Synthesis of Highly Functionalized
(*E*)-Alkene Dipeptide Isosteres and Application
to Conformational Studies on Cyclic Peptides

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Shinya Oishi

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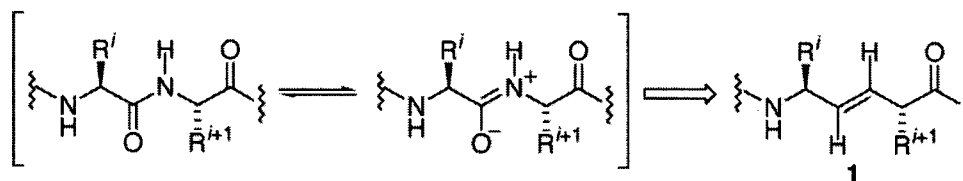

Shinya Oishi

Contents

Preface

Figure 1

Exponentially increasing information on drug-targets provided by recent advances in genome science, has amplified opportunities for medicinal chemists to develop novel pharmaceuticals. However, because methodologies for rational design of agonist/antagonist drugs from natural ligands has not matured, in order to discover lead compounds, efforts must generally rely on exhaustive random screening of extremely large compound libraries.[1]

Determination of bioactive conformations of peptides often allows identification of spatial requirements involved in pharmacophore activities. This in turn provides valuable information for rational design of non-peptidic pharmaceuticals. A number of peptidomimetics have been developed for restriction of local and/or global conformation of bioactive peptides that have been useful in conformational structure-activity relationship studies.[2,3] Mimicry of peptide backbones by replacement with functional units or addition of secondary-structure-promoting motifs represent effective strategies for conformational restriction of peptides. This can also be achieved through cyclization via covalent bond formation between side chains of neighbouring or distant residues.

(*E*)-Alkene dipeptide isosteres (EADIs) **1**, based on the concept of *ω*-angle planarity, have been used as amide bond mimetics that are stable against specific/non-specific bio-degradation and that serve as mechanistic probes lacking amide polarity (Figure 1).[4,5] For example, Miller et al. recently utilized such isosteres to verify the participation of an amide hydrogen in enantioselective acylation by peptide-based catalysts (Figure 2).[5] Replacement of a D-Pro-Aib dipeptide moiety in peptide **2**[6] by a D-Pro-*ψ*[(*E*)-CH=CH]-Aib-type EADI in **3** led to substrate-catalyst interactions that were deficient for acylation. Additionally, their conformational research based on ^{1}H-NMR also revealed that the isosteres are appropriate for (*i*+1) - (*i*+2) dipeptide motifs of type II' β-turns imparting other global conformational changes.

In their research, synthesis of the D-Pro-*ψ*[(*E*)-CH=CH]-Aib-type EADI **7** was performed following Sammes's method (Scheme 1).[7] Prolinal **4** was converted into enyne **5** by
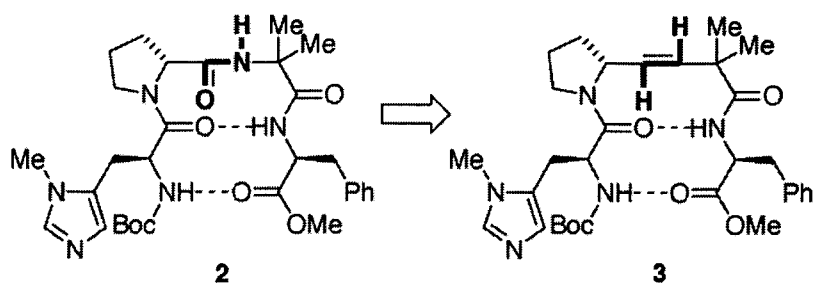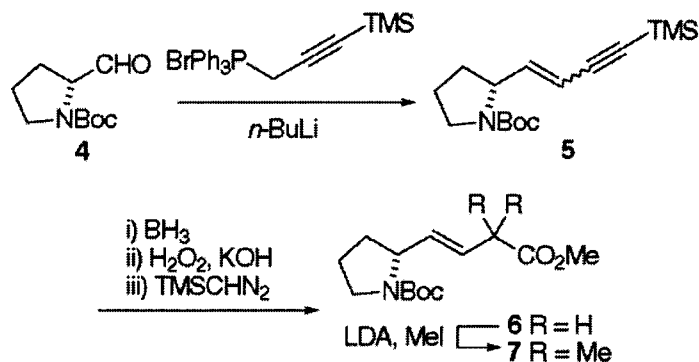
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Figure 2

Scheme 1

a Wittig reaction, then derivatized in a few steps to provide D-Pro-ψ[(E)-CH=CH]-Gly-type EADI **6**. Double alkylation of **6** afforded the expected EADI **7**. This procedure represents one of the most efficient and direct synthetic approaches to EADIs having an achiral α-carbon such as Gly and Aib. However, this methodology presents inevitable problems in terms of stereoselective synthesis of EADIs **1** that contain a chiral α-alkyl group, which would correspond to diverse chiral natural and unnatural amino acids. Stereoselective construction of two chiral centers at the α- and δ-positions adjacent to an alkene with concomitant control of olefinic geometry would be required for general synthetic procedures toward such EADIs.[8]

Ibuka et al. previously revealed that alkylation of γ-mesyloxy-α,β-enoates **8a,b** by organocopper reagents proceeds by an *anti*-S_N2' mechanism that selectively gives chiral α-alkylated products **9a,b** (Scheme 2a).[9] Application of this reaction allowed them to achieve stereoselective construction of α-alkyl groups with β,γ-E-olefinic geometry that provided the expected ψ[(E)-CH=CH]-type EADIs **11a,b** (Scheme 2b).[10] Similarly, organocopper-mediated alkylation of N-activated β-aziridino-α,β-enoates **12a,b** afforded isosteres **13a,b** having N-alkyl- or N-arylsulfonyl groups (Scheme 2c).[11] In these synthetic schemes, chirality of the δ-amino group in EADIs **11a,b** and **13a,b** is derived from the amino acid used as starting material, while chirality of the alkyl group at respective α-positions depends on the chirality of γ-leaving group in δ-aminated γ-mesyloxy-α,β-enoate substrates **10a,b** and N-activated
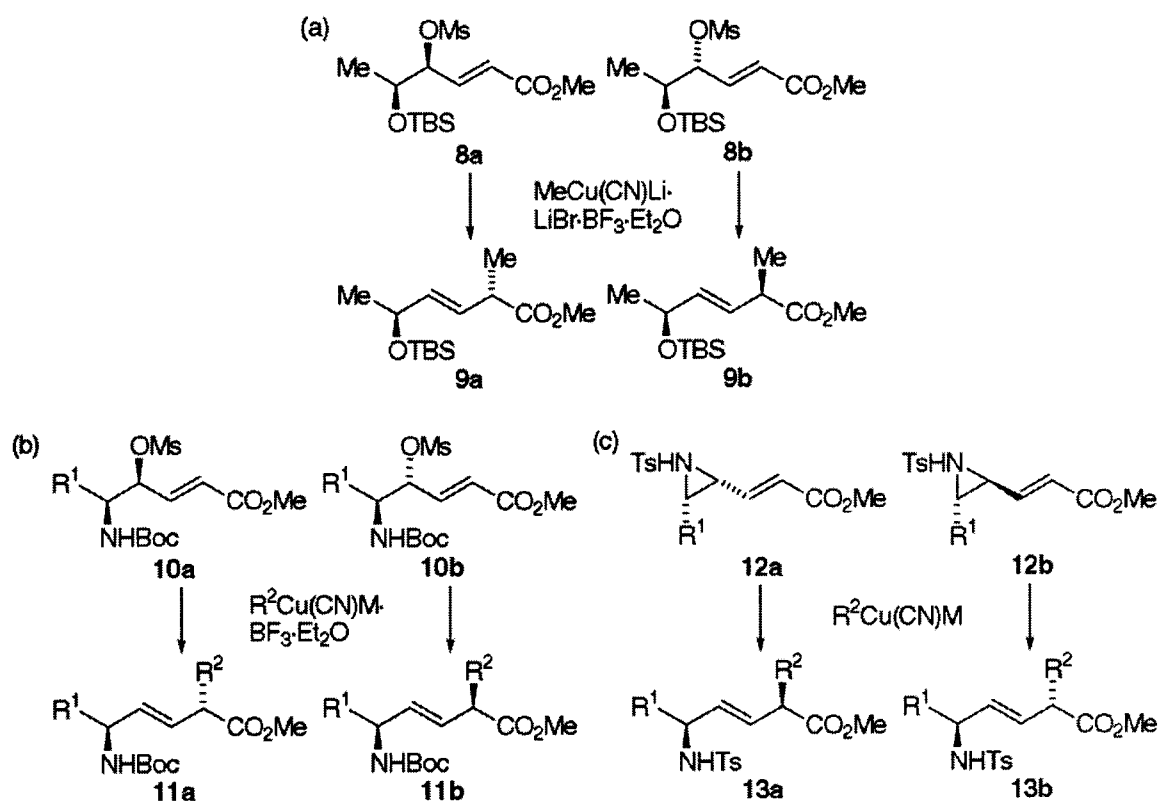
Scheme 2
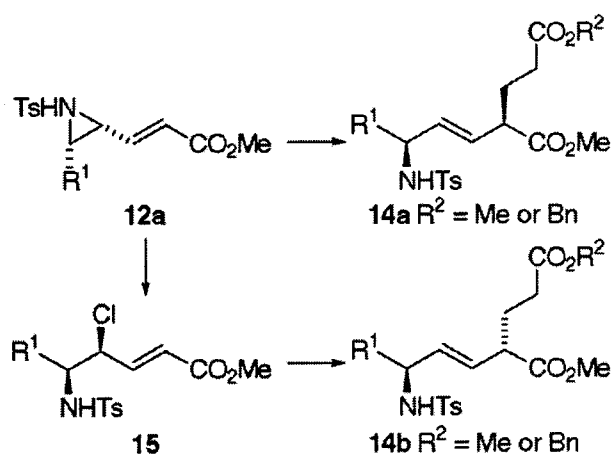
Scheme 3

aziridines **12a,b**, that can be readily prepared from chiral amino acids. On the other hand, side chain diversity at the δ- and α-positions in **11a,b** and **13a,b** is imparted from starting material and the organocopper reagents used for alkylation, respectively.

In chapter 1, section 1, the author presents stereoselective synthesis of L-Xaa-$\psi[(E)$-CH=CH]-L/D-Glu-type EADIs **14a,b** by extending these well-established procedures (Scheme 3). EADIs having side chain functional groups at the α-position have not been easily

Scheme 4

19	X = -CO-NH-
20	X = -CO-NMe-
21	X = -(E)-CH=CH-
22	X = -(E)-CH=CMe-
23	X = -(Z)-CH=CMe-
24	X = -(E)-CMe=CH-
25	X = -(E)-CMe=CMe-

Figure 3
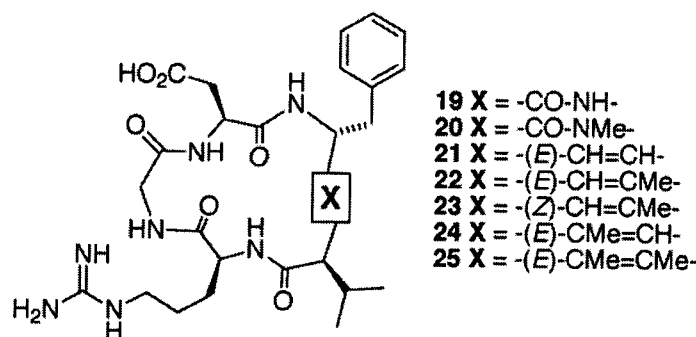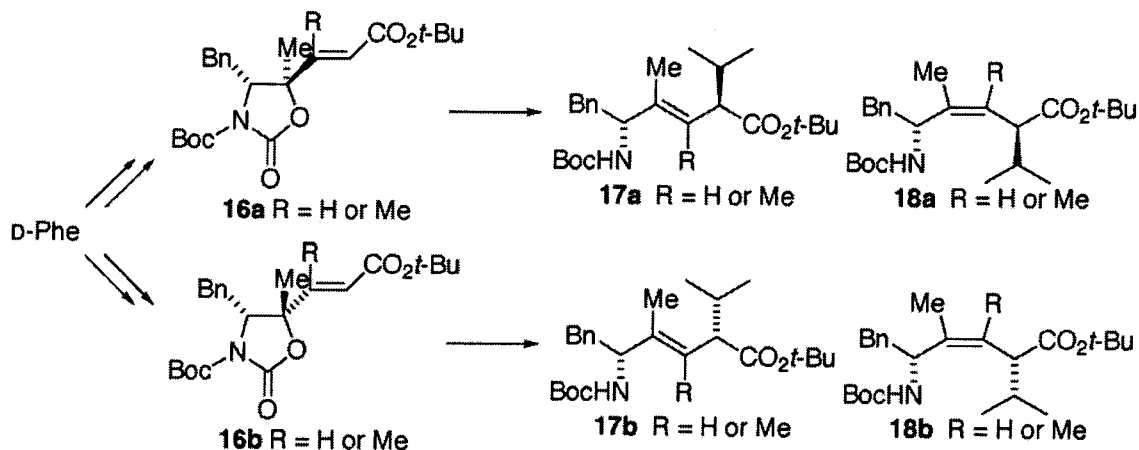
synthesized to date. This has limited the applicability of EADIs for chemical and biological research due to deficient diversity. The author demonstrates the usefulness of organozinc-copper complexes to introduce carboxylate-containing side chains at the α-position of aziridines 12a to afford EADIs 14a. Aziridine 12a can also be converted into diastereoisomeric EADIs 14b via HCl-mediated ring-opened products 15.

The author describes in chapter 1, section 2, synthesis of new $\psi[(E)$-CMe=CH]- and $\psi[(E)$-CMe=CMe]-type EADIs 17a,b having γ-methyl groups, that correspond to a peptide bond carbonyl oxygen.[12] It is supposed that the EADI γ-carbon contributes to β-turn promotion due to $A^{1,2}$- and $A^{1,3}$-strain. In a key reaction for construction of the α-alkyl group, organocopper-mediated alkylations of 1,3-oxazolidin-2-one derivatives 16a,b are utilized to afford the expected E-isomers of anti-S_N2' products 17a,b as well as the Z-congeners 18a,b in some cases (Scheme 4). As detailed in chapter 2, the resulting isosteres were utilized for conformational studies on cyclic RGD peptides 19[13] and 20[14] (Figure 3).

Recently, cyclic penta- and hexapeptides have attracted a great deal of attention as conformational templates.[15] Relative rigidity and accessibility of side chain diversity enable
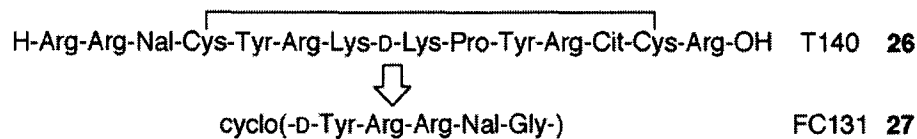
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H-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-D-Lys-Pro-Tyr-Arg-Cit-Cys-Arg-OH   T140   **26**

⇩

cyclo(-D-Tyr-Arg-Arg-Nal-Gly-)   FC131   **27**

Figure 4

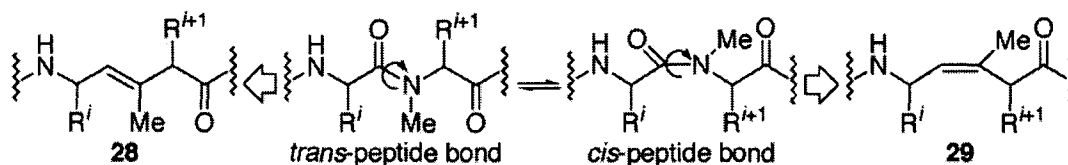28   *trans*-peptide bond   *cis*-peptide bond   29

Figure 5

their widespread utilization in drug discovery. For instance, the authors recently succeeded in molecular-size reduction of the 14-residue CXCR4 antagonist T140 (**26**) using a combination of two distinctive "conformation-based" and "sequence-based" cyclic pentapeptide libraries (Figure 4). A cyclic pentapeptide FC131 (**27**) possessing potency equal to T140 was discovered by combining the pharmacophore residues of T140 in a cyclic pentapeptide template.[16]

Meanwhile, Kessler et al. have reported cyclic pentapeptides **19** and **20** as $\alpha_v\beta_3$ integrin antagonists.[13,14] These possess a common RGD (Arg-Gly-Asp) sequence, which is a key recognition motif for interaction with integrins, as well as D-Phe and Val/MeVal residues that support the peptide backbone conformation appropriately for interaction with the receptors. They also demonstrated through structural studies that peptide **19** adopts a representative II'β/γ conformation,[13] while peptide **20** having an N-methylvaline, exhibits an alternate γ_i/γ/γ_i arrangement. N-methylation of the Val residue in **19** led to increased conformational flexibility and enhanced bioactivity in **20**.[14] As such, slight modifications of cyclic peptides often cause remarkable differences in conformation and bioactivity. However, it is extremely difficult to estimate the effects of modification on global and local peptide conformations. In practice, global conformations of the peptides **19** and **20** have been fully discussed by Kessler et al., whereas factors influenced by the MeVal N-methyl group in **20** that affect the global conformation as well as the proximal orientation of the D-Phe-Val/MeVal moieties have not been estimated. Chemical modifications of ligands through the application of peptide substructure mimetics offer powerful methodologies to understand structure-activity relationships such as these cases. The author presents conformational studies on Kessler's cyclic RGD peptides in chapter 2, wherein novel isosteres are utilized having several variations of methyl substituting groups in the D-Phe-Val/MeVal moieties found in peptides **19** and **20**.

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In chapter 2, section 1, the author investigates bioactive conformations using $\psi[(E)$-CH=CH]- **1**, $\psi[(E)$-CH=CMe]- **28** and $\psi[(Z)$-CH=CMe]-type isosteres **29**, with the intent of potentially mimicking accelerated peptide bond *cis-trans* isomerization induced by *N*-methylation (Figure 5).[17] Isostere-containing peptides **21-23** were synthesized via three separate procedures, and evaluated for their integrin antagonistic activities along with **19** and **20**.

Structure-activity relationship studies on cyclic RGD peptides are discussed in chapter 2, section 2. Additional peptides **24** and **25** containing γ-methylated EADIs were prepared and bioevaluated (Figure 3). The author also engaged in conformational studies of cyclic RGD peptides based on ^{1}H-NMR and simulated annealing/molecular dynamics calculations.

References

1   For recent reviews of drug discovery from natural/unnatural peptides, see: (a) Hruby, V. J.; Balse, P. M. *Curr. Med. Chem.* **2000**, *7*, 945. (b) Fujii, N.; Tamamura, H. *Curr. Opin. Investig. Drugs* **2001**, *2*, 1198.

2   For recent reviews of peptidomimetics, see: (a) Peptide Secondary Structure Mimetics, *Tetrahedron* (Symposia-in-Print; No. 50, ed. M. Kahn), **1993**, *49*, 3433; Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789. (b) *Peptidomimetics Protocols*, ed. Kazmiersk, W. M., Humana Press, Totowa, New Jersey, 1999 (c) Kim H.-O.; Kahn, M. *Comb. Chem. High Throughput Screen.* **2000**, *3*, 167. (d) Bursavich M. G.; Rich, D. H. *J. Med. Chem.* **2002**, *45*, 541.

3   (a) Polyak, F.; Lubell, W. D. *J. Org. Chem.*, **1998**, *63*, 5937. (b) Ogbu, C. O.; Qabar, M. N.; Boatman, P. D.; Urban, J.; Meara, J. P.; Ferguson, M. D.; Tulinsky, J.; Lum, C.; Babu, S.; Blaskovich, M. A.; Nakanishi, H.; Ruan, F.; Cao, B.; Minarik, R.; Little, T.; Nelson, S.; Nguyen, M.; Gall, A.; Kahn, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2321. (c) Kim, H.-O.; Nakanishi, H.; Lee, M. S.; Kahn, M. *Org. Lett.* **2000**, *2*, 301. (d) Hanessian, S.; Moitessier, N.; Wilmouth, S. *Tetrahedron* **2000**, *56*, 7643. (e) Smith, A. B., III; Wang, W.; Sprengeler, P. A.; Hirschmann, R. *J. Am. Chem. Soc.* **2000**, *122*, 11037. (f) Hillier, M. C.; Davidson J. P.; Martin, S. F. *J. Org. Chem.* **2001**, *66*, 1657. (g) Eguchi, M.; Lee, M. S.; Stasiak, M.; Kahn, M. *Tetrahedron Lett.* **2001**, *42*, 1237. (h) Fuchi, N.; Doi, T.; Harada, T.; Urban, J.; Cao, B.; Kahn, M.; Takahashi, T. *Tetrahedron Lett.* **2001**, *42*, 1305. (i) Belvisi, L.; Colombo, L.; Colombo, M.; Giacomo, M. D.; Manzoni, L.; Vodopivec, B.; Scolastico, C. *Tetrahedron* **2001**, *57*, 6463. (j) Alonso, E.; López-Ortiz, F.; del Pozo, C.; Peralta, E.; Macías, A.; González, J. *J. Org. Chem.*, **2001**, *66*, 6333.

4   (a) Shue, Y.-K.; Tufano, M. D.; Carrera, G. M., Jr.; Kopecka, H.; Kuyper, S. L.; Holladay, M. W.; Lin, C. W.; Witte, D. G.; Miller, T. R.; Stashko, M.; Nadzan, A. M. *Bioorg. Med. Chem.* **1993**, *1*, 161. (b) Christos, T. E.; Arvanitis, A.; Cain, G. A.; Johnson, A. L.; Pottorf, R. S.; Tam, S. W.; Schmidt, W. K. *Bioorg Med. Chem. Lett.* **1993**, *3*, 1035. (c) Bohnstedt, A. C.; Prasad, J. V. N. V.; Rich, D. H. *Tetrahedron Lett.* **1993**, *34*, 5217. (d) Wai, J. S.; Bamberger, D. L.; Fisher, T. E.; Graham, S. L.; Smith, R. L.; Gibbs, J. B.; Mosser, S. D.; Oliff, A. I.; Pompliano, D. L.; Rands, E.; Kohl, N. E. *Bioorg. Med. Chem.* **1994**, *2*, 939. (e) Wada, M.; Doi, R.; Hosotani, R.; Higashide, S.; Ibuka, T.; Habashita, H.; Nakai, K.; Fujii, N.; Imamura, M. *Pancreas* **1995**, *10*, 301. (f) Bartlett, P. A.; Otake, A. *J. Org. Chem.* **1995**, *60*, 3107. (g) Fujimoto, K.; Doi, R.; Hosotani, R.; Wada, M.; Lee, J.-U.; Koshiba, T.; Ibuka, T.; Habashita, H.; Nakai, K.; Fujii, N.; Imamura, M. *Life Sci.* **1997**, *60*, 29. (h) Miyasaka, K.; Kanai, S.; Masuda, M.; Ibuka, T.; Nakai, K.; Fujii, N.; Funakoshi, A. *J. Auton. Nerv. Syst.* **1997**, *63*, 179. (i) Kawaguchi, M.; Hosotani, R.; Ohishi, S.; Fujii, N.; Tulachan, S. S.; Koizumi, M.; Toyoda, E.; Masui, T.; Nakajima, S.; Tsuji, S.; Ida, J.; Fujimoto, K.; Wada, M.; Doi, R.; Imamura, M. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 711. (j) Tamamura, H.; Hiramatsu, K.; Miyamoto, K.; Omagari, A.; Oishi, S.; Nakashima, H.; Yamamoto, N.; Kuroda, Y.; Nakagawa, T.; Otaka A.; Fujii, N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 923.

5   Vasbinder, M. M.; Jarvo E. R.; Miller, S. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2824.

6   Copeland, G. T.; Jarvo, E. R.; Miller, S. J. *J. Org. Chem.* **1998**, *63*, 6784.

7   Miles, N. J.; Sammes, P. G.; Kennewell, P. D.; Westwood, R. *J. Chem. Soc. Perkin Trans. 1* **1985**, 2299.

8   Mitchell, H. J.; Nelson, A.; Warren, S. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1899.

9   (a) Ibuka, T.; Nakao, T.; Nishii, S.; Yamamoto, Y. *J. Am. Chem. Soc.* **1986**, *108*, 7420. (b) Ibuka, T.; Tanaka, M.; Nishii, S.; Yamamoto, Y. *J. Chem. Soc., Chem. Commun.*

1987, 1596, (c) Ibuka, T.; Akimoto, N.; Tanaka, M.; Nishii, S.; Yamamoto, Y. *J. Org. Chem.* **1989**, *54*, 4055. (d) Ibuka, T.; Tanaka, M.; Nishii, S.; Yamamoto, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4864. (e) Ibuka, T.; Tanaka, M.; Yamamoto, Y. *J. Chem. Soc., Chem. Commun.* **1989**, 967.

10 (a) Ibuka, T.; Habashita, H.; Funakoshi, S.; Fujii, N.; Oguchi, Y.; Uyehara, T.; Yamamoto, Y. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 801. (b) Ibuka, T.; Habashita, H.; Otaka, A.; Fujii, N.; Oguchi, Y.; Uyehara T.; Yamamoto, Y. *J. Org. Chem.* **1991**, *56*, 4370. (c) Ibuka, T.; Yoshizawa, H.; Habashita, H.; Fujii, N.; Chounan, Y.; Tanaka, M.; Yamamoto, Y. *Tetrahedron Lett.* **1992**, *33*, 3783. (d) Noda, M.; Ibuka, T.; Habashita, H.; Fujii, N. *Chem. Pharm. Bull.* **1997**, *45*, 1259.

11 (a) Ibuka, T.; Nakai, K.; Habashita, H.; Hotta, Y.; Fujii, N.; Mimura, N.; Miwa, Y.; Taga T.; Yamamoto, Y. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 652. (b) Wipf, P.; Fritch, P. C. *J. Org. Chem.* **1994**, *59*, 4875. (c) Fujii, N.; Nakai, K.; Tamamura, H.; Otaka, A.; Mimura, N.; Miwa, Y.; Taga, T.; Yamamoto Y.; Ibuka, T. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1359. (d) Wipf, P.; Henninger, T. C. *J. Org. Chem.* **1997**, *62*, 1586. (e) Ibuka, T.; Mimura, N.; Ohno, H.; Nakai, K.; Akaji, M.; Habashita, H.; Tamamura, H.; Miwa, Y.; Taga, T.; Fujii N.; Yamamoto, Y. *J. Org. Chem.* **1997**, *62*, 2982. (f) Tamamura, H.; Yamashita, M.; Muramatsu, H.; Ohno, H.; Ibuka, T.; Otaka, A.; Fujii, N. *Chem. Commun.* **1997**, 2327. (g) Tamamura, H.; Yamashita, M.; Nakajima, Y.; Sakano, K.; Otaka, A.; Ohno, H.; Ibuka T.; Fujii, N. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2983.

12 (a) Gardner, R. R.; Liang G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280. (b) Wipf, P.; Henninger, T. C.; Geib, S. J. *J. Org. Chem.* **1998**, *63*, 6088. (c) Gardner, R. R.; Liang G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 1806.

13 (a) Aumailley, M.; Gurrath, M.; Muller, G.; Calvete, J.; Timpl, R.; Kessler, H. *FEBS Lett.* **1991**, *291*, 50. (b) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461. (c) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374.

14 Dechantsreiter, M. A.; Planker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033.

15 (a) Nikiforovich, G. V.; Kövér, K. E.; Zhang, W.-J.; Marchall, G. R. *J. Am. Chem. Soc.* **2000**, *122*, 3262. (b) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc. Perkin Trans. 1* **2001**, 471.

16 Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z.; Peiper, S. C. *Angew. Chem. Int. Ed. submitted.*
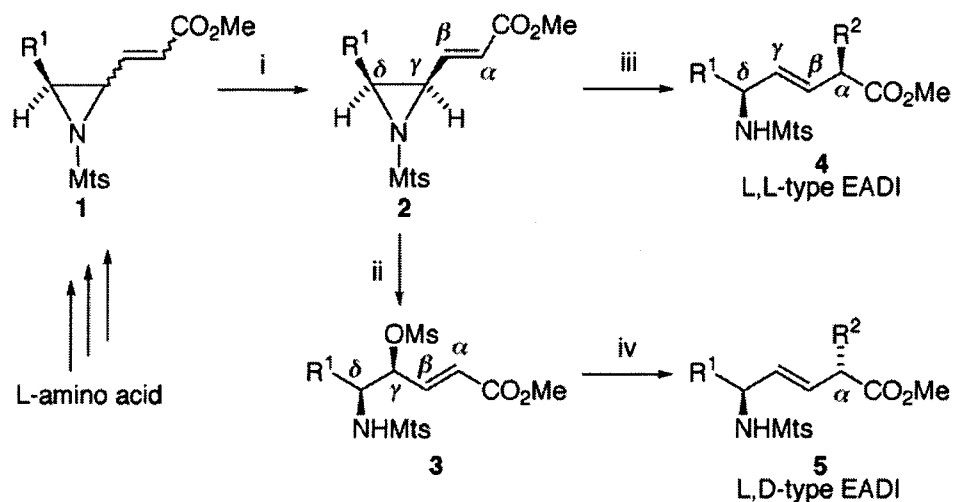
17 Fischer, G. *Chem. Soc. Rev.* **2000**, *29*, 119.

Chapter 1. Synthesis of Highly Functionalized (*E*)-Alkene Dipeptide Isosteres from Chiral Amino Acids

Section 1. Stereoselective Synthesis of a Set of Two Functionalized (*E*)-Alkene Dipeptide Isosteres of L-Amino Acid-L-Glu and L-Amino Acid-D-Glu

Summary

Treatment of *N*-arylsulfonyl-γ,δ-*cis*- or -*trans*-γ,δ-epimino (*E*)-α,β-enoates with HCl/1,4-dioxane affords regio- and stereo-selective ring-opened products as δ-aminated γ-chloro-α,β-enoates. This ring-opening reaction provides a useful method for the stereoselective synthesis of a set of diastereomeric (L-Xaa, L-Glu)-type and (L-Xaa, D-Glu)-type (*E*)-alkene dipeptide isosteres (EADIs) from a single γ,δ-epimino (*E*)-α,β-enoate substrate using organozinc-copper reagents.

A number of synthetic methodologies for the preparation of EADIs[1] have been developed by many researchers.[2-4] In each process, construction of a chiral side chain at the α-position is the most critical key reaction. For such purpose, Ibuka et al. utilized a 1,3-chirality transfer reaction that employed organocopper reagents of α,β-enoates having leaving groups at the γ-position. For example, γ-mesyloxy-α,β-enoates[3] and *N*-activated β-aziridino-α,β-enoates,[4] which can be derived from chiral amino acids, were shown to be feasible for the synthesis of diverse EADIs. However, the use of this reaction is potentially limited to the introduction of α-alkyl groups lacking side chain functional groups. To overcome this limitation, the author realized that utilization of suitably functionalized organocopper reagents for the alkylation could provide highly functionalized EADIs corresponding to natural dipeptide motifs. As a model study, the author investigated the synthesis of L-Xaa-ψ[(*E*)-CH=CH]-L/D-Glu-type EADIs, which possess a carboxylate functional group corresponding to a glutamic acid residue at the α-position. Use of organozinc-copper reagents that can be readily prepared from a soluble copper salt CuCN·2LiCl and an organozinc reagent IZnCH$_2$CH$_2$CO$_2$R, could provide entry to these EADIs (R = Me or Bn).[5]

R¹ ... CO₂Me

i

R¹ β CO₂Me
δ γ α

iii

R¹ δ γ β R² α CO₂Me
NHMts
4
L,L-type EADI

H N Mts
1

H N H Mts
2

L-amino acid

ii

OMs α
R¹ δ β
γ CO₂Me
NHMts
3

iv

R² R¹ α CO₂Me
NHMts
5
L,D-type EADI

Scheme 1. R^1, R^2 = alkyl; Ms = methanesulfonyl; Mts = 2,4,6-trimethylphenylsulfonyl. *Reagents*: i, Pd(PPh₃)₄; ii, MsOH in CHCl₃; iii, R²Cu(CN)MgCl·2LiCl; iv, R²Cu(CN)MgCl·BF₃. D-Amino acids lead to D,D-type and D,L-type EADIs in the same way.

In this section, the author describes regio- and stereo-selective ring-opening reactions of N-arylsulfonyl-γ,δ-*cis*- and -*trans*-γ,δ-epimino (E)-α,β-enoates (enamides) with HCl/1,4-dioxane to yield δ-aminated γ-chloro-α,β-enoates (enamides). Furthermore, the author also reports the stereoselective synthesis of a set of two functionalized L-Xaa-ψ[(E)-CH=CH]-L-Glu- and L-Xaa-ψ[(E)-CH=CH]-D-Glu-type diastereomeric EADIs from a single substrate of γ,δ-epimino (E)-α,β-enoate (enamide) using an organozinc-copper reagent IZn(CN)CuCH₂CH₂CO₂R (R = Me or Bn).

Initial Studies on Synthesis of Ser(O-Bn)-ψ[(E)-CH=CH]-Glu(OMe)-type EADIs in Accordance with the Established Synthetic Procedure. Recently, Tamamura et al. established a completely stereocontrolled synthetic process for L,L-type, L,D-type, D,D-type and D,L-type EADIs starting from L-amino acids or D-amino acids as chiral pools (Scheme 1).[6] In this process, regio- and stereo-selective ring-opening reactions of N-(2,4,6-trimethylphenylsulfonyl)-γ,δ-*cis*-γ,δ-epimino (E)-α,β-enoates with methanesulfonic acid (MSA) were utilized for the stereoselective synthesis of a set of two diastereomeric EADIs from a single substrate of γ,δ-epimino (E)-α,β-enoate. As shown in Scheme 1, four stereoisomeric γ,δ-epimino-α,β-enoates **1**, which are obtained from an L-amino acid, can be convergently transformed into the single *cis*-(E)-isomer **2** by exposure to a Pd(0)-catalyst.[7] The aziridine **2** provides an L,L-type EADI **4** by treatment with organocopper reagents. On the other hand, MSA treatment of **2** gives the γ-mesylate **3**, which can be converted into an L,D-
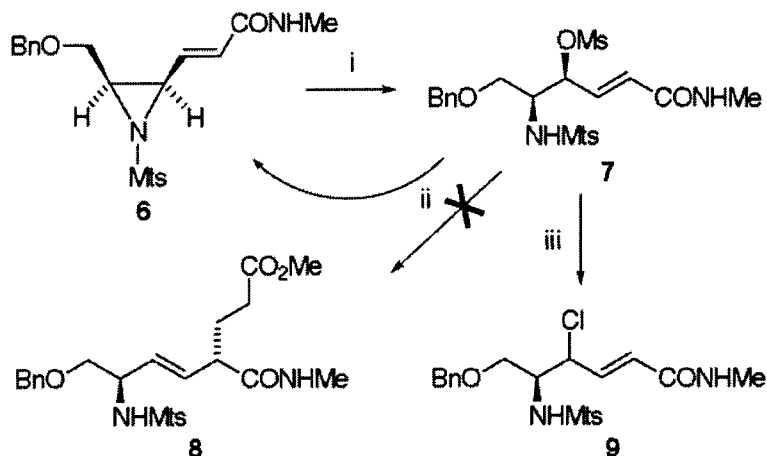
Scheme 2. *Reagents*: i, MsOH in $CHCl_3$; ii, $IZn(CN)CuCH_2CH_2CO_2Me \cdot 2LiCl$; iii, $IZn(CN)CuCH_2CH_2CO_2Me \cdot BF_3 \cdot 2LiCl$.

type EADI **5** by treatment with organocopper reagents. In the initial effort to prepare an L-Ser-$\psi[(E)$-CH=CH]-D-Glu-type EADI, the author attempted S_N2'-substitution of a $CH_2CH_2CO_2Me$ group onto δ-aminated γ-mesyloxy-α,β-enamide **7**, which was previously synthesized by the ring-opening reaction of N-Mts-γ,δ-*cis*-γ,δ-epimino (E)-α,β-enamide **6** with MSA, using organozinc-copper reagent $IZn(CN)CuCH_2CH_2CO_2Me \cdot 2LiCl$ (Scheme 2).[5] However, the reaction quantitatively regenerated aziridine **6** without forming the desired compound **8**. Since the aziridinyl ring was considered to be formed due to the basicity of the organocopper reagent, $BF_3 \cdot Et_2O$ was added to the above organocopper reagent. Unexpectedly, this reaction afforded δ-aminated γ-chloro-α,β-enamide **9** (the absolute configuration of the γ-carbon center of **9** was not identified). Nakamura et al. reported a stereoselective S_N2' reaction mediated by organometallics of zinc and copper when using allylic chlorides as substrates.[5] Thus, the author attempted to utilize γ-chloro-α,β-enamide **9** as a substrate of the S_N2'-type organometallic reaction to yield an L-Ser-$\psi[(E)$-CH=CH]-D-Glu-type EADI, and initially to find effective methods for affording diastereomerically pure δ-aminated γ-chloro-α,β-enoates (enamides).

