Designing an Organocatalyst-Driven Peptide Synthesis

Catalyst-Se-Se-Catalyst
(2.5-5.0 % mol)

Catalyst-Se-PR₃

Catalyst-SeH

Air

82-99% yield (0.5-2 h)

FmocHN

O

N

R

R

H₂N

N

R

R

O

PR₃

FmocHN

O

R

R

FmocHN

O

R

R

O

PR₃

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.)
• 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.

Low atom economy of amide forming reaction has been recognized as one of the most important issues in green chemistry field.  

“Non-Classical” Amidation Approaches

5) Blaise, E. E. Compt. Rend. 1901, 132, 38–41
Direct Catalytic Amidation

- boronic acid derivatives
  - Yamamoto (1996)
- metal catalyst
  - Williams (2012)
  - Adolfsson (2012)
- organocatalyst
  - Schmidt (2005), Awasthi (2015)

Contents

• Introduction

• Main Paper
“Rational Design of an Organocatalyst for Peptide Bond Formation”
2019, 141, 15977.)
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)
Catalytic design was inspired by two biosynthetic precedents.

1. mimicking oxyanion holes in enzymes by urea catalysts

![Diagram of Biomimetic Catalyst Design Strategy]


**generally accepted mechanism for serine proteases**

**idealized depiction of amide bond formation catalyst**

X/Y → = reversible covalent bond or noncovalent interactions
Biomimetic Catalyst Design Strategy

2. utilizing amino acid thioesters to access peptide

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

0. phosphopantetheinylation

1. adenylation

2. thiolation

3. condensation

4. release

utilizing amino acid thioesters to access peptide biosynthesis of non-ribosomal peptides synthetases (NRPS)\textsuperscript{1)}

\begin{center}
\includegraphics[width=\textwidth]{diagram.png}
\end{center}

\textgreater{} design biomimetic catalyst that efficiently
\begin{itemize}
  \item activates carboxylic acids to the more electrophilic thioesters.
  \item would couple amino acid thioesters to a growing peptide chain.
\end{itemize}

Design of Urea-based Catalyst

A (10 mM) + H₂N-CH₂-Ph (20 mM) → catalyst (1 mM) in MeCN, rt → C (X % conversion after 2 h)

Catalyst:

1. X = 13
2. X = 23
3. X = 55

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

- 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

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
Catalyst design was optimized as **4a**.
Application of Catalyst to Conversions of Fmoc-Amino Acid Thioesters to Dipeptide

\[
\begin{align*}
\text{FmocHN} & \text{\textsubscript{O}} \text{\textsubscript{SPh}} & \text{H\textsubscript{2}N} & \text{\textsubscript{O}} & \text{Me} & \rightarrow & \text{FmocHN} & \text{\textsubscript{O}} \text{\textsubscript{R\textsubscript{1}}} & \text{\textsubscript{N}} & \text{\textsubscript{R\textsubscript{2}}} & \text{\textsubscript{O}} & \text{Me} \\
(10 \ \mu\text{M}) & & (20 \ \mu\text{M}) & & & & & t : \text{time for >98\% conversion} \\
\end{align*}
\]

\[
\begin{align*}
\text{FmocHN} & \text{\textsubscript{O}} \text{\textsubscript{N}} \text{\textsubscript{Me}} & \rightarrow & \text{FmocHN} & \text{\textsubscript{O}} \text{\textsubscript{Me}} \\
X = 0, \ t > 20 \ \text{days (estimated)} & \quad X = 0, \ t > 50 \ \text{days (estimated)} & \quad X = 10, \ t = 4 \ h^a & \quad X = 10, \ t = 4.5 \ h & \quad X = 20, \ t = 2 \ h \\
X = 10, \ t = 10 \ \text{min} & \quad X = 10, \ t = 7 \ h^a & \quad X = 20, \ t = 3 \ h^a & \quad X = 20, \ t = 2 \ h \\
\end{align*}
\]

\[
\begin{align*}
\text{FmocHN} & \text{\textsubscript{N}} \text{\textsubscript{Me}} & \rightarrow & \text{FmocHN} & \text{\textsubscript{O}} \text{\textsubscript{Me}} \\
X = 0, \ t > 50 \ \text{days (estimated)} & \quad X = 10, \ t = 10 \ \text{min} & \quad X = 10, \ t = 30 \ \text{min} & \quad X = 10, \ t = 60 \ \text{min} \\
X = 10, \ t = 40 \ \text{min} & \quad & \quad & \\
\end{align*}
\]

