Convergent synthesis of peptidomimetic based on a chloroalkene dipeptide isostere and its application to inhibition against amyloid β aggregation

Takuya Kobayakawa, Chika Azuma, Kenichi Yamamoto, Kohei Tsuji, Hirokazu Tamamura
Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University

https://doi.org/10.17952/37EPS.2024.P1002

Introduction

~Background~

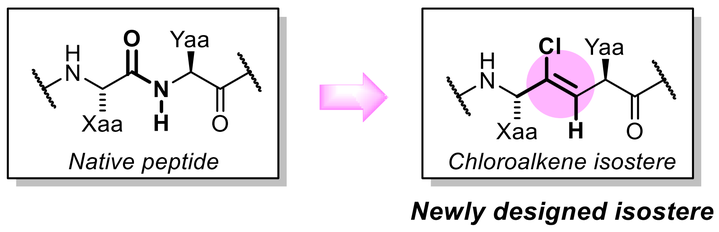
Alkene type dipeptide isosteres (ADIs), which have been designed based on a native peptide bond in a ground state conformation, are expected as “peptide bond isosteres” because of high structural homology with natural dipeptides. Practically, many groups have investigated to replace amide bonds in peptides with ADIs, and their positive effects have been demonstrated in biological molecules.[1] However, there are several problems in diversity, synthetic efficiency toward ADIs, etc.

~This Study~

In this situation, we have developed an efficient synthetic method for chloroalkene dipeptide isosteres (CADIs) as peptide bond equivalents.[2-6]

In a convergent synthetic method, Fmoc-protected carboxylic acids can be synthesized via Evans *syn* aldol reaction followed by [3.3] sigmatropic rearrangement from *N*- and *C*-terminal analogs corresponding to each amino acid as starting materials. By the synthetic strategy, an Fmoc-protected CADI can be applied for solid-phase peptide synthesis.

Design of chloroalkene dipeptide isostere (CADI)

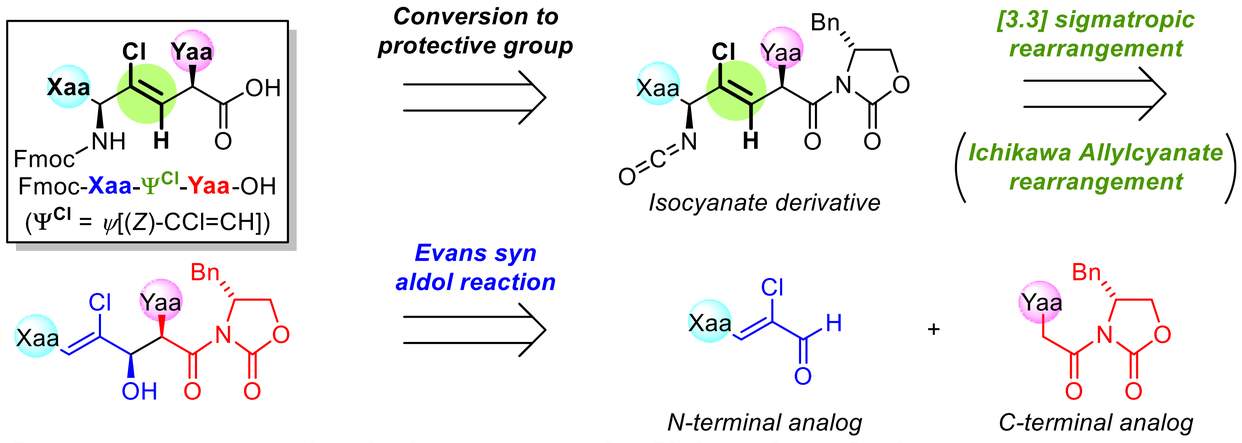


X	VDR[c] (pm)	Electronegativity
O	152	3.4[a] (3.5)[b]
H	120	2.2[a] (2.3)[b]
F	147	4.0[a] (4.1)[b]
Cl	175	3.2[a] (2.8)[b]