Ring-opening Reactions of N-(4-Methylphenylsulfonyl) (Ts)- or N-(2,4,6-Trimethylphenylsulfonyl) (Mts)-γ,δ-epimino (E)-α,β-Enoates with HCl/1,4-dioxane. To date, many precedents for aziridine ring-opening, namely, that several nucleophilic reagents,[8] including acids such as AcOH,[9] TFA[10] and toluene-p-sulfonic acid in aqueous acetone,[11] attack simple aziridines[12] at either of the two carbon atoms, to yield the corresponding ring-opened products, have been described. Tamamura et al. have also reported TFA- or MSA-mediated ring-opening reactions of N-Mts-protected (and activated) aziridines bearing α,β-

CO_2Me

R^2

H   H

N

R^1

i → R^2 ⎯ Cl ⎯ CO_2Me

NHR[1]

10 R^1 = Ts, R^2 = Me
11 R^1 = Mts, R^2 = Pr^i
12 R^1 = Mts, R^2 = Bn

14 R^1 = Ts, R^2 = Me
15 R^1 = Mts, R^2 = Pr^i
16 R^1 = Mts, R^2 = Bn

ii or iii

ii or iii

CO_2R^3

R^2 ⎯ CO_2Me

NHR[1]

24 R^1 = Ts, R^2 = R^3 = Me
25 R^1 = Ts, R^2 = Me, R^3 = Bn
26 R^1 = Mts, R^2 = Pr^i, R^3 = Me
27 R^1 = Mts, R^2 = Pr^i, R^3 = Bn
28 R^1 = Mts, R^2 = Bn, R^3 = Me
29 R^1 = Mts, R^2 = R^3 = Bn

CO_2R^3

R^2 ⎯ CO_2Me

NHR[1]

18 R^1 = Ts, R^2 = R^3 = Me
19 R^1 = Ts, R^2 = Me, R^3 = Bn
20 R^1 = Mts, R^2 = Pr^i, R^3 = Me
21 R^1 = Mts, R^2 = Pr^i, R^3 = Bn
22 R^1 = Mts, R^2 = Bn, R^3 = Me
23 R^1 = Mts, R^2 = R^3 = Bn

ii or iii

ii or iii

Cl

R^2 ⎯ CO_2Me

NHR[1]

17 R^1 = Ts, R^2 = Me

CO_2Me

R^2

H   H

N

R^1

13 R^1 = Ts, R^2 = Me

i ←

Scheme 3. *Reagents*: i, 4 M HCl/1,4-dioxane; ii, IZn(CN)CuCH$_2$CH$_2$CO$_2$Me·2LiCl; iii, IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl.

unsaturated esters.[6] Thus, the author initially examined ring-opening reactions of *N*-Ts- or *N*-Mts-γ,δ-epimino (*E*)-α,β-enoates with HCl/1,4-dioxane to afford δ-aminated γ-chloro-α,β-enoates. Exposure of *N*-Ts-γ,δ-*cis*-γ,δ-epimino (*E*)-α,β-enoate **10**, derived from L-threonine,[7] to 4 M HCl/1,4-dioxane (10 equiv.) at rt for 30 min afforded exclusively *syn*-δ-aminated γ-chloro-α,β-enoate **14** in an essentially quantitative yield (Scheme 3 and Table 1). Since X-ray analysis of **14** showed that it has (4*S*)-configuration, this ring-opening reaction was confirmed to operate via the regio- and stereo-selective S$_N$2 reaction at the γ-carbon position. The author investigated ring-opening of other aziridines, *N*-Mts-γ,δ-*cis*-γ,δ-epimino (*E*)-α,β-enoates, **11** and **12**, derived from L-valine and L-phenylalanine, respectively, according to the reported methods.[7] The regiospecific ring-opening reactions were successfully carried out to yield γ-chloro-α,β-enoates **15** and **16**. The *trans*-(*E*)-isomer **13** afforded the γ,δ-*anti*-isomer **17** by a similar S$_N$2 ring-opening reaction. In all cases, ring-opened products generated by nucleophilic attack at the α-, β- or δ-carbon position could not be detected. Regiochemical assignments for the γ-chloro-α,β-enoates **14-17** were readily made by ^{1}H-NMR spectroscopy

Table 1. Ring-opening of N-Ts- or N-Mts-γ,δ-epimino (E)-α,β-enoates with HCl/1,4-dioxane and the following addition of $CH_2CH_2CO_2R$ groups using organozinc-copper reagents.

Entry	Substrate	Ring-opened product (yield %)	Organozinc-copper reagent	Addition product (yield %)
1	**10**	**14** (92)	IZnCu(CN)CH$_2$CH$_2$CO$_2$Me·2LiCl	**18** (93)
2	**10**		IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl	**19** (91)
3	**11**	**15** (92)	IZnCu(CN)CH$_2$CH$_2$CO$_2$Me·2LiCl	**20** (99)
4	**11**		IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl	**21** (99)
5	**12**	**16** (96)	IZnCu(CN)CH$_2$CH$_2$CO$_2$Me·2LiCl	**22** (81)
6	**12**		IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl	**23** (89)
7	**13**	**17** (91)	IZnCu(CN)CH$_2$CH$_2$CO$_2$Me·2LiCl	**24** (91)
8	**13**		IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl	**25** (92)

(^{1}H-^{1}H COSY). The γ,δ-*syn* stereochemistry of **14-16** and the γ,δ-*anti* stereochemistry of **17** are based on X-ray analysis of **14**.

Synthesis of (L-Xaa, D-Glu)-type and (L-Xaa, L-Glu)-type EADIs from δ-Aminated γ-Chloro-α,β-enoates. The author examined the feasibility of the stereoselective synthesis of L-Xaa-$\psi[(E)$-CH=CH]-D-Glu- and L-Xaa-$\psi[(E)$-CH=CH]-L-Glu-type EADIs by treatment of the ring-opened products with organozinc-copper reagents. Treatment of the above γ-chloro-α,β-enoate **14** with IZnCu(CN)CH$_2$CH$_2$CO$_2$Me·2LiCl (4 equiv.) in THF at 0 °C for 30 min yielded the protected (L-Ala, D-Glu)-type (4S,7S)-EADI, Ts-L-Ala-$\psi[(E)$-CH=CH]-D-Glu(OMe)-OMe, **18** in 93% yield (diastereoselection > 99 : 1 from NMR analysis) as shown in Scheme 3 and Table 1. This reaction occurred by a sole *anti*-S$_N$2' mechanism. In contrast, an *anti*-S$_N$2' reaction of the *cis*-(E)-enoate **10** under the same reaction conditions afforded the protected (L-Ala, L-Glu)-type (4R,7S)-EADI, Ts-L-Ala-$\psi[(E)$-CH=CH]-L-Glu(OMe)-OMe, **24** in 99% yield as shown in Scheme 3 and Table 2. In a similar way, treatment of **14** with IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl yielded the (4S,7S)-EADI, Ts-L-Ala-$\psi[(E)$-CH=CH]-D-Glu(OBn)-OMe, **19**, whereas treatment of **10** with IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl afforded the (4R,7S)-EADI, Ts-L-Ala-$\psi[(E)$-CH=CH]-L-Glu(OBn)-OMe, **25**. The most important point of the HCl-mediated ring-opening reactions is the inversion of configuration at the C-γ carbon via an S$_N$2 mechanism. Thus, *cis*-(E)-enoates lead to *syn*-(E)-chlorides, which are converted into (L-Xaa, D-Glu)-type EADIs upon treatment of organozinc-copper reagents. On the other hand, *cis*-(E)-enoates themselves provide (L-Xaa, L-Glu)-type EADIs with organozinc-copper reagents.

Table 2. Direct addition of $CH_2CH_2CO_2R$ groups into N-Ts- or N-Mts-γ,δ-epimino (E)-α,β-enoates using organozinc-copper reagents.

Entry	Substrate	Organozinc-copper reagent	Product (yield %)
1	**10**	$IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$	**24** (99)
2	**10**	$IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$	**25** (95)
3	**11**	$IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$	**26** (52)
4	**11**	$IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$	**27** (45)
5	**12**	$IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$	**28** (97)
6	**12**	$IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$	**29** (99)
7	**13**	$IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$	**18** (99)
8	**13**	$IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$	**19** (92)

The author investigated the applicability of these synthetic procedures to other aziridine *cis*-(E)-enoates, **11** and **12**. Treatment of the γ-chloride **15** with $IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$ and $IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$ yielded the (4*S*,7*S*)-EADIs, Mts-L-Val-$\psi[(E)$-CH=CH]-D-Glu(OMe)-OMe, **20** and Mts-L-Val-$\psi[(E)$-CH=CH]-D-Glu(OBn)-OMe, **21**, respectively, whereas treatment of the *cis*-(E)-enoate **11** with $IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$ and $IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$ yielded the (4*R*,7*S*)-EADIs, Mts-L-Val-$\psi[(E)$-CH=CH]-L-Glu(OMe)-OMe, **26** and Mts-L-Val-$\psi[(E)$-CH=CH]-L-Glu(OBn)-OMe, **27**, respectively. In the same manner, the γ-chloride **16** provided the corresponding (4*S*,7*S*)-EADIs, **22** and **23**, whereas the *cis*-(E)-enoate **12** afforded the corresponding (4*R*,7*S*)-EADIs, **28** and **29**, respectively. In a comparative study, the *anti*-(E)-chloride **17**, derived from the *trans*-(E)-enoate **13**, was converted into the protected (L-Ala, L-Glu)-type (4*R*,7*S*)-EADIs, Ts-L-Ala-$\psi[(E)$-CH=CH]-L-Glu(OMe)-OMe, **24** and Ts-L-Ala-$\psi[(E)$-CH=CH]-L-Glu(OBn)-OMe, **25** with $IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$ and $IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$, respectively. On the other hand, treatment of the *trans*-(E)-enoate **13** with $IZnCu(CN)CH_2CH_2CO_2Me\cdot2LiCl$ and $IZnCu(CN)CH_2CH_2CO_2Bn\cdot2LiCl$ afforded the protected (L-Ala, D-Glu)-type (4*S*,7*S*)-EADIs, Ts-L-Ala-$\psi[(E)$-CH=CH]-D-Glu(OMe)-OMe, **18** and Ts-L-Ala-$\psi[(E)$-CH=CH]-D-Glu(OBn)-OMe, **19**, respectively. Thus, two types of EADIs were stereoselectively synthesized from either the *cis*- or *trans*-(E)-enoate. These synthetic procedures are applicable to aziridinyl *cis*- or *trans*-(E)-enoates. The (E)-geometry of the double bond in the synthesized EADIs was assigned based on the coupling constants of the two olefinic protons on ^{1}H-NMR analysis. The absolute
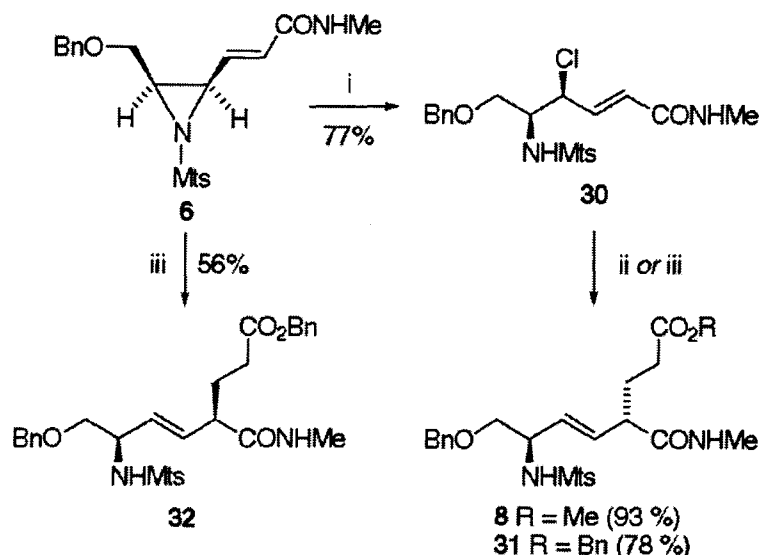
Scheme 4. *Reagents*: i, 4 M HCl/1,4-dioxane; ii, IZn(CN)CuCH$_2$CH$_2$CO$_2$Me·2LiCl; iii, IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl.

configuration of the α-alkylated carbon center in the EADI **22** was confirmed by X-ray analysis as 4*S*.

Next, the author attempted to synthesize highly functionalized (L-Ser, D-Glu)-type and (L-Ser, L-Glu)-type EADIs, as the initial synthetic targets. The ring-opening reaction of the *cis*-(*E*)-enamide **6** with HCl/1,4-dioxane yielded the corresponding γ-chloro-α,β-enamide **30**, which was converted into the protected (L-Ser, D-Glu)-type (4*S*,7*S*)-EADIs, Mts-L-Ser(*O*-Bn)-ψ[(*E*)-CH=CH]-D-Glu(OMe)-NHMe, **8** and Mts-L-Ser(*O*-Bn)-ψ[(*E*)-CH=CH]-D-Glu(OBn)-NHMe, **31** with IZnCu(CN)CH$_2$CH$_2$CO$_2$Me·2LiCl and IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl, respectively, as shown in Scheme 4. In contrast, treatment of the *cis*-(*E*)-enamide **6** with IZnCu(CN)CH$_2$CH$_2$CO$_2$Bn·2LiCl afforded the protected (L-Ser, L-Glu)-type (4*R*,7*S*)-EADI, Mts-L-Ser(*O*-Bn)-ψ[(*E*)-CH=CH]-L-Glu(OBn)-NHMe, **32**.

In conclusion, the author has found regio- and stereo-specific ring-opening reactions of *N*-Ts- and *N*-Mts-protected aziridines bearing α,β-unsaturated esters (amides) [*cis*-(*E*)- and *trans*-(*E*)-α,β-enoates (enamides)] using HCl/1,4-dioxane. The resulting HCl-mediated ring-opened products (γ,δ-*syn*-δ-aminated γ-chloro-α,β-enoates or enamides) yield L-Xaa-ψ[(*E*)-CH=CH]-D-Glu-type EADIs by reaction with organozinc-copper reagents. On the other hand, aziridines [*cis*-(*E*)-α,β-enoates (enamides)] afford L-Xaa-ψ[(*E*)-CH=CH]-L-Glu-type EADIs upon reaction with organozinc-copper reagents. The ring-opening reactions presented herein provide useful methodologies for stereoselective synthesis of both L-Xaa-ψ[(*E*)-CH=CH]-

L/D-Glu-type EADIs from a single substrate of either a γ,δ-cis- or -trans-γ,δ-epimino (E)-α,β-unsaturated ester (amide). Four stereoisomers of γ,δ-epimino-α,β-unsaturated esters (amides), that can be prepared from the corresponding chiral amino aldehydes, are converted into cis-(E)-isomers by Pd(0)-catalyzed equilibration.[7] Taken together, a completely stereocontrolled synthetic process for preparation of a set of L-Xaa-ψ[(E)-CH=CH]-L/D-Glu-type EADIs starting from L-amino acids has been established.

Experimental Section

General. ^{1}H-NMR spectra were recorded using a JEOL EX-270 or a Bruker AC 300 spectrometer at 270 or 300 MHz ^{1}H frequency for samples in CDCl$_3$. Chemical shifts (δ) are reported in parts per million downfield from internal tetramethylsilane. J-Values are in Hz. Nominal (LRMS) and exact mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 or JMS-HX/HX 110A mass spectrometer. Optical rotations were measured in CHCl$_3$ with a JASCO DIP-360 digital polarimeter (Tokyo, Japan) or a Horiba high-sensitive polarimeter SEPA-200 (Kyoto, Japan). [α]$_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. X-Ray analyses were made on a Rigaku AFC5R diffractometer with graphite-monochromated Cu-Kα radiation and a rotating anode generator. Mps were measured by a hot-stage melting-point apparatus and are uncorrected. For flash chromatography, silica gel 60 H (silica gel for TLC, Merck) and Wakogel C-200 (silica gel for column chromatography) were employed.

Methyl (2E,4R,5S)-6-phenyl-4,5-[N-(2,4,6-trimethylphenylsulfonyl)epimino]hex-2-enoate 12. According to our previous procedure,[7] the cis-(E)-enoate **12** (2.23 g, 5.58 mmol, 93%) was prepared from the corresponding vinylaziridine[7] (2.04 g, 6.00 mmol) as colorless crystals, mp 51-53 °C [from n-hexane-Et$_2$O (3:1)] (Found: C, 66.26; H, 6.46; N, 3.51. Calc. for C$_{22}$H$_{25}$NO$_4$S: C, 66.14; H, 6.31; N, 3.51%); [α]$_D$^{26} - 63.75 (c 1.19); δ_H (300 MHz) 2.30 (3 H, s, CMe), 2.56 (6 H, s, 2 × CMe), 2.64 (1 H, dd, J 14.5 and 8.0, CHH), 2.76 (1 H, dd, J 14.5 and 5.3, CHH), 3.18 (1 H, ddd, J 7.9, 7.2 and 5.3, 5-H), 3.55 (1 H, m, 4-H), 3.76 (3 H, s, OMe), 6.19 (1 H, dd, J 15.5 and 1.0, CH=), 6.84 (1 H, dd, J 15.5 and 6.6, CH=), 6.85 (2 H, s, ArH), 6.94 (2 H, m, ArH), 7.04-7.15 (3 H, m, ArH).

General procedure for the ring-opening reaction of N-Ts- or N-Mts-γ,δ-epimino (E)-α,β-enoates (enamides) by treatment with HCl/1,4-dioxane. Representative: methyl (2E,4S,5S)-4-chloro-5-(4-methylphenylsulfonylamino)hex-2-enoate 14. The cis-(E)-enoate **10**[7] (295 mg, 1.00 mmol) was dissolved in 4 M HCl solution in 1,4-dioxane (10.0 mmol, 2.5 cm^3) at rt and the mixture was stirred for 30 min at this temperature. Concentration under reduced pressure gave an oily residue, which was purified by flash chromatography over silica gel with n-hexane-EtOAc (4:1) to yield 306 mg (0.922 mmol, 92%) of the title compound **14** as colorless crystals, mp 168-169 °C [from n-hexane-EtOAc (1:1)] (Found: C, 50.74; H, 5.54; N, 4.17. C$_{14}$H$_{18}$ClNO$_4$S requires C, 50.68; H, 5.47; N, 4.22%); [α]$_D$^{21} - 78.75 (c 0.965); δ_H (300 MHz) 1.16 (3 H, d, J 6.7, CMe), 2.43 (3 H, s, CMe), 3.69 (1 H, m, 5-H), 3.74 (3 H, s OMe), 4.51 (1 H, ddd, J 7.4, 3.5 and 1.2, 4-H), 4.85 (1 H, d, J 8.5, NH), 6.02 (1 H, dd, J 15.4 and 1.2, CH=), 6.80 (1 H, dd, J 15.4 and 7.4, CH=), 7.30 (2 H, m, ArH), 7.74 (2 H, m, ArH).

Crystal structure determination of compound 14. Crystal data. C$_{14}$H$_{18}$ClNO$_4$S, M = 331.81, orthorhombic, a = 9.685(4), b = 21.585(5), c = 7.745(4) Å, V = 1619(1) Å^3, T = 296 K, space group $P2_12_12_1$ (no. 19), Z = 4, μ(Cu-Kα) = 34.27 mm^{-1}, 1580 reflections measured, 1484 [I > 3 σ(I)] were used in all calculations. The final wR was 0.066.

Methyl (2E,4S,5S)-4-chloro-6-methyl-5-(2,4,6-trimethylphenylsulfonylamino)-hept-2-enoate 15. By use of a procedure identical with that described for the preparation of **14** from **10**, the cis-(E)-enoate **11**[7] (351 mg, 1.00 mmol) was converted into the title compound **15** (360 mg, 0.928 mmol, 92%) as colorless crystals, mp 111-113 °C [from n-hexane-EtOAc (2:1)] (Found: C, 55.71; H, 6.95; N, 3.56. C$_{18}$H$_{26}$ClNO$_4$S requires C, 55.73; H, 6.76; N, 3.61%); [α]$_D$^{27} - 13.3 (c 0.822); δ_H (300 MHz) 0.97 (6 H, m, 2 × CMe), 1.88-2.05 (1

H, m, 6-H), 2.28 (3 H, s, CMe), 2.61 (6 H, s, 2 × CMe), 3.45 (1 H, ddd, J 9.5, 7.8 and 2.4, 5-H), 3.64 (3 H, s, OMe), 4.66 (1 H, ddd, J 6.4, 2.4 and 1.5, 4-H), 4.73 (1 H, d, J 9.5, NH), 5.87 (1 H, dd, J 15.2 and 1.5, CH=), 6.56 (1 H, dd, J 15.2 and 6.4, CH=), 6.90 (2 H, s, ArH).

Methyl (2E,4S,5S)-4-chloro-6-phenyl-5-(2,4,6-trimethylphenylsulfonylamino)hex-2-enoate 16. By use of a procedure identical with that described for the preparation of **14** from **10**, the *cis*-(E)-enoate **12** (399 mg, 1.00 mmol) was converted into the title compound **16** (422 mg, 0.967 mmol, 96%) as colorless crystals, mp 108-109 °C [from *n*-hexane-EtOAc (2:1)] (Found: C, 60.59; H, 6.07; N, 3.20. $C_{22}H_{26}ClNO_4S$ requires C, 60.61; H, 6.01; N, 3.21%); $[\alpha]_D^{28}$ - 57.8 (c 0.570); δ_H (300 MHz) 2.27 (3 H, s, CMe), 2.53 (6 H, s, 2 × CMe), 2.79 (1 H, dd, J 13.7 and 6.8, CHH), 3.04 (1 H, dd, J 13.7 and 8.2, CHH), 3.65 (1 H, m, 5-H), 3.70 (3 H, s, OMe), 4.59 (1 H, J 6.4, 2.5 and 1.5, 4-H), 4.86 (1 H, d, J 8.6, NH), 5.95 (1 H, dd, J 15.3 and 1.5, CH=), 6.67 (1 H, dd, J 15.3 and 6.4, CH=), 6.85 (2 H, m, ArH), 7.01-7.07 (2 H, m, ArH), 7.17-7.23 (3 H, m, ArH).

Methyl (2E,4R,5S)-4-chloro-5-(4-methylphenylsulfonylamino)hex-2-enoate 17. By use of a procedure identical with that described for the preparation of **14** from **10**, the *cis*-(E)-enoate **13** (295 mg, 1.00 mmol) was converted into the title compound **17** (303 mg, 0.913 mmol, 91%) as a colorless crystalline mass, mp 69-71 °C (from *n*-hexane) (Found: C, 50.63; H, 5.60; N, 3.95. $C_{14}H_{18}ClNO_4S$ requires C, 50.68; H, 5.47; N, 4.22%); $[\alpha]_D^{25}$ - 7.92 (c 1.00); δ_H (300 MHz) 1.07 (3 H, d, J 6.6, CMe), 2.43 (3 H, s, CMe), 3.69 (1 H, m, 5-H), 3.74 (3 H, s, OMe), 4.59 (1 H, ddd, J 6.6, 3.3 and 1.4, 4-H), 4.94 (1 H, d, J 9.1, NH), 6.06 (1 H, dd, J 15.3 and 1.4, CH=), 6.77 (1 H, dd, J 15.3 and 6.7, CH=), 7.32 (2 H, m, ArH), 7.76 (2 H, m, ArH).

General procedure for the preparation of (L-Xaa, D-Glu)-type EADIs from γ-chloro-α,β-enoates. Representative: Ts-L-Ala-ψ[(E)-CH=CH]-D-Glu(OMe)-OMe 18. To a supension of zinc dust (157 mg, 2.41 mmol) in dry THF (0.20 cm^3), which was subjected to treatment for activation, was added methyl 3-iodopropionate (257 mg, 1.20 mmol) [obtained by treatment of methyl 3-bromopropionate in acetone with sodium iodide followed by distillation] in dry THF (1.00 cm^3) at rt, and the mixture was stirred for 1 h at this temperature. The organozinc reagent was added to a stirred suspension of CuCN (107 mg, 1.20 mmol) and LiCl (102 mg, 2.41 mmol) in dry THF (1.20 cm^3) under argon at - 78 °C, and the mixture was allowed to warm to 0 °C and was stirred at this temperature for 10 min. To the solution of organozinc-copper reagent was added dropwise a solution of the ester **14** (100 mg, 0.301 mmol) in dry THF (1.00 cm^3) at - 78 °C with stirring, and the mixture was stirred at 0 °C for 30 min followed by quenching with 1:1 saturated aq. NH_4Cl-28% NH_4OH (2 cm^3). The mixture was extracted with Et_2O, and the extract was washed with water, and dried over $MgSO_4$. Concentration under reduced pressure gave a colorless oil, which was purified by flash chromatography over silica gel with *n*-hexane-EtOAc (3:1) to yield the title compound **18** (108 mg, 0.282 mmol, 93%) as a colorless oil [Found (FAB): (M + H)$^+$, 384.1470. $C_{18}H_{26}NO_6S$ requires M + H, 384.1481]; $[\alpha]_D^{27}$ - 4.06 (c 1.47); δ_H (300 MHz) 1.16 (3 H, d, J 6.7, CMe), 1.70 (1 H, dq, J 13.8 and 7.6, CHH), 1.89-2.03 (1 H, m, CHH), 2.21 (2 H, t, J 7.6, CH2), 2.41 (3 H, s, CMe), 2.91 (1 H, m, 4-H), 3.65 (3 H, s, OMe), 3.66 (3 H, s, OMe), 3.84-3.96 (1 H, m, 7-H), 4.84 (1 H, d, J 7.6, NH), 5.34-5.50 (2 H, m, 2 × CH=), 7.28 (2 H, m, ArH), 7.73 (2 H, m, ArH); m/z (FAB-LRMS) 384 (MH$^+$, base peak), 213, 198, 181, 155, 149, 121, 91.

Ts-L-Ala-ψ[(E)-CH=CH]-D-Glu(OBn)-OMe 19. By use of a procedure identical with that described for the preparation of **18** from **14**, treatment of the ester **14** (100 mg, 0.301 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl [benzyl 3-iodopropionate was obtained by successive treatment of 3-bromopropionic acid with benzyl alcohol-benzene-cat. TsOH and sodium iodide-acetone] in THF gave the title compound **19** (127 mg, 0.276 mmol, 91%) as a colorless oil [Found (FAB): (M + H)$^+$, 460.1785. C$_{24}$H$_{30}$NO$_6$S requires M + H, 460.1794]; [α]$_D^{26}$ - 3.62 (c 1.38); δ_H (300 MHz) 1.14 (3 H, d, J 6.7, CMe), 1.65-1.79 (1 H, m, CHH), 1.91-2.04 (1 H, m, CHH), 2.25 (2 H, m, CH$_2$), 2.39 (3 H, s, CMe), 2.91 (1 H, m, 4-H), 3.65 (3 H, s, OMe), 3.88 (1 H, m, 7-H), 4.59 (1 H, d, J 7.6, NH), 5.10 (2 H, s, OCH$_2$), 5.33 (1 H, dd, J 15.5 and 5.2, CH=), 5.43 (1 H, m, CH=), 7.23-7.29 (2 H, m, ArH), 7.31-7.38 (5 H, m, ArH), 7.69-7.75 (2 H, m, ArH); m/z (FAB-LRMS) 460 (MH$^+$, base peak), 391, 352, 304, 289, 257, 239, 198, 181, 167, 155, 149.

Mts-L-Val-ψ[(E)-CH=CH]-D-Glu(OMe)-OMe 20. By use of a procedure identical with that described for the preparation of **18** from **14**, treatment of the ester **15** (100 mg, 0.257 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Me·2LiCl in THF gave the title compound **20** (113 mg, 0.257 mmol, 99%) as a colorless oil [Found (FAB): (M + H)$^+$, 440.2100. C$_{22}$H$_{34}$NO$_6$S requires M + H, 440.2107]; [α]$_D^{29}$ + 20.4 (c 1.07); δ_H (300 MHz) 0.82 (3 H, d, J 6.7, CMe), 0.85 (3 H, d, J 6.8, CMe), 1.53-1.66 (1 H, m, CHH), 1.69-1.81 (1 H, m, CH), 1.82-1.96 (1 H, m, CHH), 2.16 (2 H, m, CH$_2$), 2.28 (3 H, s, CMe), 2.60 (6 H, s, 2 × CMe), 2.83 (1 H, m, 4-H), 3.55 (1 H, m, 7-H), 3.65 (6 H, s, 2 × OMe), 4.63 (1 H, d, J 7.7, NH), 5.20-5.35 (2 H, m, 2 × CH=), 6.90 (2 H, s, ArH); m/z (FAB-LRMS) 440 (MH$^+$), 396 (base peak), 364, 254, 241, 209, 183, 181, 177, 167, 149, 119.

Mts-L-Val-ψ[(E)-CH=CH]-D-Glu(OBn)-OMe 21. By use of a procedure identical with that described for the preparation of **18** from **14**, treatment of the ester **15** (100 mg, 0.257 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl in THF gave the title compound **21** (132 mg, 0.255 mmol, 99%) as a colorless oil [Found (FAB): (M + H)$^+$, 516.2408. C$_{28}$H$_{38}$NO$_6$S requires M + H, 516.2420]; [α]$_D^{29}$ - 13.3 (c 1.04); δ_H (300 MHz) 0.80 (3 H, d, J 6.7, CMe), 0.83 (3 H, d, J 6.8, CMe), 1.53-1.79 (2 H, m, CHH and CH), 1.84-1.97 (1 H, m, CHH), 2.20 (2 H, m, CH$_2$), 2.25 (3 H, s, CMe), 2.58 (6 H, s, 2 × CMe), 2.82 (1 H, m, 4-H), 3.53 (1 H, m, 7-H), 3.64 (3 H, s, OMe), 4.56 (1 H, d, J 7.7, NH), 5.09 (2 H, s, OCH$_2$), 5.14-5.33 (2 H, m, 2 × CH=), 6.87 (2 H, s, ArH), 7.29-7.40 (5 H, m, ArH); m/z (FAB-LRMS) 516 (MH$^+$, base peak), 472, 440, 332, 317, 285, 263, 254, 209.

Mts-L-Phe-ψ[(E)-CH=CH]-D-Glu(OMe)-OMe 22. By use of a procedure identical with that described for the preparation of **18** from **14**, treatment of the ester **16** (100 mg, 0.229 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Me·2LiCl in THF gave the title compound **22** (97 mg, 0.200 mmol, 81%) as colorless crystals, mp 98-100 °C [from n-hexane-EtOAc (5:1)] (Found: C, 63.88; H, 6.87; N, 2.65. C$_{26}$H$_{33}$NO$_6$S requires C, 64.04; H, 6.82; N, 2.87%); [α]$_D^{23}$ +12.1 (c 1.64); δ_H (300 MHz) 1.54-1.67 (1 H, m, CHH), 1.81-1.94 (1 H, m, CHH), 2.09 (2 H, m, CH$_2$), 2.27 (3 H, s, CMe), 2.48 (6 H, s, 2 × CMe), 2.70-2.91 (3 H, m, PhCH$_2$ and 4-H), 3.65 (6 H, m, 2 × OMe), 3.92 (1 H, m, 7-H), 4.51 (1 H, d, J 6.4, NH), 5.34 (2 H, m, 2 × CH=), 6.87 (2 H, s, ArH), 7.00-7.05 (2 H, m, ArH), 7.15-7.25 (3 H, m, ArH).

Crystal structure determination of compound 22. Crystal data. C$_{26}$H$_{33}$NO$_6$S, M = 487.61, orthorhombic, a = 11.780(8), b = 35.92(1), c = 6.24(1) Å, V = 2639(4) Å^3, T = 296 K,

space group $P2_12_12_1$ (no. 19), $Z = 4$, μ(Cu-Kα) = 14.14 mm^{-1}, 2628 reflections measured, 869 [$I > 3\ \sigma(I)$] were used in all calculations. The final wR was 0.101.

Mts-L-Phe-ψ[(E)-CH=CH]-D-Glu(OBn)-OMe 23. By use of a procedure identical with that described for the preparation of **18** from **14**, treatment of the ester **16** (100 mg, 0.229 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl in THF gave the title compound **23** (115 mg, 0.204 mmol, 89%) as a colorless oil [Found (FAB): (M + H)$^+$, 564.2415. C$_{32}$H$_{38}$NO$_6$S requires M + H, 564.2420]; [α]$_D^{29}$ + 12.2 (c 1.22); δ_H (300 MHz) 1.54-1.69 (1 H, m, CHH), 1.81-1.95 (1 H, m, CHH), 2.12 (2 H, m, CH$_2$), 2.25 (3 H, s, CMe), 2.47 (6 H, s, 2 × CMe), 2.68-2.89 (3 H, m, PhCH$_2$ and 4-H), 3.63 (3 H, s, OMe), 3.90 (1 H, m, 7-H), 4.54 (1 H, d, J 6.4, NH), 5.09 (2 H, s, OCH$_2$), 5.30 (2 H, m, 2 × CH=), 6.84 (2 H, s, ArH), 6.99 (2 H, m, ArH), 7.13-7.23 (3 H, m, ArH), 7.31-7.39 (5 H, m, ArH); m/z (FAB-LRMS) 586 (MNa$^+$), 564 (MH$^+$, base peak), 472, 440, 392, 365, 333, 315, 302, 289, 257, 225, 183.

General procedure for the preparation of (L-Xaa, L-Glu)-type EADIs from N-Ts- or N-Mts-γ,δ-epimino (E)-α,β-enoates. Representative: Ts-L-Ala-ψ[(E)-CH=CH]-L-Glu(OMe)-OMe 24 prepared from 10. To a suspension of zinc dust (177 mg, 2.70 mmol) in dry THF (0.60 cm^3), which was subjected to treatment for activation, was added methyl 3-iodopropionate (288 mg, 1.35 mmol) in dry THF (0.75 cm^3) at room temperature, and the mixture was stirred for 1 h at this temperature. The organozinc reagent was added to a stirred suspension of CuCN (121 mg, 1.35 mmol) and LiCl (114 mg, 2.70 mmol) in dry THF (1.35 cm^3) under argon at - 78 °C, and the mixture was allowed to warm to 0 °C and was stirred at this temperature for 15 min. To the solution of organozinc-copper reagent was added dropwise a solution of the cis-(E)-enoate **10** (100 mg, 0.338 mmol) in dry THF (1.00 cm^3) at - 78 °C with stirring, and the mixture was stirred at 0 °C for 30 min followed by quenching with 1:1 saturated aq. NH$_4$Cl-28% NH$_4$OH (2 cm^3). The mixture was extracted with Et$_2$O, and the extract was washed with water, and dried over MgSO$_4$. Concentration under reduced pressure gave a colorless oil, which was purified by flash chromatography over silica gel with n-hexane-EtOAc (3:1) to yield the title compound **24** (129 mg, 0.336 mmol, 99%) as a colorless oil [Found (FAB): (M + H)$^+$, 384.1488. C$_{18}$H$_{26}$NO$_6$S requires M + H, 384.1481]; [α]$_D^{24}$ - 74.3 (c 1.38); δ_H (300 MHz) 1.16 (3 H, d, J 6.7, CMe), 1.71 (1 H, dq, J 13.9 and 7.5, CHH), 1.88-2.02 (1 H, m, CHH), 2.19-2.27 (2 H, m, CH$_2$), 2.42 (3 H, s, CMe), 2.92 (1 H, m, 4-H), 3.64 (3 H, s, OMe), 3.66 (3 H, s, OMe), 3.81-3.93 (1 H, m, 7-H), 4.78 (1 H, d, J 7.4, NH), 5.35-5.49 (2 H, m, 2 × CH=), 7.29 (2 H, m, ArH), 7.74 (2 H, m, ArH); m/z (FAB-LRMS) 384 (MH$^+$, base), 228, 213, 198, 181.

