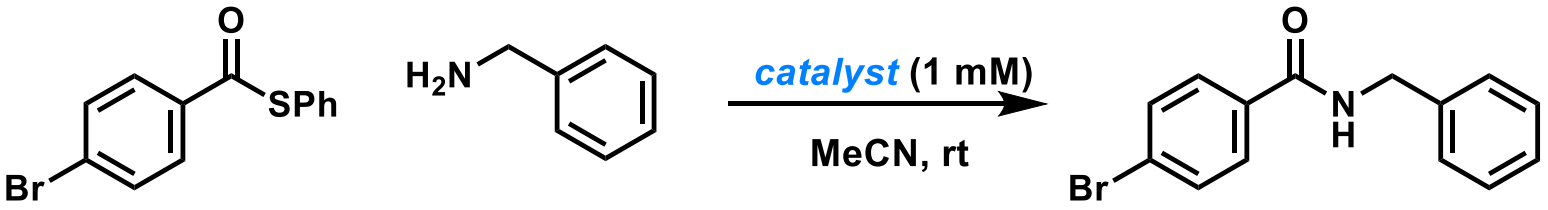
*PCP = Peptydil Carrier Protein

- **design biomimetic catalyst that efficiently**
  - **activates carboxylic acids to the more electrophilic thioesters.**
  - **would couple amino acid thioesters to a growing peptide chain.**



1) Franke, J.; Hertweck, C.; *Cell Chem. Biol.* **2016**, *23*, 1179.

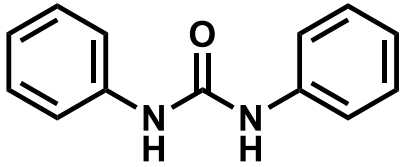
Design of Urea-based Catalyst



A (10 mM) B (20 mM) C

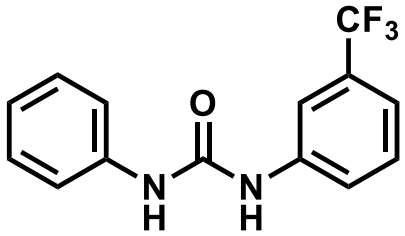
X % conversion after 2 h

catalyst



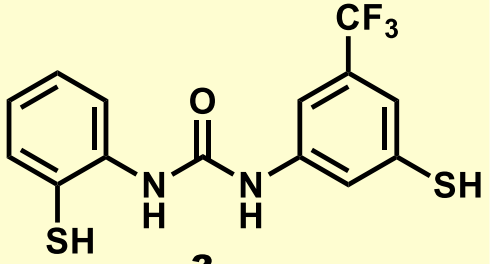
1

X = 13



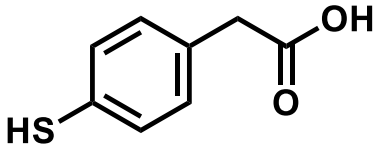
2

X = 23



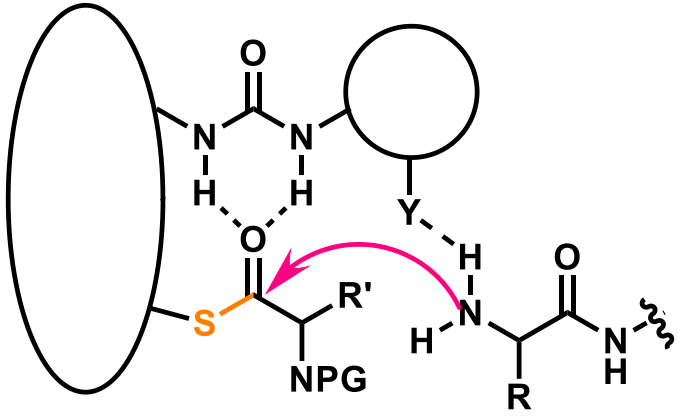
3

X = 55



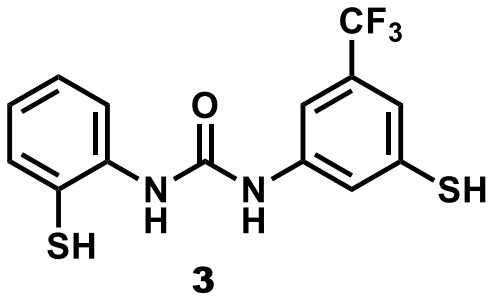
4-mercaptophenylacetic acid
(MPAA, control)
X = 11

no catalyst
X = 10

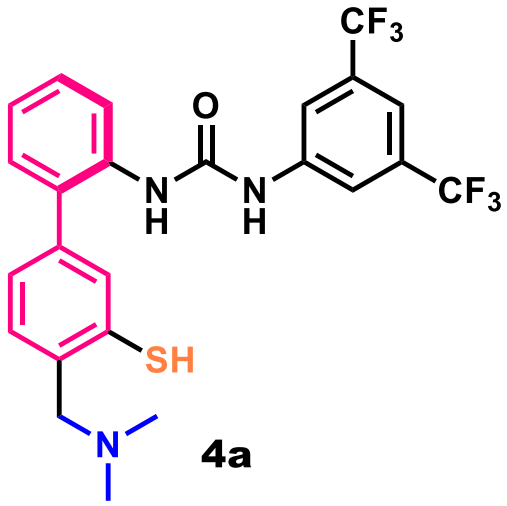


- Simple urea scaffolds are not sufficient to activation.
- A thiol group was included in 1b to engage a thioester through a thioester-exchange reaction.

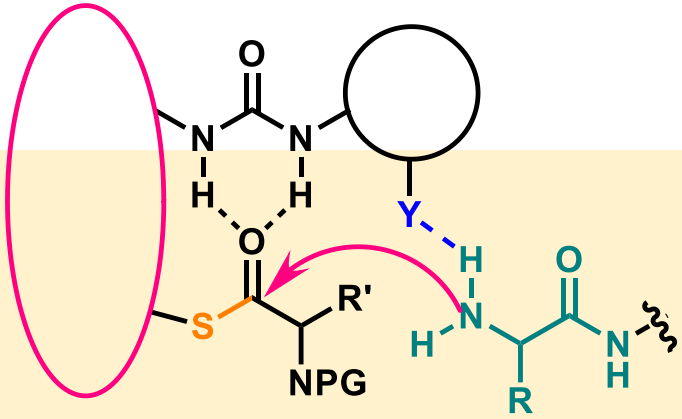
Working Hypothesis for Enhancing Efficiency

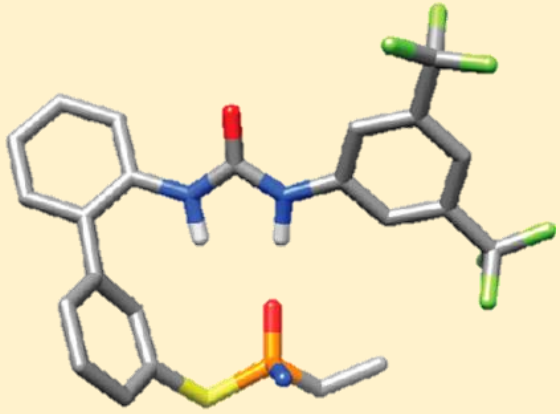


3

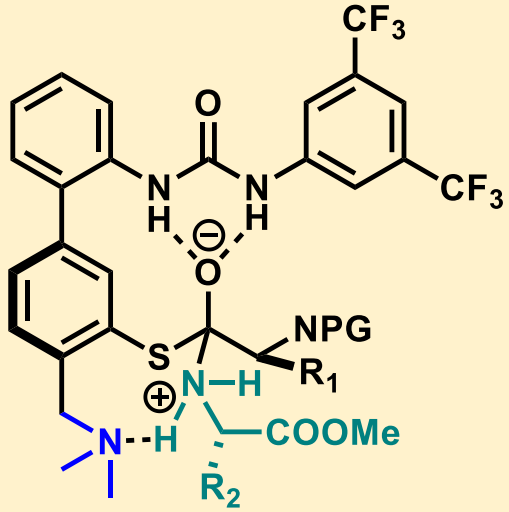


4a





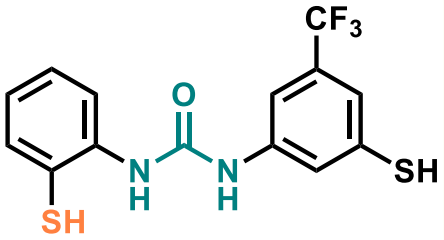
positioning thiol group on a biphenyl moiety for better captures of tetrahedral transition-state



introducing tertiary amine as Y scaffold for inducing a reversible catalyst-amine complex formation