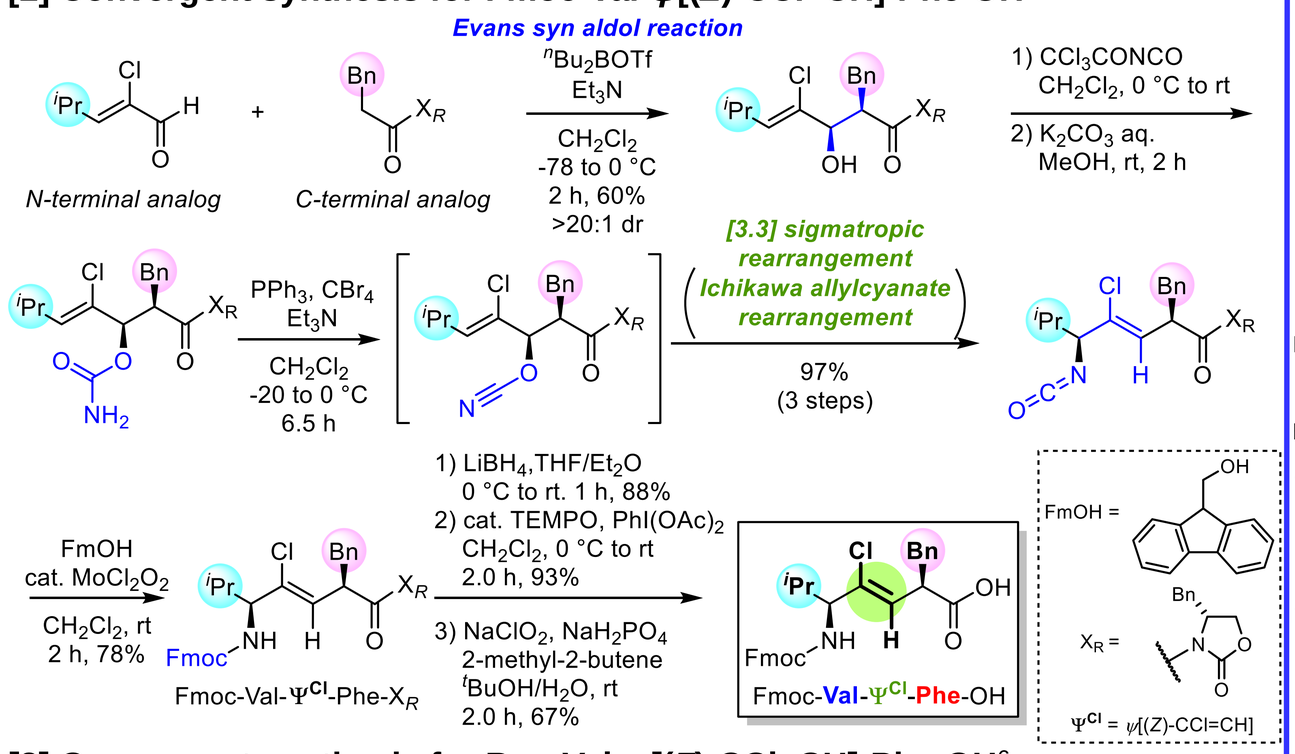
[a] Pauling value, [b] Allred-Rochow value
[c] VDR is Van der Waals Radius.

Results and discussion

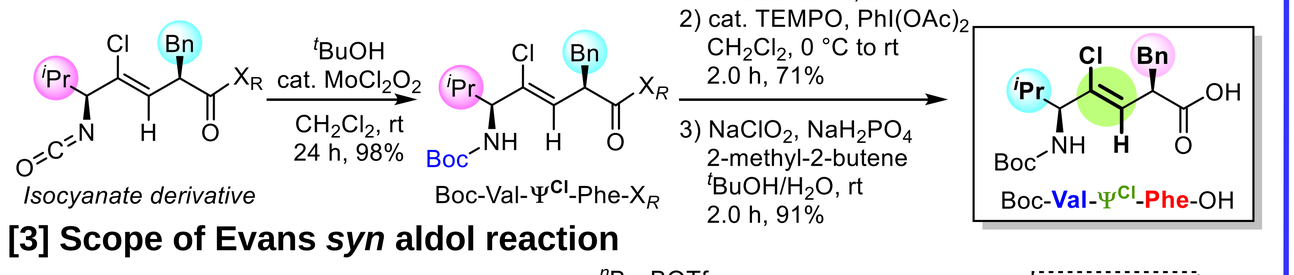
[1] Retrosynthetic analysis for chloroalkene dipeptide isostere (CADI)