Ts-L-Ala-ψ[(E)-CH=CH]-L-Glu(OBn)-OMe 25 prepared from 10. By use of a procedure identical with that described for the preparation of **24** from **10**, treatment of the cis-(E)-enoate **10** (100 mg, 0.338 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl in THF gave the title compound **25** (148 mg, 0.322 mmol, 95%) as a colorless oil [Found (FAB): (M + H)$^+$, 460.1804. C$_{24}$H$_{30}$NO$_6$S requires M + H, 460.1794]; [α]$_D^{28}$ - 52.0 (c 1.17); δ_H (300 MHz) 1.14 (3 H, d, J 6.7, CMe), 1.66-1.79 (1 H, m, CHH), 1.90-2.23 (1 H, m, CHH), 2.26 (2 H, m, CH$_2$), 2.38 (3 H, s, CMe), 2.91 (1 H, m, 4-H), 3.63 (3 H, s, OMe), 3.85 (1 H, m, 7-H), 4.69 (1 H, d, J 7.4, NH), 5.10 (2 H, m, OCH$_2$), 5.31-5.47 (2 H, m, 2 × CH=), 7.23-7.28 (2 H, m, ArH), 7.29-7.39 (5 H, m, ArH), 7.72 (2 H, m, ArH); m/z (FAB-LRMS) 460 (MH$^+$), 304, 289, 257, 239 (base peak), 198, 181, 167, 155, 149.

Mts-L-Val-ψ[(*E*)-CH=CH]-L-Glu(OMe)-OMe 26. By use of a procedure identical with that described for the preparation of **24** from **10**, treatment of the *cis*-(*E*)-enoate **11** (100 mg, 0.284 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Me·2LiCl in THF gave the title compound **26** (65 mg, 0.149 mmol, 52%) as a colorless oil [Found (FAB): (M + H)$^+$, 440.2103. C$_{22}$H$_{34}$NO$_6$S requires *M* + H, 440.2107]; [α]$_D^{22}$ - 43.2 (*c* 2.17); δ$_H$ (300 MHz) 0.80 (3 H, d, *J* 6.7, CMe), 0.85 (3 H, d, *J* 6.8, CMe), 1.54-1.93 (3 H, m, CH$_2$ and CH), 2.17 (2 H, m, CH$_2$), 2.28 (3 H, s, CMe), 2.61 (6 H, s, 2 × CMe), 2.85 (1 H, m, 4-H), 3.49 (1 H, m, 7-H), 3.63 (3 H, s, OMe), 3.67 (3 H, s, OMe), 4.56 (1 H, d, *J* 7.9, NH), 5.19-5.34 (2 H, m, 2 × CH =), 6.92 (2 H, s, ArH); *m/z* (FAB-LRMS) 440 (MH$^+$, base peak), 396, 364, 254, 241, 209.

Mts-L-Val-ψ[(*E*)-CH=CH]-L-Glu(OBn)-OMe 27. By use of a procedure identical with that described for the preparation of **24** from **10**, treatment of the *cis*-(*E*)-enoate **11** (100 mg, 0.284 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl in THF gave the title compound **27** (66 mg, 0.129 mmol, 45%) as a colorless oil [Found (FAB): (M + H)$^+$, 516.2439. C$_{28}$H$_{38}$NO$_6$S requires *M* + H, 516.2420]; [α]$_D^{27}$ - 35.5 (*c* 1.68); δ$_H$ (300 MHz) 0.79 (3 H, d, *J* 6.7, CMe), 0.84 (3 H, d, *J* 6.8, CMe), 1.54-1.77 (2 H, m, C*H*H and CH), 1.80-1.94 (1 H, m, CH*H*), 2.18-2.28 (5 H, m, CH$_2$ and CMe), 2.59 (6 H, s, 2 × CMe), 2.84 (1 H, m, 4-H), 3.47 (1 H, m, 7-H), 3.61 (3 H, s, OMe), 4.52 (1 H, d, *J* 7.9, NH), 5.11 (2 H, m, OCH$_2$), 5.21-5.26 (2 H, m, 2 × CH=), 6.88 (2 H, s, ArH), 7.31-7.39 (5 H, m, ArH); *m/z* (FAB-LRMS) 516 (MH$^+$, base peak), 514, 472, 440, 408, 380, 332, 317, 289, 285, 254, 209, 183, 167, 149.

Mts-L-Phe-ψ[(*E*)-CH=CH]-L-Glu(OMe)-OMe 28. By use of a procedure identical with that described for the preparation of **24** from **10**, treatment of the *cis*-(*E*)-enoate **12** (100 mg, 0.250 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Me·2LiCl in THF gave the title compound **28** (119 mg, 0.244 mmol, 97%) as a colorless oil [Found (FAB): (M + H)$^+$, 488.2123. C$_{26}$H$_{34}$NO$_6$S requires *M* + H, 488.2107]; [α]$_D^{27}$ - 56.4 (*c* 0.957); δ$_H$ (300 MHz) 1.59-1.73 (1 H, m, C*H*H), 1.83-1.97 (1 H, m, CH*H*), 2.19 (2 H, m, CH$_2$), 2.27 (3 H, s, CMe), 2.48 (6 H, s, 2 × CMe), 2.78 (2 H, d, *J* 6.7, PhCH$_2$), 2.88 (1 H, m, 4-H), 3.61 (3 H, s, OMe), 3.66 (3 H, s, OMe), 3.89 (1 H, m, 7-H), 4.57 (1 H, d, *J* 6.6, NH), 5.30 (1 H, dd, *J* 15.5 and 7.8, CH=), 5.41 (1 H, dd, *J* 15.5 and 6.8, CH=), 6.87 (2 H, s, ArH), 6.98-7.04 (2 H, m, ArH), 7.15-7.27 (3 H, m, ArH); *m/z* (FAB-LRMS) 488 (MH$^+$, base peak), 396, 364, 302, 289, 257, 225.

Mts-L-Phe-ψ[(*E*)-CH=CH]-L-Glu(OBn)-OMe 29. By use of a procedure identical with that described for the preparation of **24** from **10**, treatment of the *cis*-(*E*)-enoate **12** (100 mg, 0.250 mmol) with IZn(CN)CuCH$_2$CH$_2$CO$_2$Bn·2LiCl in THF gave the title compound **29** (141 mg, 0.250 mmol, 99%) as a colorless oil [Found (FAB): (M + H)$^+$, 564.2432. C$_{32}$H$_{38}$NO$_6$S requires *M* + H, 564.2420]; [α]$_D^{28}$ - 40.8 (*c* 1.29); δ$_H$ (300 MHz) 1.60-1.74 (1 H, m, C*H*H), 1.85-1.98 (1 H, m, CH*H*), 2.20-2.27 (5 H, m, CH$_2$ and CMe), 2.46 (6 H, s, 2 × CMe), 2.77 (2 H, d, *J* 6.7, PhCH$_2$), 2.88 (1 H, m, 4-H), 3.60 (3 H, s, OMe), 3.88 (1 H, m, 7-H), 4.50 (1 H, d, *J* 6.7, NH), 5.11 (2 H, m, OCH$_2$), 5.25-5.41 (2 H, m, 2 × CH=), 6.84 (2 H, s, ArH), 6.98-7.03 (2 H, m, ArH), 7.15-7.24 (3 H, m, ArH), 7.31-7.38 (5 H, m, ArH); *m/z* (FAB-LRMS) 564 (MH$^+$, base peak), 474, 472, 440, 391, 365, 333, 315, 302, 289, 257, 225, 183.

(2*E*,4*S*,5*S*)-6-Benzyloxy-4-chloro-*N*-methyl-5-(2,4,6-trimethylphenylsulfonyl-amino)hex-2-enamide 30. By use of a procedure identical with that described for the preparation of **14** from **10**, the *cis*-(*E*)-enamide **6** (11.5 g, 26.7 mmol) was converted into the title compound **30** (9.53 g, 20.5 mmol, 77%) as colorless crystals, mp 131-133 °C (from Et$_2$O) (Found: C, 59.18; H, 6.17; N, 5.79. C$_{23}$H$_{29}$ClN$_2$O$_4$S requires C, 59.41; H, 6.29; N, 6.02%);

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$[\alpha]_D{}^{28}$ - 26.0 (c 0.50); δ_H (300 MHz) 2.29 (3 H, s, CMe), 2.58 (6 H, s, 2 × CMe), 2.85 (3 H, d, J 4.9, NMe), 3.45 (1 H, m, 5-H), 3.53-3.64 (2 H, m, OCH_2), 4.39 (2 H, s, OCH_2Ph), 4.81 (1 H, ddd, J 7.4, 3.4 and 1.2, 4-H), 5.08 (1 H, d, J 8.6, NH), 5.53 (1 H, br s, CONH), 5.94 (1 H, dd, J 15.1 and 1.2, CH=), 6.56 (1 H, dd, J 15.1 and 7.4, CH=), 6.91 (2 H, s, ArH), 7.19-7.38 (5 H, m, Ph).

Mts-L-Ser(O-Bn)-ψ[(E)-CH=CH]-D-Glu(OMe)-NHMe 8. By use of a procedure identical with that described for the preparation of **18** from **14**, treatment of the enamide **30** (2.32 g, 4.98 mmol) with $IZn(CN)CuCH_2CH_2CO_2Me\cdot2LiCl$ in THF gave the title compound **8** (2.40 g, 4.64 mmol, 93%) as a colorless oil [Found (FAB): $(M + H)^+$, 517.2368. $C_{27}H_{37}N_2O_6S$ requires M + H, 517.2372]; $[\alpha]_D{}^{28}$ - 28.0 (c 0.50); δ_H (300 MHz) 1.70 (1 H, m, CHH), 2.19 (1 H, m, CHH), 2.23-2.26 (2 H, m, CH_2), 2.30 (3 H, s, CMe), 2.56 (6 H, s, 2 × CMe), 2.75 (3 H, d, J 4.7, NMe), 2.83 (1 H, m, 4-H), 3.33-3.43 (2 H, m, OCH_2), 3.62 (3 H, s, OMe), 3.69 (1 H, m, 7-H), 4.40 (2 H, s, OCH_2Ph), 5.22 (1 H, d, J 4.4, NH), 5.47 (1 H, dd, J 15.4 and 8.2, CH=), 5.55 (1 H, dd, J 15.5 and 7.2, CH=), 6.30 (1 H, br s, CONH), 6.92 (2 H, s, ArH), 7.19-7.38 (5 H, m, ArH); m/z (FAB-LRMS) 539 (MNa^+), 517 (MH^+), 485, 318, 228 (base peak), 196, 183, 119, 91.

Mts-L-Ser(O-Bn)-ψ[(E)-CH=CH]-D-Glu(OBn)-NHMe 31. By use of a procedure identical with that described for the preparation of **18** from **14**, treatment of the enamide **30** (600 mg, 1.29 mmol) with $IZn(CN)CuCH_2CH_2CO_2Bn\cdot2LiCl$ in THF gave the title compound **31** (595 mg, 1.01 mmol, 78%) as a colorless oil [Found (FAB): $(M - H)^-$, 591.2547. $C_{33}H_{39}N_2O_6S$ requires M - H, 591.2528]; $[\alpha]_D{}^{28}$ - 11.9 (c 0.50); δ_H (270 MHz) 1.73 (1 H, m, CHH), 2.23 (1 H, m, CHH), 2.28 (2 H, m, CH_2), 2.29 (3 H, s, CMe), 2.54 (6 H, s, 2 × CMe), 2.73 (3 H, d, J 4.7, NMe), 2.80 (1 H, m, 4-H), 3.30-3.40 (2 H, m, OCH_2), 3.62 (1 H, m, 7-H), 4.37 (2 H, s, OCH_2Ph), 5.07 (2 H, s, OCH_2Ph), 5.21 (1 H, d, J 4.0, NH), 5.43 (1 H, dd, J 15.1 and 7.0, CH=), 5.51 (1 H, dd, J 15.1 and 7.0, CH=), 6.30 (1 H, br s, CONH), 6.92 (2 H, s, ArH), 7.18-7.39 (10 H, m, 2 × Ph); m/z (FAB-LRMS) 591 $[(M - H)^-]$, 483 (base peak), 305, 199, 168, 153, 122.

Mts-L-Ser(O-Bn)-ψ[(E)-CH=CH]-L-Glu(OBn)-NHMe 32. By use of a procedure identical with that described for the preparation of **24** from **10**, treatment of the cis-(E)-enamide **6** (327 mg, 0.763 mmol) with $IZn(CN)CuCH_2CH_2CO_2Bn\cdot2LiCl$ in THF gave the title compound **32** (255 mg, 0.430 mmol, 56%) as a colorless oil [Found (FAB): $(M + H)^+$, 593.2700. $C_{33}H_{41}N_2O_6S$ requires M + H, 593.2685]; $[\alpha]_D{}^{28}$ - 54.0 (c 0.50); δ_H (270 MHz) 1.74 (1 H, m, CHH), 2.13 (1 H, m, CHH), 2.28 (3 H, s, CMe), 2.34 (2 H, t, J 7.5, CH_2), 2.55 (6 H, s, 2 × CMe), 2.70 (3 H, d, J 4.7, NMe), 2.80 (1 H, m, 4-H), 3.28-3.40 (2 H, m, OCH_2), 3.69 (1 H, m, 7-H), 4.36 (2 H, s, OCH_2Ph), 5.10 (2 H, s, OCH_2Ph), 5.15 (1 H, d, J 4.7, NH), 5.43 (1 H, dd, J 15.5 and 8.7, CH=), 5.70 (1 H, dd, J 15.5 and 6.7, CH=), 6.07 (1 H, m, CONH), 6.91 (2 H, s, ArH), 7.17-7.38 (10 H, m, 2 × Ph); m/z (FAB-LRMS) 593 (MH^+), 485 (base peak), 465, 394, 332, 196, 183, 119, 91.

References

1   (a) T. E. Christos, A. Arvanitis, G. A. Cain, A. L. Johnson, R. S. Pottorf, S. W. Tam, W. K. Schmidt, *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 1035. (b) B. Beresis, J. S. Panek, *Bioorg. Med. Chem. Lett.*, **1993**, *3*, 1609. (c) J. S. Wai, D. L. Bamberger, T. E. Fisher, S. L. Graham, R. L. Smith, J. B. Gibbs, S. D. Mosser, A. I. Oliff, D. L. Pompliano, E. Rands, N. E. Kohl, *Bioorg. Med. Chem.*, **1994**, *2*, 939. (d) M. Wada, R. Doi, R. Hosotani, S. Higashide, T. Ibuka, H. Habashita, K. Nakai, N. Fujii, M. Imamura, *Pancreas*, **1995**, *10*, 301. (e) P. A. Bartlett, A. Otake, *J. Org. Chem.*, **1995**, *60*, 3107. (f) K. Fujimoto, R. Doi, R. Hosotani, M. Wada, J.-U. Lee, T. Koshiba, T. Ibuka, H. Habashita, K. Nakai, N. Fujii, M. Imamura, *Life Sci.*, **1997**, *60*, 29. (g) K. Miyasaka, S. Kanai, M. Masuda, T. Ibuka, K. Nakai, N. Fujii, A. Funakoshi, *J. Auton. Nerv. Syst.*, **1997**, *63*, 179.

2   (a) J. S. Kaltenbronn, J. P. Hudspeth, E. A. Lunney, B. M. Michniewicz, E. D. Nicolaides, J. T. Repine, W. H. Roark, M. A. Steir, F. J. Tinney, P. K. W. Woo, A. D. Essenburg, *J. Med. Chem.*, **1990**, *33*, 838. (b) D. Tourwe, J. Couder, M. Ceusters, D. Meert, T. F. Burks, T. H. Kramer, P. Davis, R. Knapp, H. I. Yamamura, J. E. Leysen, G. van Binst, *Int. J. Pept. Protein Res.*, **1992**, *39*, 131. (c) M. J. Daly, R. A. Ward, D. F. Thompson, G. Procter, *Tetrahedron Lett.*, **1995**, *36*, 7545.

3   (a) T. Ibuka, H. Habashita, A. Otaka, N. Fujii, Y. Oguchi, T. Uyehara, Y. Yamamoto, *J. Org. Chem.*, **1991**, *56*, 4370. (b) for a review of syntheses of (*E*)-alkene dipeptide isosteres up to May 1992, see: T. Ibuka, *J. Synth. Org. Chem. Jpn.*, **1992**, *50*, 953. (c) T. Ibuka, T. Taga, H. Habashita, K. Nakai, H. Tamamura, N. Fujii, Y. Chounan, H. Nemoto, Y. Yamamoto, *J. Org. Chem.*, **1993**, *58*, 1207.

4   (a) T. Ibuka, K. Nakai, H. Habashita, Y. Hotta, N. Fujii, N. Mimura, Y. Miwa, T. Taga, Y. Yamamoto, *Angew. Chem., Int. Ed. Engl.*, **1994**, *33*, 652. (b) P. Wipf, P. C. Fritch, *J. Org. Chem.*, **1994**, *59*, 4875. (c) N. Fujii, K. Nakai, H. Tamamura, A. Otaka, N. Mimura, Y. Miwa, T. Taga, Y. Yamamoto, T. Ibuka, *J. Chem. Soc., Perkin Trans. 1*, **1995**, 1359. (d) P. Wipf and T. Henninger, *J. Org. Chem.*, **1997**, *62*, 1586.

5   (a) E. Nakamura, S. Aoki, K. Sekiya, H. Oshino, I. Kuwajima, *J. Am. Chem. Soc.*, **1987**, *109*, 8056. (b) H. Ochiai, Y. Tamaru, K. Tsubaki, Z. Yoshida, *J. Org. Chem.*, **1987**, *52*, 4418. (c) M. C. P. Yeh, P. Knochel, *Tetrahedron Lett.*, **1988**, *29*, 2395. (d) P. Knochel, M. C. P. Yeh, S. C. Berk, J. Talbert, *J. Org. Chem.*, **1988**, *53*, 2390. (e) L. Zhu, R. M. Wehmeyer, R. D. Rieke, *J. Org. Chem.*, **1991**, *56*, 1445.

6   (a) H. Tamamura, M. Yamashita, H. Muramatsu, H. Ohno, T. Ibuka, A. Otaka, N. Fujii, *Chem. Commun.*, **1997**, 2327. (b) H. Tamamura, M. Yamashita, Y. Nakajima, K. Sakano, A. Otaka, H. Ohno, T. Ibuka, N. Fujii, *J. Chem. Soc., Perkin Trans. 1*, **1999**, 2983.

7   (a) T. Ibuka, N. Mimura, H. Aoyama, M. Akaji, H. Ohno, Y. Miwa, T. Taga, K. Nakai, H. Tamamura, N. Fujii, Y. Yamamoto, *J. Org. Chem.*, **1997**, *62*, 999. (b) T. Ibuka, N. Mimura, H. Ohno, K. Nakai, M. Akaji, H. Habashita, H. Tamamura, Y. Miwa, T. Taga, N. Fujii, Y. Yamamoto, *J. Org. Chem.*, **1997**, *62*, 2982.

8   (a) M. E. Duggan, D. S. Karanewsky, *Tetrahedron Lett.*, **1983**, *24*, 2935. (b) S. Itsuno, K. Tanaka, K. Ito, *Chem. Lett.*, **1986**, 1133. (c) D. Tanner, P. Somfai, *Tetrahedron Lett.*, **1987**, *28*, 1211. (d) S. Matsubara, T. Kodama, K. Utimoto, *Tetrahedron Lett.*, **1990**, *31*, 6379. (e) J. Legters, J. G. H. Willems, L. Thijs, B. Zwanenburg, *Recl. Trav. Chim. Pays-Bas*, **1992**, *111*, 59. (f) D. Tanner, H. M. He, P. Somfai, *Tetrahedron*, **1992**, *48*, 6069. (g) T. Kawabata, Y. Kiryu, Y. Sugiura, K. Fuji, *Tetrahedron Lett.*, **1993**, *34*, 5127. (h) N. J. Church, D. W. Young, *Tetrahedron Lett.*, **1995**, *36*, 151; and references cited.

(i) B. G. M. Burgaud, D. C. Horwell, A. Padova, M. C. Pritchard, *Tetrahedron*, **1996**, *52*, 13035. (j) C. M. Rayner, *Synlett*, **1997**, 11. (k) H. Ohno, N. Mimura, A. Otaka, H. Tamamura, N. Fujii, T. Ibuka, I. Shimizu, A. Satake, Y. Yamamoto, *Tetrahedron*, **1997**, *53*, 12933. (l) A. A. Cantrill, H. M. I. Osborn, J. Sweeney, *Tetrahedron*, **1998**, *54*, 2181. (m) I. Fleming, J. Frackenpohl, H. Ila, *J. Chem. Soc., Perkin Trans. 1*, **1998**, 1229.

9   (a) A. Bongini, G. Cardillo, M. Orena, S. Sandri, C. Tomasini, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 1339. (b) A. Bongini, G. Cardillo, M. Orena, S. Sandri, C. Tomasini, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 1345.

10   (a) D. A. Evans, M. M. Faul, M. T. Bilodeau, B. A. Anderson, D. M. Barnes, *J. Am. Chem. Soc.*, **1993**, *115*, 5328. (b) I. Coldham, A. J. Collis, R. J. Mould, R. E. Rathmell, *J. Chem. Soc., Perkin Trans. 1*, **1995**, 2739. (c) F. A. Davis, G. V. Reddy, *Tetrahedron Lett.*, **1996**, *37*, 4349.

11   F. A. Davis, P. Zhou, *Tetrahedron Lett.*, **1994**, *35*, 7525.

12   G. E. Ham, *J. Org. Chem.*, **1964**, *29*, 3052.

Section 2. Regio- and Stereoselective Ring-opening of Chiral 1,3-Oxazolidin-2-one Derivatives by Organocopper Reagents: A Novel Access to Di-, Tri- and Tetra-substituted Alkene Dipeptide Isosteres

Summary

Organocopper-mediated alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates has been intensively investigated. Alkylation proceeds regio- and stereoselectively by *anti*-S_N2' ring-opening, providing a new route to $\psi[(E)$-CH=CH]-, $\psi[(E)$-CMe=CH]- and $\psi[(E)$-CMe=CMe]-type alkene dipeptide isosteres from chiral amino acid derivatives. These resulting agents constitute potential mimetics of type II and type II' β-turn substructures.
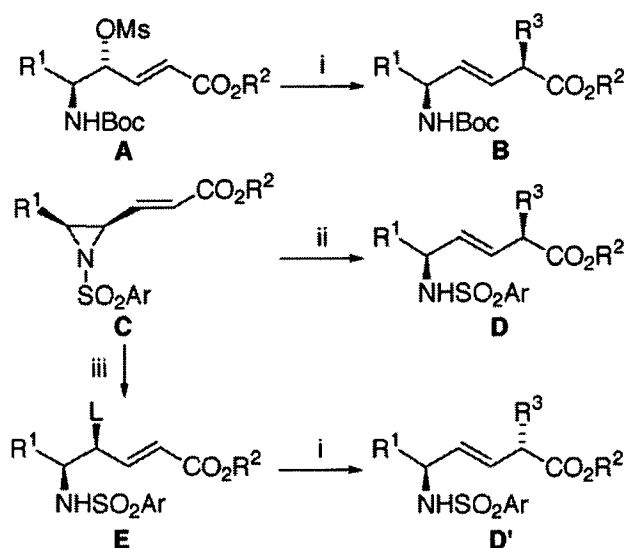
Scheme 1. R^1, R^2, R^3 = alkyl; L = OMs or Cl; Ms = methanesulfonyl. *Reagents*: i, R^3Cu(CN)MgCl·BF$_3$; ii, R^3Cu(CN)MgCl·2LiCl; iii, MsOH or HCl.

In section 1, the author described the stereoselective synthesis of a set of L-Xaa^1-$\psi[(E)$-CH=CH]-L-Glu^2- and L-Xaa^1-$\psi[(E)$-CH=CH]-D-Glu^2-type EADIs from a common key intermediate, N-activated β-aziridino-α,β-enoates.[1] Among the synthetic processes, used was the HCl-mediated ring-opening of the aziridines and the subsequent treatment with organocopper reagents to give (L,D)-type EADIs, which are thought to be potential surrogates of (i+1) - (i+2) dipeptides in type II β-turns. Similarly, preparation of (D,L)-type EADIs, which are type II' β-turn congeners, can be achieved from the enantiomeric aziridines. Recently, it has been demonstrated that EADIs having a γ-carbon corresponding to a carbonyl
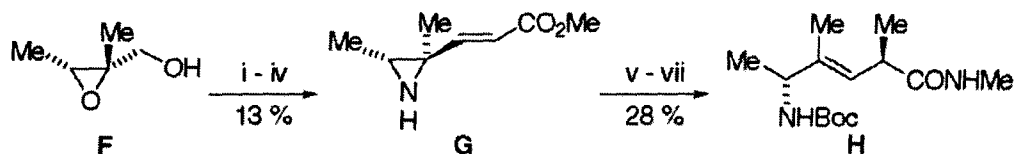
Scheme 2. *Reagents*: i, DMSO, (COCl)$_2$, Et$_3$N; ii, Ph$_3$P=CHCO$_2$Me; iii, NaN$_3$, NH$_4$Cl, iv, Ph$_3$P; v, Boc$_2$O, Et$_3$N; vi, Me$_2$Zn·2LiCl, CuCN; vii, MeNH$_2$, NaCN.

oxygen may be more appropriate than Xaa^1-ψ[(E)-CH=CH]-Xaa^2-type EADIs having disubstituted alkenes, due to rigidity of ϕ- and ψ-dihedral angles. Wipf et al. reported that, Xaa^1-ψ[(E)-CMe=CH]-Xaa^2- and Xaa^1-ψ[(Z)-CCF$_3$=CH]-Xaa^2-type EADIs having trisubstituted alkenes are effective β-turn promoters in the solid state.[2] Gardner et al. have also shown that peptides containing a tetrasubstituted alkene such as a Gly-ψ[(E)-CMe=CMe]-Gly-type EADI, induce β-hairpin formation.[3]

Several researchers have developed practical methodologies for the stereoselective synthesis of EADIs starting from chiral amino acid derivatives (Scheme 1),[1,4-9] wherein key reactions for the construction of side chain functionality at the α-position, utilize organocopper-mediated alkylation of α,β-enoates with a leaving group at the γ-position. This affords E-isomers of α-alkylated products regio- and stereoselectively. For example, treatment of γ-mesyloxy-α,β-enoates **A**[4,5] and *N*-activated γ,δ-epimino-α,β-enoates **C**[6,7] with organocopper reagents gives solely *anti*-S$_N$2' products **B** and **D**, respectively. In addition, alkylation of γ-mesyloxy- or γ-chloro-α,β-enoates **E**, which can be obtained by MsOH- or HCl-treatment of *N*-activated γ,δ-epimino-α,β-enoates **C**, can also give *anti*-S$_N$2' products **D'**, which are diastereomers of **D**.[1,8] As such, 1,3-chirality transfer by organocopper-mediated alkylation offers an efficient approach for the synthesis of molecules having two remote chiral centers adjacent to an olefin, such as Xaa^1-ψ[(E)-CH=CH]-Xaa^2-type EADIs.[10] The author speculated that ψ[(E)-CMe=CH]- and ψ[(E)-CMe=CMe]-type EADIs could be synthesized utilizing the same strategy as that used for the synthesis of ψ[(E)-CH=CH]-type EADIs. However, it was inferred that γ-methylated γ-mesyloxy-α,β-enoates or γ-methylated γ,δ-epimino-α,β-enoates might not be obtainable through known synthetic schemes, owing to difficulties in derivatization of tertiary hydroxy groups. Examplary of this D-Ala-ψ[(E)-CMe=CH]-L-Ala-type EADI **H** was prepared only in low overall yield by Wipf et al. from a chiral epoxide **F**, wherein transformation of the epoxide **F** to a γ-methylated γ,δ-epimino-α,β-enoate **G** was not straightforward (Scheme 2). Therefore, the author sought to develop a new general synthesis of diverse ψ[(E)-CMe=CH]- and ψ[(E)-CMe=CMe]-type EADIs using organocopper-mediated alkylation of new key intermediates.

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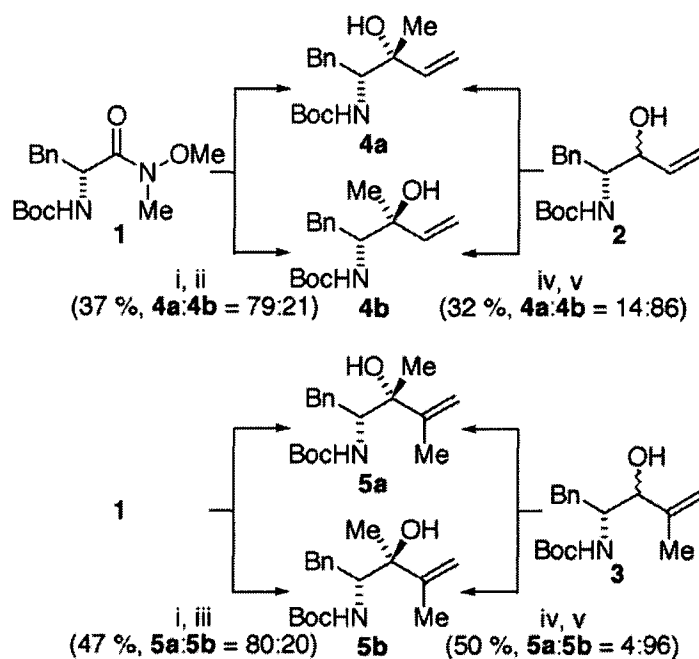
Scheme 3. *Reagents*: i, MeMgCl, THF; ii, CH₂=CH-MgCl, CeCl₃, THF; iii, CH₂=CMe-MgBr, CeCl₃, THF; iv, (COCl)₂, DMSO, (Prⁱ)₂NEt, CH₂Cl₂; v, MeMgCl, CeCl₃, THF.
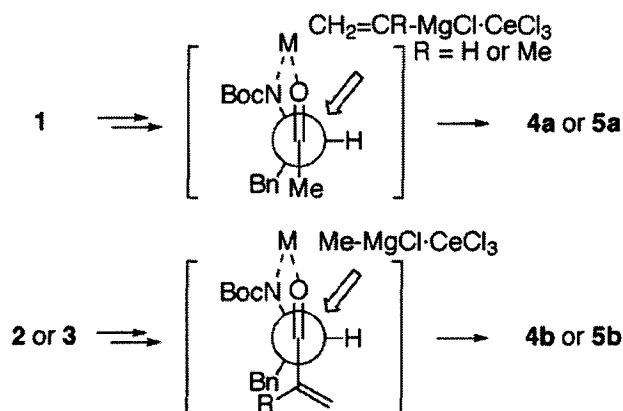
Figure 1

Accordingly, 5-vinyl-1,3-oxazolidin-2-ones, having hydroxy groups protected and activated as cyclic carbamates, were subjected to various modifications using organometallic reagents with accompanying ring-opening and decarboxylation.[11-14] Ibuka et al. had previously reported that alkylation of 5-vinyl-1,3-oxazolidin-2-ones with various organocopper reagents proceeds regio- and stereoselectively to give vinylglycine derivatives.[11] Therefore, the author examined the alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates as an approach to precursors for the synthesis of EADIs. In this section, the author reports the organocopper-mediated alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates to afford ψ[(E)-

Scheme 4. *Reagents*: i, NaH, THF; ii, (Boc)$_2$O; iii, O$_3$ gas, EtOAc or CH$_2$Cl$_2$; iv, Me$_2$S; v, (EtO)$_2$P(O)CH$_2$CO$_2$Bu^t, LiCl, (Pr^i)$_2$NEt, MeCN; vi, Ph$_3$P=CHCO$_2$Bu^t, CHCl$_3$; vii, Me$_3$SiCH$_2$CO$_2$Bu^t, (Cy)$_2$NLi, THF.

CH=CH]-type EADIs having disubstituted alkenes. The author further describes the application of this methodology to the synthesis of ψ[(*E*)-CMe=CH]- and ψ[(*E*)-CMe=CMe]-type EADIs having multisubstituted alkenes.

Synthesis of Diastereomerically Pure β-(*N*-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates. In the synthesis of EADIs based on the *anti*-S$_N$2' reaction of organocopper reagents, the chirality of the leaving group at the γ-position and the geometry of the olefin are

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responsible for the chirality of alkyl groups introduced at the α-position of the product. As such, the efficient synthesis of enantiomerically and diastereomerically pure substrates is critical. The author therefore investigated the preparation of chiral β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates, including mono- and dimethylated derivatives. These were selected as chiral key intermediates for the preparation of diverse EADIs from α-amino alcohol or α-amino acid derivatives **1-3**.[12,15,16] (Schemes 3 and 4). Vinyl and isopropenyl Grignard addition in the presence of anhydrous $CeCl_3$ to a methyl ketone, which was prepared by treatment of Weinreb amide **1** with MeMgCl, preferentially afforded the *syn*-allyl alcohols **4a** and **5a** (**4a**:**4b** = 79:21 and **5a**:**5b** = 80:20), respectively. Meanwhile, Swern oxidation of allyl alcohols **2** and **3** followed by addition of MeMgCl in the presence of $CeCl_3$ predominantly yielded *anti*-allyl alcohols **4b** and **5b** (**4a**:**4b** = 14:86 and **5a**:**5b** = 4:96), respectively. The ratio of isomers formed can be explained by chelation control during addition of alkenyl or methyl groups to methyl ketones or enones (Figure 1). Each isomer of the allyl alcohols **4b** and **5a,b** was obtained as a single diastereomer following purification by flash chromatography and repeated recrystallization. The *syn*-allyl alcohol **4a** containing the minor isomer **4b** was used for next step without further purification.

Sodium hydride-mediated cyclization of allyl alcohols **6a,b**,[15] **4a,b** and **5a,b** followed by N-protection with $(Boc)_2O$ efficiently gave the respective 1,3-oxazolidin-2-ones **7a,b**, **8a,b** and **9a,b**. Removal of the minor isomer **8b** from **8a** was carried out at this step by repeated recrystallization. Ozone-dimethyl sulfide treatment of the 5-vinyl derivatives **7a,b**, **8a,b** followed by modified Horner-Wadsworth-Emmons reaction (HWE reaction)[17] gave α,β-enoates **10a,b** and **11a,b** E-selectively. β-Methylated analogues **12a,b** were also obtained E-selectively following Wittig reaction with $Ph_3P=CHCO_2Bu^t$ in $CHCl_3$ of methyl ketones prepared by ozonolysis and successive reductive treatment of 5-isopropenyl derivatives **9a,b**. Peterson olefination of the methyl ketone derived from **9b** gave an unexpected Z-isomer of α,β-enoates **12c**, although in low yield.