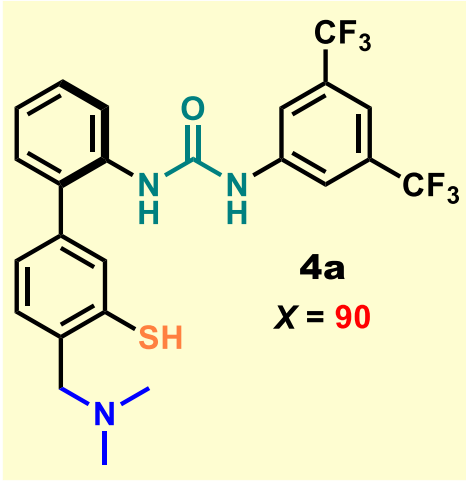
Optimization of Catalyst

catalyst



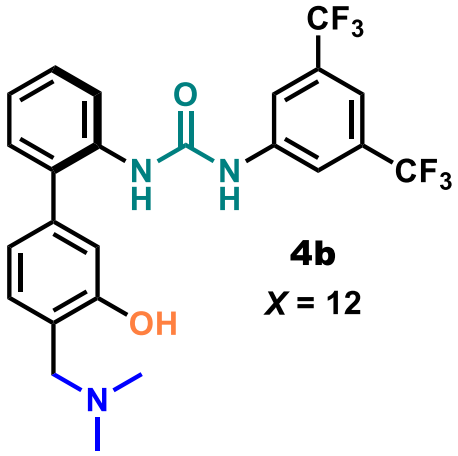
3

X = 55



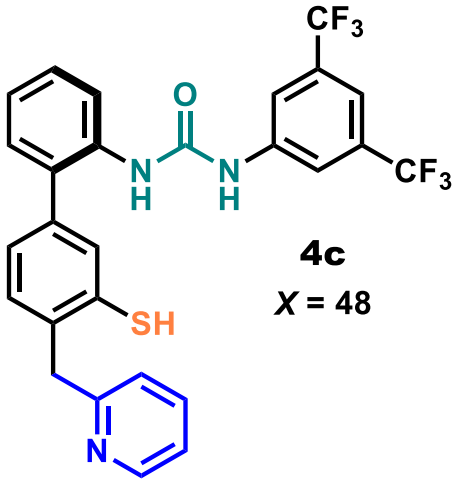
4a

X = 90



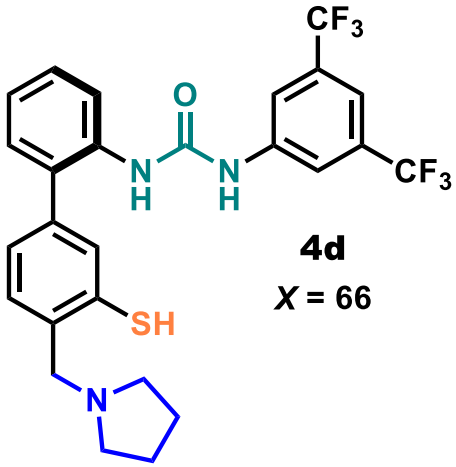
4b

X = 12



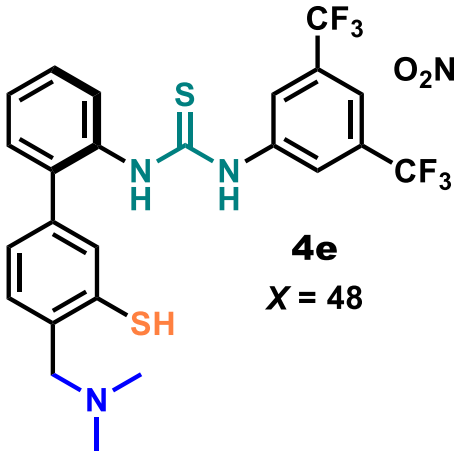
4c

X = 48



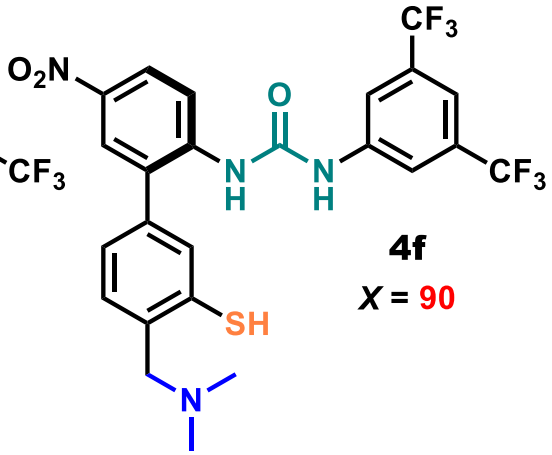
4d

X = 66



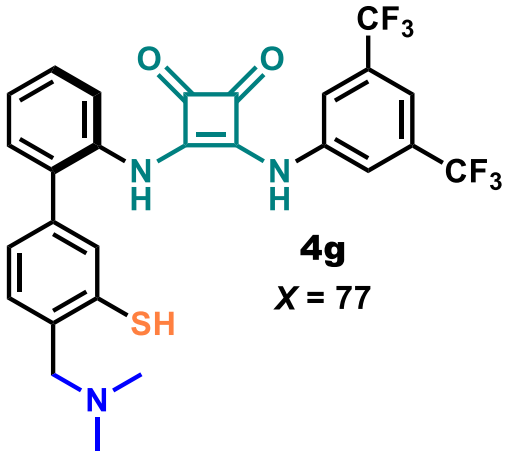
4e

X = 48



4f

X = 90

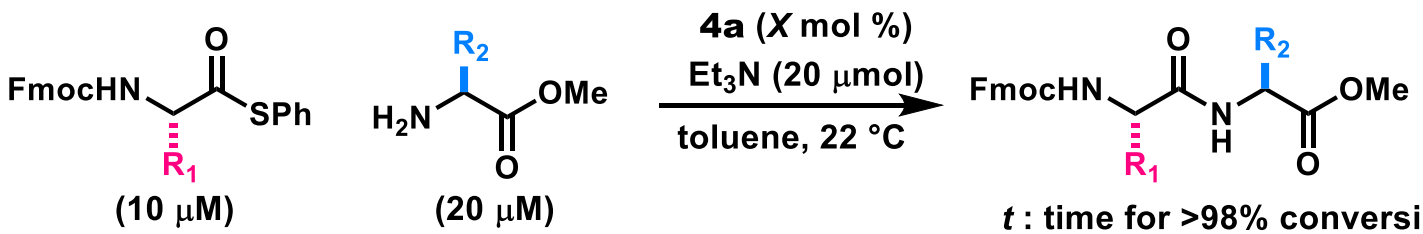


4g

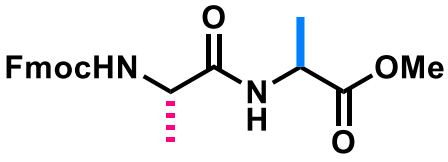
X = 77

Catalyst design was optimized as 4a.

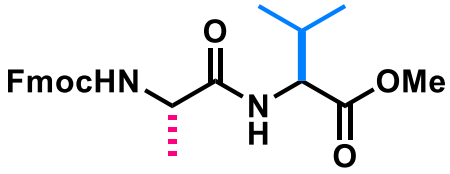
Application of Catalyst to Conversions of Fmoc-Amino Acid Thioesters to Dipeptide



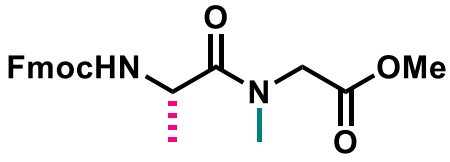
t : time for >98% conversion



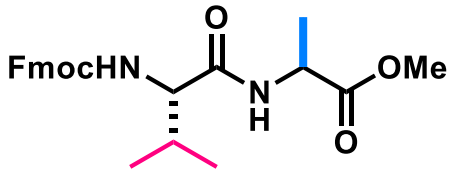
X = 0, *t* > 20 days (estimated)
X = 10, *t* =10 min



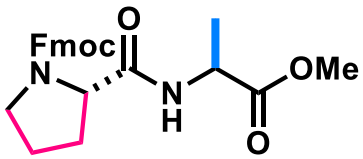
X = 0, *t* > 50 days (estimated)
X = 10, *t* = 7 h[a]
X = 20, *t* = 3 h[a]



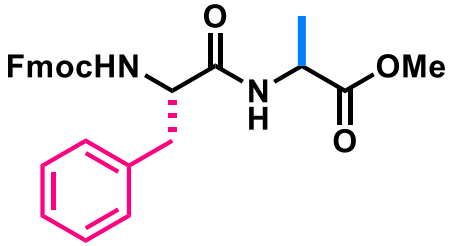
X = 10, *t* = 4 h[a]
X = 20, *t* = 2 h[a]



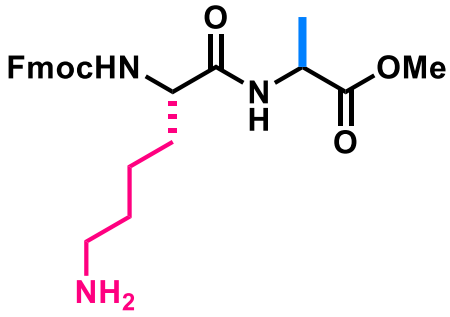
X = 10, *t* = 4.5 h
X = 20, *t* = 2 h



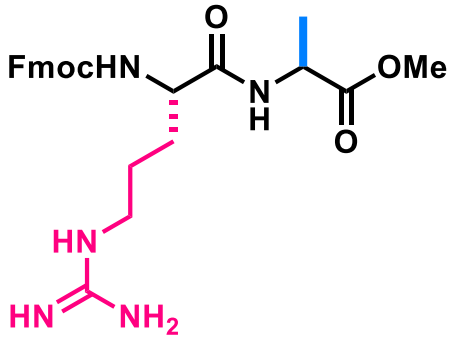
X = 0, *t* > 50 days (estimated)
X = 10, *t* = 40 min



X = 10, *t* = 10 min



X = 10, *t* = 30 min

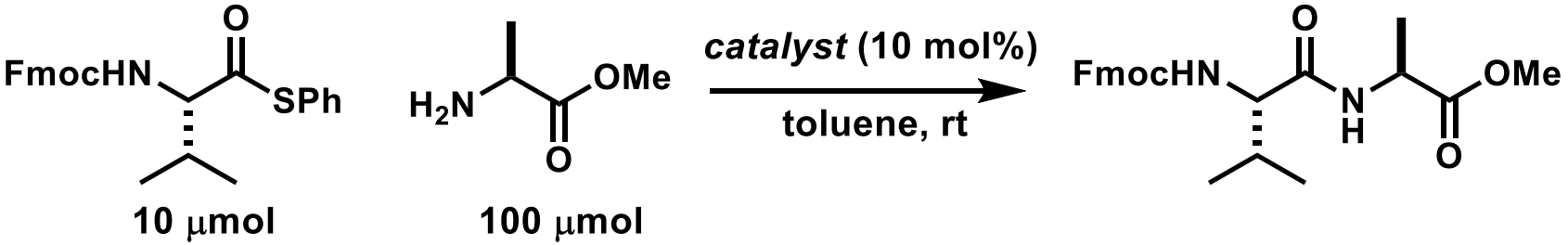


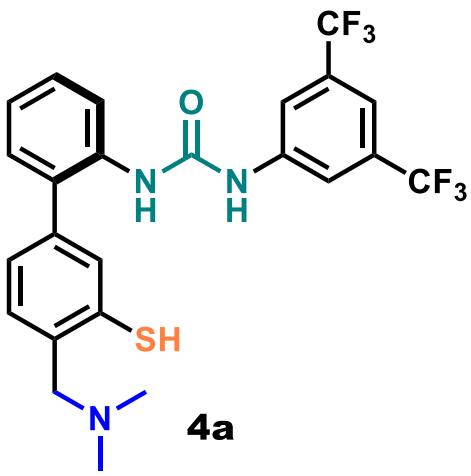
X = 10, *t* = 60 min

No epimerization was observed

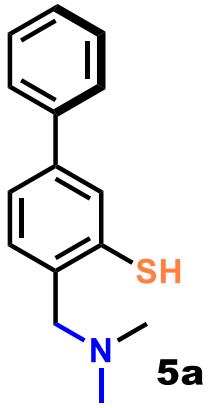
[a] 10-15% hydrolysis of Fmoc-Ala-SPh was observed

