Synthesis of a Chloroalkene Dipeptide Isostere-Containing Peptidomimetic and Its Biological Application

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Supporting Information

ABSTRACT: The first rapid and efficient chemical synthesis of a cyclic Arg-Gly-Asp (RGD) peptide containing a chloroalkene dipeptide isostere (CADI) is reported. By a developed synthetic method, an *N*-tert-butylsulfonyl protected CADI was obtained utilizing diastereoselective allylic alkylation as a key reaction. This CADI was also transformed into an *N*-Fmoc protected CADI in a few steps. The CADI was used in Fmoc-based solid-phase peptide synthesis. The first synthesis of a CADI-containing cyclic RGD peptide was successful, and the synthesized CADI-containing peptidomimetic was found to be a more potent inhibitor against integrin-mediated cell attachment than the parent cyclic peptide.

KEYWORDS: Chloroalkene dipeptide isostere, peptidomimetic, solid-phase peptide synthesis, cyclic RGD peptide

During the last quarter-century, various biologically active peptides have been discovered and characterized. These bioactive peptides influence and control physiological functions through interaction with their various receptors, and the number of natural and modified peptides that are used as therapeutics continues to increase. Many bioactive peptides have been developed and have been involved in the discovery of novel therapies. However, the use of peptides as therapeutics is limited by several factors, including low metabolic stability toward proteolysis and undesired activity resulting from interactions of peptides with various receptors.^{1,2}

Alkene dipeptide isosteres (ADIs), which are designed based on the partial double-bond character of the native peptide bond in its ground state conformation, have been expected to be structure units as they have ideal amide bond mimetics in the original dipeptides. Practically, many groups have attempted to replace the amide bonds in peptides with several types of dipeptide isosteres.^{3–11} In addition, the metabolic stability of ADIs was improved.⁵ However, bioactive peptides containing ADIs do not always function effectively as peptidomimetics because they may possess a smaller dipole moment as a result of changes in the electronegativity. Furthermore, these ADIs lack the steric restriction between the carbonyl oxygen and the side chain of the amino acid due to their van der Waals radius (VDR), which is smaller than that of the original amide bond. In addition, many ADIs cannot be

supplied efficiently due to problems associated with their synthesis.

Our research group has focused on the chloroolefin structures in chloroalkene dipeptide isosteres (CADIs), which can be used to replace an amide bond in peptides as shown in Figure 1. Replacement of a peptide bond by the chloroolefin moiety can also be considered as mimicking steric restriction resulting from the pseudo-1,3-allylic strain by a chlorine atom, which is larger than a carbonyl oxygen.^{11,12}

In addition, while the direction of the vector of the dipole moment in the chloroolefin is similar to that of an amide, the vector of the dipole moment in the fluoroolefin is significantly

Figure 1. Native peptide bonds and chloroalkene dipeptide isosteres.

Received: June 11, 2017

Accepted: December 27, 2017

Published: December 27, 2017

different.¹³ Thus, it is expected that CADIs might compensate for the drawbacks associated with ADIs. Few reports, however, have been available on application of chloroalkene structures as peptidomimetics.^{14,15} This is possibly due to the lack of efficient methods or limitation of substrates for synthesis of CADIs.

Our group has developed synthetic methods for various type CADIs (Bus-Xaa- $\psi[(Z)\text{-CCl=CH}]$ -Yaa-OEt) utilizing organocopper reagents and switching the olefin geometry of the allylic *gem*-dichlorides that are used as chloroalkene precursors.^{16–19} In addition, a Boc- or Fmoc-protected dipeptide (Boc- or Fmoc-Xaa- $\psi[(Z)\text{-CCl=CH}]$ -Yaa-OH) can be easily prepared for peptide synthesis from a common intermediate Bus-protected dipeptide (Bus-Xaa- $\psi[(Z)\text{-CCl=CH}]$ -Yaa-OH) in a few steps and with high total yield. In this report, we describe the introduction of a CADI into a cyclic pentapeptide, *cyclo*[-Arg-Gly-Asp-D-Phe-Val-] **1**, which was reported by Kessler et al. as a highly bioactive $\alpha_v\beta_3$ integrin antagonist.^{20,21} We report the first chemical synthesis and biological evaluation of a CADI-containing cyclic RGD peptide **2** utilizing Fmoc-based solid-phase peptide synthesis (SPPS),²² and the peptidomimetic was biologically evaluated (Figure 2).