The $2E$-geometry of **10a,b** and **11a,b** was established based on the large ${}^1H$-coupling constants between the two olefinic protons (15.5-15.6 Hz). The geometry of **12a,b** was established by the absence of NOESY cross-peaks between the olefinic proton and the β-methyl protons, while the $2Z$-geometry of **12c** was established by NOE enhancement (19%) of the olefinic proton resonance upon irradiation of the β-methyl protons. Relative configurations of 1,3-oxazolidin-2-ones **11a,b** were established by NOE experiments. No NOE enhancement of the 4-H resonance was observed by irradiation of the 5-methyl protons in the *trans*-isomer **11a**, while an NOE enhancement in the *cis*-isomer **11b** was observed
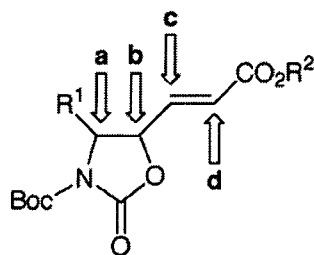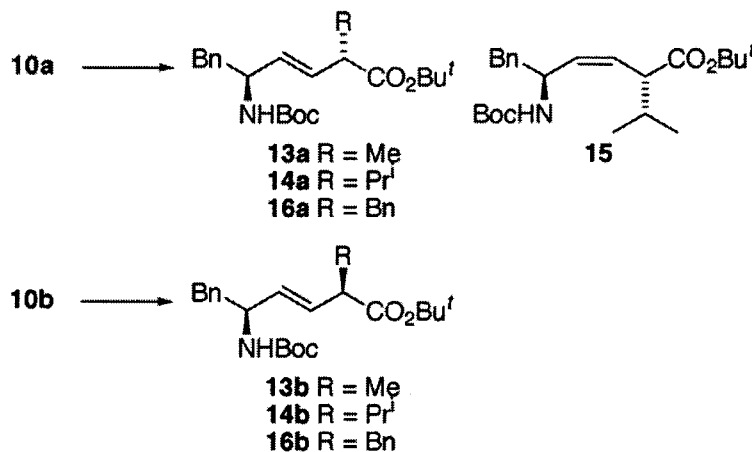
Figure 2

Scheme 5

(12%). The *trans*-configuration of **12a** was established by a NOESY cross-peak between the 5-methyl protons and one benzyl proton, while the *cis*-configuration of **12b** was established by cross-peaks between the 4-*H* proton and the 5-methyl protons.
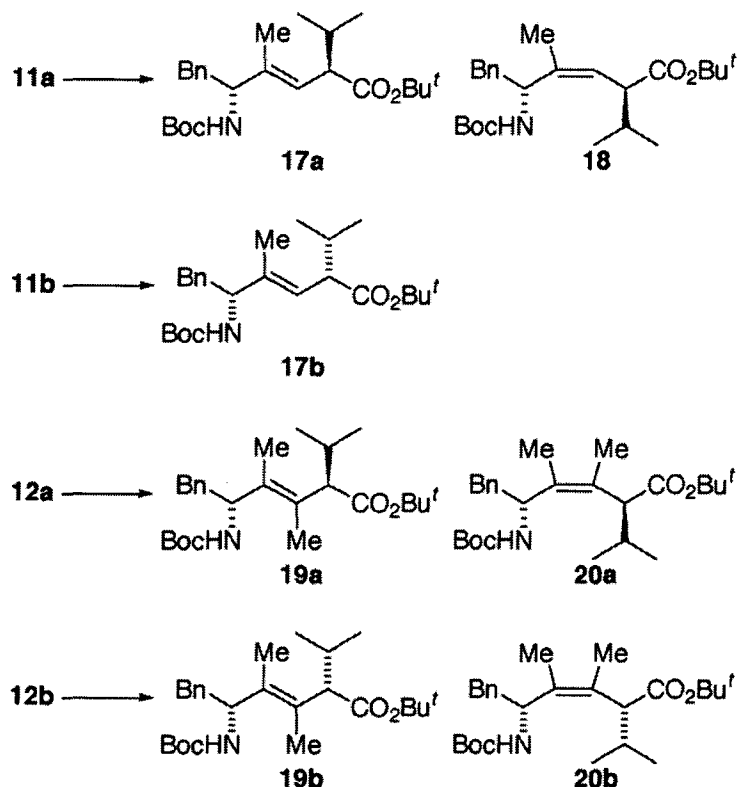
Synthesis of ψ[(E)-CH=CH]-type EADIs by Alkylation with Ring-opening of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates Using Organocopper Reagents. For β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates, at least four alkylation sites are possible: S_N2-type substitution sites (Figure 2, sites a and b) and a likely S_N2'-type substitution site (site d) with ring-opening, and a 1,4-addition site (site c). Alkylation of α,β-enoates having a leaving group at the γ-position is known to proceed principally via an S_N2' mechanism. However, the author was afraid that 1,4-adducts might be obtained without ring-opening, due to the poor leaving-group ability of cyclic carbamates. Therefore the author investigated in detail alkylations of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates **10a,b** by various organocopper reagents (Scheme 5 and Table 1). Here, expected α-alkylations of **10a,b** by an *anti*-S_N2' mechanism should give ψ[(E)-CH=CH]-type EADIs.

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Table 1. Alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates **10a** and **10b** by organocopper reagents.

Entry	Substrate	Reagent (4 equiv.)	Solvent	Conditions	Products (Yield %)
1	**10a**	MeCu·LiI·LiBr	THF-Et$_2$O (10 : 1)	- 78 °C, 30 min, then 0 °C, 1 h	-[a]
2	**10a**	Me$_2$CuLi·LiI·2LiBr	THF-Et$_2$O (5 : 1)	- 78 °C, 1 h	-[a]
3	**10a**	MeCu(CN)MgCl·BF$_3$·2LiCl	THF	- 78 °C, 30 min	**13a** (77)
4	**10a**	Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl	THF	- 78 °C, 30 min	**14a** (85) + **15** (14)[b]
5	**10a**	Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl	THF	- 78 °C, 30 min	**14a** (50) + **15** (27)[b]
6	**10a**	BnCu(CN)MgCl·BF$_3$·2LiCl	THF	- 78 °C, 30 min	**16a** (99)
7	**10b**	MeCu(CN)Li·BF$_3$·LiBr	THF-Et$_2$O (10 : 1)	- 78 °C, 30 min, then 0 °C, 3 h	**13b** (24)[c]
8	**10b**	Me$_2$Cu(CN)Li$_2$·BF$_3$·2LiBr	THF-Et$_2$O (5 : 1)	- 78 °C, 30 min	-[a]
9	**10b**	MeCu(CN)MgCl·BF$_3$·2LiCl	THF	- 78 °C, 30 min	**13b** (90)
10	**10b**	Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl	THF	- 78 °C, 30 min	**14b** (96)
11	**10b**	BnCu(CN)MgCl·BF$_3$·2LiCl	THF	- 78 °C, 30 min	**16b** (99)

[a]α-Alkylated products were not isolated. [b]The product ratios were determined by reverse phase HPLC. [c]The starting material was recovered (58%).

Treatment of *trans*-1,3-oxazolidin-2-one **10a** with either a methyl copper reagent (MeCu·LiI·LiBr) or Gilman-type reagent (Me$_2$CuLi·LiI·2LiBr) in THF gave a complex mixture of products (Table 1, entries 1 and 2). On the other hand, "lower-order" organocyanocuprate-BF$_3$ complexes prepared from methyl Grignard reagents [MeCu(CN)MgCl·BF$_3$·2LiCl] in THF at - 78 °C for 30 min gave an α-alkylated product, L-Phe-ψ[(E)-CH=CH]-D-Ala **13a**, regio- and stereoselectively (entry 3). Other organocyanocuprate-BF$_3$ complexes [RCu(CN)MgCl·BF$_3$·2LiCl (R = Pr^i and Bn)] also provided the respective α-alkylated products, L-Phe-ψ[(E)-CH=CH]-D-Xaa **14a** and **16a** (Xaa = Val and Phe, respectively), in excellent yields (entries 4 and 6). Interestingly, unforeseen formation of the Z-isomer of the *anti*-S$_N$2' product, L-Phe-ψ[(Z)-CH=CH]-L-Val **15**, was observed only in the reaction of **10a** with Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl (entry 4), although the reason for this is unclear.[18] An α-alkylated product **14a** and Z-isomer **15** were also afforded in moderate yields using a "higher-order" organocyanocuprate-BF$_3$ complex [Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl, entry 5]. This is an inappropriate reagent for alkylation of N-

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11a ⟶

Me

Bn ⟍ CO₂Buᵗ

BocHN

17a

Me

Bn ⟍ CO₂Buᵗ

BocHN

18

11b ⟶

Me

Bn ⟍ CO₂Buᵗ

BocHN

17b

12a ⟶

Me

Bn ⟍ CO₂Buᵗ

BocHN   Me

19a

Me   Me

Bn ⟍ CO₂Buᵗ

BocHN

20a

12b ⟶

Me

Bn ⟍ CO₂Buᵗ

BocHN   Me

19b

Me   Me

Bn ⟍ CO₂Buᵗ

BocHN

20b

Scheme 6

activated β-aziridinyl-α,β-enoates, owing to the formation of unwanted reductive products.[6] Alkylation of *cis*-1,3-oxazolidin-2-one **10b** with the "lower-order" methyl cyanocuprate-BF_3 complex [MeCu(CN)Li·BF₃·LiBr] derived from methyllithium in THF-Et₂O, resulted in the production of small amounts of α-alkylated product **13b** with recovery of starting material **10b** (entry 7), whereas use of a "higher-order" complex [Me₂Cu(CN)Li₂·BF₃·2LiBr] gave a number of uncharacterized products (entry 8). In contrast, alkylation of **10b** proceeded favourably using organocyanocuprate-BF_3 complexes [RCu(CN)MgCl·BF₃·2LiCl, R = Me, Prⁱ and Bn] derived from Grignard reagents, to afford L-Phe-ψ[(E)-CH=CH]-L-Xaa **13b**, **14b** and **16b** (Xaa = Ala, Val and Phe), respectively, in excellent yields (entries 9-11). This was similar to reactions of the *trans*-isomer **10a**. In contrast to the *trans*-isomer **10a** (entry 4), reaction of *cis*-1,3-oxazolidin-2-one **10b** with PrⁱCu(CN)MgCl·BF₃·2LiCl gave exclusively the E-isomer of the α-alkylated product **14b** without production of the Z-isomer (entry 10).

The regiochemistry of the resulting EADIs, **13a,b**, **14a,b** and **16a,b** was readily assigned by ¹H-¹H COSY spectra, large coupling constants between the olefinic protons (15.4-15.7 Hz) and/or comparison with the ¹H NMR spectrum of an authentic sample.[5] The stereochemical assignments of the α-alkyl groups in **13a,b**, **14a,b** and **16a,b** could be deduced from well-established organocopper-mediated *anti*-S_N2' alkylations. Additionally, the ¹H

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Table 2. Alkylation of mono- and dimethylated β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates **11a,b** and **12a,b** by organocopper reagents.

Entry	Substrate	Reagent	Conditions	Products (Yield %)
1	**11a**	Pr^{i}Cu(CN)MgCl·BF$_3$ (4.2 equiv.)	- 78 °C, 30 min	**17a** (70) + **18** (22)^a
2	**11a**	Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$ (4.2 equiv.)	- 78 °C, 30 min	**17a** (38) + **18** (26)^a
3	**11a**	Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl (4.2 equiv.)	- 78 °C, 30 min	**17a** (68) + **18** (31)^a
4	**11a**	Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl (4.2 equiv.)	- 78 °C, 30 min	**17a** (58) + **18** (27)^a
5	**11b**	Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl (4 equiv.)	- 78 °C, 30 min	**17b** (95)
6	**11b**	Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl (4 equiv.)	- 78 °C, 30 min	**17b** (95)
7	**12a**	Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl (4 equiv.)	- 78 °C, 30 min, then 0 °C, 3 h	-^{b}
8	**12a**	Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl (4 equiv.)	- 78 °C, 30 min, then 0 °C, 3 h	**19a** (75) + **20a** (20)^a
9	**12b**	Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl (4 equiv.)	- 78 °C, 30 min, then 0 °C, 3 h	-^{b}
10	**12b**	Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl (4 equiv.)	- 78 °C, 30 min, then 0 °C, 3 h	**19b** (37) + **20b** (11)^a
11	**12b**	Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl (6 equiv.)	- 78 °C, 30 min, then 0 °C, 3 h	**19b** (55) + **20b** (19)^a
12	**12b**	Pr$^i_3$Cu(CN)(MgCl)$_3$·BF$_3$·2LiCl (4 equiv.)	- 78 °C, 30 min, then 0 °C, 3 h	**19b** (52) + **20b** (25)^a

^{a}The product ratios were determined by reverse phase HPLC. ^{b}The starting material was recovered.

NMR spectrum of **14a** was in good accordance with that of the enantiomer[5] derived from another precursor. Based both on a 220 nm negative Cotton effect in its CD spectrum[19] and on regiochemical assignment by ^{1}H NMR spectroscopy, the α-adduct **15** was proved to be the Z-isomer of an *anti*-S$_N$2' product, L-Phe-ψ[(Z)-CH=CH]-L-Val-type isostere. As such, except for the formation of a Z-isomer **15** from **10a**, each reaction using organocopper reagents derived from Grignard reagents yielded the respective single diastereomers regio- and stereoselectively out of four possible α-alkylated products.

Synthesis of ψ[(E)-CMe=CH]- and ψ[(E)-CMe=CMe]-type Alkene Dipeptide Isosteres via Organocopper-mediated Alkylation. To extend the application of regio- and stereoselective alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates by organocopper reagents, the author sought to synthesize ψ[(E)-CMe=CH]- and ψ[(E)-

CMe=CMe]-type EADIs having multisubstituted alkenes (Scheme 5 and Table 2). As a model system, syntheses of D-Phe-ψ[(*E*)-CMe=CH]-L-Val-, D-Phe-ψ[(*E*)-CMe=CMe]-L-Val-type EADIs and their diastereomers were attempted. Such structures represent a potential (*i*+1) - (*i*+2) scaffold of type II' β-turn found in the cyclic RGD peptide, cyclo(-Arg-Gly-Asp-D-Phe-Val-).[20] As employed for the synthesis of ψ[(*E*)-CH=CH]-type EADIs from 1,3-oxazolidin-2-ones **10a,b**, isopropylcyanocuprate-BF$_3$ complexes were prepared from Pr^{i}MgCl and used for alkylations.

Similarly to the reaction of *trans*-1,3-oxazolidin-2-one **10a**, treatment of monomethylated (γ-methylated) *trans*-1,3-oxazolidin-2-one **11a** with Pr^{i}Cu(CN)MgCl·BF$_3$ in THF at - 78 °C for 30 min afforded α-alkylated products as both the *E*-isomer, D-Phe-ψ[(*E*)-CMe=CH]-L-Val **17a**, and the *Z*-isomer, D-Phe-ψ[(*Z*)-CMe=CH]-D-Val **18** (**17a:18** = 76:24, Table 2, entry 1). Additionally, reaction of **11a** with Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$ also gave α-alkylated products **17a** and **18** in low selectivity (entry 2). The use of LiCl-containing complexes such as Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl and Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl in the reaction of **11a** somewhat improved the combined yield of α-alkylated products (entries 3 and 4).[21] From the *cis*-isomer **11b**, only the *E*-isomer of the α-alkylated product, D-Phe-ψ[(*E*)-CMe=CH]-D-Val **17b**, was obtained in excellent yields either by treatment with Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl or with Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl in THF at - 78 °C for 30 min (entries 5 and 6).

In contrast, dimethylated (β,γ-dimethylated) 1,3-oxazolidin-2-ones **12a,b** displayed different reactivity to organocopper reagents than those of the non-methylated **10a,b** and monomethylated ones **11a,b**. For both isomers of 1,3-oxazolidin-2-ones **12a,b**, no reaction was observed with the "lower-order" cyanocuprate-BF$_3$ complex [Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl], rather, starting materials were recovered (entries 7 and 9). This was probably due to the low reactivity of the reagent. Alternatively, alkylation of *trans*-1,3-oxazolidin-2-one **12a** with the "higher-order" cyanocuprate-BF$_3$ complex [Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl] at - 78 °C for 30 min then at 0 °C for 3 h, afforded two isomeric α-alkylated products, D-Phe-ψ[(*E*)-CMe=CMe]-L-Val **19a** and D-Phe-ψ[(*Z*)-CMe=CMe]-D-Val **20a**. The selectivity of this reaction was similar to that in reactions of *trans*-1,3-oxazolidin-2-ones **10a** and **11a** (**19a:20a** = 79:21, entry 8). On the other hand, treatment of the *cis*-isomer **12b** with four equivalents of Pr$^i_2$Cu(CN)(MgCl)$_2$·BF$_3$·2LiCl gave both the expected *E*-isomer, D-Phe-ψ[(*E*)-CMe=CMe]-D-Val **19b**, as well as the *Z*-isomer, D-Phe-ψ[(*Z*)-CMe=CMe]-L-Val **20b**, in low yield (**19b:20b** = 76:24). Substrate **12b** was recovered in 13% yield (entry 10). Use of six equivalents of the same reagent or four equivalents of Pr$^i_3$Cu(CN)(MgCl)$_3$·BF$_3$·2LiCl improved the combined
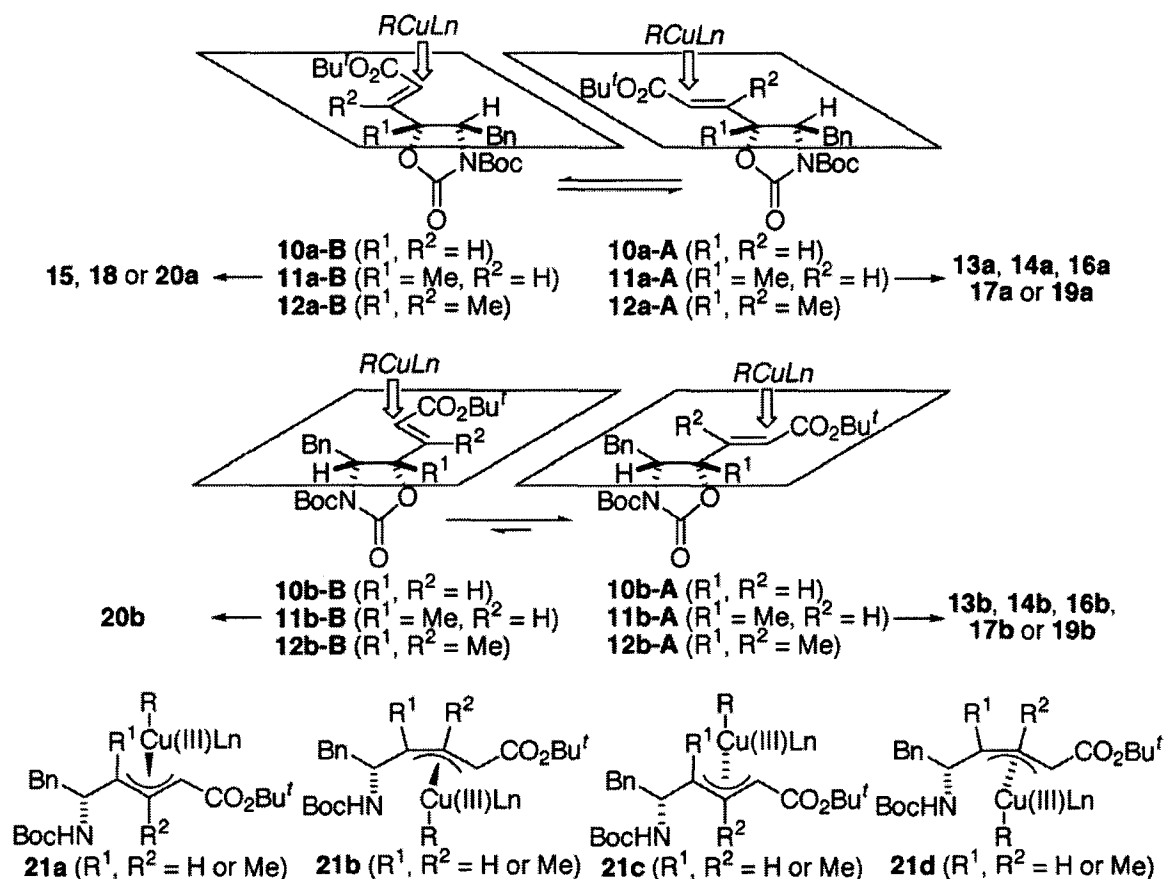
Figure 3. Feasible reaction mechanism in the alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates.[23]

yields of the reaction to 74 or 77%, respectively, although trace amounts of reactant **12b** still remained (entries 11 and 12).

Regiochemical assignments of EADIs **17a,b**, **18**, **19a,b** and **20a,b** were accurately established by ^{1}H NMR (^{1}H-^{1}H COSY, NOE experiments and NOESY). For example, an NOE enhancement of the olefinic proton signal upon irradiation of the 5-H resonance in the 3E-isomer **17a** (7%) as well as by irradiation of the 4-methyl proton signal in the 3Z-isomer **18** (15%) were observed. The presence of a cross-peak between the olefinic proton and the 5-H proton and the absence of a cross-peak between the olefinic proton and the 4-methyl protons in the NOESY spectrum of **17b**, suggested 3E-geometry. The lack of NOE enhancement of the 5-H proton signal of **19b** following irradiation of the 2-H proton signal, along with an NOE enhancement of the same signal of **20b** (14%), suggested that **19b** and **20b** had 3E- and 3Z-geometries, respectively. The stereochemistry of the α-alkyl groups of **17a,b**, **18**, **19a,b** and **20a,b** was established by the CD spectra.[19] EADIs **17a**, **19a** and **20b**, which showed negative Cotton effects, were regarded as 2R-isomers, while **17b**, **18**, **19b** and

20a, which showed positive Cotton effects around 220 nm, were regarded as 2S-isomers. Crystal structures of **18**, **19a** and **19b** also supported these assignments. Based on the above, it was concluded that all α-alkylated products **17a,b**, **18**, **19a,b** and **20a,b** were obtained by organocopper-mediated $anti$-S_N2'-type alkylation.

A feasible mechanism for organocopper-mediated alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates was inferred based on the precedence of γ-mesyloxy-α,β-enoates[4] or N-activated γ,δ-epimino-α,β-enoates[6] as shown in Figure 3. Assuming direct alkylation by the organocopper reagents, examination of two possible conformers during the reaction facilitates interpretation of the diastereoselective formation of E- and Z-isomers. For example, alkylation of conformers **10a,b-A**, **11a,b-A** and **12a,b-A** can yield only E-isomers **13a,b**, **14a,b**, **16a,b**, **17a,b** and **19a,b**, while conformers **10a,b-B**, **11a,b-B** and **12a,b-B** would yield only Z-isomers such as **15**, **18** and **20a,b**. It might be supposed that the product ratios of E- and Z-isomers depend on the proportions of these two conformers, the stability of which is based on the cis/$trans$-configuration of the 1,3-oxazolidin-2-ones and the presence of methyl groups.[22] In addition, the unlikely formation of reactive conformers **12b-A** and **12b-B** of dimethylated cis-1,3-oxazolidin-2-one **12b** due to 1,3-allylic strain, may cause low reactivity to organocopper reagents. This could result in the production of Z-isomer **20b**, which does not occur with the non- and monomethylated substrates **10b** and **11b**. On the other hand, the formation of copper-π-allyl complexes as reactive intermediates such as **21a-d** cannot be ruled out. In such a mechanism, two complexes **21a** and **21b**, which can afford E- and Z-isomers of α-alkylated products, respectively, would be required to form from $trans$-1,3-oxazolidin-2-ones **10-12a** in a ratio dependent on their stability. Similarly, two complexes **21c** and **21d** should be formed from cis-1,3-oxazolidin-2-ones **10-12b**. In fact, in the alkylation of the non-methylated $trans$-1,3-oxazolidin-2-one **10a**, formation of the Z-isomer **15** of the α-alkylated product was observed only by treatment with Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl (but not the methyl and benzyl counterparts). Bulky alkyl groups such as an isopropyl group, may lead to favourable formation of other copper-π-allyl complexes such as **21b**, which can provide the Z-isomer **15**. In both mechanisms, organocopper-mediated alkylation of 1,3-oxazolidin-2-ones **10a,b**, **11a,b** and **12a,b** can afford only $anti$-S_N2'-type products.

In conclusion, the author found that alkylation of β-(N-Boc-2-oxo-1,3-oxazolidin-5-yl)-α,β-enoates with organocyanocuprates derived from Grignard reagents proceeds with ring-opening and decarboxylation to afford α-alkylated products in regio- and stereoselective

fashion. The stereochemistry at the α-positions of the α-alkylated products depends on chirality at the 5-position of the 1,3-oxazolidin-2-one derivatives. The reactions proceed with complete *anti*-S_N2'-type chirality transfer regardless of E/Z-products. Finally, the author achieved the first synthesis of $\psi[(E)$-CMe=CH]- and $\psi[(E)$-CMe=CMe]-type alkene dipeptide isosteres starting from chiral amino acid derivatives by application of organocopper-mediated alkylation. To summarize, the reaction of 1,3-oxazolidin-2-one derivatives with organocopper reagents yields (L,D)-/(D,L)-type E- and (L,L)-/(D,D)-type Z-alkene isosteres from *trans*-starting material, while *cis*-isomers yield (L,L)-/(D,D)-type E- and (L,D)-/(D,L)-type Z-alkene isosteres.

Experimental Section

General. ^{1}H NMR spectra were recorded in CDCl$_3$ using a JEOL EX-270, a Bruker AC 300 or a Bruker AM 600 spectrometer at 270, 300 or 600 MHz. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane and coupling constants (J) are given in Hz. Nominal (LRMS) and exact mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 or JMS-HX/HX 110A mass spectrometer. Optical rotations were measured for samples in CHCl$_3$ with a Horiba high-sensitivity polarimeter SEPA-200 (Kyoto, Japan). CD spectra were recorded on a JASCO J-720 spectropolarimeter. The X-ray analysis was carried out on a Rigaku AFC5R-RU200 four-circle diffractometer. Melting points were measured on a hot stage melting point apparatus and are uncorrected. For flash chromatography, Silica Gel 60 H (silica gel for thin-layer chromatography, Merck) and Wakogel C-200 (silica gel for column chromatography) were employed. For HPLC separations, a Cosmosil 5C18-ARII analytical (4.6 × 250 mm) column was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA aqueous solution (v/v) and 0.1% TFA in MeCN (v/v) was used for HPLC elution.

General procedure for *syn*-selective synthesis of amino alcohols 4a and 5a from amide 1: (3*R*,4*R*)-4-(*tert*-butoxycarbonylamino)-3-methyl-5-phenylpent-1-en-3-ol 4a. To a stirred solution of Boc-D-Phe-NMe(OMe) 1 (25.0 g, 81.1 mmol) in dry THF (250 cm^3) was added dropwise a solution of MeMgBr (1.00 mol dm^{-3}, 243 cm^3, 243 mmol) in dry THF at - 78 °C under argon, and the mixture was stirred for 1.5 h at - 78 °C and for 1.5 h at 0 °C. The reaction was quenched with saturated citric acid at - 78 °C and the whole was extracted with EtOAc. The extract was washed successively with water, 5% NaHCO$_3$ and brine, and dried over MgSO$_4$. Concentration under reduced pressure gave a crude ketone, which was used immediately in next step without further purification. To a stirred suspension of anhydrous CeCl$_3$ (59.9 g, 243 mmol) and the above ketone in dry THF (625 cm^3) was added dropwise a solution of vinyl magnesium chloride (2.60 mol dm^{-3}, 62.3 cm^3, 162 mmol) in dry THF at 0 °C under argon. After 1.5 h, the reaction was quenched with saturated citric acid at - 78 °C. The mixture was concentrated under reduced pressure and extracted with EtOAc. The extract was washed successively with water, 5% NaHCO$_3$ and brine, and dried over MgSO$_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (4:1) gave the amino alcohol 4a and its isomer 4b (8.76 g, 30.0 mmol, 37%). Compound 4a was not isolated by recrystallization and flash chromatography over silica gel, 4a containing small amount of 4b was used for the next step without further purification.

(3*R*,4*R*)-4-(*tert*-Butoxycarbonylamino)-2,3-dimethyl-5-phenylpent-1-en-3-ol   5a. By use of a procedure similar to that described for the preparation of the amino alcohol 4a and 4b from 1, the amide 1 (25.0 g, 81.1 mmol) was converted into the title compound 5a and its isomer 5b (11.8 g, 38.6 mmol, 47%). The major isomer 5a was isolated by repeated recrystallization.

Compound **5a**, colourless crystals, mp 107-109 °C [from *n*-hexane-Et$_2$O (3:1)] (Found: C, 70.55; H, 8.71; N, 4.64. C$_{18}$H$_{27}$NO$_3$ requires C, 70.79; H, 8.91; N, 4.59%); [α]$_D^{26}$ + 95.0 (*c* 1.04); δ$_H$ (270 MHz) 1.30 (9 H, s, CMe$_3$), 1.39 (3 H, s, CMe), 1.82 (3 H, s, CMe), 2.74 (1 H, m, C*H*H), 3.02 (1 H, dd, *J* 14.1 and 3.3, CH*H*), 3.36 (1 H, s, OH), 3.79 (1 H, m, CH), 4.60 (1 H, d, *J* 8.5, NH), 4.90 (1 H, m, C*H*H=), 5.08 (1 H, m, CH*H*=), 7.15-7.35 (5 H, m, Ph).

General procedure for synthesis of *anti*-amino alcohols 4b and 5b from amino alcohols 2 and 3: (3*S*,4*R*)-4-(*tert*-butoxycarbonylamino)-3-methyl-5-phenylpent-1-en-3-ol

4b. To a stirred solution of oxalyldichloride (9.44 cm^3, 108 mmol) in CH_2Cl_2 (60 cm^3) was added dropwise a solution of DMSO (15.3 cm^3, 216 mmol) in CH_2Cl_2 (30 cm^3) at - 78 °C under argon. After 30 min, a solution of the alcohol **2** (15.0 g, 54.1 mmol) in CH_2Cl_2 (60 cm^3) was added to the above mixture at - 78 °C, and the mixture was stirred for 30 min at this temperature. To the mixture was added dropwise (Pr^i)$_2$NEt (75.3 cm^3, 432 mmol) at - 78 °C, and the stirring was continued for 1 h at 0 °C. The reaction was quenched with saturated NH_4Cl (40 cm^3), and the whole was extracted with Et_2O. The extract was washed successively with 5% citiric acid and brine, and dried over $MgSO_4$. Concentration under reduced pressure gave the corresponding enone, which was used for the next reaction without further purification. To a stirred suspension of anhydrous $CeCl_3$ (62.2 g, 252 mmol) in dry THF (600 cm^3) was added MeMgCl (3.00 mol dm^{-3}, 72.1 cm^3, 216 mmol) in dry THF at 0 °C under argon, and the mixture was stirred for 1.5 h. A solution of the above enone in dry THF (100 cm^3) was added dropwise to the mixture at 0 °C. After 2 h, the reaction was quenched with saturated citric acid. The mixture was concentrated under reduced pressure and extracted with EtOAc. The extract was washed successively with water, 5% $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexane-EtOAc (4:1) gave the mixture of the amino alcohols **4a** and **4b** (5.05 g, 17.3 mmol, 32%). The major isomer **4b** was isolated by repeated recrystallization.

Compound **4b**, colourless crystals, mp 138-139 °C [from n-hexane-Et$_2$O (3:1)] [Found (FAB): (M + H)$^+$, 292.1909. $C_{17}H_{26}NO_3$ requires M + H, 292.1913]; [α]$_D^{21}$ + 24.0 (c 0.982); δ_H (300 MHz, at 320 K) 1.28 (9 H, br, CMe$_3$), 1.34 (3 H, s, CMe), 2.41 (1 H, m, OH), 2.55 (1 H, m, CHH), 3.01 (1 H, dd, J 14.2 and 3.3, CHH), 3.75 (1 H, m, CH), 4.54 (1 H, m, NH), 5.20 (1 H, dd, J 10.7 and 1.2, CHH=) 5.37 (1 H, dd, J 17.2 and 1.2, CHH=), 5.98 (1 H, dd, J 17.2 and 10.7, CH=), 7.12-7.29 (5 H, m, Ph); m/z (FAB-LRMS) 292 (MH$^+$, base peak), 236, 218, 164, 157, 120, 91, 73 and 57.

(3S,4R)-4-(*tert*-Butoxycarbonylamino)-2,3-dimethyl-5-phenylpent-1-en-3-ol 5b.

By use of a procedure similar to that described for the preparation of the amino alcohol **4a** and **4b** from **2**, the amino alcohol **3** (17.0 g, 58.3 mmol) was converted into the title compound **5a** and **5b** (9.00 g, 29.4 mmol, 50%).

Compound **5b**, colourless crystals, mp 130-132 °C [from n-hexane-Et$_2$O (3:1)] (Found: C, 70.91; H, 9.14; N, 4.54. $C_{18}H_{27}NO_3$ requires C, 70.79; H, 8.91; N, 4.59%); [α]$_D^{27}$ + 16.4 (c 0.426); δ_H (300 MHz, at 320 K) 1.29 (9 H, br, CMe$_3$), 1.34 (3 H, s. CMe), 1.85 (3 H, m, CMe), 1.94 (1 H, br, OH), 2.54 (1 H, m, CHH), 2.85 (1 H, dd, J 14.4 and 3.1, CHH), 3.95 (1 H, m, CH), 4.67 (1 H, m, NH), 4.95 (1 H, m, CHH=), 5.17 (1 H, m, CHH=), 7.11-7.28 (5 H, m, Ph).

(4S,5S)-4-Benzyl-*N*-(*tert*-butoxycarbonyl)-5-ethenyl-1,3-oxazolidin-2-one 7a.

To a stirred suspension of NaH (1.58 g, 66.0 mmol) in dry THF (40 cm^3) was added dropwise a solution of the known allyl alcohol **6a**^{15} (4.58 g, 16.5 mmol) in dry THF (60 cm^3) at 0 °C under argon, and the mixture was heated under reflux for 15 min. (Boc)$_2$O (7.20 g, 33.0 mmol) was added to the mixture at 0 °C, and the mixture was stirred for 1.5 h with warming to room temperature. The mixture was poured into a saturated NH_4Cl solution at 0 °C, and the whole was extracted with EtOAc. The extract was washed successively with water, saturated $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexane-EtOAc (4:1) gave the title compound **7a** (4.36 g, 14.3 mmol, 87%) as colourless crystals, mp 89-91 °C [from n-hexane-Et$_2$O (3:1)] (Found: C, 67.39; H, 6.96; N, 4.50. $C_{17}H_{21}NO_4$ requires C, 67.31; H, 6.98; N, 4.62%); [α]$^{21}_D$ -

33.8 (c 0.708); δ_H (300 MHz) 1.58 (9 H, s, CMe$_3$), 2.84 (1 H, dd, J 13.4 and 9.6, CHH), 3.34 (1 H, dd, J 13.4 and 3.6, CHH), 4.17 (1 H, dt, J 9.6 and 3.1, NCH), 4.62 (1 H, m, OCH), 5.10-5.21 (2 H, m, CH$_2$=), 5.62 (1 H, ddd, J 17.2, 10.4 and 5.6, CH=), 7.15-7.38 (5 H, m, Ph).