Investigation of Critical Components for Catalytic Mechanism

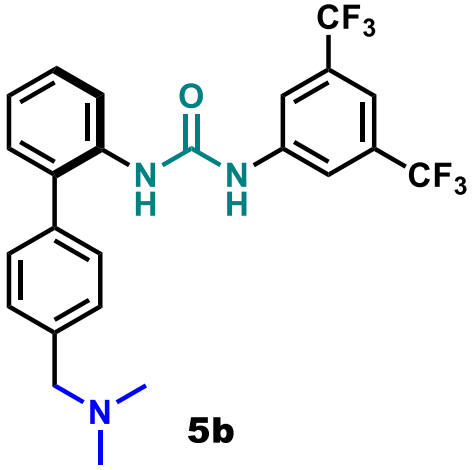




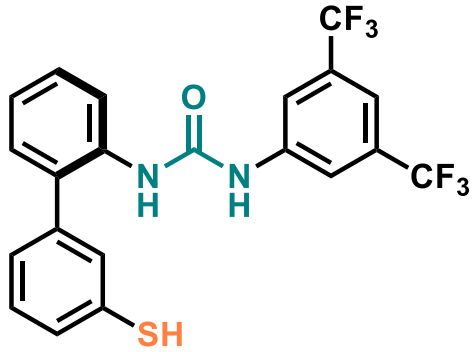
4a



5a



5b

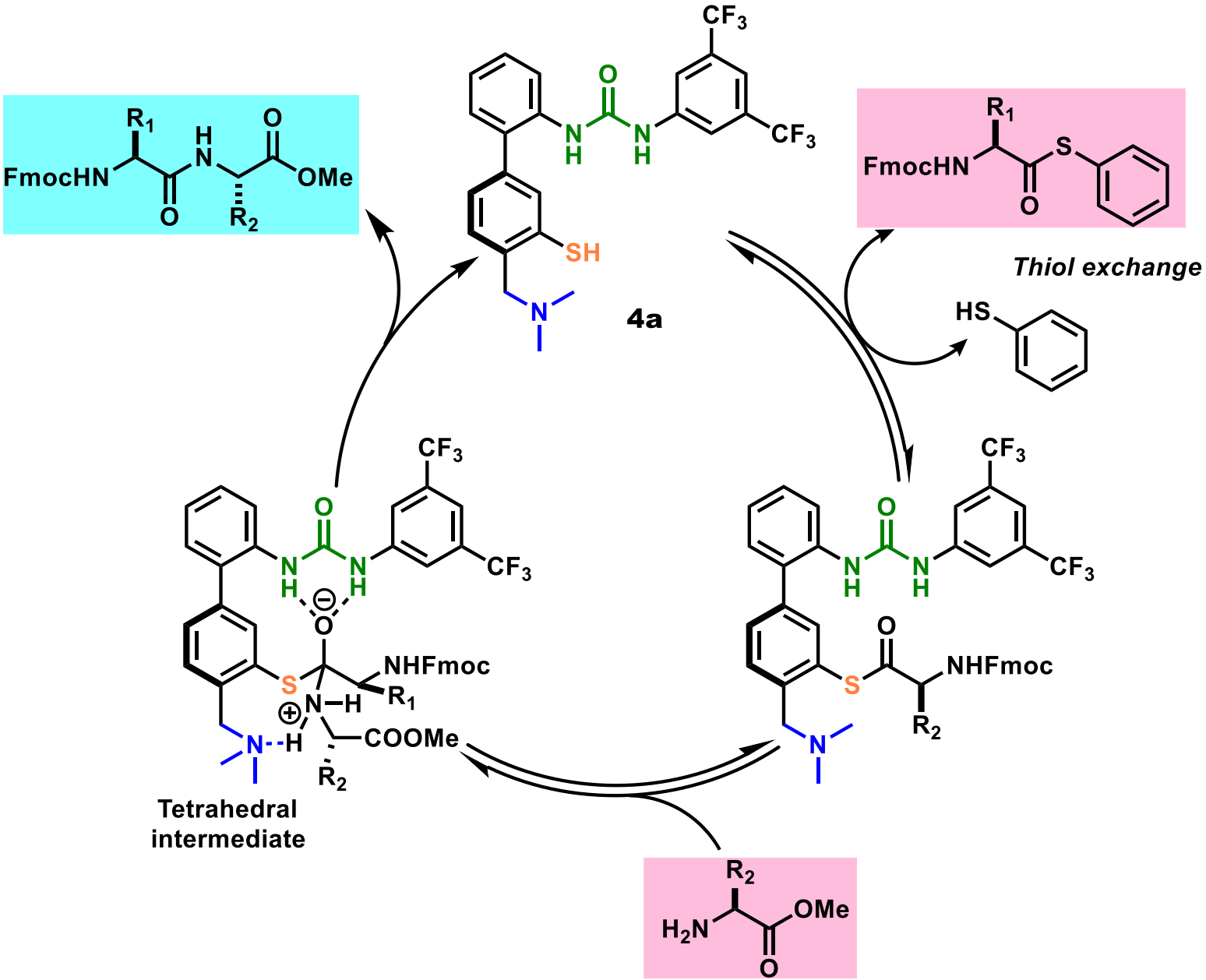


5c

catalyst	*k* (min^{-1})	k_{rel}
4a	5.22 × 10^{-2}	10,000
5a	1.50 × 10^{-5}	3
5b	1.18 × 10^{-5}	2
5c	2.98 × 10^{-5}	6
no catalyst	5.03 × 10^{-6}	1

- Removal of either of three functional groups (thiol, urea, or tertiary amine) leads to a significant loss in the observed activity.
- It is suggested that the functional groups are participating in a cooperative mechanism.

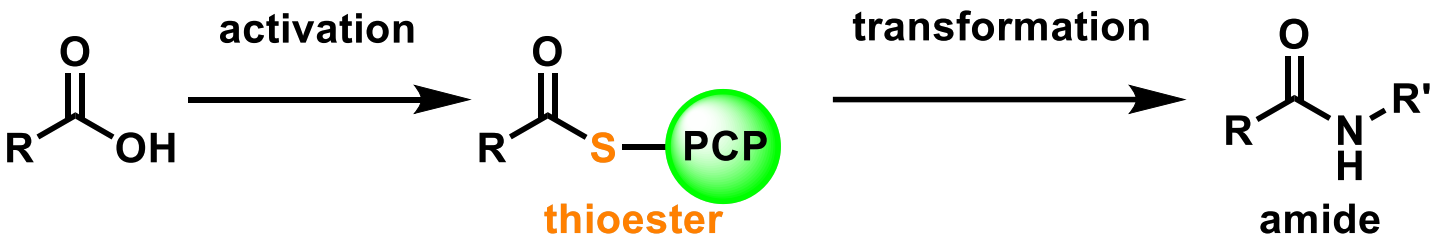
Proposed Catalytic Mechanism



Biomimetic Catalyst Design Strategy

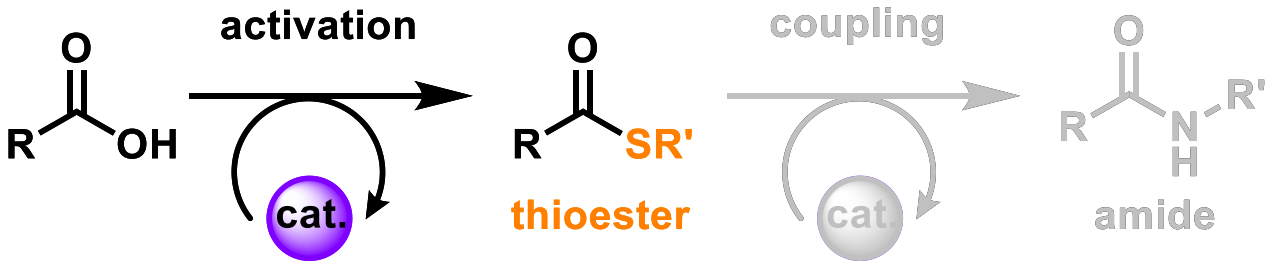
utilizing amino acid thioesters to access peptide

biosynthesis of non-ribosomal peptides synthetases (NRPS)[1)]



*PCP = Peptydil Carrier Protein

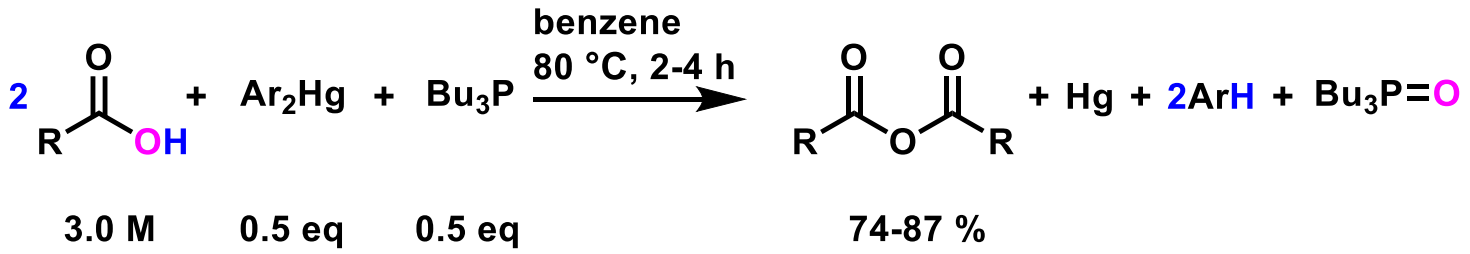
- **design biomimetic catalyst that efficiently**
  - **activates carboxylic acids to the more electrophilic thioesters.**
  - **would couple amino acid thioesters to a growing peptide chain.**



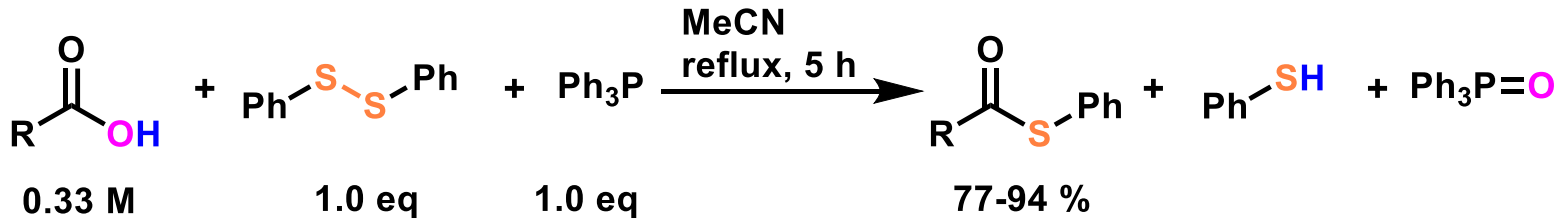
[1)] Franke, J.; Hertweck, C.; *Cell Chem. Biol.* **2016**, *23*, 1179.

Mukaiyama Reduction-Oxidation Condensation

concept:
perform a dehydration condensation by removing H_2O as 2[H] and [O] by the use of a combination of a weak reductant and oxidant[1)]



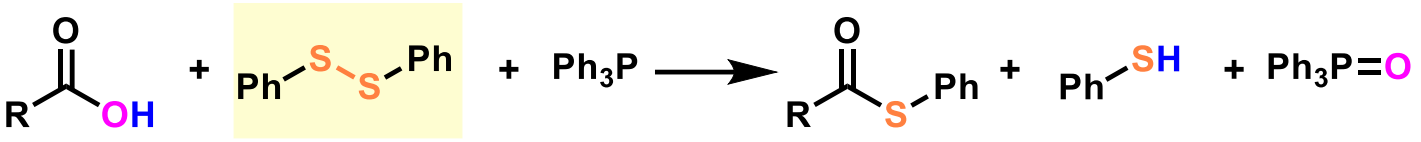
condensation of carboxylic acid to thioester with disulfides and phosphine reagents[2)]

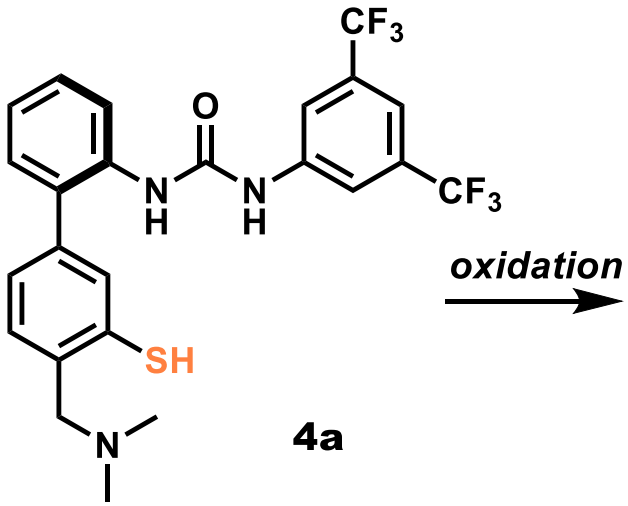


1) Mukaiyama, T.; Kuwajima, I.; Suzuki, Z. *J. Org. Chem.* **1963**, *28*, 2024.
2) Endo, T.; Ikenaga, S.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 2632.