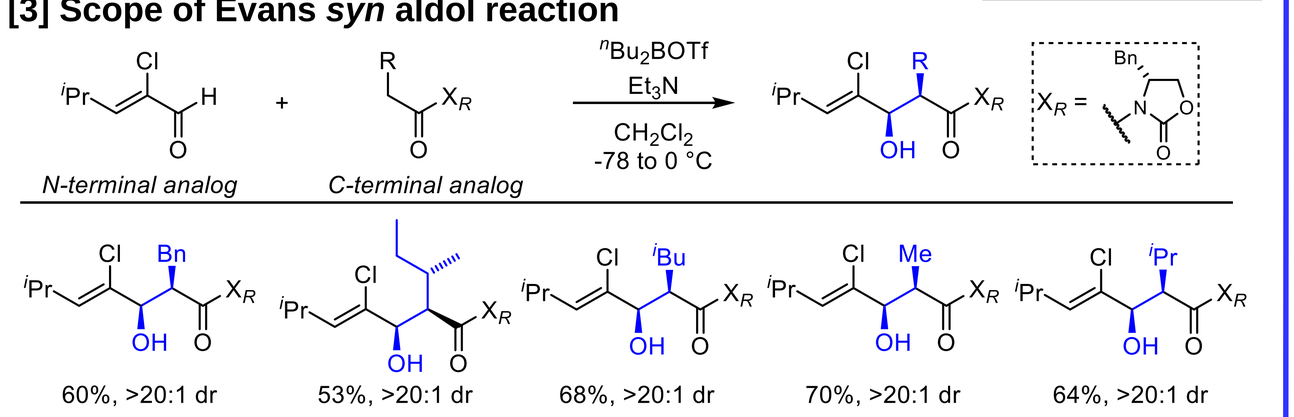
[2] Convergent synthesis for Fmoc-Val-ψ[(*Z*)-CCl=CH]-Phe-OH[6]



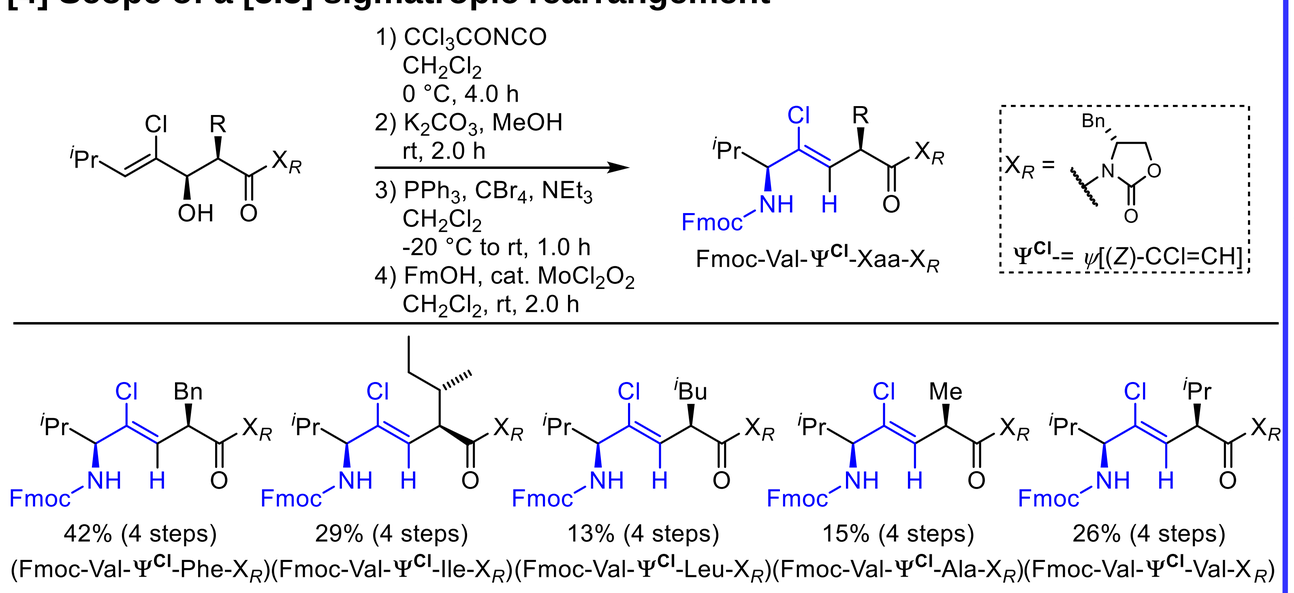
[2] Convergent synthesis for Boc-Val-ψ[(*Z*)-CCl=CH]-Phe-OH[6]