(4S,5R)-4-Benzyl-N-(tert-butoxycarbonyl)-5-ethenyl-1,3-oxazolidin-2-one 7b. By use of a procedure similar to that described for the preparation of the 1,3-oxazolidin-2-one 7a from 6a, the alcohol 6b[15] (1.77 g, 6.38 mmol) was converted into the title compound 7b (1.53 g, 5.04 mmol, 79%) as colourless crystals, mp 64-66 °C [from n-hexane-Et$_2$O (3:1)] (Found: C, 67.15; H, 6.98; N, 4.57. C$_{17}$H$_{21}$NO$_4$ requires C, 67.31; H, 6.98; N, 4.62%); [α]$_D^{20}$ - 15.6 (c 0.957); δ_H (300 MHz) 1.42 (9 H, s, CMe$_3$), 2.96-3.02 (2 H, m, CH$_2$), 4.56-4.67 (1 H, m, NCH), 4.98-5.06 (1 H, m, OCH), 5.36 (1 H, dt, J 10.6 and 1.3, CHH=), 5.49 (1 H, dt, J 17.1 and 1.3, CHH=), 5.76 (1 H, ddd, J 17.1, 10.6 and 5.69, CH=), 7.15-7.35 (5 H, m, Ph).

(4R,5R)-4-Benzyl-N-(tert-butoxycarbonyl)-5-ethenyl-5-methyl-1,3-oxazolidin-2-one 8a. By use of a procedure similar to that described for the preparation of the 1,3-oxazolidin-2-one 7a from 6a, the alcohol 4a (9.16 g, 31.4 mmol) was converted into the title compound 8a (8.57 g, 27.0 mmol, 85%) as colourless crystals, mp 147-148 °C (from EtOAc) (Found: C, 68.00; H, 7.35; N, 4.38. C$_{18}$H$_{23}$NO$_4$ requires C, 68.12; H, 7.30; N, 4.41%); [α]$_D^{19}$ + 33.6 (c 0.802); δ_H (300 MHz) 1.39 (3 H, s, CMe), 1.41 (9 H, s, CMe$_3$), 2.96 (1 H, dd, J 14.2 and 8.3, CHH), 3.12 (1 H, dd, J 14.2 and 5.7, CHH), 4.37 (1 H, dd, J 8.3 and 5.7, CH), 5.16 (1 H, d, J 10.8, CHH=), 5.34 (1 H, d, J 17.1, CHH=), 5.76 (1 H, dd, J 17.1 and 10.8, CH=), 7.20-7.36 (5 H, m, Ph).

(4R,5S)-4-Benzyl-N-(tert-butoxycarbonyl)-5-ethenyl-5-methyl-1,3-oxazolidin-2-one 8b. By use of a procedure similar to that described for the preparation of the 1,3-oxazolidin-2-one 7a from 6a, the alcohol 4b (1.80 g, 6.18 mmol) was converted into the title compound 8b (1.80 g, 5.67 mmol, 92%) as colourless crystals, mp 116-117 °C [from n-hexane-Et$_2$O (3:1)] (Found: C, 68.12; H, 7.27; N, 4.17. C$_{18}$H$_{23}$NO$_4$ requires C, 68.12; H, 7.30; N, 4.41%); [α]$_D^{20}$ + 38.7 (c 1.00); δ_H (270 MHz) 1.41-1.50 (12 H, m, CMe and CMe$_3$), 2.82 (1 H, dd, J 13.8 and 8.5, CHH), 2.99 (1 H, dd, J 14.1 and 5.2, CHH), 4.28 (1 H, m, CH), 5.27 (1 H, dd, J 10.2 and 1.9, CHH=), 5.50 (1 H, dd, J 17.1 and 1.9, CHH=), 5.61 (1 H, dd, J 17.1 and 10.2, CH=), 7.14-7.33 (5 H, m, Ph).

(4R,5R)-4-Benzyl-N-(tert-butoxycarbonyl)-5-methyl-5-(propen-2-yl)-1,3-oxazolidin-2-one 9a. By use of a procedure similar to that described for the preparation of the 1,3-oxazolidin-2-one 7a from 6a, the alcohol 5a (4.81 g, 15.7 mmol) was converted into title compound 9a (5.12 g, 15.4 mmol, 98%) as colourless crystals, mp 130-132 °C [from n-hexane-Et$_2$O (3:1)] (Found: C, 68.65; H, 7.64; N, 4.02. C$_{19}$H$_{25}$NO$_4$ requires C, 68.86; H, 7.60; N, 4.23%); [α]$_D^{27}$ + 40.1 (c 1.02); δ_H (270 MHz) 1.39 (3 H, s, CMe), 1.43 (9 H, s, CMe$_3$), 1.64 (3 H, s, CMe), 2.96 (1 H, dd, J 14.1 and 8.5, CHH), 3.13 (1 H, dd, J 14.1 and 5.2, CHH), 4.46 (1 H, dd, J 8.5 and 5.2, CH), 4.90 (1 H, s, CHH=), 5.08 (1 H, s, CHH=), 7.20-7.37 (5 H, m, Ph).

(4R,5S)-4-Benzyl-N-(tert-butoxycarbonyl)-5-methyl-5-(propen-2-yl)-1,3-oxazolidin-2-one 9b. By use of a procedure similar to that described for the preparation of the 1,3-oxazolidin-2-one 7a from 6a, the alcohol 5b (8.50 g, 27.8 mmol) was converted into the title compound 9b (8.67 g, 26.1 mmol, 94%) as colourless crystals, mp 106-107 °C [from n-hexane-Et$_2$O (3:1)] (Found: C, 68.88; H, 7.83; N, 4.41. C$_{19}$H$_{25}$NO$_4$ requires C, 68.86; H, 7.60;

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N, 4.23%); $[\alpha]_D^{20}$ + 78.0 (c 1.05); δ_H (270 MHz) 1.37 (9 H, s, CMe$_3$), 1.46 (3 H, s, CMe), 1.50 (3 H, s, CMe), 2.80 (2 H, d, J 6.9, CH$_2$), 4.27 (1 H, t, J 6.9, CH), 5.07 (1 H, m, CHH=), 5.34 (1 H, m, CHH=), 7.15-7.32 (5 H, m, Ph).

***tert*-Butyl (2*E*)-3-[(4*S*,5*S*)-4-benzyl-*N*-(*tert*-butoxycarbonyl)-2-oxo-1,3-oxazolidin-5-yl]prop-2-enoate 10a.** Ozone gas was bubbled through a stirred solution of the 1,3-oxazolidin-2-one **7a** (4.26 g, 14.0 mmol) in EtOAc (150 cm^3) at - 78 °C until a blue colour persisted. Me$_2$S (20.6 cm^3, 280 mmol) was added to the solution at - 78 °C. After being stirred for 30 min at 0 °C, the mixture was dried over MgSO$_4$, and concentrated under reduced pressure to give the crude aldehyde, which was used for the next reaction without further purification. To a stirred suspension of LiCl (1.48 g, 35.1 mmol) in MeCN (50 cm^3) was added *tert*-butyl diethyl phosphonoacetate (8.34 cm^3, 35.1 mmol) and (Pr^i)$_2$NEt (6.11 cm^3, 35.1 mmol) at 0 °C under argon. After 1 h, the above aldehyde in MeCN (35 cm^3) was added to the mixture at 0 °C, and the stirring was continued for 2 h. The mixture was concentrated under reduced pressure and extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, 5% NaHCO$_3$ and brine, and dried over MgSO$_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (6:1) gave the title compound **10a** (3.98 g, 9.86 mmol, 70%) as colourless crystals, mp 157-159 °C [from *n*-hexane-Et$_2$O (3:1)] (Found: C, 65.44; H, 7.21; N, 3.34. C$_{22}$H$_{29}$NO$_6$ requires C, 65.49; H, 7.24; N, 3.47%); $[\alpha]_D^{20}$ - 74.7 (c 0.976); δ_H (300 MHz) 1.44 (9 H, s, CMe$_3$), 1.59 (9 H, s, CMe$_3$), 2.82 (1 H, dd, J 13.4 and 9.8, CHH), 3.37 (1 H, dd, J 13.4 and 3.6, CHH), 4.22 (1 H, ddd, J 9.8, 3.5 and 2.9, NCH), 4.74 (1 H, ddd, J 4.5, 2.8 and 1.7, OCH), 5.86 (1 H, dd, J 15.6 and 1.7, CH=), 6.42 (1 H, dd, J 15.6 and 4.5, CH=), 7.15-7.40 (5 H, m, Ph).

***tert*-Butyl (2*E*)-3-[(4*S*,5*R*)-4-benzyl-*N*-(*tert*-butoxycarbonyl)-1,3-oxazolidin-2-on-5-yl]prop-2-enoate 10b.** By use of a procedure similar to that described for the preparation of the ester **10a** from **7a**, the 1,3-oxazolidin-2-one **7b** (1.48 g, 4.87 mmol) was converted into the title compound **10b** (800 mg, 1.98 mmol, 40%) as colourless crystals, mp 163-165 °C [from *n*-hexane-EtOAc (3:1)] (Found: C, 65.22; H, 7.09; N, 3.41. C$_{22}$H$_{29}$NO$_6$ requires C, 65.49; H, 7.24; N, 3.47%); $[\alpha]_D^{24}$ + 22.4 (c 1.02); δ_H (300 MHz) 1.48 (9 H, s, CMe$_3$), 1.49 (9 H, s, CMe$_3$), 2.84 (1 H, dd, J 14.1 and 8.8, CHH), 3.03 (1 H, dd, J 14.1 and 4.7, CHH), 4.68 (1 H, ddd, J 8.8, 7.1 and 4.7, NCH), 5.12 (1 H, ddd, J 7.0, 4.7 and 1.8, OCH), 6.11 (1 H, dd, J 15.6 and 1.8, CH=), 6.53 (1 H, dd, J 15.6 and 4.6, CH=), 7.12-7.34 (5 H, m, Ph).

***tert*-Butyl (2*E*)-3-[(4*R*,5*R*)-4-benzyl-*N*-(*tert*-butoxycarbonyl)-5-methyl-1,3-oxazolidin-2-on-5-yl]prop-2-enoate 11a.** By use of a procedure similar to that described for the preparation of the ester **10a** from **7a**, the 1,3-oxazolidin-2-one **8a** (3.93 g, 12.3 mmol) was converted into the title compound **11a** (3.45 g, 8.26 mmol, 66%) as colourless crystals, mp 175-177 °C [from *n*-hexane-Et$_2$O (3:1)]; (Found: C, 65.91; H, 7.53; N, 3.20. C$_{23}$H$_{31}$NO$_6$ requires C, 66.17; H, 7.48; N, 3.35%); $[\alpha]_D^{26}$ + 122.8 (c 0.692); δ_H (300 MHz) 1.41 (3 H, s, CMe), 1.45 (9 H, s, CMe$_3$), 1.46 (9 H, s, CMe$_3$), 2.97 (1 H, dd, J 14.4 and 9.0, CHH), 3.19 (1 H, dd, J 14.4 and 4.8, CHH), 4.43 (1 H, dd, J 9.0 and 4.8, CH), 6.00 (1 H, d, J 15.5, CH=), 6.62 (1 H, d, J 15.5, CH=), 7.21-7.36 (5 H, m, Ph).

***tert*-Butyl (2*E*)-3-[(4*R*,5*S*)-4-benzyl-*N*-(*tert*-butoxycarbonyl)-5-methyl-1,3-oxazolidin-2-on-5-yl]prop-2-enoate 11b.** By use of a procedure similar to that described for the preparation of the ester **10a** from **7a**, the 1,3-oxazolidin-2-one **8b** (1.52 g, 4.78 mmol) was

converted into the title compound **11b** (1.07 g, 2.56 mmol, 53%) as colourless crystals, mp 169-171 °C [from *n*-hexane-Et$_2$O (3:1)]; (Found: C, 66.17; H, 7.58; N, 3.33. C$_{23}$H$_{31}$NO$_6$ requires C, 66.17; H, 7.48; N, 3.35%); [α]$_D^{19}$ + 5.08 (*c* 0.984); δ$_H$ (300 MHz) 1.45 (3 H, s, CMe), 1.50 (18 H, s, 2 × CMe$_3$), 2.75 (1 H, dd, *J* 14.1 and 9.1, C*H*H), 3.06 (1 H, dd, *J* 14.1 and 4.3, CH*H*), 4.34 (1 H, dd, *J* 9.1 and 4.3, CH), 6.11 (1 H, d, *J* 15.6, CH=), 6.46 (1 H, d, *J* 15.6, CH=), 7.13-7.34 (5 H, m, Ph).

tert-Butyl (2*E*)-3-[(4*R*,5*R*)-4-benzyl-*N*-(*tert*-butoxycarbonyl)-5-methyl-2-oxo-1,3-oxazolidin-5-yl]but-2-enoate 12a. By use of a procedure similar to that described for the successive treatment of the 1,3-oxazolidin-2-one **7a** with ozone gas and Me$_2$S, the 1,3-oxazolidin-2-one **9a** (3.33 g, 10.0 mmol) was converted into the corresponding ketone. To a solution of the above ketone in CHCl$_3$ (20 cm^3) was added Ph$_3$P=CHCO$_2$Bu^t (11.3 g, 30.1 mmol), and the mixture was gently refluxed for 24 h. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (6:1) gave the title compound **12a** (3.08 g, 7.13 mmol, 71%) as colourless crystals, mp 158-159 °C [from *n*-hexane-Et$_2$O (3:1)]; (Found: C, 66.82; H, 7.71; N, 3.24. C$_{24}$H$_{33}$NO$_6$ requires C, 66.80; H, 7.71; N, 3.25%); [α]$_D^{24}$ + 78.3 (*c* 1.06); δ$_H$ (300 MHz) 1.40 (3 H, s, CMe), 1.45 (9 H, s, CMe$_3$), 1.47 (9 H, s, CMe$_3$), 1.90 (3 H, d, *J* 1.2, CMe), 2.98 (1 H, dd, *J* 14.3 and 9.1, C*H*H), 3.18 (1 H, dd, *J* 14.3 and 4.5, CH*H*), 4.48 (1 H, dd, *J* 9.1 and 4.5, CH), 5.95 (1 H, m, CH=), 7.23-7.38 (5 H, m, Ph).

tert-Butyl (2*E*)-3-[(4*R*,5*S*)-4-benzyl-*N*-(*tert*-butoxycarbonyl)-5-methyl-1,3-oxazolidin-2-on-5-yl]but-2-enoate 12b. By use of a procedure identical with that described for the preparation of the α,β-enoate **12a** from **9a**, the 1,3-oxazolidin-2-one **9b** (1.60 g, 4.82 mmol) was quantitatively converted to the corresponding ketone (1.60 g, 4.79 mmol) as colourless oil by successive treatment with ozone gas and Me$_2$S, and purification by flash chromatography over silica gel with *n*-hexane-EtOAc (6:1). To a solution of the above ketone (790 mg, 2.37 mmol) in CHCl$_3$ (5 cm^3) was added Ph$_3$P=CHCO$_2$Bu^t (4.02 g, 10.6 mmol), and the mixture was gently refluxed for 4 days. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (6:1) gave the title compound **12b** (412 mg, 0.954 mmol, 40%) as colourless crystals, mp 184-186 °C [from *n*-hexane-Et$_2$O (3:1)]; (Found: C, 66.56; H, 7.61; N, 3.31. C$_{24}$H$_{33}$NO$_6$ requires C, 66.80; H, 7.71; N, 3.25%); [α]$_D^{20}$ + 50.6 (*c* 1.06); δ$_H$ (300 MHz) 1.38 (9 H, s, CMe$_3$), 1.50 (9 H, s, CMe$_3$), 1.53 (3 H, s, CMe), 1.76 (3 H, d, *J* 1.2, CMe), 2.76 (1 H, dd, *J* 14.2 and 6.8, C*H*H), 2.82 (1 H, dd, *J* 14.2 and 6.8, CH*H*), 4.35 (1 H, t, *J* 6.8, CH), 6.16 (1 H, d, *J* 1.2, CH=), 7.10-7.32 (5 H, m, Ph).

tert-Butyl (2*Z*)-3-[(4*R*,5*S*)-4-benzyl-*N*-(*tert*-butoxycarbonyl)-5-methyl-1,3-oxazolidin-2-on-5-yl]but-2-enoate 12c. To a stirred solution of (Cy)$_2$NH (0.268 cm^3, 1.35 mmol) in THF (2.1 cm^3) was added dropwise a solution *n*-BuLi in *n*-hexane (1.56 mol dm^{-3}, 0.865 cm^3, 1.35 mmol) at - 78 °C, and the mixture was stirred for 15 min at this temperature. To the mixture was added Me$_3$SiCH$_2$CO$_2$Bu^t (0.295 cm^3, 1.35 mmol) at - 78 °C. After 10 min, a solution of the ketone (150 mg, 0.450 mmol, prepared from **9b** by the similar procedure described above) in THF (0.5 cm^3) was added dropwise to the mixture, and the mixture was stirred overnight with warming up to room temperature. The reaction was quenched with saturated citric acid and the whole was extracted with EtOAc. The extract was washed successively with brine, saturated NaHCO$_3$ and brine, and dried over MgSO$_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (6:1) gave the title compound **12c** (26.0 mg, 0.0602 mmol, 13%) as colourless crystals,

mp 139-141 °C [from n-hexane-Et$_2$O (3:1)]; (Found: C, 66.69; H, 7.77; N, 3.28. C$_{24}$H$_{33}$NO$_6$ requires C, 66.80; H, 7.71; N, 3.25%); $[\alpha]_D^{20}$ + 57.1 (c 0.385); δ_H (300 MHz) 1.29 (9 H, s, CMe$_3$), 1.52 (9 H, s, CMe$_3$), 1.65 (3 H, s, CMe), 1.94 (3 H, d, J 1.3, CMe), 2.52 (1 H, dd, J 13.9 and 8.6, CHH), 3.10 (1 H, dd, J 13.8 and 3.7, CHH), 5.15 (1 H, dd, J 8.4 and 3.6, CH), 5.82 (1 H, d, J 1.4, CH=), 7.08-7.30 (5 H, m, Ph).

General procedure for the synthesis of $\psi[(E)$-CH=CH]- and $\psi[(E)$-CMe=CH]-type alkene dipeptide isosteres via "lower-order" cyanocuprate-mediated alkylation: synthesis of *tert*-butyl (2S,5S,3E)-5-(*tert*-butoxycarbonylamino)-2-methyl-6-phenylhex-3-enoate (Boc-L-Phe-$\psi[(E)$-CH=CH]-D-Ala-OBu^t) 13a. To a stirred suspension of CuCN (44.4 mg, 0.496 mmol) and LiCl (42.0 mg, 0.992 mmol) in dry THF (1.5 cm^3) was added MeMgCl in dry THF (2.92 mol dm^{-3}, 0.169 cm^3, 0.496 mmol) under argon at -78 °C, and the mixture was stirred for 10 min at 0 °C. BF$_3$·Et$_2$O (0.0628 cm^3, 0.496 mmol) was added to the above mixture at - 78 °C, and the mixture was stirred for 5 min. To the solution of organocopper reagent was added dropwise a solution of the ester 10a (50.0 mg, 0.124 mmol) in dry THF (1.8 cm^3) at - 78 °C. After being stirred for 30 min, the reaction mixture was quenched with a 1:1 saturated NH$_4$Cl-28% NH$_4$OH solution (2 cm^3). The mixture was extracted with Et$_2$O, and then the extract was washed with water, and dried over MgSO$_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexane-EtOAc (15:1) yielded the title compound 13a (36.1 mg, 0.0961 mmol, 77%) as colourless crystals, mp 64-66 °C [from n-hexane-Et$_2$O (10:1)]; (Found: C, 70.63; H, 8.83; N, 3.66. C$_{22}$H$_{33}$NO$_4$ requires C, 70.37; H, 8.86; N, 3.73%); $[\alpha]_D^{25}$ +19.2 (c 0.987); δ_H (600 MHz) 1.14 (3 H, d, J 6.9, CMe), 1.40 (9 H, s, CMe$_3$), 1.41 (9 H, s, CMe$_3$), 2.79 (1 H, dd, J 13.3 and 6.9, CHH), 2.82-2.88 (1 H, m, CHH), 2.97 (1 H, dd, J 14.2 and 7.1, 2-H), 4.38 (1 H, m, 5-H), 4.45 (1 H, m, NH), 5.47 (1 H, dd, J 15.5 and 5.5, CH=), 5.57 (1 H, dd, J 15.5 and 7.5, CH=), 7.13-7.30 (5 H, m, Ph).

tert-Butyl (2R,5S,3E)-5-(*tert*-butoxycarbonylamino)-2-methyl-6-phenylhex-3-enoate (Boc-L-Phe-$\psi[(E)$-CH=CH]-L-Ala-OBu^t) 13b. By use of a procedure similar to that described for the preparation of the ester 13a from 10a, treatment of the ester 10b (50.0 mg, 0.124 mmol) with MeCu(CN)MgCl·BF$_3$·2LiCl in dry THF gave the title compound 13b (42.3 mg, 0.112 mmol, 90%) as a colourless oil, [Found (FAB): (M + H)$^+$, 376.2474. C$_{22}$H$_{34}$NO$_4$ requires M + H, 376.2488]; $[\alpha]_D^{23}$ - 17.4 (c 0.747); δ_H (300 MHz) 1.16 (3 H, d. J 7.0, CMe), 1.40 (9 H, s, CMe$_3$), 1.41 (9 H, s, CMe$_3$), 2.82 (2 H, d, J 6.0, CH$_2$), 2.97 (1 H, m, 2-H), 4.33-4.47 (2 H, m, 5-H and NH), 5.47 (1 H, m, CH=), 5.60 (1 H, m, CH=), 7.12-7.32 (5 H, m, Ph); m/z (FAB-LRMS) 376 (MH$^+$, base peak), 320, 284, 264, 228, 218, 184, 172, 157, 129, 91, 57.

tert-Butyl (2S,5S,3E)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-6-phenylhex-3-enoate (Boc-L-Phe-$\psi[(E)$-CH=CH]-D-Val-OBu^t) 14a and *tert*-butyl (2R,5S,3Z)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-6-phenylhex-3-enoate (Boc-L-Phe-$\psi[(Z)$-CH=CH]-L-Val-OBu^t) 15. By use of a procedure similar to that described for the preparation of the ester 13a from 10a, treatment of the ester 10a (50.0 mg, 0.124 mmol) with Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl in dry THF gave the title compound 14a and 15 (50.3 mg, 0.124 mmol, 99%).

Compound 14a, colourless crystals, mp 78-80 °C [from n-hexane-Et$_2$O (10:1)]; (Found: C, 71.18; H, 9.50; N, 3.42. C$_{24}$H$_{37}$NO$_4$ requires C, 71.43; H, 9.24; N, 3.47%); $[\alpha]_D^{24}$ + 42.0 (c 1.42); δ_H (300 MHz) 0.74 (3 H, d, J 6.7, CMe), 0.86 (3 H, d, J 6.7, CMe), 1.40 (9 H, s, CMe), 1.42 (9 H, s, CMe), 1.86 (1 H, m, CH), 2.50 (1 H, m, 2-H), 2.77 (1 H, dd, J 13.4 and

7.0, C*H*H), 2.87 (1 H, dd, *J* 13.6 and 5.4, CH*H*), 4.32-4.52 (2 H, m, 5-H and NH), 5.39-5.55 (2 H, m, 2 × CH=), 7.12-7.30 (5 H, m, Ph).

Compound **15**, colourless crystals, mp 74-75 °C [from *n*-hexane-Et$_2$O (10:1)]; (Found: C, 71.68; H, 9.16; N, 3.28. C$_{24}$H$_{37}$NO$_4$ requires C, 71.43; H, 9.24; N, 3.47%); Δε = - 11.98 (221 nm, isooctane); [α]$_D$^{27} - 97.1 (*c* 0.422); δ$_H$ (600 MHz) 0.64 (3 H, d, *J* 6.4, CMe), 0.81 (3 H, d, *J* 6.5, CMe), 1.42 (18H, s, 2 × CMe$_3$), 1.74-1.84 (1 H, m, CH), 2.74 (1 H, dd, *J* 13.2 and 7.3, C*H*H), 2.91 (1 H, t, *J* 8.6, 2-H), 2.97 (1 H, m, CH*H*), 4.36-4.60 (2 H, m, 5-H and NH), 5.41 (1 H, dd, *J* 10.8 and 8.5, CH=), 5.45 (1 H, dd, *J* 10.8 and 9.8, CH=), 7.15-7.28 (5 H, m, Ph).

***tert*-Butyl   (2*R*,5*S*,3*E*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-6-phenylhex-3-enoate (Boc-L-Phe-ψ[(*E*)-CH=CH]-L-Val-OBu^t) 14b.** By use of a procedure similar to that described for the preparation of the ester **13a** from **10a**, treatment of the ester **10b** (50.0 mg, 0.124 mmol) with Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl in dry THF gave the title compound **14b** (48.3 mg, 0.119 mmol, 96%), mp 52-54 °C [from *n*-hexane-Et$_2$O (10:1)]; (Found: C, 71.50; H, 9.05; N, 3.33. C$_{24}$H$_{37}$NO$_4$ requires C, 71.43; H, 9.24; N, 3.47%); [α]$_D$^{24} - 42.9 (*c* 1.142); δ$_H$ (300 MHz) 0.79 (3 H, d, *J* 6.7, CMe), 0.88 (3 H, d, *J* 6.6, CMe), 1.40 (9 H, s, CMe$_3$), 1.41 (9 H, s, CMe$_3$), 1.80-1.98 (1 H, m, CH), 2.49 (1 H, t, *J* 8.5, 2-H), 2.75-2.91 (2 H, m, CH$_2$), 4.30-4.50 (2 H, m, 5-H and NH), 5.42 (1 H, dd, *J* 15.5 and 5.3, CH=), 5.51 (1 H, dd, *J* 15.5 and 8.4, CH=), 7.16-7.31 (5 H, m, Ph).

***tert*-Butyl   (2*S*,5*S*,3*E*)-2-benzyl-5-(*tert*-butoxycarbonylamino)-6-phenylhex-3-enoate (Boc-L-Phe-ψ[(*E*)-CH=CH]-D-Phe-OBu^t) 16a.** By use of a procedure similar to that described for the preparation of the ester **13a** from **10a**, treatment of the ester **10a** (50.0 mg, 0.124 mmol) with BnCu(CN)MgCl·BF$_3$·2LiCl in dry THF gave the title compound **16a** (55.4 mg, 0.122 mmol, 99%) as a colourless oil, [Found (FAB): (M + H)$^+$, 452.2790. C$_{28}$H$_{38}$NO$_4$ requires *M* + H, 452.2801]; [α]$_D$^{26} + 22.7 (*c* 1.27); δ$_H$ (600 MHz) 1.32 (9 H, s, CMe$_3$), 1.40 (9 H, s, CMe$_3$), 2.71 (1 H, dd, *J* 13.6 and 6.9, C*H*H), 2.77 (2 H, m, 6-CH$_2$), 2.95 (1 H, dd, *J* 13.6 and 8.3, CH*H*), 3.15 (1 H, m, 2-H), 4.20-4.60 (2 H, m, 5-H and NH), 5.42 (1 H, m, CH=), 5.56 (1 H, dd, *J* 15.7 and 8.2, CH=), 7.05-7.28 (10 H, m, Ph) ; *m/z* (FAB-LRMS) 452 (MH$^+$, base peak), 340, 294, 261, 248, 233, 204, 117, 91, 57, 41.

***tert*-Butyl   (2*R*,5*S*,3*E*)-2-benzyl-5-(*tert*-butoxycarbonylamino)-6-phenylhex-3-enoate (Boc-L-Phe-ψ[(*E*)-CH=CH]-L-Phe-OBu^t) 16b.** By use of a procedure similar to that described for the preparation of the ester **13a** from **10a**, treatment of the ester **10b** (50.0 mg, 0.124 mmol) with BnCu(CN)MgCl·BF$_3$·2LiCl in dry THF gave the title compound **16b** (55.4 mg, 0.122 mmol, 99%) as a colourless oil, [Found (FAB): (M + H)$^+$, 452.2815. C$_{28}$H$_{38}$NO$_4$ requires *M* + H, 452.2801]; [α]$_D$^{26} - 44.7 (*c* 1.02); δ$_H$ (600 MHz) 1.32 (9 H, s, CMe$_3$), 1.40 (9 H, s, CMe$_3$), 2.70 (2 H, m, C*H*H and 6-C*H*H), 2.79 (1 H, m, 6-CH*H*), 2.97 (1 H, dd, *J* 13.6 and 8.0, CH*H*), 3.13 (1 H, m, 2-H), 4.37 (2 H, m, 5-H and NH), 5.38 (1 H, dd, *J* 15.5 and 5.4, CH=), 5.55 (1 H, dd, *J* 15.4 and 8.4, CH=), 7.06-7.36 (10 H, m, Ph); *m/z* (FAB-LRMS) 452 (MH$^+$, base peak), 340, 294, 248, 233, 204, 164, 129, 117, 91, 57, 41.

***tert*-Butyl   (2*R*,5*R*,3*E*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-4-methyl-6-phenylhex-3-enoate   (Boc-D-Phe-ψ[(*E*)-CMe=CH]-L-Val-OBu^t)   17a   and   *tert*-butyl (2*S*,5*R*,3*Z*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-4-methyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*Z*)-CMe=CH]-D-Val-OBu^t) 18.** By use of a procedure similar to that described for the preparation of the ester **13a** from **10a**, treatment of the ester **11a** (50.0 mg,

0.119 mmol) with Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl in dry THF gave the title compound **17a** (34.0 mg, 0.0816 mmol, 68%) and **18** (15.6 mg, 0.0375 mmol, 31%),

Compound **17a**, colourless crystals, mp 81-83 °C (from *n*-hexane); (Found: C, 71.76; H, 9.45; N, 3.36. C$_{25}$H$_{39}$NO$_4$ requires C, 71.91; H, 9.41; N, 3.35%); Δε = - 8.32 (218 nm, isooctane); [α]$_D$^{26} - 40.1 (*c* 1.14); δ$_H$ (300 MHz) 0.66 (3 H, d, *J* 6.7, CMe), 0.85 (3 H, d, *J* 6.6, CMe), 1.38 (9 H, s, CMe$_3$), 1.42 (9 H, s, CMe$_3$), 1.66 (3 H, d, *J* 1.1, CMe), 1.83 (1 H, m, CH), 2.76 (1 H, dd, *J* 9.8 and 8.8, 2-H), 2.82 (2 H, d, *J* 7.1, CH$_2$), 4.25 (1 H, m, 5-H), 4.56 (1 H, m, NH), 5.23 (1 H, m, CH=), 7.11-7.29 (5 H, m, Ph).

Compound **18**, colourless crystals, mp 112-113 °C (from *n*-hexane); (Found: C, 71.89; H, 9.35; N, 3.37. C$_{25}$H$_{39}$NO$_4$ requires C, 71.91; H, 9.41; N, 3.35%); Δε = + 17.01 (222 nm, isooctane); [α]$_D$^{27} + 92.4 (*c* 1.44); δ$_H$ (300 MHz) 0.63 (3 H, d, *J* 5.3, CMe), 0.81 (3 H, d, *J* 6.6, CMe), 1.38 (9 H, s, CMe$_3$), 1.43 (9 H, s, CMe$_3$), 1.63-1.78 (4 H, m, CMe and CH), 2.75 (1 H, dd, *J* 13.5 and 7.2, C*H*H), 2.86-2.98 (2 H, m, CH*H* and 2-H), 4.58 (1 H, m, NH), 4.67 (1 H, m, 5-H), 5.24 (1 H, d, *J* 9.3, CH=), 7.14-7.29 (5 H, m, Ph).

Crystal structure determination of compound 18. Crystal data. C$_{25}$H$_{39}$NO$_4$, *M* = 417.59, orthorhombic, *a* = 12.672(8), *b* = 18.64(1) Å, *c* = 11.128(5) Å, Space Group P2$_1$2$_1$2$_1$ (No.19) , *Z* = 4, μ(CuKα)=5.64 cm^{-1}, 2543 reflections measured, 1177 (*I* > 3.00 σ(I)) were used in all calculations. The final *wR* was 0.115.

***tert*-Butyl (2*S*,5*R*,3*E*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-4-methyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*E*)-CMe=CH]-D-Val-OBu^t) 17b.** By use of a procedure similar to that described for the preparation of the ester **13a** from **10a**, treatment of the ester **11b** (50.0 mg, 0.119 mmol) with Pr^{i}Cu(CN)MgCl·BF$_3$·2LiCl in dry THF gave the title compound **17b** (47.6 mg, 0.113 mmol, 95%) as colourless crystals, mp 69-71 °C (from *n*-hexane); (Found: C, 71.71; H, 9.25; N, 3.29. C$_{25}$H$_{39}$NO$_4$ requires C, 71.91; H, 9.41; N, 3.35%); Δε = + 6.17 (225 nm, isooctane); [α]$_D$^{24} + 55.6 (*c* 0.953); δ$_H$ (600 MHz) 0.79 (3 H, d, *J* 6.6, CMe), 0.89 (3 H, d, *J* 6.6, CMe), 1.37 (9 H, s, CMe$_3$), 1.38 (9 H, s, CMe$_3$), 1.66 (3 H, d, *J* 1.1, CMe), 1.89 (1 H, m, CH), 2.72-2.92 (3 H, m, CH$_2$ and 2-H), 4.32 (1 H, m, 5-H), 4.55 (1 H, m, NH), 5.28 (1 H, d, *J* 9.9, CH=), 7.12-7.32 (5 H, m, Ph).

General procedure for the synthesis of ψ[(*E*)-CMe=CMe]-type alkene dipeptide isosteres via "higher-order" cyanocuprate-mediated alkylation: *tert*-butyl (2*R*,5*R*,3*E*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-3,4-dimethyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*E*)-CMe=CMe]-L-Val-OBu^t) **19a** and *tert*-butyl (2*S*,5*R*,3*Z*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-3,4-dimethyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*Z*)-CMe=CMe]-D-Val-OBu^t) **20a.** To a stirred solution of LiCl (32.2 mg, 0.760 mmol) and CuCN (34.0 mg, 0.380 mmol) in dry THF (1.5 cm^3) was added dropwise Pr^{i}MgCl (1.50 mol dm^{-3}, 0.506 cm^3, 0.760 mmol) in dry THF at - 78 °C under argon, and the mixture was stirred for 10 min at 0 °C. BF$_3$·Et$_2$O (0.0481 cm^3, 0.380 mmol) was added to the mixture at - 78 °C. After 5 min, to the above reagent was added dropwise a solution of the ester **12a** (41.0 mg, 0.0950 mmol) in dry THF (1.5 cm^3) at - 78 °C, and stirring was continued for 30 min at - 78 °C. The reaction was quenched with saturated NH$_4$Cl-28% NH$_4$OH (1:1, 2 cm^3), and the whole was extracted with Et$_2$O. The extract was washed with water, and dried over MgSO$_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with *n*-hexane-EtOAc (25:1) gave the title compound **20a** (8.1 mg, 0.0187 mmol, 20%) and **19a** (30.8 mg, 0.0713 mmol, 75%), in order of elution.

Compound **19a**, colourless crystals, mp 128-130 °C (from *n*-hexane); (Found: C, 72.40; H, 9.85; N, 3.26. $C_{26}H_{41}NO_4$ requires C, 72.35; H, 9.57; N, 3.25%); $\Delta\varepsilon = -36.54$ (229 nm, isooctane); $[\alpha]_D^{26} - 176.3$ (*c* 0.726); δ_H (300 MHz) 0.32 (3 H, d, *J* 6.2, CMe), 0.87 (3 H, d, *J* 6.3, CMe), 1.38 (9 H, s, CMe$_3$), 1.39 (9 H, s, CMe$_3$), 1.55 (3 H, s, CMe), 1.68 (3 H, m, CMe), 1.93 (1 H, m, CH), 2.66 (1 H, dd, *J* 13.3 and 8.8, C*H*H), 2.86-2.97 (2 H, m, CH*H* and 2-H), 4.70 (1 H, m, NH), 4.89 (1 H, m, 5-H), 7.12-7.28 (5 H, m, Ph).