Mukaiyama Reduction-Oxidation Condensation

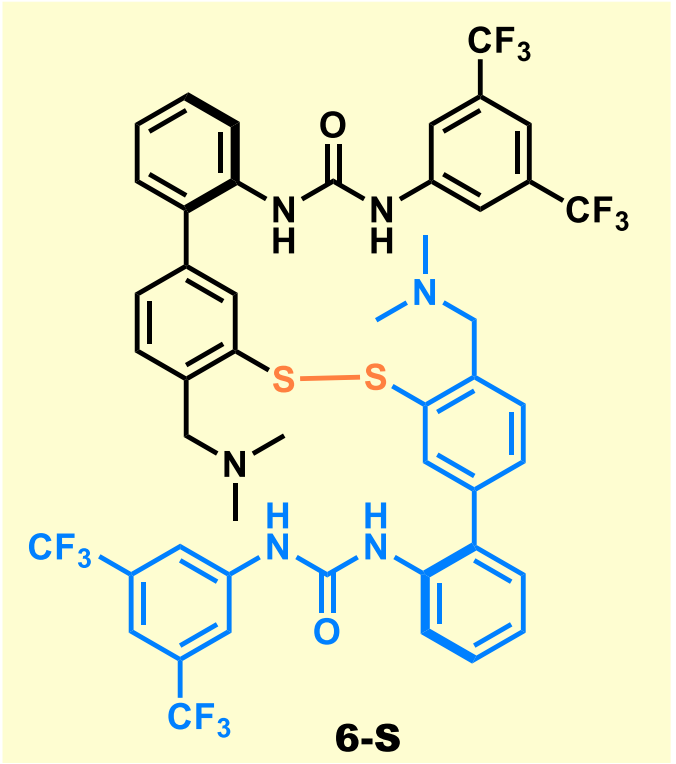
condensation of carboxylic acid to thioester with disulfides and phosphine reagents





4a

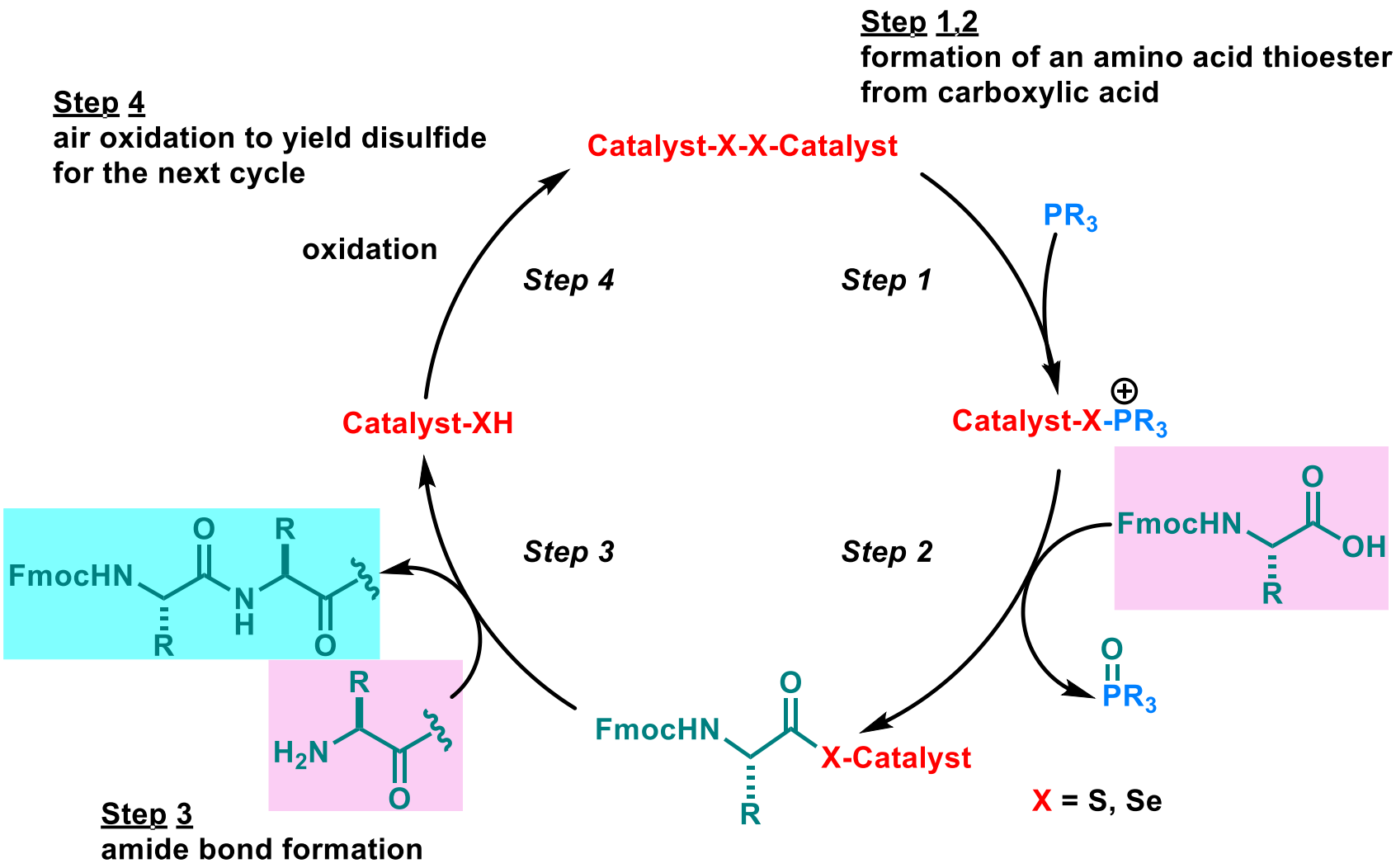
oxidation



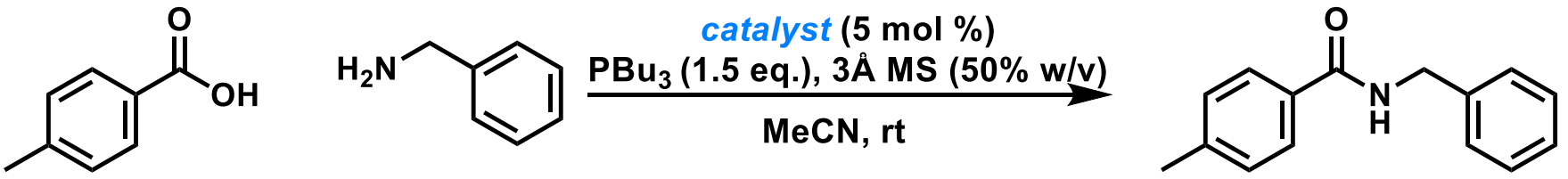
6-S

Oxidized dimer of **4a** (denoted as **6-S**) can be utilized as disulfides.

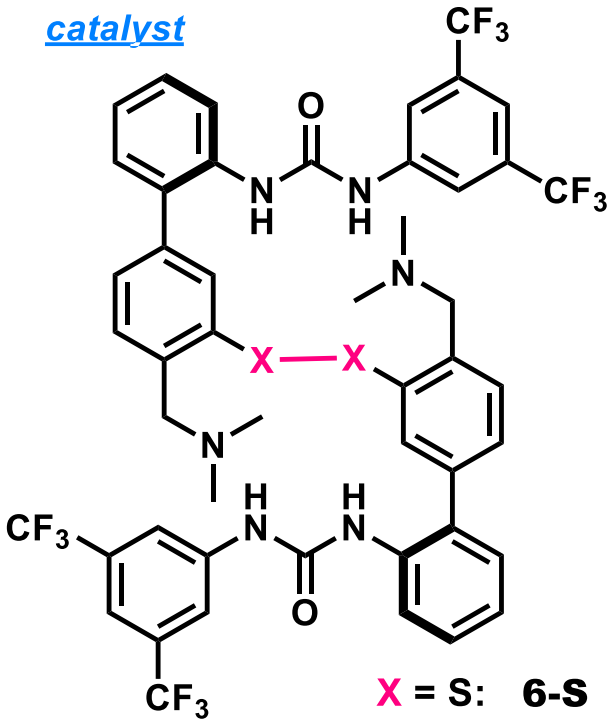
Working Hypothesis



Development of Dimerized Catalyst



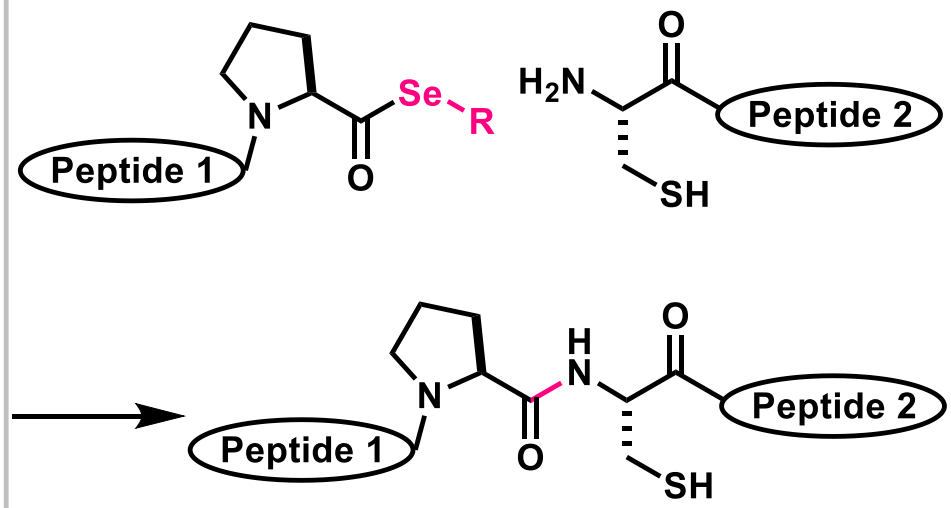
catalyst



X = S: **6-S** *Y* = 9

X = Se: **6-Se** *Y* = 36

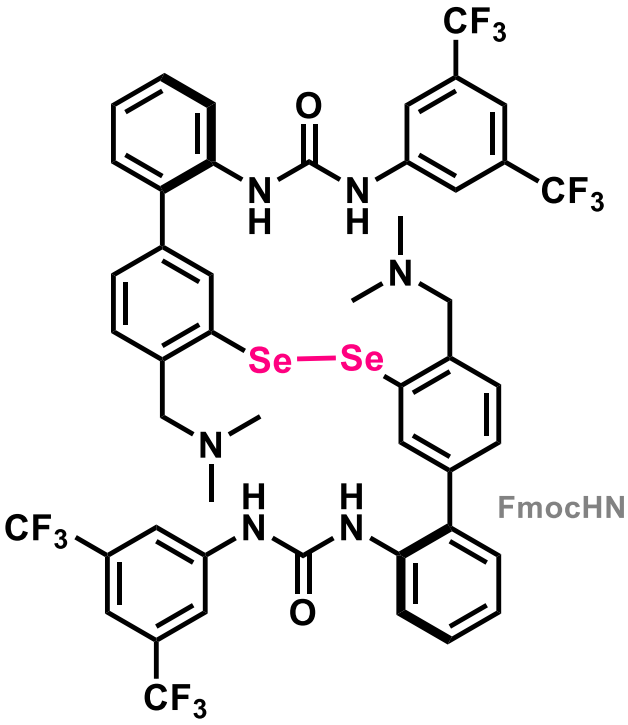
Pro-Cys ligation using selenoester[1)]