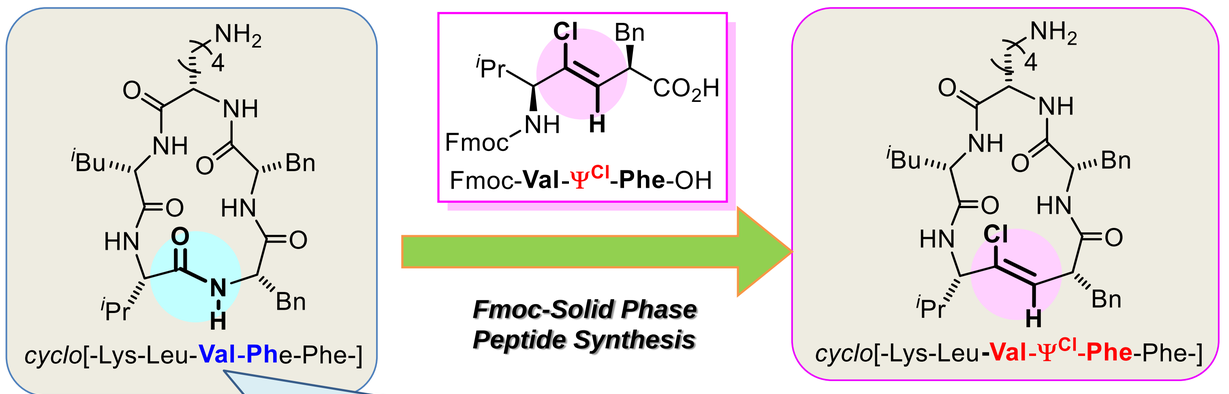
[3] Scope of Evans *syn* aldol reaction



[4] Scope of a [3.3] sigmatropic rearrangement



[5] Application of CADI for cyclic KLVFF peptide as Aβ aggregation inhibitor[6]

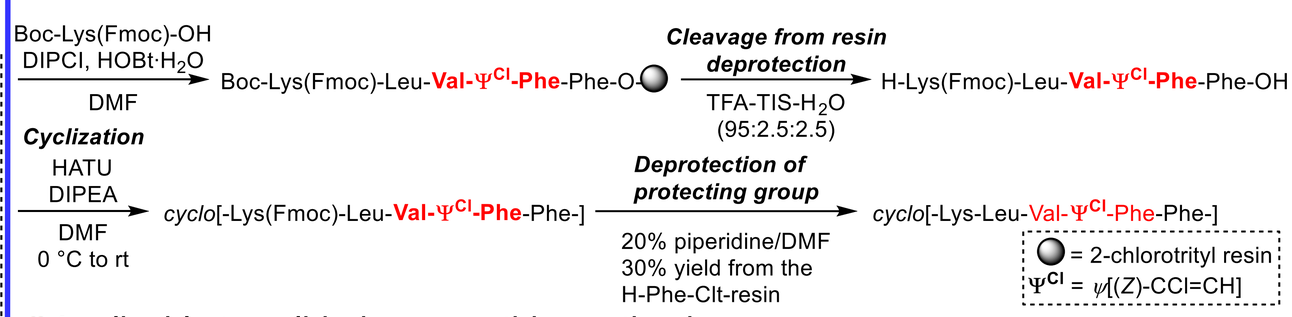


Aβ[1–42] :DAEFRHDSGYEVHHQ**KLVFF**AEDVGSNKGAIIGLMVGGVVIA
(16 20)