Figure 2. Newly designed RGD peptidomimetic.

Initially, Fmoc-D-Phe- $\psi[(Z)\text{-CCl=CH}]$ -Val-OH **3** was produced by published synthetic methods.^{16–19} As shown in Scheme 1, the γ,γ -dichloro- α,β -unsaturated ester **6**, which has been reported as a precursor in CADI synthesis,¹³ was prepared.

Scheme 1. Synthesis of Fmoc-D-Phe- $\psi[(Z)\text{-CCl=CH}]$ -Val-OH

Diastereoselective allylic alkylation utilizing organocopper reagents, prepared from 30 mol % CuCl and 2-propylzinc bromide, afforded the desired chloroalkene product **7** in high yield and with excellent diastereoselectivity. Deprotection of the Bus group with AlCl₃ and anisole, followed by the Fmoc protection, led to the ester **8**. The ester group of **8** was reduced to the corresponding aldehyde with DIBAL at $-78\text{ }^{\circ}\text{C}$, and this was followed by Pinnick oxidation to provide the desired Fmoc-protected carboxylic acid **3** in 81% yield from the Bus-protected ester **7** without decrease in diastereoselectivity or appreciable olefin isomerization. These 10 steps proceeded smoothly to provide the desired compound from starting materials **4** and **5**,²³ and in this way, the Fmoc-protected carboxylic acid **3** became available on a gram-scale synthesis in 38% overall yield.¹³

Finally, the synthesis of the CADI-containing RGD peptide was performed by established protocols (Scheme 2).²⁴ A

Scheme 2. Synthesis of the CADI-Containing Cyclic RGD Peptide Utilizing Fmoc-Based Solid-Phase Peptide Synthesis

protected peptide resin **10** was constructed by Fmoc-based SPPS on a glycyl 2-chlorotrityl (Clt) resin **9**, which can provide side chain-protected peptides by subsequent mild acidic treatment.²⁴ Exposing the resin **10** to AcOH/TFE/ CH_2Cl_2 (1:1:3) provided the protected peptide **11** without removal of the protecting groups in the aspartic acid and arginine residues, which was then cyclized using HATU and HOAt²⁵ to give the protected CADI-containing cyclic pentapeptide **12**. In the final step, deprotection of the cyclic peptide **12** with 87% TFA was carried out, and the obtained crude peptide was then purified by HPLC to provide the desired cyclic peptide **2** containing a D-Phe- $\psi[(Z)\text{-CCl=CH}]$ -Val-type isostere, in 72% yield from the resin **9**. The metabolic stability of the obtained peptidomimetic **2** was shown by no detectable decomposition in human serum.¹³

The CADI-containing cyclic RGD peptide **2** was evaluated for its inhibitory effect against integrin-mediated cell attachment, and the results are shown in Table 1. The CADI-containing peptide **2** showed approximately 20-fold higher inhibitory activity ($\text{IC}_{50} = 0.497\text{ nM}$, entry 2) compared with Kessler's peptide **1** ($\text{IC}_{50} = 10.9\text{ nM}$, entry 1). This result shows that the CADI-containing cyclic RGD peptides have 7.74–11.5-fold higher inhibitory activity than other pseudopeptides, the ADI-containing peptide **13**, and a (*E*)-tetrasubstituted alkene dipeptide isostere (TADI)-containing peptide **14** (entries 3, 4).²⁶ According to this result, the stabilized structure of the CADI-containing cyclic RGD

Table 1. Inhibitory Effect of Cyclic RGD Peptides against Human Dermal Fibroblast (HDF) Attachment to Vitronectin

entry	cyclo[-Arg-Gly-Asp-D-Phe- Ψ -Val-]	IC ₅₀ (nM)
1	$\Psi = -C(O)NH-$ (1)	10.8 $\pm$ 6.76 ^a (6.80 $\pm$ 2.70) ^b
2	$\Psi = -\psi[(Z)-CCl=CH]-$ (2)	0.497 $\pm$ 0.149 ^a
3	$\Psi = -\psi[(E)-CH=CH]-$ (13)	3.60 $\pm$ 1.30 ^b
4	$\Psi = -\psi[(E)-CMe=CH]-$ (14)	2.4 $\pm$ 0.33 ^b