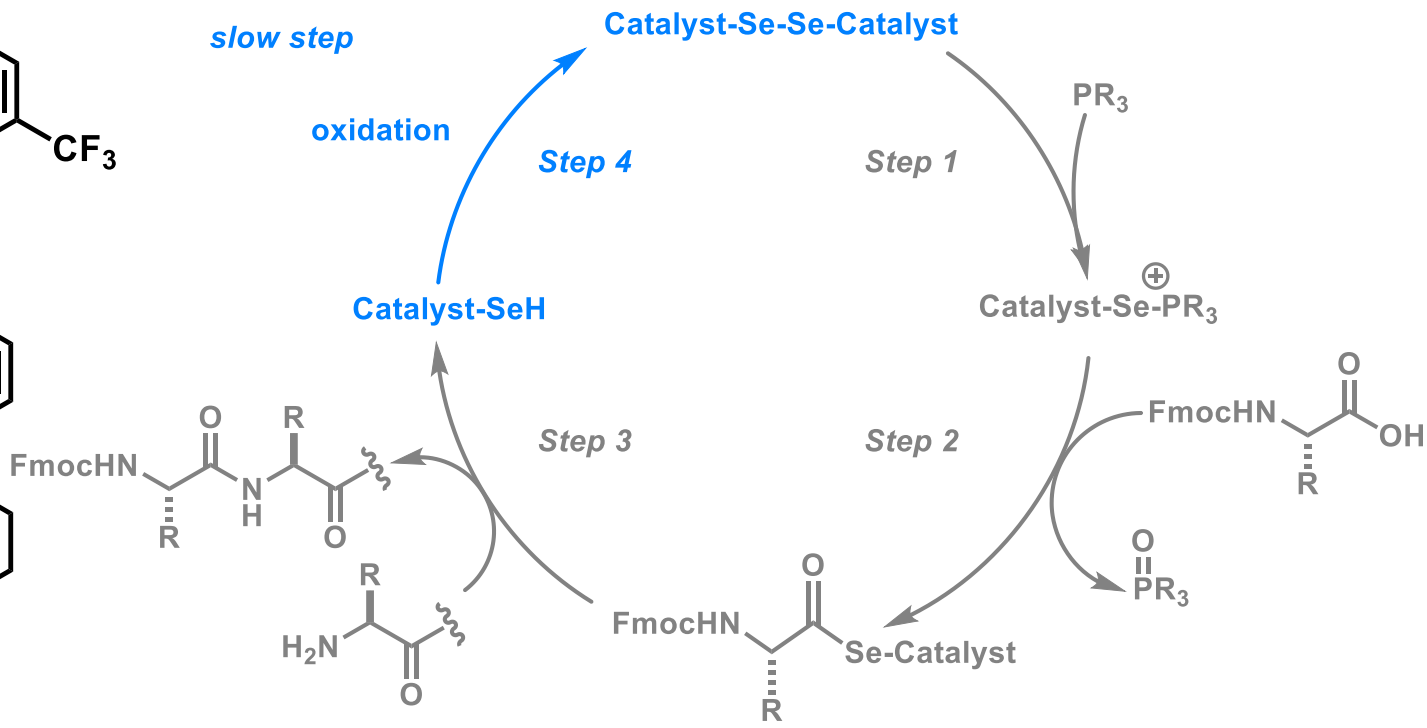
Diselenide 6-Se exhibited a significant overall rate enhancement over 6-S.

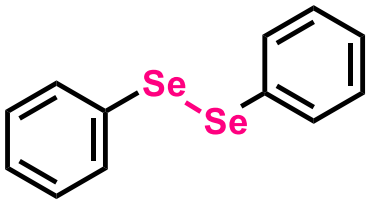
1) Durek, T.; Alewood, P. F. *Angew. Chem., Int. Ed.* **2011**, *50*, 12042

Limiting Factor of Catalyst Availability



6-Se *Y* = 36



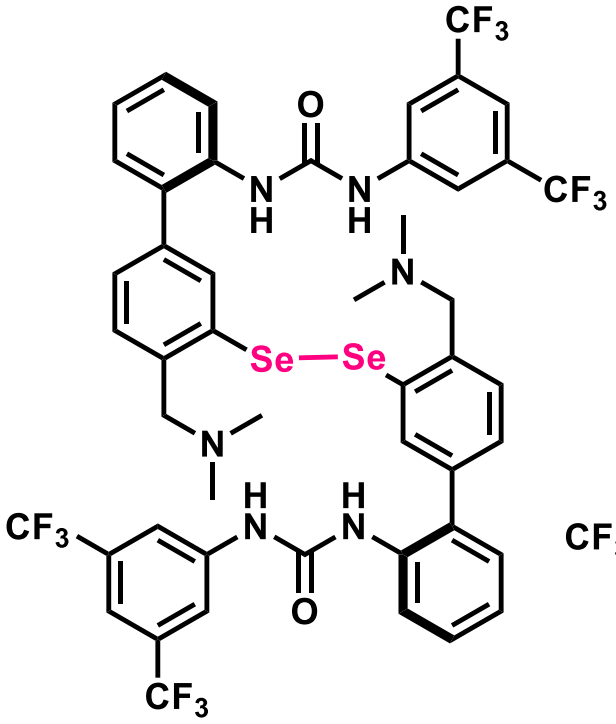


6' *Y* = 22

not significant difference between 6-Se and 6

- **It is postulated that the oxidation of the selenol to the diselenide might be slow, thus limiting catalyst availability for the subsequent steps.**

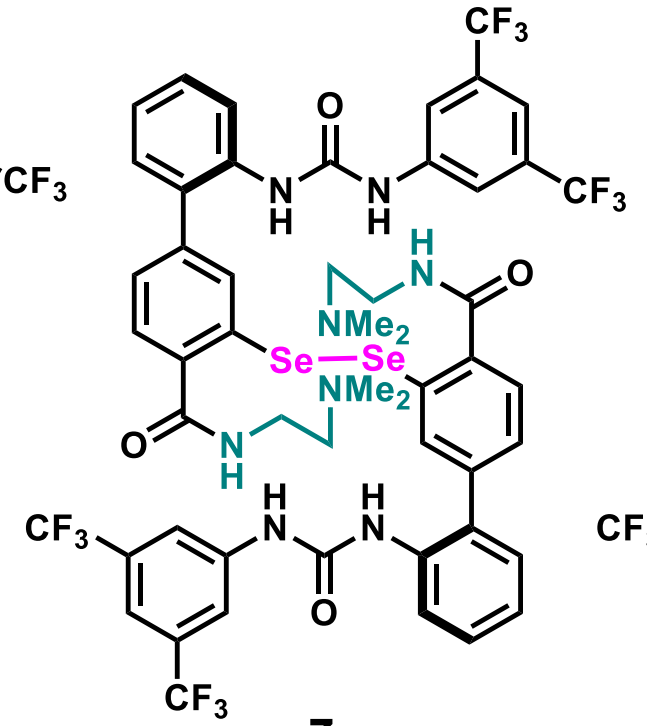
Linker Design for Accelerating Reoxidation



6-Se

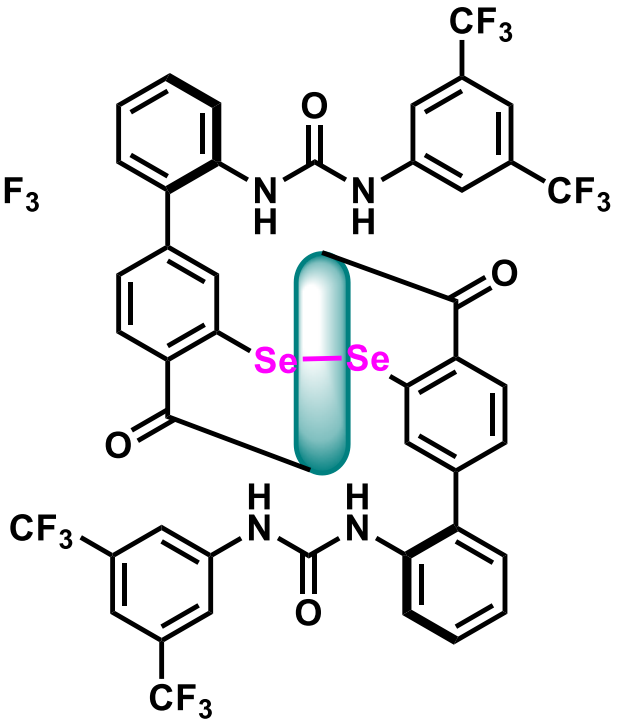
Y % conversion after 20 min

Z % conversion after 240 min



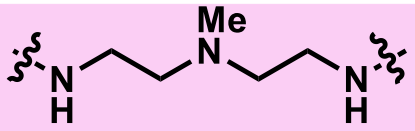
7

catalyst	*Y* (%)	*Z* (%)
6-Se	36	40
7	34	58
8a	42	73
8b	40	67
8c	32	62

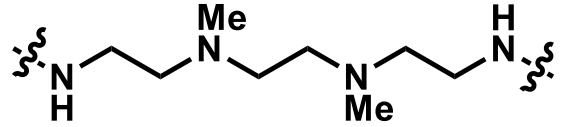


Linker :