Compound **20a**, colourless crystals, mp 59-61 °C (from *n*-hexane); (Found: C, 72.25; H, 9.56; N, 3.12. $C_{26}H_{41}NO_4$ requires C, 72.35; H, 9.57; N, 3.25%); $\Delta\varepsilon = +32.47$ (228 nm, isooctane); $[\alpha]_D^{26} +182.9$ (*c* 1.72); δ_H (300 MHz) 0.31 (3 H, d, *J* 6.1, CMe), 0.85 (3 H, d, *J* 6.3, CMe), 1.38 (9 H, s, CMe$_3$), 1.39 (9 H, s, CMe$_3$), 1.59 (3 H, d, *J* 0.9, CMe), 1.66 (3 H, d, *J* 0.9, CMe), 2.03 (1 H, m, CH), 2.78 (1 H, dd, *J* 13.3 and 7.4, C*H*H), 2.95-3.05 (2 H, m, CH*H* and 2-H), 4.53 (1 H, m, NH), 4.92 (1 H, m, 5-H), 7.12-7.28 (5 H, m, Ph).

Crystal structure determination of compound 19a. Crystal data. $C_{26}H_{41}NO_4$, $M = 431.61$, orthorhombic, $a = 8.570(6)$ Å, $b = 47.121(7)$ Å, $c = 6.602(8)$ Å, Space Group $P2_12_12_1$ (No.19), $Z = 4$, $\mu(CuK\alpha) = 5.64$ cm^{-1}, 2708 reflections measured, 999 ($I > 3.00$ $\sigma(I)$) were used in all calculations. The final *wR* was 0.043.

tert-**Butyl** **(2*S*,5*R*,3*E*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-3,4-dimethyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*E*)-CMe=CMe]-D-Val-OBu^t) 19b and *tert*-butyl (2*R*,5*R*,3*Z*)-5-(*tert*-butoxycarbonylamino)-2-isopropyl-3,4-dimethyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*Z*)-CMe=CMe]-L-Val-OBu^t) 20b.** By use of a procedure similar to that described for the preparation of the ester **19a** and **20a** from **12a**, treatment of the ester **12b** (50.0 mg, 0.115 mmol) with $Pr^i_3Cu(CN)(MgCl)_3·BF_3·2LiCl$ in dry THF at - 78 °C for 30 min and at 0 °C for 3 h gave the title compound **19b** (26.0 mg, 0.0603 mmol, 52%) and **20b** (12.3 mg, 0.0286 mmol, 25%).

Compound **19b**, colourless crystals, mp 111-113 °C (from *n*-hexane); (Found: C, 72.32; H, 9.48; N, 3.12. $C_{26}H_{41}NO_4$ requires C, 72.35; H, 9.57; N, 3.25%); $\Delta\varepsilon = +18.09$ (230 nm, isooctane); $[\alpha]_D^{25} + 82.4$ (*c* 0.752); δ_H (600 MHz) 0.72 (3 H, d, *J* 6.3, CMe), 0.92 (3 H, d, *J* 6.3, CMe), 1.36 (9 H, s, CMe$_3$), 1.38 (9 H, s, CMe$_3$), 1.53 (3 H, s, CMe), 1.66 (3 H, s, CMe), 2.06 (1 H, m, CH), 2.70 (1 H, m, C*H*H), 2.81 (1 H, m, CH*H*), 2.97 (1 H, d, *J* 10.7, 2-H), 4.62 (1 H, m, NH), 4.86 (1 H, m, 5-H), 7.14-7.28 (5 H, m, Ph).

Compound **20b**, colourless oil, [Found (FAB): (M + H)$^+$, 432.3112. $C_{26}H_{42}NO_4$ requires *M* + H, 432.3114]; $\Delta\varepsilon = -29.22$ (226 nm, isooctane); $[\alpha]_D^{23} - 188.2$ (*c* 0.696); δ_H (600 MHz) 0.76 (3 H, d, *J* 6.6, CMe), 0.99 (3 H, d, *J* 6.4, CMe), 1.27 (9 H, s, CMe$_3$), 1.42 (9 H, s, CMe$_3$), 1.59 (3 H, s, CMe), 1.66 (3 H, s, CMe), 2.16 (1 H, m, CH), 2.72 (1 H, m, C*H*H), 2.87 (1 H, m, CH*H*), 3.40 (1 H, m, 2-H), 4.55 (1 H, m, NH), 5.09 (1 H, m, 5-H), 7.17-7.32 (5 H, m, Ph); *m/z* (FAB-LRMS) 432 (MH$^+$, base peak), 340, 284, 259, 228, 184, 164, 91, 57.

Crystal structure determination of compound 19b. Crystal data. $C_{26}H_{41}NO_4$, $M = 431.62$, monoclinic, $a = 10.483(3)$, $b = 10.019(2)$, $c = 13.104(4)$ Å, $\beta = 90.12(2)°$, $U = 1376.3(5)$ Å^3, $T = 295$ K, space group $P2_1$ (No. 4), $Z = 2$, $\mu(Cu-K\alpha) = 0.55$ mm^{-1}, 2229 reflections measured, 1897 [$I > 3.00$ $\sigma(I)$] were used in all calculations. The final $R(F^2)$ was 0.061.

References and Footnotes

1 S. Oishi, H. Tamamura, M. Yamashita, Y. Odagaki, N. Hamanaka, A. Otaka, N. Fujii, *J. Chem. Soc., Perkin Trans. 1*, **2001**, 2445.

2 P. Wipf, T. C. Henninger, S. J. Geib, *J. Org. Chem.*, **1998**, *63*, 6088.

3 (a) R. R. Gardner, G.-B. Liang, S. H. Gellman, *J. Am. Chem. Soc.*, **1995**, *117*, 3280. (b) R. R. Gardner, G.-B. Liang, S. H. Gellman, *J. Am. Chem. Soc.*, **1999**, *121*, 1806.

4 (a) T. Ibuka, H. Habashita, S. Funakoshi, N. Fujii, Y. Oguchi, T. Uyehara, Y. Yamamoto, *Angew. Chem., Int. Ed. Engl.*, **1990**, *29*, 801. (b) T. Ibuka, H. Habashita, A. Otaka, N. Fujii, Y. Oguchi, T. Uyehara, Y. Yamamoto, *J. Org. Chem.*, **1991**, *56*, 4370. (c) T. Ibuka, H. Yoshizawa, H. Habashita, N. Fujii, Y. Chounan, M. Tanaka, Y. Yamamoto, *Tetrahedron Lett.*, **1992**, *33*, 3783. (d) M. Noda, T. Ibuka, H. Habashita, N. Fujii, *Chem. Pharm. Bull.*, **1997**, *45*, 1259.

5 S. Oishi, T. Kamano, A. Niida, M. Kawaguchi, R. Hosotani, M. Imamura, N. Yawata, K. Ajito, H. Tamamura, A. Otaka and N. Fujii, in *Peptide Science 2000*, ed. T. Shioiri, The Japanese Peptide Society, Osaka, 2001, p. 249.

6 (a) T. Ibuka, K. Nakai, H. Habashita, Y. Hotta, N. Fujii, N. Mimura, Y. Miwa, T. Taga, Y. Yamamoto, *Angew. Chem., Int. Ed. Engl.*, **1994**, *33*, 652. (b) N. Fujii, K. Nakai, H. Tamamura, A. Otaka, N. Mimura, Y. Miwa, T. Taga, Y. Yamamoto, T. Ibuka, *J. Chem. Soc., Perkin Trans. 1*, **1995**, 1359. (c) T. Ibuka, N. Mimura, H. Ohno, K. Nakai, M. Akaji, H. Habashita, H. Tamamura, Y. Miwa, T. Taga, N. Fujii, Y. Yamamoto, *J. Org. Chem.*, **1997**, *62*, 2982.

7 (a) P. Wipf, P. C. Fritch, *J. Org. Chem.*, **1994**, *59*, 4875. (b) P. Wipf, T. C. Henninger, *J. Org. Chem.*, **1997**, *62*, 1586.

8 (a) H. Tamamura, M. Yamashita, H. Muramatsu, H. Ohno, T. Ibuka, A. Otaka, N. Fujii, *Chem. Commun.*, **1997**, 2327. (b) H. Tamamura, M. Yamashita, Y. Nakajima, K. Sakano, A. Otaka, H. Ohno, T. Ibuka, N. Fujii, *J. Chem. Soc., Perkin Trans. 1*, **1999**, 2983.

9 (a) N. Fujii, H. Habashita, N. Shigemori, A. Otaka, T. Ibuka, M. Tanaka, Y. Yamamoto, *Tetrahedron Lett.*, **1991**, *32*, 4969. (b) A. Otaka, H. Watanabe, E. Mitsuyama, A. Yukimasa, H. Tamamura, N. Fujii, *Tetrahedron Lett.*, **2001**, *42*, 285. (c) A. Otaka, H. Watanabe, A. Yukimasa, S. Oishi, H. Tamamura, N. Fujii, *Tetrahedron Lett.*, **2001**, *42*, 5443. (d) S. Oishi, T. Kamano, A. Niida, Y. Odagaki, H. Tamamura, A. Otaka, N. Hamanaka, N. Fujii, *Org. Lett.*, **2002**, *4*, 1051.

10 H. J. Mitchell, A. Nelson, S. Warren, *J. Chem. Soc., Perkin Trans. 1*, **1999**, 1899.

11 T. Ibuka, K. Suzuki, H. Habashita, A. Otaka, H. Tamamura, N. Mimura, Y. Miwa, T. Taga, N. Fujii, *J. Chem. Soc., Chem. Commun.*, **1994**, 2151.

12 T. Ibuka, N. Mimura, H. Aoyama, M. Akaji, H. Ohno, Y. Miwa, T. Taga, K. Nakai, H. Tamamura, N. Fujii, Y. Yamamoto, *J. Org. Chem.*, **1997**, *62*, 999.

13 T. Xin, S. Okamoto, F. Sato, *Tetrahedron Lett.*, **1998**, *39*, 6927.

14 J. G. Knight, S. W. Ainge, A. M. Harm, S. J. Harwood, H. I. Maughan, D. R. Armour, D. M. Hollinshead, A. A. Jaxa-Chamiec, *J. Am. Chem. Soc.*, **2000**, *122*, 2944.

15 G. J. Hanson, T. Lindberg, *J. Org. Chem.*, **1985**, *50*, 5399.

16 S. J. Hocart, M. V. Nekola, D. H. Coy, *J. Med. Chem.*, **1988**, *31*, 1820.

17 M. A. Blanchette, W. Choy, J. T. Davis, A. P. Essenfeld, S. Masamune, W. R. Roush, T. Sakai, *Tetrahedron Lett.*, **1984**, *25*, 2183.

18 A small amount of Z-isomer formation by organocopper-mediated alkylation has previously been reported: H. Yang, X. C. Sheng, E. M. Harrington, K. Ackermann, A. M. Garcia, M. D. Lewis, *J. Org Chem.*, **1999**, *64*, 242.

19 T. Ibuka, H. Habashita, S. Funakoshi, N. Fujii, K. Baba, M. Kozawa, Y. Oguchi, T. Uyehara, Y. Yamamoto, *Tetrahedron: Asymmetry*, **1990**, *1*, 379.

20 R. Haubner, D. Finsinger, H. Kessler, *Angew. Chem., Int. Ed. Engl.*, **1997**, *36*, 1374.

21 (a) G. Hallnemo, C. Ullenius, *Tetrahedron Lett.*, **1986**, *27*, 395. (b) B. H. Lipshutz, S. Whitney, J. A. Kozlowski, C. M. Breneman, *Tetrahedron Lett.*, **1986**, *27*, 4273. (c) B. H. Lipshutz, E. L. Ellsworth, S. H. Dimock, *J. Am. Chem. Soc.*, **1990**, *112*, 5869.

22 In this plausible mechanism, the nearly equal rates in the direct alkylations of the two conformers of 1,3-oxazolidin-2-ones or in reactions via two copper-π-allyl complexes are assumed. Taking the Curtin-Hammett principle into consideration, product distribution may depend on the relative rates of reaction of the two conformers.

23 Enantiomers of **10a,b** are depicted for easy understanding.

Section 1. Synthesis and Evaluation of Cyclic Peptidomimetics Having ψ[(E)-CH=CH]-, and ψ[(E/Z)-CH=CMe]-type Alkene Dipeptide Isosteres

Summary

The first application to conformational research on bioactive peptides of ψ[(E)-CH=CMe]- and ψ[(Z)-CH=CMe]-type alkene dipeptide isosteres corresponding to dipeptides having one N-methylamino acid are described. The two isosteres, D-Phe-ψ[(E)-CH=CMe]-L-Val and D-Phe-ψ[(Z)-CH=CMe]-L-Val, which corresponded to trans- and cis-conformers of D-Phe-L-MeVal, respectively, were utilized in a structure-activity relationship (SAR) study on cyclic RGD peptides 1 and 2, in conjunction with a ψ[(E)-CH=CH]-type isostere, D-Phe-ψ[(E)-CH=CH]-L-Val. The cyclic isostere-containing pseudopeptides 3, 4, and 5 were synthesized and biological activity against integrin $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptors were evaluated.

1 X = -CO-NH-
2 X = -CO-NMe-
3 X = -(E)-CH=CH-
4 X = -(E)-CH=CMe-
5 X = -(Z)-CH=CMe-
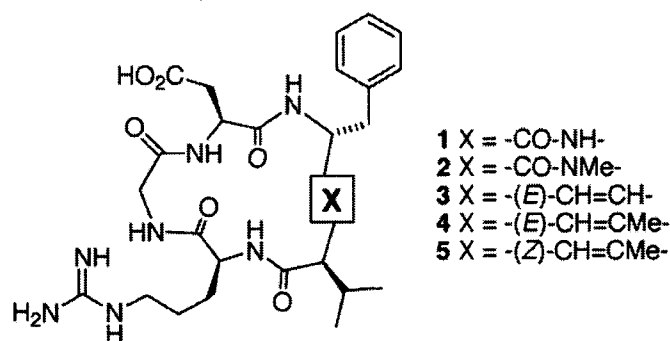
Figure 1

Integrins are α and β subunit-containing cell membrane receptors that participate in cell-cell and cell-matrix adhesive interactions.[1] Among these receptors, $\alpha_v\beta_3$ integrin receptors are particularly involved in a number of biological processes, including tumor-induced angiogenesis[2] and adhesion of osteoclasts to bone matrix.[3] Thus, $\alpha_v\beta_3$ integrin antagonists are being developed as potential therapeutic agents for tumor metastasis, osteoporosis, and other diseases. A significant number of drug candidates have been designed based on the RGD
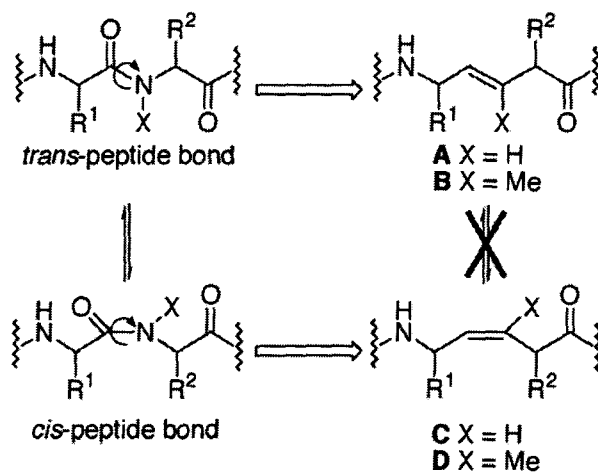
Figure 2

(Arg-Gly-Asp) sequence, which is a key integrin receptor recognition motif.[4] In 1991, Kessler et al. reported that the cyclic pentapeptide, cyclo(-Arg-Gly-Asp-D-Phe-Val-) **1**, was a potent integrin $\alpha_v\beta_3$ antagonist.[5] Their extensive conformational research also revealed that peptide **1** adopts a representative type II' β/γ structure, in which the D-Phe and Gly residues were located at the $i + 1$ positions of β- and γ-turns in dimethyl sulfoxide, respectively (Figure 1). Additionally, N-methylamino acid scanning of **1** demonstrated that substitution of Val with N-methylvaline remarkably increased antagonistic activity against $\alpha_v\beta_3$ integrin, resulting in improved selectivity relative to $\alpha_{IIb}\beta_3$ integrin.[6] The selective $\alpha_v\beta_3$ antagonist, cyclo(-Arg-Gly-Asp-D-Phe-MeVal-) **2**, is now in phase III clinical trial. In general, imidic peptide bonds N-terminal to N-alkylamino acids such as proline, possess similar energy barriers for cis-trans imide isomerization. This results in greater flexibility that provides for a higher population of cis-peptide bonds ($\omega = 0°$), as compared to usual amidic peptide bonds.[7] It is noteworthy that in H_2O and dimethyl sulfoxide the N-methyl group of L-MeVal in the peptide **2** induces a more flexible $\gamma_i/\gamma/\gamma_i$ arrangement that consists of two inverse γ-turns (γ_i-turns) having Arg and Asp residues located at the $i + 1$ positions with one γ-turn having a Gly residue at the $i + 1$ position.[6] Although peptide backbone secondary structure was discussed in detail, no suggestion was offered whether the flexible conformation in **2** was derived from induction of rotation of the peptide bond between D-Phe and L-MeVal by N-methylation or from altered distribution of side chains proximal to the N-methyl group with the maintenance of a trans-amide conformer of D-Phe-L-MeVal ($\omega = 180°$).

To characterize bioactive conformations of cyclic RGD peptides **1** and **2**, particularly in D-Phe-L-Val and D-Phe-L-MeVal moieties, the author designed two cyclic pseudopeptides **3** and **4** that contain $\psi[(E)\text{-CH=CH}]$- **A** and $\psi[(E)\text{-CH=CMe}]$- **B** type EADIs, respectively
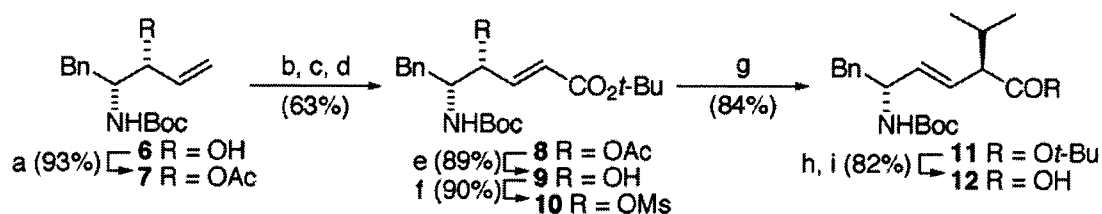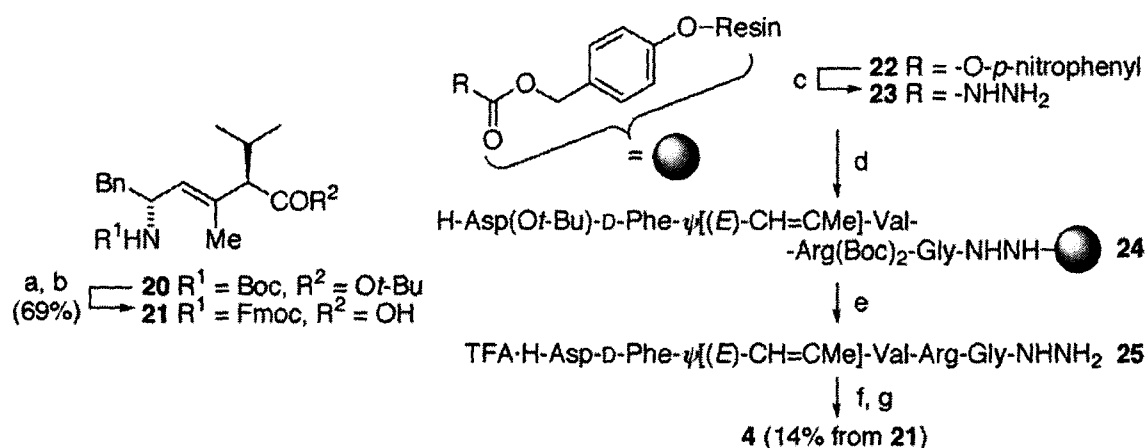
Scheme 1. *Reagents*: (a) Ac₂O, pyridine, DMAP; (b) O₃ gas; (c) Me₂S; (d) (EtO)₂P(O)CH₂CO₂t-Bu, (i-Pr)₂NEt, LiCl; (e) Na₂CO₃, MeOH; (f) MsCl, pyridine; (g) i-PrCu(CN)MgCl·BF₃; (h) TFA; (i) (Boc)₂O, Et₃N.
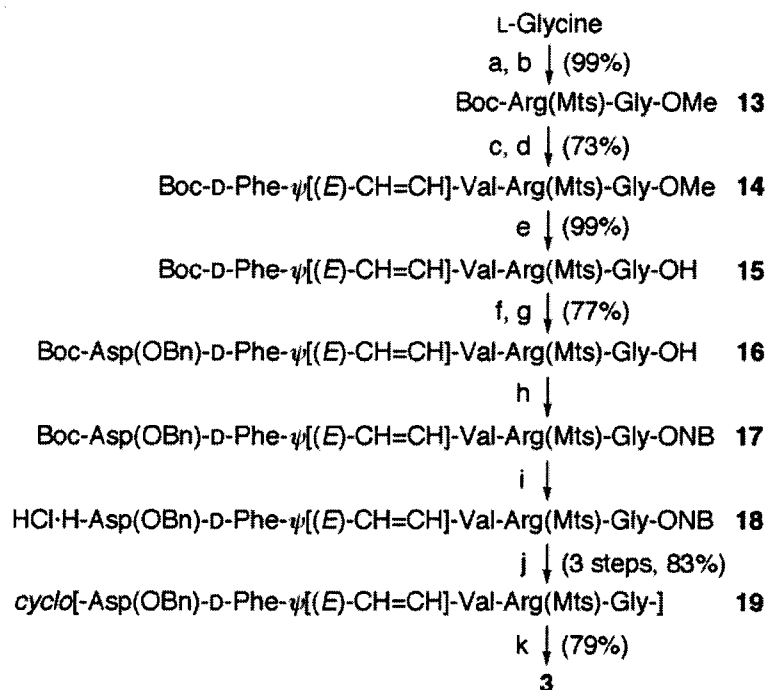
Scheme 2. *Reagents*: (a) SOCl₂, MeOH; (b) Boc-Arg(Mts)-OH, DCC, HOBt, (i-Pr)₂NEt; (c) 4 M HCl/1,4-dioxane, anisole; (d) 12, DCC, HOBt, (i-Pr)₂NEt; (e) LiOH; (f) TFA, anisole; (g) Boc-Asp(OBn)-ONB, (i-Pr)₂NEt; (h) DCC, HONB; (i) 4 M HCl/1,4-dioxane; (j) N-methylmorpholine; (k) 1 M TMSBr-thioanisole/TFA, m-cresol, 1,2-ethanedithiol. *Abbreviations*: HOBt = 1-hydroxybenzotriazole; HONB = N-hydroxy-5-norbornene-2,3-dicarboximide; Mts = 2,4,6-trimethylphenylsulfonyl.

(Figure 2). It was assumed that the D-Phe-ψ[(E)-CH=CH]-L-Val-type EADI in **3** could mimic the *i* + 1 and *i* + 2 sites of β-turn in **1**. The methylated D-Phe-ψ[(E)-CH=CMe]-L-Val-type EADI in **4** can be viewed similarly as an equivalent of the D-Phe-L-MeVal moiety in **2**.[8] In addition, the cyclic pseudopeptide **5** was also designed that possesses a D-Phe-ψ[(Z)-CH=CMe]-L-Val-type isostere representing a potential mimetic of the *cis*-peptide bond between D-Phe and L-MeVal.[8,9] In pseudopeptides **3**, **4** and **5**, alkene isostere-mediated restriction of ω-angle rotations between D-Phe and L-Val/MeVal residues may permit rational evaluation of the N-methyl group's effects on conformations of peptide **2**.
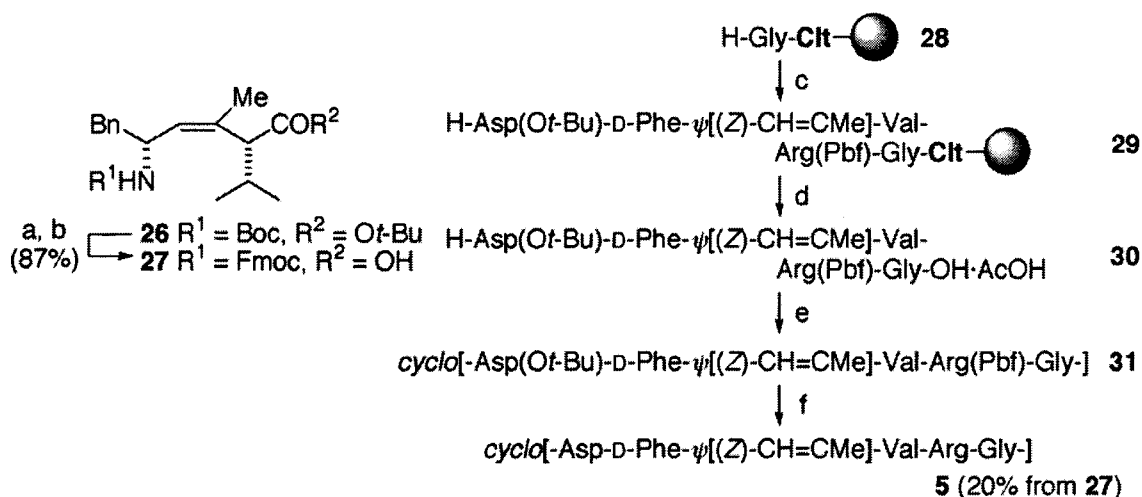
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Scheme 3 structure region:

R—C(=O)—O—CH₂—(benzyl)—O—Resin

$$c \begin{cases} \textbf{22 } R = \text{-}O\text{-}p\text{-nitrophenyl} \\ \textbf{23 } R = \text{-NHNH}_2 \end{cases}$$

(structure at left)

Bn, R¹HN, Me, COR²

$$\begin{array}{l} a, b \\ (69\%) \end{array} \begin{cases} \textbf{20 } R^1 = Boc,\ R^2 = O\text{-}t\text{-Bu} \\ \textbf{21 } R^1 = Fmoc,\ R^2 = OH \end{cases}$$

| d

H-Asp(O*t*-Bu)-D-Phe-ψ[(*E*)-CH=CMe]-Val-Arg(Boc)₂-Gly-NHNH—(resin) **24**

| e

TFA·H-Asp-D-Phe-ψ[(*E*)-CH=CMe]-Val-Arg-Gly-NHNH₂ **25**

| f, g

4 (14% from 21)

Scheme 3. *Reagents*: (a) TFA; (b) Fmoc-OSu, Et₃N; (c) NH₂NH₂·H₂O; (d) Fmoc-based SPPS; (e) TFA; (f) HCl, isoamyl nitrite; (g) (*i*-Pr)₂NEt.

In this section, the author describes an exploration of bioactive conformations of Kessler's cyclic RGD peptides using pseudopeptides containing new ψ[(*E*)-CH=CMe]-type **B** and ψ[(*Z*)-CH=CMe]-type alkene dipeptide isosteres **D** as well as a ψ[(*E*)-CH=CH]-type isostere **A**.

Synthesis of a Cyclic RGD Pseudopeptide Containing D-Phe-ψ[(*E*)-CH=CH]-L-Val-type Alkene Dipeptide Isostere. Initially, the author undertook the synthesis of pseudopeptide **3**, which contains a D-Phe-ψ[(*E*)-CH=CH]-L-Val-type EADI, that is a potential equivalent of the cyclic peptide **1**. Stereoselective synthesis from chiral amino acid derivatives utilizing 1,3-chirality transfer of ψ[(*E*)-CH=CH]-type EADIs **A** having a disubstituted alkene has been fully documented by many researchers. Here reaction of γ-mesyloxy-α,β-enoates using organocopper reagents gave α-alkylated products regio- and stereoselectively by an *anti*-S$_N$2' mechanism.[10] A protected D-Phe-ψ[(*E*)-CH=CH]-L-Val-type EADI **12** was synthesized according to established procedures (Scheme 1).

After protection with an acetyl group of the known allyl alcohol **6**,[11] the resulting acetate **7** was subjected to a sequence of reactions consisting of ozonolysis followed by modified Horner-Wadsworth-Emmons olefination[12] to give γ-acetoxy-α,β-enoate **8** *E*-selectively in 63% yield, which was readily isolated by flash chromatography over silica gel. Alcoholysis of the acetyl group followed by mesylation yielded γ-mesyloxy-α,β-enoate **10**, which is a key substrate for organocopper-mediated alkylation. Treatment of α,β-enoate **10** with *i*-PrCu(CN)MgCl·BF₃ afforded an α-alkylated product, Boc-D-Phe-ψ[(*E*)-CH=CH]-L-Val-O*t*-Bu **11**, as a single diastereomer in 84% yield. Successive TFA treatment and *N*-

H-Gly-Clt—● **28**

$\downarrow$ c

H-Asp(Ot-Bu)-D-Phe-ψ[(Z)-CH=CMe]-Val-Arg(Pbf)-Gly-Clt—● **29**

$\downarrow$ d

H-Asp(Ot-Bu)-D-Phe-ψ[(Z)-CH=CMe]-Val-Arg(Pbf)-Gly-OH·AcOH **30**

$\downarrow$ e

cyclo[-Asp(Ot-Bu)-D-Phe-ψ[(Z)-CH=CMe]-Val-Arg(Pbf)-Gly-] **31**

$\downarrow$ f

cyclo[-Asp-D-Phe-ψ[(Z)-CH=CMe]-Val-Arg-Gly-]
5 (20% from **27**)

Bn, Me, COR², R¹HN

a, b (87%) ⌐ **26** R¹ = Boc, R² = Ot-Bu
           └→ **27** R¹ = Fmoc, R² = OH

Scheme 4. *Reagents*: (a) TFA; (b) Fmoc-OSu, Et₃N; (c) Fmoc-based SPPS; (d) AcOH-TFE-CH₂Cl₂ (1:1:3); (e) DPPA, NaHCO₃; (f) 95% TFA-H₂O. *Abbreviations*: Clt resin = 2-chlorotrityl resin; Pbf = 2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl; DPPA = diphenylphosphoryl azide.

reprotection of **11** provided *N*-Boc-protected EADI **12**, which was utilized for Boc-based solution-phase peptide synthesis of pseudopeptide **3** (Scheme 2). EADI **12** was converted to protected pseudotetrapeptide **14** by DCC condensation with a HCl-treated sample of Boc-Arg(Mts)-Gly-OMe **13**, which was prepared by coupling of Boc-Arg(Mts)-OH and glycine methyl ester. With the intention of avoiding succinimide formation of an aspartic acid moiety by basic treatment, the methyl ester of protected peptide **14** was saponified at this stage to give the corresponding acid, Boc-D-Phe-ψ[(E)-CH=CH]-Val-Arg(Mts)-Gly-OH **15**. TFA treatment of protected peptide **15** and coupling with Boc-Asp(OBn)-ONB provided the linear pseudopentapeptide, Boc-Asp(OBn)-D-Phe-ψ[(E)-CH=CH]-Val-Arg(Mts)-Gly-OH **16**. To facilitate efficient intramolecular peptide bond formation, acid **16** was quantitatively converted to the activated ester, Boc-Asp(OBn)-D-Phe-ψ[(E)-CH=CH]-Val-Arg(Mts)-Gly-ONB **17**. This was subjected to Boc group deprotection using HCl in 1,4-dioxane followed by cyclization in highly dilute solution. The resulting protected cyclic peptide **19** was treated with 1 M TMSBr-thioanisole in TFA in the presence of *m*-cresol and 1,2-ethanedithiol[13] to afford expected cyclic peptide **3**, which was fully characterized by ¹H NMR and mass spectra.

Synthesis of Cyclic RGD Pseudopeptides Containing D-Phe-ψ[(E)-CH=CMe]-L-Val- and D-Phe-ψ[(Z)-CH=CMe]-L-Val-type Alkene Dipeptide Isosteres. The isosteres Boc-D-Phe-ψ[(E)-CH=CMe]-L-Val-Ot-Bu **20** and Boc-D-Phe-ψ[(Z)-CH=CMe]-L-Val-Ot-Bu **26** were utilized for the synthesis of cyclic RGD pseudopeptides **4** and **5**, respectively

(Schemes 3 and 4). These were prepared via *anti*-S_N2' alkylation of β-methylated γ-mesyloxy-α,β-enoates using organocopper reagents.[8] Fmoc-based solid-phase peptide synthesis (SPPS) was employed in order to prevent trisubstituted alkene moieties from isomerizing during strong acid treatments needed for final deprotection in Boc-based synthesis. Isosteres **20** and **26** were converted to the corresponding Fmoc-amino acids **21** and **27** beforehand. For the synthesis of **4**, cyclization of linear peptide precursor was attempted without protection of side chain functional group. A hydrazino linker was constructed on a solid-support prior to peptide synthesis since the C-terminal peptide hydrazide **25** should be selectively activated by the azide method[14] without modification of a carboxylic acid functionality of Asp (Scheme 3). Accordingly, *p*-nitrophenyl carbonate Wang resin **22** was treated with hydrazine hydrate in DMF to afford a resin possessing a hydrazino linker **23**, which could be easily cleaved by TFA treatment to yield a peptide hydrazide. Fmoc-amino acids containing **21** were successively coupled onto the linker using normal Fmoc-based SPPS. Following 95% TFA treatment of the protected peptidyl resin **24**, the resulting peptide hydrazide **25** was cyclized by the azide method in highly diluted DMF solution to yield the cyclic pseudopeptide **4**,[15] which contains a D-Phe-ψ[(*E*)-CH=CMe]-L-Val-type isostere, in 14% yield from **21**.

In contrast, cyclic pseudopeptide **5** was synthesized by well-established cyclic peptide synthesis protocols (Scheme 4). A protected peptide resin **29** was constructed by Fmoc-based SPPS on glycinyl 2-chlorotrityl (Clt) resin **28**, which can provide side chain-protected peptides following mild acidic treatment.[16] Exposing resin **29** to AcOH-TFE-CH_2Cl_2 (1:1:3) provided protected peptide **30**, which was subjected to cyclization using DPPA and $NaHCO_3$ in DMF.[17] Deprotection of protected cyclic peptide **31** using 95% TFA, followed by HPLC purification yielded the expected cyclic pseudopeptide **5** having a D-Phe-ψ[(*Z*)-CH=CMe]-L-Val-type isostere, in 20% yield from the isostere **27**.