^aIC₅₀ values are the concentrations for 50% inhibition of the against integrin-mediated cell attachment. ^bFujii and co-workers' reported values.²⁶

peptide 2 interacts with $\alpha_v\beta_3$ integrin more strongly than the parent RGD peptide. This might be due to the more highly rigid structure of the chloroalkene (Figure 3a) or the 1,3-allylic

Figure 3. Stabilized whole structure by the chloroalkene structure.

strain exerted by the chlorine atom, which is higher than that associated with the parent amide bond (Figure 3b).¹³ The structural analysis of the CADI-containing peptide 2 was performed utilizing a LowModeMD method in Molecular Operating Environment (MOE) with reflection of the data from 2D NMR spectra (Supporting Information) (Figure 4).^{27,28} As shown in Figure 4a, the superimposed peptide 1 and CADI-containing peptide 2 have similar conformations. Furthermore, comparison of the superimposed 10 lowest energy structures of peptide 1 and peptidomimetic 2 demonstrated a more rigid structure of 2 (Figure 4b,c). In fact, the root-mean-square deviation (rmsd) values of peptide 1 and peptidomimetic 2 were 1.91 and 1.45, respectively. It is expected that the CADI moiety might be mainly contributed to the restriction of the other region involving the RGD sequence because the D-Phe-Val sequence is located outside of the interactions between the RGD sequence and $\alpha_v\beta_3$.^{13,29} In addition, some groups have reported that hydrogen bond-like properties can be observed in O–H...Cl or N–H...Cl interactions.^{30,31} An intermolecular hydrogen bond or H₂O-mediated hydration with a chlorine atom might occur. Therefore, it is considered that the CADI-containing RGD peptide was more potent than the TADI-containing peptide.

In this Letter, we describe utilization of an Fmoc-based SPPS to complete a Gram-scale synthesis of Fmoc-D-Phe- $\psi[(Z)-CCl=CH]-$ Val-OH 3, the first chemical synthesis of a CADI-containing cyclic RGD peptide. In addition, the synthetic CADI-containing cyclic RGD peptide as a peptidomimetic was shown to be an effective inhibitor of integrin-mediated cell attachment, superior to the parent peptide.

(a)

(b)

(c)

Figure 4. (a) Superimposition of the structures of peptide 1 and peptidomimetic 2, which are shown as orange and green colors, respectively. (b) Superimposition of the 10 lowest energy structures of peptide 1. (c) Superimposition of the 10 lowest energy structures of peptidomimetic 2.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.7b00234.

Experimental procedures and characterization data including NMR charts (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Dr. Takaaki Mizuguchi (Tokyo Medical and Dental University) for his assistance in peptide synthesis. We would like to extend our thanks to Prof. Yoshio Hayashi, Dr. Kentaro Takayama (Tokyo University of Pharmacy and Life Sciences), and Ms. Kei Toyama (Tokyo Medical and Dental University) for their assistance in behavior experiments in human serum. This work was supported in part by KAKENHI, Grant-in-Aid for Scientific Research (B) (15H04652 to H.T.); Research Program on HIV/AIDS, Japan Agency for Medical Research and Development (AMED); JSPS Core-to-Core Program, A. Advanced Research Networks; and the Platform for Drug Discovery, Informatics, and Structural Life Science of MEXT, Japan. T.K. was supported by JSPS Research Fellowships for Young Scientists (15J04754).

■ ABBREVIATIONS

ADI, alkene dipeptide isostere; Bus, *N*-tert-butylsulfonyl; CADI, chloroalkene dipeptide isostere; Clt, 2-chlorotriptyl; DIBAL, diisobutylaluminum hydride; DIPEA, *N,N*-diisopropylethylamine; Fmoc, 9-fluorenylmethyloxycarbonyl; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; HDF, human dermal fibroblast; HOAt, 1-hydroxy-7-azabenzotriazole; MOE, Molecular Operating Environment; MS, mass spectrometry; SPPS, solid-phase peptide synthesis; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; TADI, (*E*)-tetrasubstituted alkene dipeptide isostere; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; THF, tetrahydrofuran

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