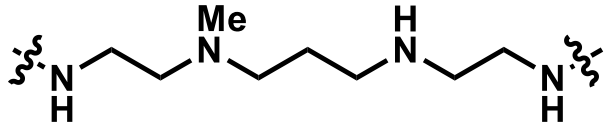
8a



8b



8c



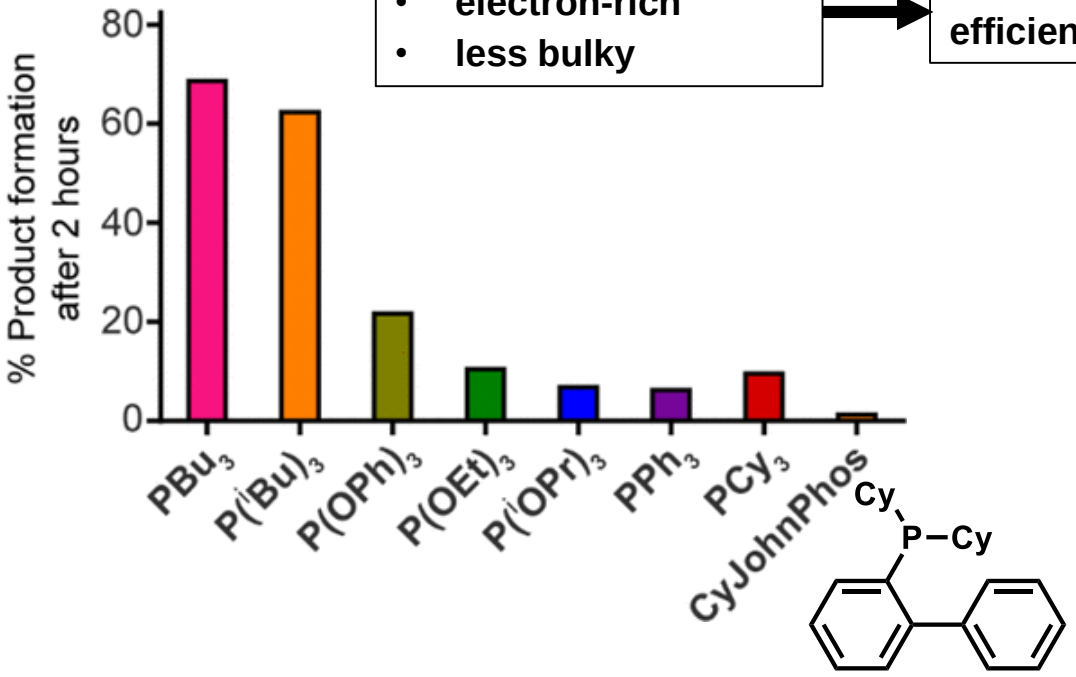
Catalyst design was optimized as **8a**

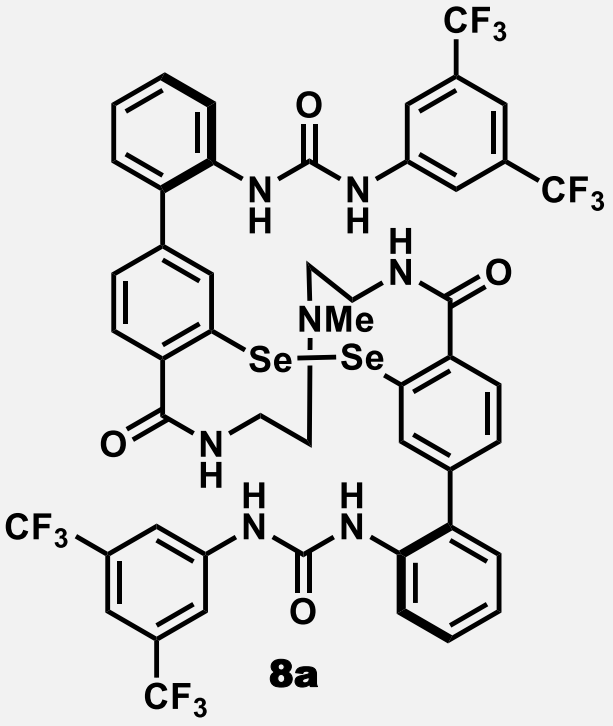
Optimization of Phosphine Reagent

optimization of phosphine

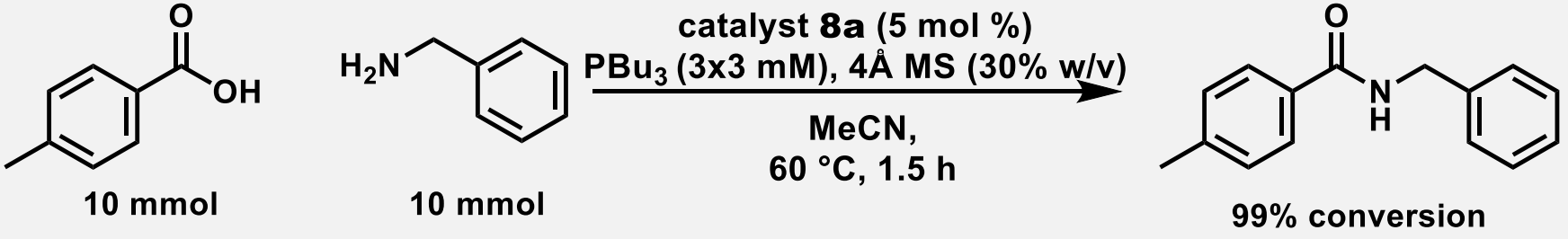
phosphine reagent
- **electron-rich**
- **less bulky**

→ **air-sensitive and yet efficient catalyst turnover**





optimized conditions



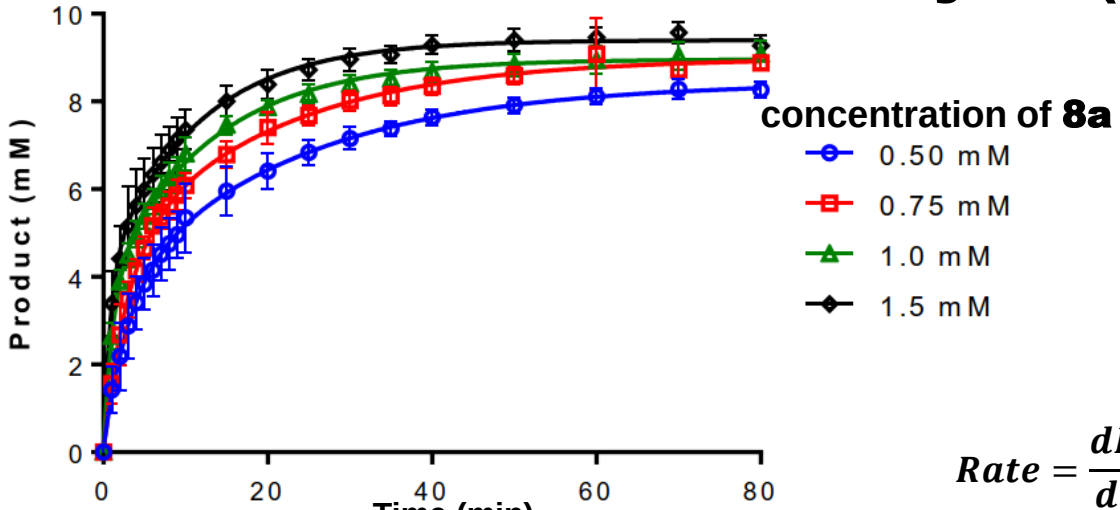
10 mmol

10 mmol

99% conversion

*PBu_3 was added in portions every 30 min.

Time Normalization Analysis (Burés Method [1])



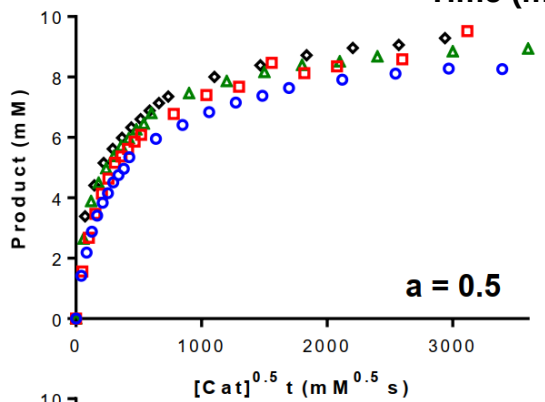
Find a value of *a* (reaction order) so that reaction curve overlays

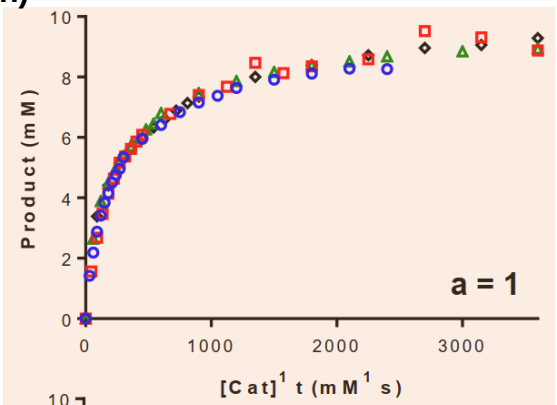
$$Rate = \frac{dP}{dt} = k[\mathbf{8a}]^a[acid]^b[amine]^c[PBu_3]^d[O_2]^e$$
$$= k'[\mathbf{8a}]^a[acid]^b[amine]^c[PBu_3]^d$$

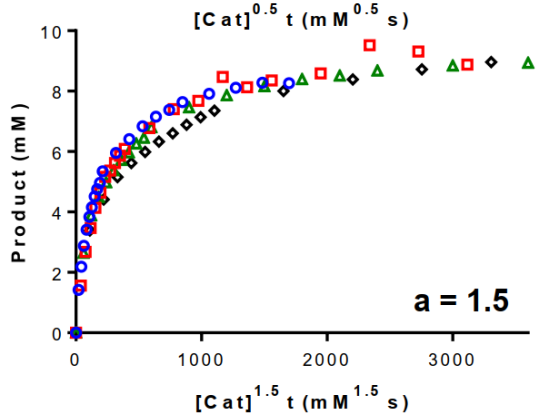
$$\frac{dP}{df(t)} = k'[acid]^b[amine]^c[PBu_3]^d$$

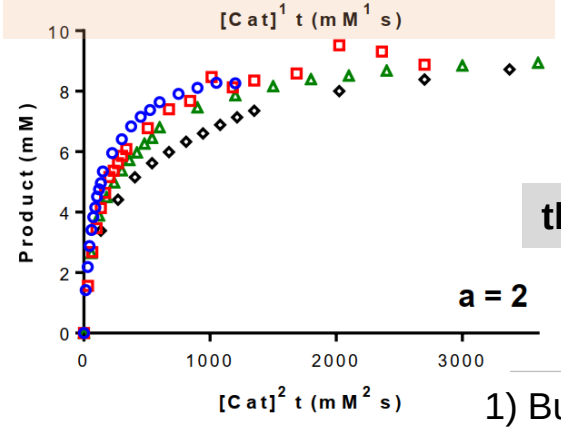
$$df(t) = [\mathbf{8a}]^a dt$$

$$f(t) = \int [\mathbf{8a}]^a dt = [\mathbf{8a}]^a t$$





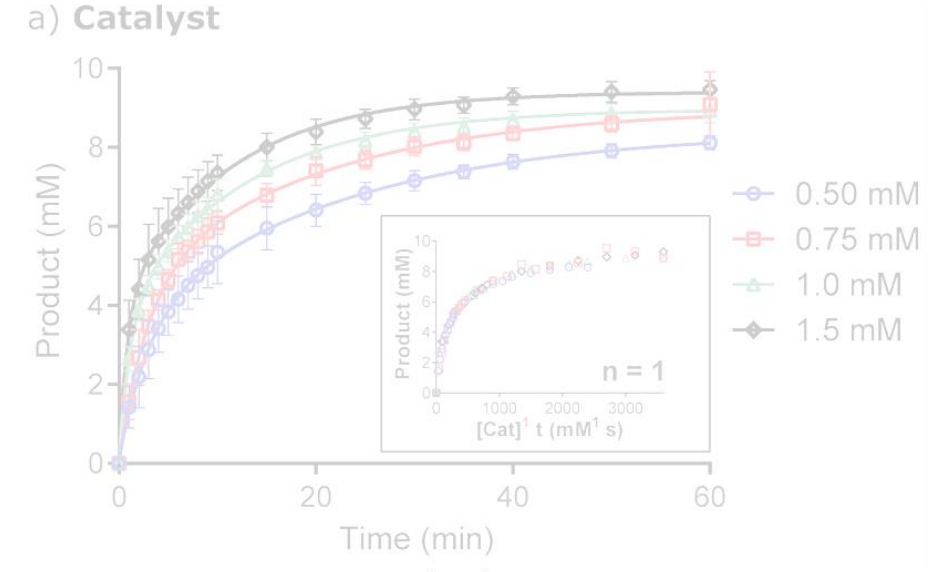


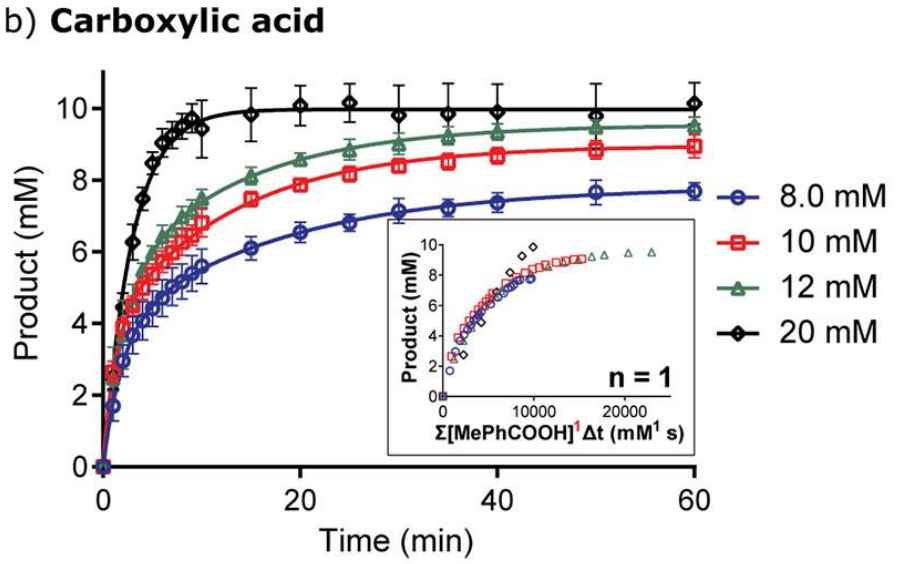


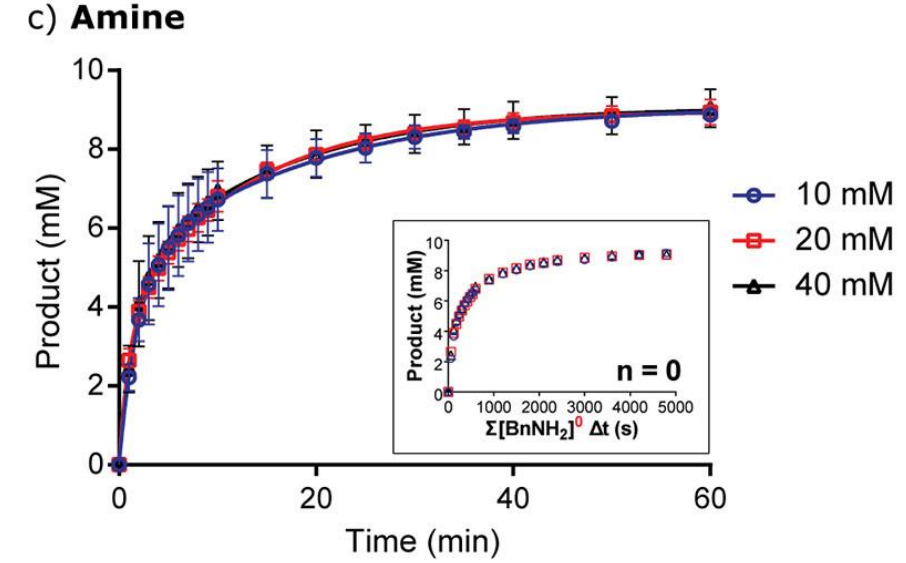
the reaction is first-order with respect to 8a