No epimerization was observed

\[^a\ 10-15\% \text{ hydrolysis of Fmoc-Ala-SPh was observed}\]
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)\(^1\)

\[ \text{activation} \quad \text{R} \quad \text{OH} \quad \rightarrow \quad \text{R} \quad \text{SO}_{\text{PCP}} \quad \text{(thioester)} \quad \text{transformation} \quad \rightarrow \quad \text{R} \quad \text{NH} \quad \text{R}' \quad \text{(amide)} \]

\*PCP = Peptidyl 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.

\[ \text{activation} \quad \text{R} \quad \text{OH} \quad \rightarrow \quad \text{R} \quad \text{SR}' \quad \text{(thioester)} \quad \text{coupling} \quad \rightarrow \quad \text{R} \quad \text{NH} \quad \text{R}' \quad \text{(amide)} \]

Mukaiyama Reduction-Oxidation Condensation

concept:
perform a dehydration condensation by removing $\text{H}_2\text{O}$ as $2[\text{H}]$ and $[\text{O}]$ by the use of a combination of a weak reductant and oxidant$^1$)

$$
\begin{align*}
2 \ce{R\text{COOH}} + \ce{Ar_2\text{Hg}} + \ce{Bu_3\text{P}} & \xrightarrow{\text{benzene, } 80^\circ\text{C, } 2-4\ \text{h}} \ce{R\text{COOR}} + \ce{Hg} + 2\ce{ArH} + \ce{Bu_3\text{P}=O} \\
3.0\ \text{M} & \quad 0.5\ \text{eq} & \quad 0.5\ \text{eq} & \quad 74-87\ %
\end{align*}
$$

condensation of carboxylic acid to thioester with disulfides and phosphine reagents$^2$)

$$
\begin{align*}
\ce{R\text{COOH}} + \ce{Ph-S-S-Ph} + \ce{Ph_3\text{P}} & \xrightarrow{\text{MeCN reflux, } 5\ \text{h}} \ce{R\text{C(SPh)}_2} + \ce{PhSH} + \ce{Ph_3\text{P}=O} \\
0.33\ \text{M} & \quad 1.0\ \text{eq} & \quad 1.0\ \text{eq} & \quad 77-94\ %
\end{align*}
$$

Mukaiyama Reduction-Oxidation Condensation

condensation of carboxylic acid to thioester with disulfides and phosphine reagents

\[
\text{R-OH} + \text{PhSSPh} + \text{Ph}_3\text{P} \rightarrow \text{R-SSPh} + \text{PhSH} + \text{Ph}_3\text{P}=\text{O}
\]

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

Step 1
formation of an amino acid thioester from carboxylic acid

Step 2

Step 3
amide bond formation

Step 4
air oxidation to yield disulfide for the next cycle
Diselenide $\textit{6-Se}$ exhibited a significant overall rate enhancement over $6-\text{S}$.

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

Catalyst design was optimized as 8a.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Y (%)</th>
<th>Z (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Se</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>58</td>
</tr>
<tr>
<td>8a</td>
<td>42</td>
<td>73</td>
</tr>
<tr>
<td>8b</td>
<td>40</td>
<td>67</td>
</tr>
<tr>
<td>8c</td>
<td>32</td>
<td>62</td>
</tr>
</tbody>
</table>

6-Se  
Y % conversion after 20 min  
Z % conversion after 240 min
Optimization of Phosphine Reagent

Optimization of phosphine reagent:
- electron-rich
- less bulky

Air-sensitive and yet efficient catalyst turnover

Optimized conditions:

10 mmol

\[
\text{ArCOH} + \text{H}_{2}\text{N-Ph} \rightarrow \text{PhCONHPh}
\]

Catalyst 8a (5 mol %) with P(But)_3 (3x3 mM), 4Å MS (30% w/v) in MeCN, 60 °C, 1.5 h yields 99% conversion.