Biological Activities of Cyclic RGD Peptides and Pseudopeptides Containing Alkene Dipeptide Isosteres. Alkene moieties of pseudopeptides **3** and **4**, which include ψ[(*E*)-CH=CH]- and ψ[(*E*)-CH=CMe]-type EADIs, respectively, were assumed to exhibit the similar structures ($\omega = 180°$), corresponding to the peptide bonds between D-Phe and L-Val/MeVal in **1** and **2**. It was presumed that a higher potency of methylated analogue **4** as compared to **3** should enable an interpretation of the contribution of conformation to the increased bioactivity of **2**. On the other hand, if bioactivities of pseudopeptides **3** and **4** were equivalent, this showed that comparatively free rotation about the D-Phe-L-MeVal peptide bond might result in increased bioactivity of **2**. Evaluation of the biological activities of the

Table 1. Biological effects of cyclic RGD peptides and pseudopeptides against integrin receptors.

peptide	$\alpha_v\beta_3$ IC$_{50}$ (nM)	Q^b	$\alpha_{IIb}\beta_3$ IC$_{50}$ (nM)	Q^b
RGDS[a]	98	1	270	1
1	6.8	0.069	770	2.9
2	1.4	0.014	280	1.0
3	3.6	0.037	140	0.53
4	3.3	0.034	100	0.37
5	18000	180	370000	1400

[a]A linear peptide RGDS (H-Arg-Gly-Asp-Ser-OH) was used as a standard peptide.
[b]Q values were calculated as $Q = $ IC$_{50}$(peptide)/IC$_{50}$(RGDS).

cyclic pseudopeptides **3**, **4** and **5** against integrin receptors ($\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$) was performed using a competitive binding assay with immobilized $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins, in comparison with Kessler's cyclic RGD peptides **1** and **2** (Table 1). More potent antagonistic activities of pseudopeptides **3** and **4** were observed in comparison with **1** against on $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins. In contrast, pseudopeptides **5**, which contained a $\psi[(Z)$-CH=CMe]-type isostere, showed exceedingly low potency against these receptors. Furthermore, the difference between the activities of the pseudopeptides **3** and **4** was minimal. These results suggest that a *cis*-conformation for the peptide bond in D-Phe-L-Val/MeVal is inappropriate for interaction of cyclic peptides **1** and **2** with integrins. Taken together, it can be concluded that the *N*-methyl group in **2** influences conformation not by simple addition of a methyl group followed by redistribution of close side chains, but by a subtle rotation of the ω-angle of D-Phe-L-MeVal away from an exact *trans*-conformation.[7] From the viewpoint of selectivity against the two integrin receptors, peptide **2** was the most potent agent against $\alpha_v\beta_3$ integrin despite being comparatively less potent against $\alpha_{IIb}\beta_3$ integrin. Overall, it exhibited the best profile. Limited rotation or loss of polarity in the D-Phe-L-Val/MeVal peptide bonds of pseudopeptides **3** and **4** seemed to induce increased affinity for $\alpha_{IIb}\beta_3$ integrin, resulting in the lower selectivities ($\alpha_{IIb}\beta_3/\alpha_v\beta_3$) relative to their peptidic counterparts **1** and **2**.

In conclusion, the synthesis of pseudopeptides **3**, **4**, and **5**, which contain D-Phe-$\psi[(E)$-CH=CH]-L-Val, D-Phe-$\psi[(E)$-CH=CMe]-L-Val, and D-Phe-$\psi[(Z)$-CH=CMe]-L-Val-type isosteres, respectively, was carried out utilizing three separate routes for conformational analysis of Kessler's cyclic RGD peptides **1** and **2**. Antagonistic potencies against integrins were also evaluated. These isosteres are potential dipeptide mimetics of ω-constrained *trans*-

and *cis*-peptide bonds between an amino acid and an *N*-methyl amino acid, respectively. The reduced difference in potencies against $\alpha_v\beta_3$ integrin between cyclic pseudopeptides **3** and **4**, as compared to between cyclic peptides **1** and **2**, suggests that the high potency of **2** may be derived from relatively free rotation about the D-Phe-L-MeVal ω-angle caused by *N*-methylation. These distinctive alkene dipeptide isosteres may be useful tools for exploration of bioactive conformations of peptides containing *N*-methylamino acids.

Experimental Section

General Methods. Melting points are uncorrected. Chemical shifts of the compounds, of which ^{1}H NMR spectra were recorded in CDCl$_3$, are reported in parts per million downfield from internal Me$_4$Si (s = singlet, d = doublet, dd = double doublet, ddd = doublet of double doublet, t = triplet, m = multiplet). Those of the compounds measured in DMF-d_7 and DMSO-d_6 are calibrated to the solvent signal (2.75 and 2.50 ppm, respectively). For flash chromatographies, silica gel 60 H (silica gel for thin-layer chromatography, Merck) and Wakogel C-200 (silica gel for column chromatography) were employed. For HPLC separations, a Cosmosil 5C18-ARII analytical (4.6 × 250 mm, flow rate 1 mL/min) column or a Cosmosil 5C18-ARII preparative (20 × 250 mm, flow rate 11 mL/min) column was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA solution (v/v, solvent A) and 0.1% TFA in MeCN (v/v, solvent B) were used for HPLC elution.

(3R,4R)-O-Acetyl-4-(tert-butoxycarbonylamino)-5-phenylpent-1-en-3-ol (7). To a stirred solution of the alcohol 6[11] (12.9 g, 46.5 mmol), pyridine (75.2 mL, 930 mmol), and DMAP (568 mg, 4.65 mmol) in CHCl$_3$ (50 mL) at 0 °C was added Ac$_2$O (43.8 mL, 465 mmol). The mixture was stirred for 3 h with warming to room temperature. The mixture was poured into water at 0 °C, and the whole was extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, saturated NaHCO$_3$, and brine and dried over MgSO$_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexanes-EtOAc (3:1) gave the title compound 7 (13.8 g, 93% yield) as colorless crystals: mp 60-63 °C (n-hexane/Et$_2$O = 5:1); $[\alpha]_D^{24}$ + 55.2 (c 0.977, CHCl$_3$); ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.38 (s, 9 H), 2.10 (s, 3 H), 2.78 (d, J = 6.9 Hz, 2 H), 4.07 (m, 1 H), 4.67 (m, 1 H), 5.15-5.30 (m, 3 H), 5.79 (ddd, J = 17.4, 10.5, 6.2 Hz, 1 H), 7.08-7.32 (m, 5 H). Anal. Calcd for C$_{18}$H$_{25}$NO$_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.81; H, 8.04; N, 4.31.

tert-Butyl (4R,5R,2E)-4-Acetoxy-5-(tert-butoxycarbonylamino)-6-phenylhex-2-enoate (8). To a solution of the acetate 7 (6.46 g, 20.2 mmol) in EtOAc (250 mL) was bubbled O$_3$ gas at - 78 °C until a blue color persisted. To the above solution was added Me$_2$S (29.7 mL, 404 mmol), and the mixture was stirred for 30 min at 0 °C. The mixture was dried over MgSO$_4$. Concentration under reduced pressure gave an oily aldehyde, which was used immediately in the next step without further purification. To a stirred suspension of LiCl (2.14 g, 50.5 mmol) in MeCN (120 mL) under argon were added (EtO)$_2$P(O)CH$_2$CO$_2$$t$-Bu (12.0 mL, 50.5 mmol) and (i-Pr)$_2$NEt (8.79 mL, 50.5 mmol) at 0 °C. After 20 min, the above aldehyde in MeCN (60 mL) was added to the above mixture at 0 °C, and the mixture was stirred at this temperature for 4 h. The mixture was concentrated under reduced pressure, and the residue was extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, 5% NaHCO$_3$, and brine and dried over MgSO$_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexanes-EtOAc (5:1) gave the title compound 8 (5.38 g, 63% yield) as colorless crystals: mp 101-105 °C (n-hexane/Et$_2$O = 3:1); $[\alpha]_D^{29}$ + 47.8 (c 1.06, CHCl$_3$); ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.38 (s, 9 H), 1.45 (s, 9 H), 2.14 (s, 3 H), 2.78 (d, J = 7.2 Hz, 2 H,), 4.14 (m, 1 H), 4.68 (m, 1 H), 5.37 (m, 1 H), 5.80 (d, J = 15.4 Hz, 1 H), 6.70 (dd, J = 15.4, 5.2 Hz, 1 H), 7.08-7.34 (m, 5 H). Anal. Calcd for C$_{23}$H$_{33}$NO$_6$: C, 65.85; H, 7.93; N, 3.34. Found: C, 65.79; H, 8.23; N, 3.31.

tert-Butyl (4R,5R,2E)-5-(tert-Butoxycarbonylamino)-4-hydroxy-6-phenylhex-2-enoate (9). Powdered Na$_2$CO$_3$ (5.44 g, 51.3 mmol) was added to a solution of the ester 8 (5.38

g, 12.8 mmol) in dry MeOH (30 mL) at room temperature, and the mixture was stirred for 1 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed with water and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexanes-EtOAc (3:1) gave the title compound **9** (4.35 g, 89% yield) as colorless crystals: mp 91-95 °C (n-hexane/Et_2O = 3:1); $[\alpha]_D^{30}$ + 59.3 (c 0.977, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.38 (s, 9 H), 1.46 (s, 9 H), 2.94 (m, 2 H), 3.05 (m, 1 H), 3.81 (m, 1 H), 4.27 (m, 1 H), 4.82 (m, 1 H), 5.99 (dd, J = 15.4, 1.9 Hz, 1 H), 6.81 (dd, J = 15.4, 4.6 Hz, 1 H), 7.18-7.35 (m, 5 H). Anal. Calcd for $C_{21}H_{31}NO_5$: C, 66.82; H, 8.28; N, 3.71. Found: C, 66.71; H, 8.44; N, 3.60.

***tert*-Butyl (4*R*,5*R*,2*E*)-5-(*tert*-Butoxycarbonylamino)-4-methylsulfonyloxy-6-phenylhex-2-enoate (10).** To a stirred mixture of the alcohol **9** (4.35 g, 11.5 mmol) and pyridine (18.5 mL, 230 mmol) in $CHCl_3$ (18 mL) was added dropwise MsCl (8.92 mL, 115 mmol) at 0 °C, and the mixture was stirred for 1 h at this temperature. The mixture was poured into water at 0 °C. The whole was extracted with EtOAc, and the extract was washed successively with saturated citric acid, brine, 5% $NaHCO_3$, brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexanes-EtOAc (3:1) gave the title compound **10** (4.75 g, 90% yield) as colorless crystals: mp 132-135 °C (n-hexane/Et_2O = 3:1); $[\alpha]_D^{27}$ + 58.6 (c 1.07, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.37 (s, 9 H), 1.47 (s, 9 H), 2.79 (dd, J = 13.8, 7.9 Hz, 1 H), 2.96 (dd, J = 13.8, 6.5 Hz, 1 H), 3.06 (s, 3 H), 4.14 (m, 1 H), 4.69 (d, J = 9.2 Hz, 1 H), 5.22 (m, 1 H), 6.02 (d, J = 15.8 Hz, 1 H), 6.79 (dd, J = 15.8, 6.2 Hz, 1 H), 7.20-7.35 (m, 5 H). Anal. Calcd for $C_{22}H_{33}NO_7S$: C, 58.00; H, 7.30; N, 3.07. Found: C, 58.14; H, 7.22; N, 2.86.

***tert*-Butyl (2*R*,5*R*,3*E*)-5-(*tert*-Butoxycarbonylamino)-2-isopropyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*E*)-CH=CH]-L-Val-O*t*-Bu, 11).** To a stirred slurry of CuCN (3.73 g, 41.7 mmol) in THF (50 mL) was added a solution of i-PrMgCl in THF (1.5 M, 27.8 mL, 41.7 mmol) at - 78 °C, and the mixture was stirred for 15 min at 0 °C. BF_3·Et_2O (5.28 mL, 41.7 mmol) was added to the above mixture at - 78 °C. After 5 min, a solution of the mesylate **10** (4.75 g, 10.4 mmol) in dry THF (20 mL) was added dropwise to the above reagent at - 78 °C, and the stirring was continued for 30 min followed by quenching with 20 mL of a 1:1 saturated NH_4Cl-28% NH_4OH solution. The mixture was extracted with Et_2O, and the extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with n-hexanes-EtOAc (5:1) yielded the title compound **11** (3.56 g, 84% yield) as colorless crystals: mp 78-81 °C (n-hexane/Et_2O = 10:1); $[\alpha]_D^{26}$ - 43.0 (c 0.999, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.74 (d, J = 6.7 Hz, 3 H), 0.86 (d, J = 6.6 Hz, 3 H), 1.40 (s, 9 H), 1.42 (s, 9 H), 1.86 (m, 1 H), 2.50 (m, 1 H), 2.77 (dd, J = 13.5, 7.0 Hz, 1 H), 2.87 (dd, J = 13.5, 5.8 Hz, 1 H), 4.33-4.52 (m, 2 H), 5.44-5.50 (m, 2 H), 7.13-7.30 (m, 5 H). Anal. Calcd for $C_{24}H_{37}NO_4$: C, 71.43; H, 9.24; N, 3.47. Found: C, 71.16; H, 9.06; N, 3.42.

(2*R*,5*R*,3*E*)-5-(*tert*-Butoxycarbonylamino)-2-isopropyl-6-phenylhex-3-enoic acid (Boc-D-Phe-ψ[(*E*)-CH=CH]-L-Val-OH, 12). The ester **11** (533 mg, 1.32 mmol) was dissolved in TFA (10 mL) at 0 °C, and the mixture was stirred overnight at room temperature. Concentration under reduced pressure gave an oily residue, which was dissolved in $CHCl_3$-DMF-H_2O (50:9:1, 6 mL). To the mixture were added Et_3N (0.552 mL, 3.96 mmol) and $(Boc)_2O$ (864 mg, 3.96 mmol) at 0 °C, and the mixture was stirred for 3 h at room temperature.

The mixture was concentrated under reduced pressure to give an oily residue, which was acidified with saturated citric acid and extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chlomatography over silica gel with n-hexanes-EtOAc (1:1) gave the title compound **12** (379 mg, 82% yield) as a colorless oil: $[\alpha]_D^{20}$ - 28.5 (c 1.54, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.75 (d, J = 6.2 Hz, 3 H), 0.90 (d, J = 6.6 Hz, 3 H), 1.39 (s, 9 H), 1.93 (m, 1 H), 2.64 (m, 1 H), 2.76 (dd, J = 13.3, 6.6 Hz, 1 H), 2.88 (dd, J = 13.3, 6.2 Hz, 1 H), 4.25-4.70 (m, 2 H), 5.49 (m, 2 H), 7.10-7.30 (m, 5 H); LRMS (FAB), m/z 348 (MH^+, base peak), 292, 256, 248, 246, 230, 200, 185, 164, 156, 149, 129, 91, 57, 41; HRMS (FAB), m/z calcd for $C_{20}H_{30}NO_4$ (MH^+) 348.2175, found: 348.2166.

Boc-Arg(Mts)-Gly-OMe (13). To a stirred dry MeOH (66 mL) was added dropwise $SOCl_2$ (9.70 mL, 133 mmol) at - 78 °C, and the mixture was stirred at room temperature for 1 h. L-Glycine (5.0 g, 66.6 mmol) was added to the mixture, and the mixture was heated under reflux for 3 h. The mixture was concentrated under reduced pressure to give a semisolid, which was dissolved in DMF (200 mL). To the above mixture were added successively (i-Pr)$_2$NEt (23.1 mL, 133 mmol), Boc-Arg(Mts)-OH (22.8 g, 49.9 mmol), HOBt·H_2O (7.64 g, 49.9 mmol), and DCC (15.5 g, 74.9 mmol) at 0 °C, and the mixture was stirred overnight. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give an oily residue, which was extracted with EtOAc. The extract was washed successively with saturated citric acid, brine, 5% $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with EtOAc gave the title compound **13** (26.1 g, 99% yield) as a colorless amorphous semisolid: $[\alpha]_D^{24}$ - 8.80 (c 1.02, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 1.40 (s, 9 H), 1.55-1.73 (m, 3 H), 1.80-1.92 (m, 1 H), 2.26 (s, 3 H), 2.65 (s, 6 H), 3.15-3.35 (m, 2 H), 3.70 (s, 3 H), 3.91 (dd, J = 17.8, 5.5 Hz, 1 H), 4.06 (dd, J = 17.8, 5.9 Hz, 1 H), 4.25 (m, 1 H), 5.55 (d, J = 7.7 Hz, 1 H), 6.19 (br, 1 H), 6.34 (m, 2 H), 6.88 (s, 2 H), 7.48 (t, J = 5.7 Hz, 1 H); LRMS (FAB), m/z 528 (MH^+), 472, 428, 346, 185, 119, 70, 57, 41 (base peak); HRMS (FAB), m/z calcd for $C_{23}H_{38}N_5O_7S$ (MH^+) 528.2492, found: 528.2505.

Boc-D-Phe-ψ[(E)-CH=CH]-Val-Arg(Mts)-Gly-OMe (14). To the protected dipeptide ester **13** (936 mg, 1.77 mmol) were added successively anisole (0.5 mL) and 4 M HCl in 1,4-dioxane (5 mL) at 0 °C, and the mixture was stirred for 1 h at room temperature. The mixture was concentrated under reduced pressure to give an oily residue, which was dissolved in DMF (5 mL). To the solution were added successively (i-Pr)$_2$NEt (0.618 mL, 3.54 mmol), Boc-D-Phe-ψ[(E)-CH=CH]-Val-OH **12** (327 mg, 0.941 mmol), HOBt·H_2O (144 mg, 0.941 mmol), and DCC (290 mg, 1.41 mmol) at 0 °C, and the mixture was stirred overnight. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed successively with 5% $NaHCO_3$, brine, 0.1 N HCl, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with EtOAc gave the title compound **14** (527 mg, 73% yield) as a colorless powder: mp 151-154 °C; $[\alpha]_D^{22}$ - 29.5 (c 1.01, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 0.67 (d, J = 6.4 Hz, 3 H), 0.79 (d, J = 6.5 Hz, 3 H), 1.34 (s, 9 H), 1.45-1.77 (m, 3 H), 1.87-2.02 (m, 2 H), 2.26 (s, 3 H), 2.50 (m, 1 H), 2.65 (s, 6 H), 2.74 (m, 1 H), 2.84 (dd, J = 13.4, 6.7 Hz, 1 H), 3.16-3.25 (m, 2 H), 3.69 (s, 3 H), 3.90 (dd, J = 17.8, 5.6 Hz, 1 H), 4.03 (dd, J = 17.8, 5.7 Hz, 1 H), 4.28 (m, 1 H), 4.50 (m, 1 H), 4.85 (m, 1 H), 5.46-5.54 (m, 2 H), 6.17 (m, 1 H), 6.34 (m, 2 H), 6.80-6.92 (m, 3 H), 7.10-7.26 (m,

5 H), 7.46 (m, 1 H). Anal. Calcd for $C_{38}H_{56}N_6O_8S$: C, 60.30; H, 7.46; N, 11.10. Found: C, 60.02; H, 7.47; N, 10.99.

Boc-D-Phe-ψ[(E)-CH=CH]-Val-Arg(Mts)-Gly-OH (15). To a stirred solution of the protected tetrapeptide ester **14** (470 mg, 0.620 mmol) in MeOH (1 mL) was added 1 N LiOH (1.24 mL, 1.24 mmol) at room temperature. After 3 h, the mixture was acidified with 1 N HCl and concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed with 0.1 N HCl and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with $CHCl_3$-MeOH (10:0 to 9:1) gave the title compound **15** (461 mg, 99% yield) as a colorless amorphous semisolid: $[\alpha]_D^{18}$ - 19.8 (c 5.79, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.63 (d, J = 6.4 Hz, 3 H), 0.75 (d, J = 6.1 Hz, 3 H), 1.32 (s, 9 H), 1.53 (m, 2 H), 1.69 (m, 1 H), 1.89 (m, 2 H), 2.24 (s, 3 H), 2.51 (m, 1 H), 2.61 (m, 7 H), 2.76 (m, 1 H), 3.17 (m, 2 H), 3.82-4.10 (m, 2 H), 4.23 (m, 1 H), 4.52 (m, 1 H), 5.46 (m, 2 H), 6.43 (m, 2 H), 6.85 (s, 2 H), 7.04-7.25 (m, 6 H), 7.74 (m, 1 H); LRMS (FAB), m/z 743 (MH^+, base peak), 681, 561, 414, 185, 119, 91, 70, 57; HRMS (FAB), m/z calcd for $C_{37}H_{55}N_6O_8S$ (MH^+) 743.3802, found: 743.3774.

Boc-Asp(OBn)-D-Phe-ψ[(E)-CH=CH]-Val-Arg(Mts)-Gly-OH (16). DCC (536 mg, 2.60 mmol) was added to the mixture of Boc-Asp(OBn)-OH (646 mg, 2.00 mmol) and N-hydroxy-5-norbornene-2,3-carboxyimide (HONB, 358 mg, 2.00 mmol) in THF (10 mL) at 0 °C, and the mixture was stirred for 1 h at room temperature. The solution was filtrated and the filtrate was concentrated reduced pressure. The residue was dissolved in EtOAc followed by addition of n-hexane to give a powder of Boc-Asp(OBn)-ONB (866 mg), which was used in next step without further purification. To the protected pseudotetrapeptide **15** (470 mg, 0.632 mmol) was added successively anisole (0.5 mL) and TFA (5 mL) at 0 °C, and the mixture was stirred for 2 h at room temperature. The mixture was concentrated under reduced pressure, and the residue was dissolved in DMF (5 mL). To the stirred solution were added successively Et_3N (0.176 mL, 1.26 mmol) and the above Boc-Asp(OBn)-ONB (353 mg, 0.728 mmol), and the stirring was continued overnight at room temperature. Concentration under reduced pressure gave an oily residue, which was acidified with saturated citric acid and extracted with EtOAc. The extract was washed with saturated citric acid, brine, and water. Concentration under reduced pressure followed by flash chromatography over silica gel with $CHCl_3$-MeOH (10:0 to 9:1) gave the title compound **16** (466 mg, 77% yield) as a colorless powder: $[\alpha]_D^{21}$ - 28.5 (c 0.980, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.63-0.92 (m, 6 H), 1.39 (s, 9 H), 1.45-2.12 (m, 5 H), 2.23 (s, 3 H), 2.55-2.87 (m, 11 H), 3.17 (m, 2 H), 3.80-4.11 (m, 2 H), 4.38-4.65 (m, 3 H), 5.00-5.09 (m, 2 H), 5.35-5.47 (m, 1 H), 5.49-5.61 (m, 1 H), 6.10 (m, 1 H), 6.83-6.94 (m, 3 H), 7.06-7.35 (m, 11 H), 7.66 (m, 1 H); LRMS (FAB), m/z 948 (MH^+), 848, 202, 154 (base peak), 119, 91, 70, 57; HRMS (FAB), m/z calcd for $C_{48}H_{66}N_7O_{11}S$ (MH^+) 948.4541, found: 948.4553.

cyclo[-Asp(OBn)-D-Phe-ψ[(E)-CH=CH]-Val-Arg(Mts)-Gly-] (19). To a stirred mixture of the protected pseudopentapeptide **16** (201 mg, 0.211 mmol) and HONB (45 mg, 0.254 mmol) in EtOAc (2 mL) was added DCC (52 mg, 0.254 mmol) at 0 °C, the mixture was stirred for 3 h at room temperature. The solution was filtrated, and the filtrate was concentrated under reduced pressure to give an oily residue, which was washed with n-hexane and Et_2O. 4 M HCl-dioxane (5 mL) was added to the residue, and the mixture was stirred for 30 min at room temperature. The mixture was concentrated under reduced pressure, and the residue was dissolved in DMF (400 mL). Subsequently, pH of the solution was adjusted to 8.0

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with 10% *N*-methylmorpholine in DMF at - 20 °C. After 3 h, the solution was concentrated under reduced pressure, and the residue was extracted with EtOAc. The extract was washed with 1 N HCl, brine, 5% $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash chromatography over silica gel with $CHCl_3$-MeOH (98:2 to 90:10) gave the title compound **19** (145 mg, 83% yield) as colorless crystals: mp 291 °C (decomp., Et_2O); $[\alpha]_D^{18}$ - 10.0 (*c* 0.497, DMF); ^{1}H NMR (300 MHz, DMF-d_7) δ 0.68 (d, *J* = 6.6 Hz, 3 H), 0.82 (d, *J* = 6.5 Hz, 3 H), 1.40-1.66 (m, 3 H), 1.80-2.01 (m, 2 H), 2.25 (s, 3 H), 2.50 (t, *J* = 9.4 Hz, 1 H), 2.59 (dd, *J* = 15.8, 6.1 Hz, 1 H), 2.66 (s, 6 H), 2.78 (m, 1 H), 2.87 (m, 1 H), 2.89-3.00 (m, 1 H), 3.19 (m, 2 H), 3.40 (dd, *J* = 15.3, 3.6 Hz, 1 H), 4.22 (dd, *J* = 15.4, 7.7 Hz, 1 H), 4.40 (m, 1 H), 4.54 (m, 1 H), 4.82 (td, *J* = 8.2, 6.2 Hz, 1 H), 5.09-5.19 (m, 2 H), 5.54-5.77 (m, 2 H), 6.65-6.85 (m, 1 H), 6.85-7.08 (m, 3 H), 7.17-7.46 (m, 10 H), 7.62 (d, *J* = 8.7 Hz, 1 H), 7.78 (m, 1 H), 7.85 (d, *J* = 7.6 Hz, 1 H), 8.13 (d, *J* = 8.5 Hz, 1 H). Anal. Calcd for $C_{43}H_{55}N_7O_8S$: C, 62.22; H, 6.68; N, 11.81. Found: C, 61.98; H, 6.70; N, 11.52.

cyclo[-Arg-Gly-Asp-D-Phe-ψ[(*E*)-CH=CH]-Val-]·TFA (3). The protected cyclic peptide **19** (145 mg, 0.174 mmol) was treated with 1 M TMSBr-thianisole/TFA (10 mL) in the presence of *m*-cresol (0.488 mL, 4.66 mmol) and 1,2-ethanedithiol (0.200 mL, 2.38 mmol) at 0 °C for 3 h. After concentration under reduced pressure, ice-cold Et_2O was added. The resulting powder was collected by centrifugation, and the powder was washed three times with Et_2O. Purification by preparative HPLC (23% B in A) gave the title cyclic peptide **3** as mono-TFA salt (93 mg, 79% yield) of colorless freeze-dried powder, $[\alpha]_D^{24}$ - 45.0 (*c* 0.636, AcOH); t_R = 14.5 min (23% B in A); ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.64 (d, *J* = 6.6 Hz, 3 H), 0.77 (d, *J* = 6.6 Hz, 3 H), 1.36-1.59 (m, 3 H), 1.75-1.91 (m, 2 H), 2.36 (dd, *J* = 16.0, 5.7 Hz, 1 H), 2.41 (t, *J* = 9.2 Hz, 1 H), 2.67-2.70 (m, 2 H) 2.80 (dd, *J* = 13.4, 6.2 Hz, 1 H), 3.09 (m, 2 H), 3.31 (m, 1 H), 4.11 (dd, *J* = 15.4, 7.9 Hz, 1 H), 4.25 (m, 1 H), 4.41 (m, 1 H), 4.59 (td, *J* = 8.4, 5.8 Hz), 5.45 (dd, *J* = 15.2, 6.2 Hz, 1 H), 5.50 (dd, *J* = 15.2, 9.3 Hz, 1 H), 7.17 (m, 3 H), 7.26 (m, 2 H), 7.47 (br, 1 H), 7.62 (d, *J* = 7.8 Hz, 1 H), 7.65 (d, *J* = 8.6 Hz, 1 H), 7.68 (dd, *J* = 7.9, 3.0 Hz, 1 H), 8.02 (d, *J* = 8.6 Hz, 1 H), 12.20 (br, 1 H); LRMS (FAB), *m/z* 558 (MH$^+$), 91, 87 (base peak), 70; HRMS (FAB), *m/z* calcd for $C_{27}H_{40}N_7O_6$ (MH$^+$) 558.3040, found: 558.3052.

(2*R*,5*R*,3*E*)-5-(9-Fluorenylmethoxycarbonylamino)-2-isopropyl-3-methyl-6-phenylhex-3-enoic Acid (Fmoc-D-Phe-ψ[(*E*)-CH=CMe]-L-Val-OH, 21). The ester **20**[8] (245 mg, 0.586 mmol) was dissolved in TFA (5 mL), and the mixture was stirred for 1.5 h at room temperature. Concentration under reduced pressure gave an oily residue, which was dissolved in MeCN-H_2O (2:1, 3.9 mL). Et_3N (0.163 mL, 1.17 mmol) and a solution of Fmoc-OSu (207 mg, 0.616 mmol) in MeCN (2.6 mL) were added to the above solution at 0 °C. After being stirred for 3.5 h, the mixture was acidified with 0.1 N HCl and was extracted with EtOAc. The extract was washed with 0.1 N HCl and brine and dried over $MgSO_4$. Concentration under reduced presssure followed by flash chromatography over silica gel with *n*-hexanes-EtOAc (2:1) gave the title compound **21** (198 mg, 69% yield) as a colorless oil: $[\alpha]_D^{22}$ - 34.0 (*c* 1.29, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$ at 328 K) δ 0.62 (d, *J* = 6.5 Hz, 3 H), 0.92 (d, *J* = 6.4 Hz, 3 H), 1.52 (br, 3 H), 2.01 (m, 1 H), 2.49 (d, *J* = 10.6 Hz, 1 H), 2.63 (m, 1 H), 2.85 (m, 1 H), 4.16 (t, *J* = 6.5 Hz, 1 H), 4.30-4.65 (m, 4 H), 5.22 (d, *J* = 8.8 Hz, 1 H), 7.05 (m, 2 H), 7.13-7.30 (m, 5 H), 7.32-7.39 (m, 2 H), 7.52 (m, 2 H), 7.72 (m, 2 H); LRMS (FAB), *m/z* 484 (MH$^+$), 440 (base peak), 245, 235, 196, 179; HRMS (FAB), *m/z* calcd for $C_{31}H_{34}NO_4$ (MH$^+$) 484.2488, found: 484.2479.

General Procedure for Synthesis of Protected Peptide Resins. Protected peptide-resins were manually constructed by Fmoc-based solid-phase peptide synthesis. t-Bu ester for Asp, (Boc)$_2$ or Pbf for Arg (for **24** or **29**, respectively) were employed for side-chain protection. Fmoc deprotection were achieved by 20% piperidine in DMF (2 × 1 min, 1 × 20 min). Fmoc-amino acids except for Fmoc-D-Phe-ψ[(E/Z)-CH=CMe]-Val-OH were coupled by treatment with 5 equiv of reagents [Fmoc-amino acid, N,N'-diisopropylcarbodiimide (DIPCDI), and HOBt·H$_2$O] to free amino group (or hydrazino group) in DMF for 1.5 h.

H-Asp(Ot-Bu)-D-Phe-ψ[(E)-CH=CMe]-Val-Arg(Boc)$_2$-Gly-NHNHCO-Wang Resin (24). p-Nitrophenyl carbonate Wang resin (0.93 mmol/g, 161 mg, 0.15 mmol) was treated with NH$_2$NH$_2$·H$_2$O (0.046 mL, 0.75 mmol) in DMF (2 mL) at room temperature for 2 h to give a hydrazino linker. Gly and Arg(Boc)$_2$ residues were coupled by general coupling protocol. Fmoc-D-Phe-ψ[(E)-CH=CMe]-L-Val-OH **21** (51.0 mg, 0.105 mmol) was incorporated by twice treatments with DIPCDI (0.019 mL, 0.126 mmol) and HOBt·H$_2$O (0.016 mg, 0.105 mmol) for 1.5 h each. After capping of free amino groups with Ac$_2$O-pyridine, Asp(Ot-Bu) residue was coupled by general coupling protocol to afford the title protected peptide resin **24**.

cyclo(-Arg-Gly-Asp-D-Phe-ψ[(E)-CH=CMe]-Val-)·TFA (4). The protected peptide resin **24** was treated with TFA for 1.5 h at room temperature, and the mixture was filtered. Concentration under reduced pressure followed by preparative HPLC (linear gradient of B in A, 14 to 20% over 60 min) gave a peptide hydrazide **25** as a colorless powder. To a stirred solution of **25** in DMF (12 mL) were added a solution of 4 M HCl in DMF (0.079 mL, 0.316 mmol) and isoamyl nitrite (0.014 mL, 0.105 mmol) at - 40 °C. After being stirred for 30 min at - 20 °C, the mixture was diluted with precooled DMF (72 mL). To the above solution was added (i-Pr)$_2$NEt (0.183 mL, 1.05 mmol) at - 40 °C, and the mixture was stirred for 24 h at - 20 °C. Concentration under reduced pressure and purification by preparative HPLC (linear gradient of B in A, 19 to 25% over 60 min) gave the cyclic pseudopeptide **4**[15] (10.3 mg, 14% yield from **21**) as freeze-dried powder: $[\alpha]_D^{21}$ - 36.8 (c 0.516, H$_2$O); t_R = 31.2 min (linear gradient of B in A, 20 to 40% over 40 min); ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.43 (d, J = 6.5 Hz, 3 H), 0.79 (d, J = 6.4 Hz, 3 H), 1.35-1.50 (m, 5 H), 1.81 (m, 2 H), 1.97 (m, 1 H), 2.33 (d, J = 11.2 Hz, 1 H), 2.45 (dd, J = 16.7, 6.1 Hz, 1 H), 2.69 (dd, J = 13.2, 9.2 Hz, 1 H), 2.78 (m, 1 H), 2.83 (dd, J = 13.2, 4.9 Hz, 1 H), 3.08 (m, 2 H), 3.39 (dd, J = 14.5, 2.5 Hz, 1 H), 3.85-3.90 (m, 2 H), 4.47 (m, 1 H), 4.62 (ddd, J = 16.9, 8.5, 5.1 Hz, 1 H), 5.25 (d, J = 8.5 Hz, 1 H), 7.13-7.17 (m, 3 H), 7.19-7.27 (m, 3 H), 7.31 (d, J = 7.9 Hz, 1 H), 7.49 (br, 1 H), 7.78 (m, 1 H), 8.69 (d, J = 7.9 Hz, 1 H), 12.26 (br, 1 H); LRMS (FAB), m/z 572 (MH$^+$), 185 (base peak), 154, 137, 93; HRMS (FAB), m/z calcd for C$_{28}$H$_{42}$N$_7$O$_6$ (MH$^+$) 572.3197, found: 572.3185.

($2R,5R,3Z$)-5-(9-Fluorenylmethoxycarbonylamino)-2-isopropyl-3-methyl-6-phenylhex-3-enoic Acid (Fmoc-D-Phe-ψ[(Z)-CH=CMe]-L-Val-OH, 27). By use of a procedure similar to that described for the preparation of Fmoc-amino acid **21** from **20**, the ester **26**[8] (98.0 mg, 0.234 mmol) was converted into the title compound **27** (99.0 mg, 87% yield) as a colorless oil: $[\alpha]^{24}_D$ - 100.4 (c 1.22, CHCl$_3$); ^{1}H NMR (300 MHz, CDCl$_3$, at 328 K) δ 0.78 (d, J = 6.6 Hz, 3 H), 0.99 (d, J = 6.4 Hz, 3 H), 1.73 (d, J = 1.3 Hz, 3 H), 2.13 (m, 1 H), 2.79 (dd, J = 13.6, 6.6 Hz, 1 H), 2.89 (m, 1 H), 3.27 (d, J = 10.8 Hz, 1 H), 4.14 (t, J = 6.8 Hz, 1 H), 4.34 (m, 2 H), 4.65-4.84 (m, 2 H), 5.28 (dd, J = 10.3, 1.2 Hz, 1 H), 7.12-7.30 (m, 7 H), 7.36 (m, 2 H), 7.50 (m, 2 H), 7.72 (m, 2 H); LRMS (FAB), m/z 484 (MH$^+$, base peak),

179, 149, 91, 69, 57, 55, 43; HRMS (FAB), *m/z* calcd for $C_{31}H_{34}NO_4$ (MH$^+$) 484.2488, found: 484.2502.

Synthesis of H-Asp(O*t*-Bu)-D-Phe-ψ[(Z)-CH=CMe]-Val-Arg(Pbf)-Gly-Clt Resin (29).

Arg(Pbf) residue was coupled by general coupling protocol on H-Gly-Clt resin (0.63 mmol/g, 216 mg, 0.136 mmol). Fmoc-D-Phe-ψ[(Z)-CH=CMe]-Val-OH **27** (55.0 mg, 0.113 mmol) was incorporated by twice treatments with DIPCDI (0.021 mL, 0.136 mmol) and HOBt·H₂O (0.017 mg, 0.113 mmol) for 1.5 h each. After capping of the remaining free amino groups with Ac₂O-pyridine, Asp(O*t*-Bu) residue was coupled by general coupling protocol to afford the title protected peptide resin **29**.

cyclo(-Arg-Gly-Asp-D-Phe-ψ[(Z)-CH=CMe]-Val-)·TFA (5).