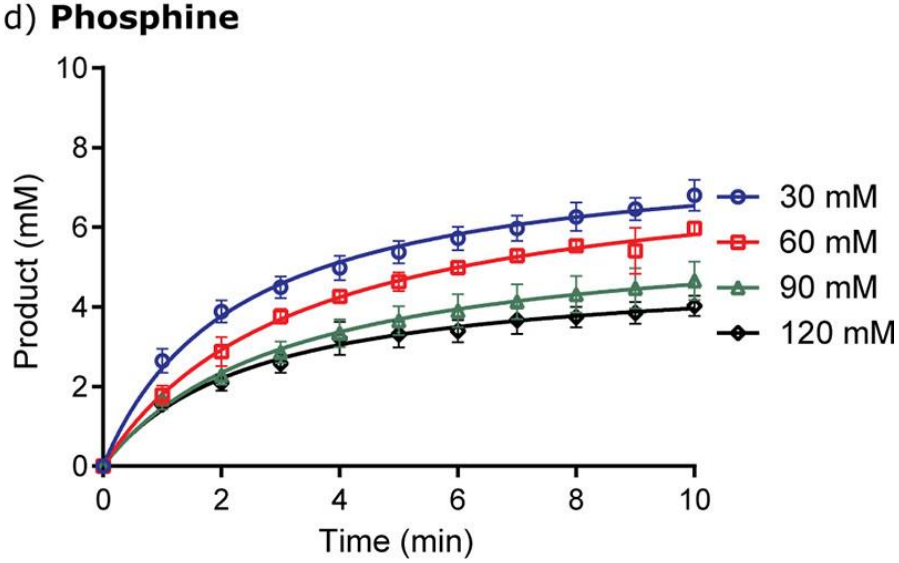
1) Burés, J. *Angew. Chem., Int. Ed.* **2016**, *55*, 16084.

Reaction Order Determination





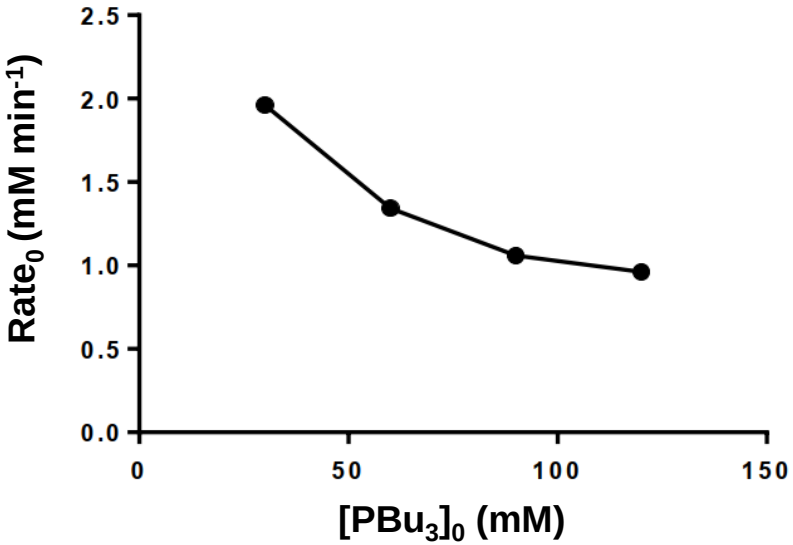




The reaction is first-order with respect to carboxylic acid and has no rate dependence on amine concentration.

Reaction Order Determination of PBu_3

Tri-*n*-butylphosphine (d = –0.5)



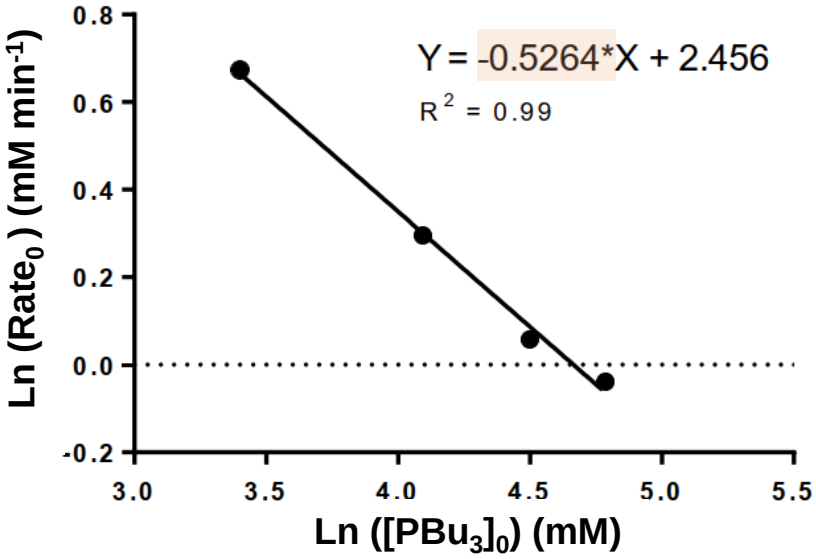
$$Rate = \frac{dP}{dt} = k'[8a]^a[acid]^b[amine]^c[PBu_3]^d$$

$$Rate_0 = k'[8a]_0^a[acid]_0^b[amine]_0^c[PBu_3]_0^d$$

In first 3-minute data, concentration of other components are regarded as constants

$$Rate = k'[8a]_0^a[acid]_0^b[amine]_0^c[PBu_3]^d$$

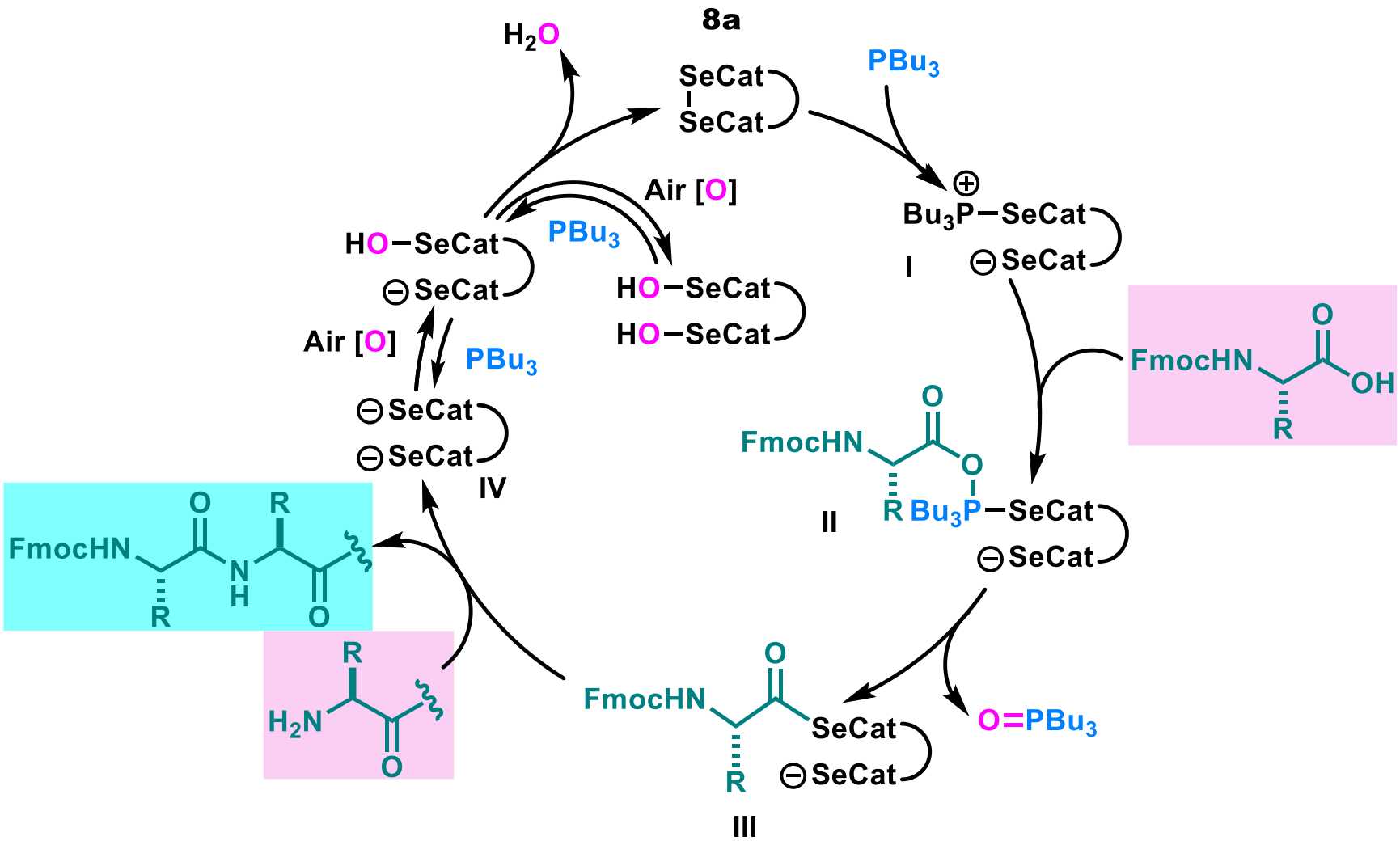
$$ln(Rate) = d\ ln([PBu_3]) + C$$



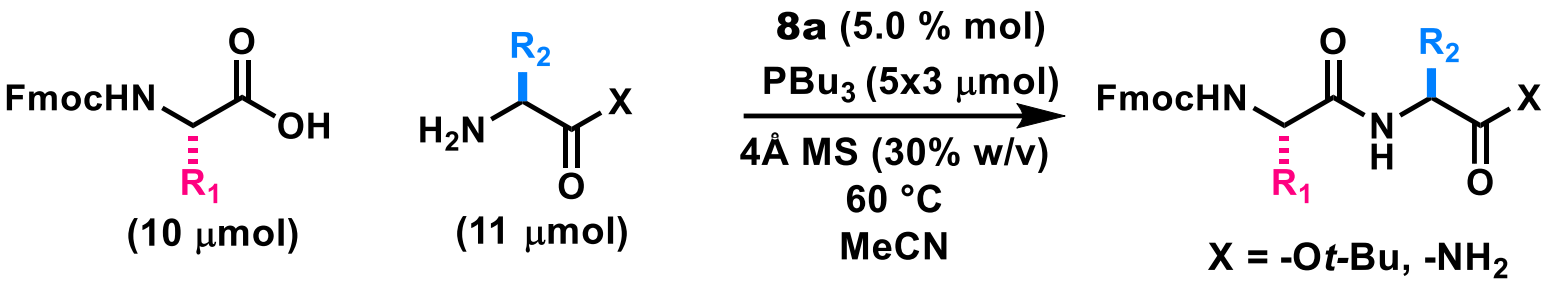
The reaction order of PBu_3 is -1/2.