*P(But)_3 was added in portions every 30 min.
concentration of $8a$

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

$$\frac{dP}{df(t)} = k'[8a]^a[\text{acid}]^b[\text{amine}]^c[PBu_3]^d$$

$$df(t) = [8a]^a dt$$

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

Find a value of $a$ (reaction order) so that reaction curve overlays the reaction is first-order with respect to $8a$

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 ($d = -0.5$)

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

\[
\text{Rate}_0 = k'[8a]_0^a[\text{acid}]_0^b[\text{amine}]_0^c[PBu_3]^d
\]

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

\[
\text{Rate} = k'[8a]^a[\text{acid}]^b[\text{amine}]^c[PBu_3]^d
\]

\[
\ln(\text{Rate}) = d \ln([PBu_3]) + C
\]

The reaction order of PBu$_3$ is -1/2.
Proposed Catalytic Mechanism
**Application to Fmoc-Amino Acids Coupling**

![Chemical structures and reaction conditions](Image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Dipeptides</th>
<th>Conversion Rate</th>
<th>Entry</th>
<th>Dipeptides</th>
<th>Conversion Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fmoc-L-Ala-L-Ala-Ot-Bu</td>
<td>97% (1 h)</td>
<td>8</td>
<td>Fmoc-L-Lys(Boc) -L-Ala-Ot-Bu</td>
<td>90% (1 h)</td>
</tr>
<tr>
<td>2</td>
<td>Fmoc-L-Ala-L-Phe-Ot-Bu</td>
<td>95% (1 h)</td>
<td>9</td>
<td>Fmoc-L-Pro-L-Ala-Ot-Bu</td>
<td>90% (2 h)</td>
</tr>
<tr>
<td>3</td>
<td>Fmoc-L-Ala-L-Lys(Cbz) -Ot-Bu</td>
<td>99% (1.5 h)</td>
<td>10</td>
<td>Fmoc-L-Arg(Pbf) -L-Ala-Ot-Bu</td>
<td>99% (1.5 h)</td>
</tr>
<tr>
<td>4</td>
<td>Fmoc-L-Ala-L-Val-Ot-Bu</td>
<td>99% (1 h)</td>
<td>11</td>
<td>Fmoc-L-Val-L-Ala-Ot-Bu</td>
<td>92% (2 h)</td>
</tr>
<tr>
<td>5</td>
<td>Fmoc-L-Ala-L-Pro-Ot-Bu</td>
<td>94% (2 h)</td>
<td>12</td>
<td>Fmoc-L-Aib-L-Ala-Ot-Bu</td>
<td>91% (2 h)</td>
</tr>
<tr>
<td>6</td>
<td>Fmoc-L-Ala-L-Trp-NH₂</td>
<td>99% (1 h)</td>
<td>13</td>
<td>Fmoc-L-Phe-L-Pro-Ot-Bu</td>
<td>82% (2 h)</td>
</tr>
<tr>
<td>7</td>
<td>Fmoc-L-Phe-L-Ala-Ot-Bu</td>
<td>90% (1 h)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PBU₃ was added in portions every 30 min

---

**: Less than 2% epimerization was observed. Aib: α-aminoisobutyric acid**
Application of Designed Catalyst to Solid-Phase Peptide Synthesis

1. 20% piperidine in DMF, 20 min
2. Fmoc-Gly-OH (1.1 eq.), 8a (5 mol %)
   PBu₃ (0.5 eq. x3), 4Å MS (30% w/v)
   MeCN, 40 °C, 1 h (2 cycles)

(Tentagel S-RAM)

3. repeat step 1, and 2 with different amino acids

HPLC traces of crude peptide

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

82-99% yield (0.5-2 h)

Catalyst-Se-Se-Catalyst

(2.5-5.0 % mol)
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.