➢ **Hydrophobic central region important for Aβ aggregation**

- # Incorporation into *cyclo*[KLVFF][7], an *anti*-aggregation peptide of amyloid-β (Aβ) peptide hypothesized to cause Alzheimer's disease
- # Synthesis of CADI based on the developed synthesis method
- # Introduction into Aβ aggregation Inhibitory peptide *cyclo*[KLVFF] by Fmoc-solid phase peptide synthesis

[6] Synthesis of cyclic KLVFF peptide including CADI structure[6]



Applicable to solid-phase peptide synthesis

[7] Evaluation of Aβ aggregation inhibition by Thioflavin-T dye assay[6]

entry	Compound (**Ψ[Cl]** = ψ[(*Z*)-CCl=CH])	ThT fluorescence intensity [%][a] inhibitor 20 μM (10 μM)
1	H-Lys-Leu-Val-Phe-Phe-OH	90.2 ± 8.3
2	H-Lys-Leu-Val-**Ψ[Cl]**-Phe-Phe-OH	79.6 ± 15.6
3	*cyclo*[-Lys-Leu-Val-Phe-Phe-]	20.3 ± 1.9 (77.9 ± 2.5)
4	*cyclo*[-Lys-Leu-Val-**Ψ[Cl]**-Phe-Phe-]	24.6 ± 0.9 (**30.1 ± 3.3**)
5	Morin	36.0 ± 8.9

[a]Relative fluorescence intensity of Aβ1–42 (20 μM) + inhibitor (20 or 10 μM) vs. Aβ1–42 (20 μM) alone incubated for 6.0 h. All data shown are mean values of at least three independent experiments.

- ✔ Higher inhibitory activity of the CADI containing KLVFF peptide than that of the parent KLVFF peptide
- ✔ Increase of interaction with Aβ by the stabilized whole structure compared to the parent KLVFF peptide

Summary

[1] Development of an efficient synthetic method for (L,L)-type CADIs

We have also succeeded to establish of the convergent synthesis for CADIs, which was used by *N*- and *C*-terminal analogs corresponding amino acids as starting materials, via Evans syn aldol reaction followed by [3.3] sigmatropic rearrangement of the allyl cyanate.

[2] Application to peptide synthesis and introduction to a bioactive peptide

Utilizing the above methodology, a CADI was incorporated into a cyclic KLVFF peptide by solid-phase peptide synthesis. As a result, the cyclic KLVFF peptidomimic has shown higher activity than the parent cyclic peptide.

Acknowledgement

We acknowledge Prof. Yoshio Hayashi and Assoc. Prof. Atsuhiko Taniguchi (School of Pharmacy, Tokyo University of Pharmacy and Life Sciences) for evaluation of inhibitory activity for Aβ aggregation.

References

[1] Tamamura H.; Kobayakawa T.; Ohashi N. Mid-size drugs based on peptides and peptidomimetics: a new drug category. In: *Springer Briefs in Pharmaceutical Science & Drug Development*, *Singapore*, **2018**, 1-100. [2] Kobayakawa, T.; Tamamura, H. *J. Synth. Org. Chem. Jpn.* **2019**, *77*, 904-911. [3] Kobayakawa, T.; Narumi, T.; Tamamura, H. *Org. Lett.* **2015**, *17*, 2302-2305. [4] Kobayakawa, T.; Tamamura, H. *Tetrahedron* **2017**, *73*, 4464-4471. [5] Kobayakawa, T.; Matsuzaki, Y.; Hozumi, K.; Nomura, W.; Nomizu, M.; Tamamura, H. *ACS Med. Chem. Lett.* **2018**, *9*, 6-10.[5] Kobayakawa, T.; Azuma, C.; Watanabe, Y.; Sawamura, S.; Taniguchi, A.; Hayashi, Y.; Tsuji, K.; Tamamura, H. *J. Org. Chem.* **2021**, *86*, 5091-5101. [6] Arai, T.; Araya, T.; Sasaki, D.; Taniguchi, A.; Sato, T.; Sohma, Y.; Kanai, M. *Angew. Chem., Int. Ed.* **2014**, *53*, 8236-8239.