Rate = k′[8a][acid][PBu_3]$^{-1/2}$

Proposed Catalytic Mechanism



Application to Fmoc-Amino Acids Coupling

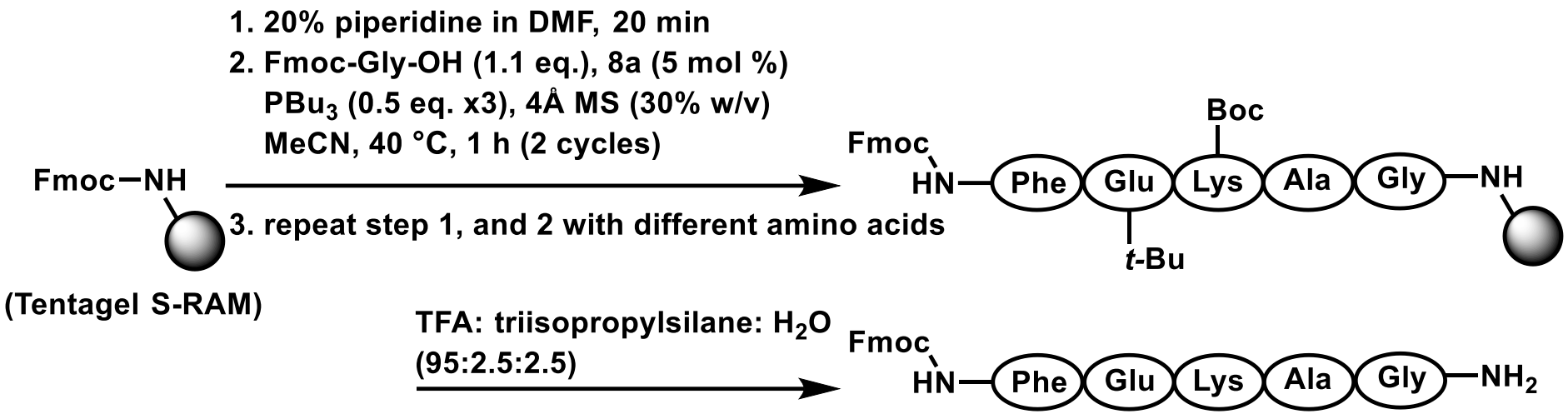


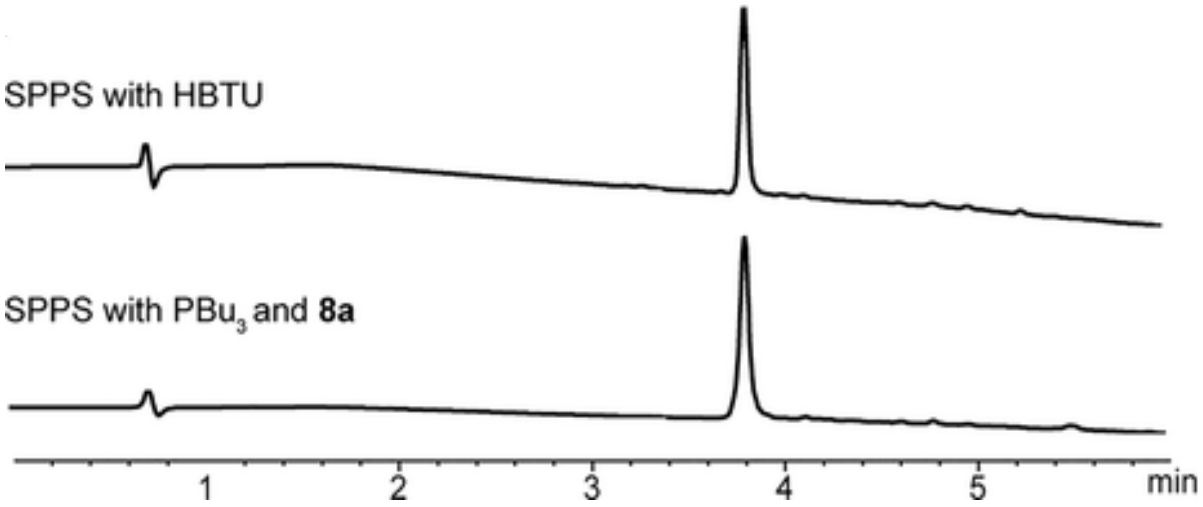
*PBu_3 was added in portions every 30 min

entry	dipeptides	conversion rate	entry	dipeptides	conversion rate
1	Fmoc-L-Ala-L-Ala-O*t*-Bu	97% (1 h)	8	Fmoc-L-Lys(Boc) -L-Ala-O*t*-Bu	90% (1 h)
2	Fmoc-L-Ala-L-Phe-O*t*-Bu	95% (1 h)	9	Fmoc-L-Pro-L-Ala-O*t*-Bu	90% (2 h)
3	Fmoc-L-Ala-L-Lys(Cbz) -O*t*-Bu	99% (1.5 h)	10	Fmoc-L-Arg(Pbf) -L-Ala-O*t*-Bu	99% (1.5 h)
4	Fmoc-L-Ala-L-Val-O*t*-Bu	99% (1 h)	11	Fmoc-L-Val-L-Ala-O*t*-Bu	92% (2 h)[a]
5	Fmoc-L-Ala-L-Pro-O*t*-Bu	94% (2 h)	12	Fmoc-L-Aib-L-Ala-O*t*-Bu	91% (2 h)
6	Fmoc-L-Ala-L-Trp-NH_2	99% (1 h)	13	Fmoc-L-Phe-L-Pro-O*t*-Bu	82% (2 h)[a]
7	Fmoc-L-Phe-L-Ala-O*t*-Bu	90% (1 h)			

[a]: Less than 2 % epimerization was observed.
Aib: α-aminoisobutyric acid

Application of Designed Catalyst to Solid-Phase Peptide Synthesis

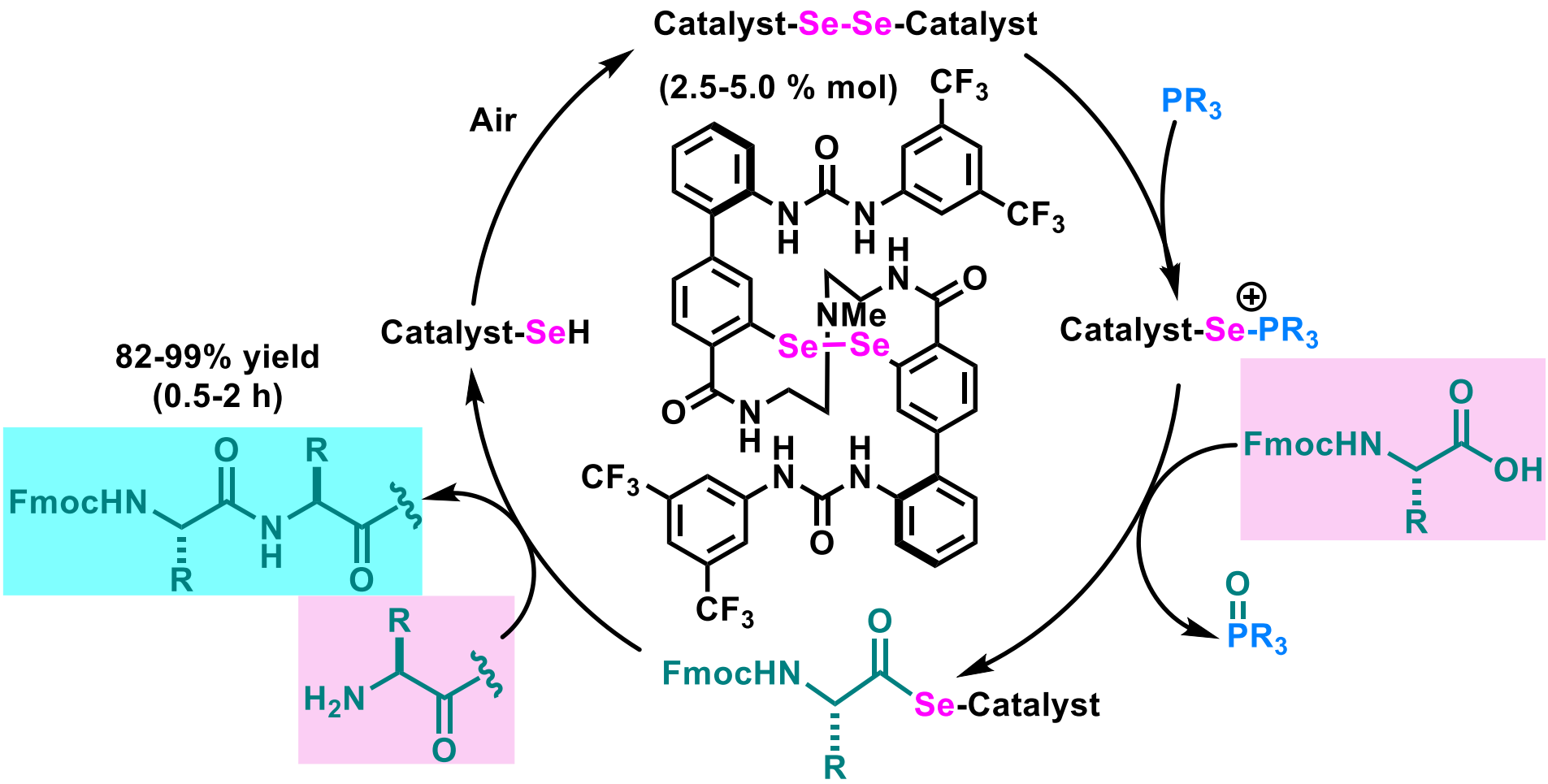




HPLC traces of crude peptide

Relatively pure peptide was obtained after iterative synthesis on the solid support.

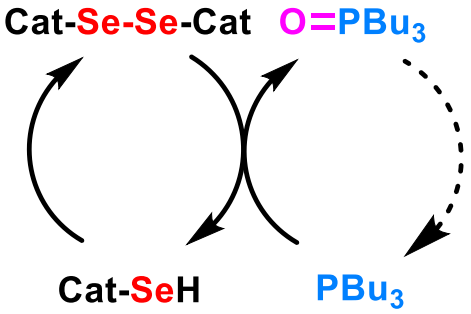
Summary



- compatible with a diverse range of Fmoc-amino acid substrates with insignificant epimerization
- provided a lead toward organocatalytic peptide synthesis without excess reagents
- exhibited a promise for solid-phase peptide synthesis

Future Perspective

- explore phosphine reagents that are less prone to oxidation
  - evaluating other phosphine derivatives
  - exploring the recycling of the phosphine oxide product



- develop solid-phase peptide synthesis(SPPS) methodology
  - further optimization of catalyst design to fit SPPS conditions
  - substrate scope
  - molecular sieves-free system

➢ The overall aim of this work is to develop organocatalysts that can replace standard coupling agents and reduce waste in peptide synthesis.