The protected peptide resin **29** was subjected to AcOH/TFE/CH₂CH₂ (1:1:3, 10 mL) treatment for 2 h at room temperature. After filtration of the residual resin, the filtrate was concentrated under reduced pressure to give the crude protected peptide **30** as a colorless powder. To a stirred suspension of **30** and NaHCO₃ (57.1 mg, 0.680 mmol) in DMF (41 mL) was added DPPA (0.0879 mL, 0.408 mmol) at - 40 °C. The mixture was stirred for 36 h with warming to room temperature and filtered. The filtrate was concentrated under reduced pressure to give an oily residue, which was subjected to solid-phase extraction over basic alumina gel with CHCl₃-MeOH (9:1) to remove inorganic salts. The resulting cyclic protected peptide **31** was treated with 95% TFA solution for 1.5 h at room temperature. Concentration under reduced pressure and purification by preparative HPLC (linear gradient of B in A, 19 to 25% over 60 min) gave the cyclic pseudopeptide **5** (15.7 mg, 20% yield from **27**) as freeze-dried powder: $[\alpha]_D^{22}$ - 5.61 (*c* 0.712, H₂O); t_R = 30.6 min (linear gradient of B in A, 20 to 35% over 30 min); ¹H NMR (600 MHz, DMSO-d_6) δ 0.08 (m, 3 H), 0.66 (d, *J* = 5.8 Hz, 3 H), 1.44 (m, 2 H), 1.70-1.79 (m, 4 H), 1.86 (m, 1 H), 1.93 (m, 1 H), 2.22 (m, 1 H), 2.42-2.58 (m, 3 H), 3.00 (m, 1 H), 3.05 (m, 1 H), 3.12 (m, 1 H), 3.39 (m, 1 H), 3.49 (dd, *J* = 16.6, 5.3 Hz, 1 H), 3.76 (dd, *J* = 16.6, 5.8 Hz, 1 H), 4.40 (m, 1 H), 4.74 (m, 1 H), 5.32 (d, *J* = 10.3 Hz, 1 H), 7.13-7.26 (m, 5 H), 7.37-7.53 (m, 3 H), 7.91 (m, 1 H), 8.55 (m, 1 H), 12.11 (br, 1 H); LRMS (FAB), *m/z* 572 (MH$^+$), 185, 154, 137, 93, 91, 70 (base peak); HRMS (FAB), *m/z* calcd for $C_{28}H_{42}N_7O_6$ (MH$^+$) 572.3197, found: 572.3218.

Integrin-Binding Assays. Compounds were evaluated for their inhibitory activities in $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$-ELISA (enzyme linked immunosorbent assay). $\alpha_v\beta_3$ was purified from human placenta, using RGDSPK-sepharose CL-4B affinity chromatography, followed by mono Q ion exchange chromatography, according to Pytela's protocol.[18] $\alpha_{IIb}\beta_3$ was purified from human platelet by RGDSPK-sepharose CL-4B as well.[18] $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ binding assays were performed according to the modified method of Kouns et al.[19] EIA plates were coated with $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$, and blocked with bovine serum albumin. In each reaction, a test sample in the reaction mixture (20 mM Tris-HCl, 150 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, pH 7.4, 0.100 mL) including vitronectin or fibrinogen was added to the receptor-coated plate and incubated for 4 h at 25 °C. Thereafter the ligand binding was measured using anti-vitronectin rabbit antibody and peroxidase-conjugated anti-rabbit IgG antibody for $\alpha_v\beta_3$, or peroxidase-conjugated anti-fibrinogen antibody for $\alpha_{IIb}\beta_3$, and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) as the substrate of peroxidase. The IC₅₀ values were determined from measurement of absorbance at 415 nm.

References and Footnotes

1   (a) Hynes, R. O. *Cell* **1992**, *69*, 11. (b) Humphries, M. J. *Biochem. Soc. Trans.* **2000**, *28*, 311.

2   (a) Brooks, P. C.; Clark, R. A.; Cheresh, D. A. *Science* **1994**, *264*, 569. (b) Varner, J. A.; Cheresh, D. A. *Curr. Opin. Cell Biol.* **1996**, *8*, 724.

3   Ross, F. P.; Chappel, J.; Alvarez, J. I.; Sander, D.; Butler, W. T.; Farach-Carson, M. C.; Mintz, K. A.; Robey, P. G.; Teitelbaum, S. L.; Cheresh, D. A. *J. Biol. Chem.* **1993**, *268*, 9901.

4   (a) Kerr, J. S.; Wexler, R. S.; Mousa, S. A.; Robinson, C. S.; Wexler, E. J.; Mohamed, S.; Voss, M. E.; Devenny, J. J.; Czerniak, P. M.; Gudzelak, A., Jr; Slee, A. M. *Anticancer Res.* **1999**, *19*, 959. (b) Miller, W. H; Alberts, D. P.; Bhatnagar, P. K.; Bondinell, W. E.; Callahan, J. F.; Calvo, R. R.; Cousins, R. D.; Erhard, K. F.; Heerding, D. A.; Keenan, R. M.; Kwon, C.; Manley, P. J.; Newlander, K. A.; Ross, S. T.; Samanen, J. M.; Uzinskas, I. N.; Venslavsky, J. W.; Yuan, C. C.-K.; Haltiwanger, R. C.; Gowen, M.; Hwang, S.-M.; James, I. E.; Lark, M. W.; Rieman, D. J.; Stroup, G. B.; Azzarano, L. M.; Salyers, K. L.; Smith, B. R.; Ward, K. W.; Johanson, K. O.; Huffman, W. F. *J. Med. Chem.* **2000**, *43*, 22. (c) Kumar, C. C.; Malkowski, M.; Yin, Z.; Tanghetti, E.; Yaremko, B.; Nechuta, T.; Varner, J.; Liu, M.; Smith, E. M.; Neustadt, B.; Presta, M.; Armstrong, L. *Cancer Res.* **2001**, *61*, 2232. (d) Coleman, P. J.; Brashear, K. M.; Hunt, C. A.; Hoffman, W. F.; Hutchinson, J. H.; Breslin, M. J.; McVean, C. A.; Askew, B. C.; Hartman, G. D.; Rodan, S. B.; Rodan, G. A.; Leu, C. T.; Prueksaritanont, T.; Fernandez-Metzler, C.; Ma, B.; Libby, L. A.; Merkle, K. M.; Stump, G. L.; Wallace, A. A.; Lynch, J. J.; Lynch, R.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 31. (e) Goodman, S. L.; Hölzemann, G.; Sulyok, G. A. G.; Kessler, H. *J. Med. Chem.* **2002**, *45*, 1045.

5   (a) Aumailley, M.; Gurrath, M.; Muller, G.; Calvete, J.; Timpl, R.; Kessler, H. *FEBS Lett.* **1991**, *291*, 50. (b) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461. (c) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374.

6   Dechantsreiter, M. A.; Planker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033.

7   Fischer, G. *Chem. Soc. Rev.* **2000**, *29*, 119.

8   Oishi, S.; Kamano, T.; Niida, A.; Odagaki, Y.; Tamamura, H.; Otaka, A.; Hamanaka, N.; Fujii, N. *Org. Lett.* **2002**, *4*, 1051.

9   (a) Bol, K. M.; Liskamp, R. M. J. *Tetrahedron* **1992**, *48*, 6425. (b) Bohnstedt, A. C.; Prasad, J.V.N. V.; Rich, D. H. *Tetrahedron Lett.* **1993**, *34*, 5217. (c) Andres, C. J.; Macdonald, T. L.; Ocain, T. D.; Longhi, D. *J. Org. Chem.* **1993**, *58*, 6609. (d) Boros, L. G.; De Corte, B.; Gimi, R. H.; Welch, J. T.; Wu, Y.; Handschumacher, R. E. *Tetrahedron Lett.* **1994**, *35*, 6033. (e) Welch, J. T.; Lin, J. *Tetrahedron* **1996**, *52*, 291. (f) Hart, S. A.; Sabat M.; Etzkorn, F. A. *J. Org. Chem.* **1998**, *63*, 7580. (g) Hart S. A.; Etzkorn, F. A. *J. Org. Chem.* **1999**, *64*, 2998. (h) Hart, S. A.; Trindle, C. O.; Etzkorn, F. A. *Org. Lett.* **2001**, *3*, 1789.

10   (a) Ibuka, T.; Habashita, H.; Funakoshi, S.; Fujii, N.; Oguchi, Y.; Uyehara, T.; Yamamoto, Y. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 801. (b) Ibuka, T.; Habashita, H.; Otaka, A.; Fujii, N.; Oguchi, Y.; Uyehara T.; Yamamoto, Y. *J. Org. Chem.* **1991**, *56*, 4370. (c) Ibuka, T.; Yoshizawa, H.; Habashita, H.; Fujii, N.; Chounan, Y.; Tanaka, M.; Yamamoto, Y. *Tetrahedron Lett.* **1992**, *33*, 3783. (d) Noda, M.; Ibuka, T.; Habashita, H.; Fujii, N. *Chem. Pharm. Bull.* **1997**, *45*, 1259.

11   Tamamura, H.; Yamashita, M.; Nakajima, Y.; Sakano, K.; Otaka, A.; Ohno, H.; Ibuka T.; Fujii, N. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2983.

12   Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183.

13   Yajima, H.; Fujii, N.; Funakoshi, S.; Watanabe, T.; Murayama, E.; Otaka, A. *Tetrahedron* **1988**, *44*, 805.

14   Honzl, J.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1961**, *26*, 2333.

15   The pseudopeptide **4** contained about 6% of an uncharacterized impurity by ^{1}H NMR analysis, which failed to be removed by HPLC.

16   (a) Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotiriu, P.; Wenqing, Y.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943. (b) Barlos, K.; Gatos, D.; Kapolos, S.; Papaphotiu, G.; Schäfer, W.; Wenqing, Y. *Tetrahedron Lett.* **1989**, *30*, 3947. (c) Barlos, K.; Gatos, D.; Kapolos, S.; Poulos, C.; Schäfer, W.; Wenqing, Y. *Int. J. Pept. Protein Res.* **1991**, *38*, 555.

17   Shioiri, T.; Yamada, S. *Chem. Pharm. Bull.* **1974**, *22*, 855.

18   Pytela, R.; Pierschbacher, M. D.; Argraves, S.; Suzuki, S.; Rouslahti, E. *Methods Enzymol.* **1987**, *144*, 475.

19   Kouns, W. C.; Kirchhofer, D.; Hadvary, P.; Edenhofer, A.; Weller, T.; Pfenninger, G.; Baumgartner, H. R.; Jennings, L. K.; Steiner, B. *Blood* **1992**, *80*, 2539.

Section 2. Structure-activity Relationship Studies on Cyclic RGD Peptides Using Multi-substituted Alkene Dipeptide Isosteres

Summary

The first application of novel $\psi[(E)$-CX=CX]-type alkene dipeptide isosteres to conformational studies of bioactive peptides was carried out where X = H or Me. For exploration of bioactive conformations of Kessler's cyclic RGD peptides, cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Val^5-) 1 and cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-MeVal^5-) 2, D-Phe-$\psi[(E)$-CX=CX]-L-Val-type dipeptide isosteres were utilized having di-, tri- and tetrasubstituted alkenes containing the γ-methylated isosteres that have been reported to be potential type II' β-turn promoters. The biological activities of the isostere-containing peptides 3-6 against $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins were comparatively evaluated along with those of peptides 1 and 2. All (E)-alkene pseudopeptides exhibited higher antagonistic potency against $\alpha_v\beta_3$ integrin than 1, although potencies were slightly lower than for 2. Detailed structural analysis using ^{1}H-NMR spectroscopy revealed that representative type II' β/γ backbone arrangements proposed for 1, were not observed in peptides 3-6. Rather on the basis of ^{1}H-NMR data, the conformations of peptides 3-6 were estimated to be more analogous to those of the N-methylated peptide 2.

1 X = -CO-NH-
2 X = -CO-NMe-
3 X = -(E)-CH=CH-
4 X = -(E)-CH=CMe-
5 X = -(E)-CMe=CH-
6 X = -(E)-CMe=CMe-
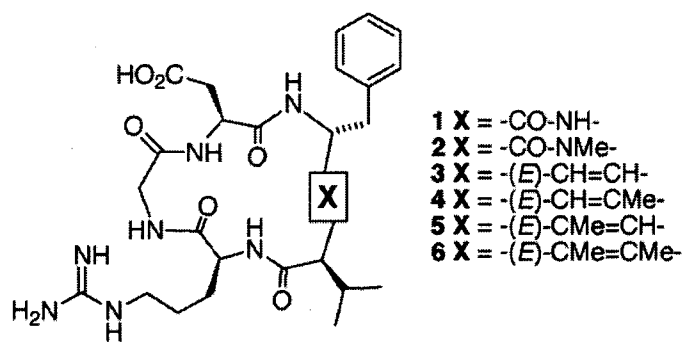
Figure 1

In chapter 2, section 1, the author describes the application of D-Phe-$\psi[(E)$-CH=CX]-L-Val- and D-Phe-$\psi[(Z)$-CH=CMe]-L-Val-type alkene dipeptide isosteres (X = H or Me) to the D-Phe^4-L-Val/MeVal^5 moieties in the Kessler cyclic RGD peptides, cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Val^5-) 1^1 and cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-MeVal^5-) 2 (Figure 1).[2] Both peptides 3 and 4 contain $\psi[(E)$-CH=CH]- and $\psi[(E)$-CH=CMe]-type isosteres, respectively, and possess potent antagonistic activity against $\alpha_v\beta_3$ integrin. In contrast, (Z)-congeners show
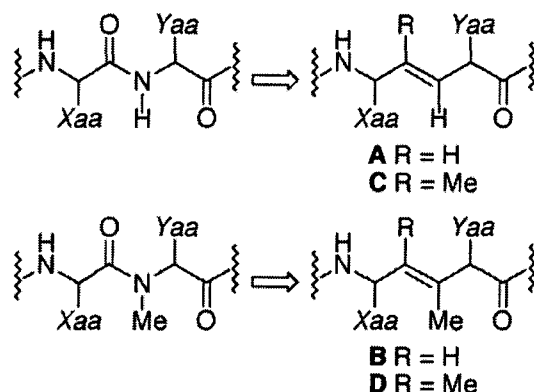
Figure 2. (E)-Alkene dipeptide isosteres having di-, tri- and tetrasubstituted alkenes; Xaa, Yaa = amino acid side chains.

extremely low potency in terms of $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ antagonism. Slight differences between the potencies of **3** and **4** independent of the presence of a β-methyl group in **4** corresponding to an N-methyl group of **2**, supported a conformational role for the N-methyl group of **2** beyond a simple steric one. It was thought that utilization of more highly functional β-turn motifs could be of value in order to facilitate a deeper understanding of the effect of N-methylation on the conformation of peptides as a whole, as well as on the topology of the pharmacophores, especially in the neighbourhood of the D-Phe^4-L-Val/MeVal^5 peptide bond.

Recently, Gellman et al. reported that Gly-$\psi[(E)$-CMe=CMe]-Gly-type isostere **D** is a potential β-hairpin promoter (Figure 2).[3] In addition, Wipf et al. have characterized D-Ala-$\psi[(E)$-CMe=CH]-L-Ala- and L-Ala-$\psi[(E)$-CCF$_3$=CH]-D-Ala-type isosteres such as **C** as promoting β-turn formation in the solid states due to $A^{1,2}$- and $A^{1,3}$-strains. This is opposed to L-Ala-$\psi[(E)$-CH=CH]-D-Ala-type motifs exemplified by **A** that have disubstituted alkenes.[4] γ-Methylated and γ-trifluoromethylated isosteres, which possess a carbon atom corresponding to a peptide bond carbonyl oxygen, are thought to be reasonable amide mimetics. However, application of such γ-methylated isosteres to turn moieties of bioactive peptides has not been reported to date. Development of organocopper-mediated stereoselective synthesis of multi-substituted (E)-alkene isosteres presented in the previous chapter, for the first time allowed the utilization of these isosteres for practical SAR studies on bioactive peptides. The author employed D-Phe-$\psi[(E)$-CMe=CX]-L-Val-type isosteres (X = H or Me), prepared in chapter 1, section 2, to the i + 1 and i + 2 sites of the type II' β-turn substructure in cyclic RGD peptide **1**.

Moreover a D-Phe-$\psi[(E)$-CMe=CMe]-L-Val-type analogue can also be regarded as a D-Phe^4-MeVal^5 dipeptide equivalent having reduced polarity. Here the β- and γ-methyl groups conceptually replicate allylic strain across peptide bonds between the D-Phe^4 carbonyl oxygen
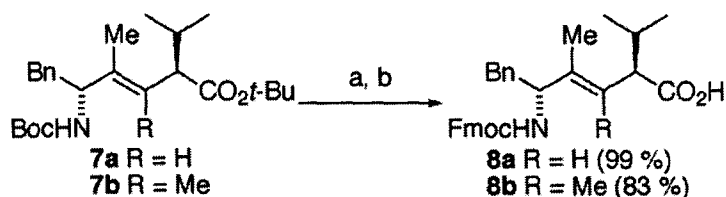
Scheme 1. *Reagents*: (a) TFA; (b) Fmoc-OSu, Et₃N.
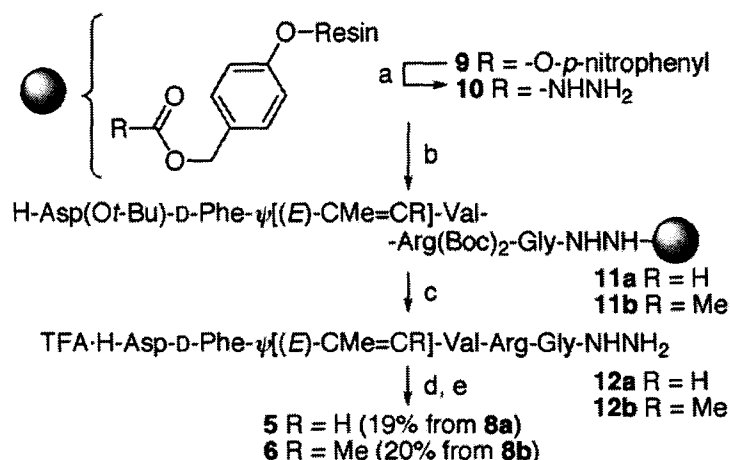
Scheme 2. *Reagents*: (a) NH₂NH₂·H₂O; (b) Fmoc-based SPPS; (c) TFA; (d) HCl, isoamyl nitrite; (e) (*i*-Pr)₂NEt.

and the MeVal[5] side chain, as well as between the *N*-methyl group and the D-Phe[4] side chain.[5] With this in mind, the synthesis and bio-evaluation of isostere-containing cyclic peptides **5** and **6** was undertaken, along with thier ¹H-NMR conformational analysis and a comparison with the previous peptides **1-4**. Reported herein are results of this first attempt to evaluate γ-methylated alkene dipeptide isosteres as type II' β-turn motifs in bioactive peptides. The author also examined SAR effects of *N*-methylation of Val[5] in cyclic RGD peptides using the (*E*)-alkene isosteres having differential substitution motifs.

Synthesis of Cyclic RGD Peptidomimetics Possessing D-Phe-ψ[(*E*)-CMe=CX]-L-Val-type Alkene Dipeptide Isosteres (X = H or Me). Preparation of cyclic RGD peptides **5** and **6** that contain D-Phe-ψ[(*E*)-CMe=CH]-L-Val- and D-Phe-ψ[(*E*)-CMe=CMe]-L-Val-type alkene dipeptide isosteres, respectively, was performed according to the synthetic scheme utilized for the synthesis of peptide **4** (Schemes 1 and 2).[6] In this process, a combination of Fmoc-based solid phase peptide synthesis (SPPS) and cyclization of linear peptide hydrazides **12a,b** without side-chain protecting groups was employed by an adapted azide method in order to avoid olefinic isomerization which would otherwise be possible during final

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Table 1. Integrin antagonistic activities of cyclic RGD peptides and peptidomimetics.

Peptide	X	$\alpha_v\beta_3$ IC$_{50}$ (nM)	Q^b	$\alpha_{IIb}\beta_3$ IC$_{50}$ (nM)	Q^b	SI^c
RGDS^a	-	98	14	270	0.35	2.7
1	-CO-NH-	6.8	1	770	1	110
2	-CO-NMe-	1.4	0.20	280	0.36	200
3	-CH=CH-	3.6	0.53	140	0.19	40
4	-CH=CMe-	3.3	0.48	100	0.13	30
5	-CMe=CH-	2.4	0.35	81	0.11	34
6	-CMe=CMe-	1.8	0.27	48	0.06	26

^{a}A linear peptide RGDS (H-Arg-Gly-Asp-Ser-OH) was used as a standard peptide. ^bQ values were calculated as Q = IC$_{50}$(peptide)/IC$_{50}$(1). ^{c}SI values were calculated as SI = IC$_{50}(\alpha_{IIb}\beta_3)$/IC$_{50}(\alpha_v\beta_3)$.

deprotection by strong acid treatment in Boc-based synthesis. For side-chain protection, *tert*-butyl ester for Asp and (Boc)$_2$ for Arg were employed, both of which are amenable to mild acidic deprotection. TFA-treatment of the *N*-Boc-protected isosteres **7a,b**, which were obtained by regio- and stereoselective alkylation of β-(1,3-oxazolidin-2-one)-5-yl-α,β-enoates by organocopper reagents,[7] followed by Fmoc-reprotection, provided building blocks **8a,b** that were suitable for SPPS. Following preparation of hydrazide linker **10** by treatment of *p*-nitrophenyl carbonate resin **9** with hydrazine hydrate in DMF, peptide chain elongation by Fmoc-based SPPS gave the expected protected peptide resins **11a,b**. Side chain deprotection and TFA-mediated cleavage from resins **11a,b** provided peptide hydrazides **12a,b**, which were subjected to successive azide formation and cyclization in highly diluted DMF solution.[8] The crude peptides were readily purified by reverse-phase HPLC to yield the expected cyclic peptides **5** and **6** in 19 % and 20 % yield, respectively, which were fully characterized by ^{1}H-NMR and mass spectra.

Structure-activity Relationship Investigation of Cyclic RGD Peptides and Peptidomimetics. Integrin antagonistic activities of the resulting peptides **5** and **6** against $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrins were comparatively evaluated along with Kessler's RGD peptides **1** and **2**, and the peptides **3** and **4** having γ-unmethylated D-Phe-$\psi[(E)$-CH=CX]-L-Val-type isosteres (X = H or Me). The results are shown in Table 1 as inhibition by the peptides **1-6** of vitronectin or fibrinogen binding to $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ integrin, respectively. Each of the isostere-containing peptides **3-6** showed strong $\alpha_v\beta_3$ integrin antagonistic activity within the range from IC$_{50}$ = 6.8 nM for **1** to IC$_{50}$ = 1.4 nM for **2**. It seemed that the amide or olefinic moiety in

the D-Phe^4-Val/MeVal^5 dipeptide portion of the peptides **1-6** was not directly involved in recognition and binding to $\alpha_v\beta_3$ integrin. This is consistent with a recent crystal structure analysis of a $\alpha_v\beta_3$ integrin-ligand complex.[9] Structure-activity relationship studies on cyclic RGD peptides investigating effects due to the *N*-methyl group of **2** using novel alkene dipeptide isosteres seemed to be highly appropriate. On the other hand, isostere-containing peptides **3-6** were less selective $\alpha_v\beta_3$ integrin antagonists than **1** or **2**, since these possessed relatively high potency against $\alpha_{IIb}\beta_3$ integrin. This indicated that hydrophobic characteristics based on the olefinic moiety of the isosteres might participate in favorable interactions between peptides **3-6** and $\alpha_{IIb}\beta_3$ integrin.

Turning attention back to the $\alpha_v\beta_3$ integrin antagonism of peptides **1-6**, it is noticeable that only minimal differences were observed between the activities of peptides **3** and **5** having β-unmethylated isosteres and the respective β-methylated isostere-containing congeners **4** and **6**. In contrast, peptide **2**, having an *N*-methylvaline, exhibited approximately five times higher potency than peptide **1**, similar to a previous report. If the presence of an *N*-methyl group in **2** could enhance potency, then perhaps either peptide **4** or **6**, which possesses an isostere β-methyl group corresponding to *N*-methyl group of **2**, could also show potencies superior to **3** or **5**, respectively. This unexpected result demonstrated that the conformational transformation from **1** to **2** and the resulting improvement of $\alpha_v\beta_3$ integrin antagonism depended on factors other than simple steric properties of the *N*-methyl group.

Additionally, since the isostere γ-methyl group corresponds to the carbonyl oxygen of D-Phe^4, peptides **5** and **6** containing such an isostere γ-methyl group had slightly higher potency against $\alpha_v\beta_3$ integrin than the γ-unmethylated congeners **3** and **4**, respectively. The improved potencies may potentially be derived from steric interactions, including allylic strain induced by the γ-methyl group. Similarly, a D-Phe^4 carbonyl oxygen of **1** and **2** may partially contribute to enhanced potencies by effects other than polar interactions such as hydrogen bonding.

Conformational Aspects of Cyclic RGD Peptides Derived from ^{1}H-NMR Spectroscopy and Validation of a Tri- or Tetra-substituted Alkene-containing Isosteres as Turn Motifs. Conformations of cyclic peptides have been intensively investigated using NMR spectroscopy and molecular dynamics calculations.[10,11] In structure-activity relationship studies on cyclic RGD peptides under "conformational control", Kessler et al. has reported that replacement at either the D-Phe^4 or Val^5 positions did not induce changes in backbone conformations.[10a] ^{1}H-NMR parameters such as chemical shifts, temperature dependence of

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Table 2. Observed ^{1}H-NMR chemical shifts and $^3J(H^N,H^\alpha)$ values of 3.

residue	chemical shifts (ppm)							$^3J(H^N,H^\alpha)$	$^3J(H^\alpha,H^\beta)$
	H^N	H^α	H^β	H^γ	H^δ	H^ϵ	other		
Arg^1	7.66	4.25	1.51 1.82	1.41 1.45	3.10	7.47		8.61	
Gly^2	7.69	3.31 4.12						3.10 7.91	
Asp^3	8.02	4.59	2.36 2.72					8.60	5.79 8.45
D-Phe^4	7.62	4.41	2.72 2.80				5.45 (CH=)	7.84	8.42 6.21
Val^5	-	2.41	1.82	0.64^{proS} 0.77^{proR}			5.50 (CH=)	9.39	9.27

Table 3. Observed ^{1}H-NMR chemical shifts and $^3J(H^N,H^\alpha)$ values of 4.

residue	chemical shifts (ppm)							$^3J(H^N,H^\alpha)$	$^3J(H^\alpha,H^\beta)$
	H^N	H^α	H^β	H^γ	H^δ	H^ϵ	other		
Arg^1	7.78	3.87	1.81	1.42	3.08	7.49		-^{a}	
Gly^2	7.23	3.39 3.89						2.53 6.76	
Asp^3	8.69	4.47	2.45 2.78					7.87	6.10 7.39
D-Phe^4	7.31	4.62	2.69 2.83				5.25 (CH=)	7.87	9.23 4.95
Val^5	-	2.33	1.97	0.43^{proS} 0.79^{proR}			1.39 (CMe)	-	11.21

^{a}The ^3J value was not determined due to the broad peak.

Table 4. Observed ^{1}H-NMR chemical shifts and $^3J(H^N,H^\alpha)$ values of 5.

residue	chemical shifts (ppm)							$^3J(H^N,H^\alpha)$	$^3J(H^\alpha,H^\beta)$
	H^N	H^α	H^β	H^γ	H^δ	H^ϵ	other		
Arg^1	7.36	4.18	1.53 1.70	1.36 1.42	3.08	7.46		8.37	
Gly^2	7.95	3.27 3.98						4.26 6.94	
Asp^3	8.12	4.55	2.40 2.67					8.33	6.62 7.82
D-Phe^4	7.97	4.30	2.75 2.84				1.58 (CMe)	7.12	9.77 5.87
Val^5	-	2.56	1.84	0.47^{proS} 0.67^{proR}			5.06 (CH=)	-	9.13

amide protons and ^3J-coupling constants support homogeneous families of cyclic peptide conformations. Based on similar concepts, using alkene isosteres, the author attempted to

residue	chemical shifts (ppm)							$^3J(H^N,H^\alpha)$	$^3J(H^\alpha,H^\beta)$
	H^N	H^α	H^β	H^γ	H^δ	H^ϵ	other		
Arg^1	7.57	3.86	1.75	1.42	3.08	7.51		6.94	
Gly^2	7.29	3.32						3.22	
		3.91						6.77	
Asp^3	8.55	4.53	2.44					8.27	6.79
			2.74						7.69
D-Phe^4	7.74	4.92	2.76				1.71	7.47	10.47
			2.85				(CMe)		5.10
Val^5	-	2.72	1.86	0.26^{proS}			1.40	-	10.73
				0.81^{proR}			(CMe)		
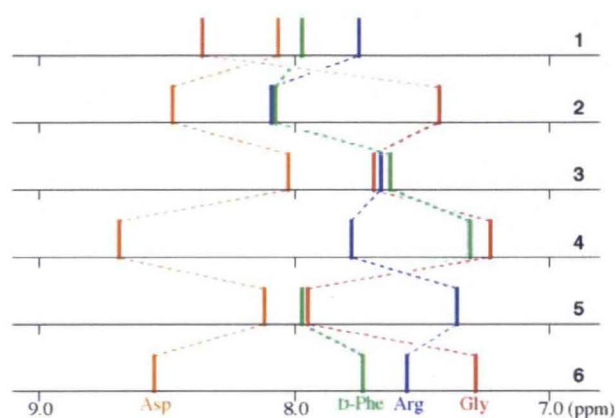
Figure 3. Chemical shifts of amide protons of cyclic peptides **1-6** in ^{1}H-NMR spectra in DMSO-d_6.
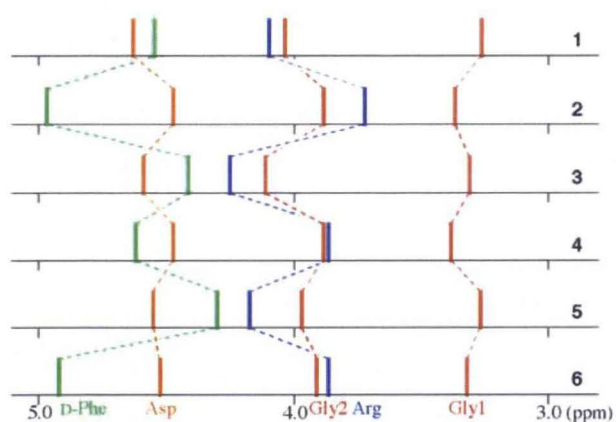
Figure 4. Chemical shifts of amide protons of cyclic peptides **1-6** in ^{1}H-NMR spectra in DMSO-d_6.

understand effects of the N-methyl groups or isostere β-methyl groups on conformations and $\alpha_v\beta_3$ integrin antagonistic activity.

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Table 6. Coupling constants, $^3J(H^N,H^\alpha)$, of cyclic peptides **1-6**.

peptide	Arg^1	Gly^2	Asp^3	D-Phe^4	Val^5
1	7.6	4.1 / 7.4	8.4	7.4	7.8
2	7.1	3.1 / 6.7	8.2	8.7	-
3	8.6	3.1 / 7.9	8.6	7.8	-
4	-^{a}	4.3 / 6.9	8.3	7.1	-
5	8.4	2.5 / 6.8	7.9	7.9	-
6	6.9	3.2 / 6.8	8.3	7.5	-

^{a} The ^3J value was not determined due to the broad peak.

Table 7. Temperature dependence of amide proton chemical shifts, $-\Delta\delta/\Delta T$ (ppb/K) of cyclic peptides **1-6**.

peptide	Arg^1	Gly^2	Asp^3	D-Phe^4	Val^5
1	1.8	5.5	5.1	3.1	3.0
2	5.5	1.0	4.7	5.1	-
3	5.4	2.2	3.0	3.5	-
4	4.8	0.9	5.5	3.3	-
5	5.7	2.5	5.5	2.7	-
6	6.8	-1.4	7.4	2.5	-

In chemical shift data of peptides **1-6** in DMSO solution, downfield shifts of Arg^1 H^N, Asp^3 H^N, one Gly^2 H$^\alpha$ (high field) and D-Phe^4 H$^\alpha$ of peptides **2**, **4** and **6** that possess MeVal^5 N-methyl groups or corresponding β-methyl groups, were comparable to those of **1**, **3** and **5**, respectively (Tables 2-5, Figures 3 and 4). On the other hand, Gly^2 H^N, Arg^1 H$^\alpha$, the other Gly^2 H$^\alpha$ (low field) and Asp^3 H$^\alpha$ of **2**, **4** and **6** were located at higher fields than those of **1**, **3** and **5**, respectively. For D-Phe^4 H^N, no significant difference was found between **1** and **2**, while similar upfield shift correlations were observed among the isostere-containing peptides **3-6**. As such, addition of a methyl group to the α-amino group of Val^5 or to the isostere β-position, equally induced the chemical shift changes, although this may not necessarily indicate similar changes in peptide backbone conformation. These observations are contrasted to the fact that among peptides **2**, **4** and **6** an increase in $\alpha_v\beta_3$ integrin antagonistic activity was observed only in **2**.

In a sharp contrast to effects on the chemical shifts of amide and α-protons, the vicinal coupling constants between amide protons and α-protons of each residue of peptides **1-6** displayed no common tendency due to N-methylation or β-methylation (Table 6). If anything,

the values of each residue were similar among all the peptides **1-6**. This revealed that methylation did not result in drastic ϕ angle changes.

Temperature coefficients in the range of 300 - 340 K of chemical shifts of amide protons in peptides **1-6** were also examined (Table 7). This parameter often indicates solvent accessibility of amide hydrogens.[11a] Kessler et al. previously reported that the temperature dependence of Arg^1 H^N in cyclo(-Arg^1-Gly^2-Asp^3-D-Xaa^4-Val^5-) and cyclo(-Arg^1-Gly^2-Asp^3-D-Phe^4-Yaa^5-) is commonly small, except in cases where cyclic amino acids such as proline are utilized for D-Xaa^4 and Yaa^5.[10a] This data supports solvent shielding of Arg^1 H^N and indicates the presence of a hydrogen bond corresponding to a type II' β-turn substructure. However, among peptides **1-6**, a small coefficient of Arg^1 H^N was observed only in peptide **1**. Interestingly, the Gly^2 H^N coefficient was small in the remaining peptides **2-6**, while those of the other residues were over 2.0 ppb/K. Thus, those amide protons shielded from the solvent appeared to be quite different for **1** as compared to the other peptides. These observations implied that conformations of isostere-containing peptides **3-6** may resemble one another in DMSO, and that these peptides may adopt flexible structures such as **2**, rather than representative type II' β/γ arrangements as seen with **1**. Alkene dipeptide isosteres, including γ-methylated D-Phe-ψ[(E)-CMe=CH]-L-Val- and D-Phe-ψ[(E)-CMe=CMe]-L-Val-type analogues, were unlikely to induce expected β-turns, at least in cyclic pentapeptides as contrasted with precedent reports by Gellman et al. and Wipf et al.[3,4]

Taking into account combined biological and ^{1}H-NMR data, it is evident that the lack of a Val^5 amide hydrogen incurred by N-methylation in **2**, contributed to conformational changes that increased $\alpha_v\beta_3$ antagonistic activity. This was common in peptides **3-6** replaced with alkene isosteres. In other words, the Val^5 amide hydrogen in **1** may contribute unfavorably to the bioactive conformation through intramolecular interactions, although such an amide hydrogen originating from the $i + 2$ residue of a β-turn normally would be indispensable to the distinctive type II' β/γ arrangement of cyclic pentapeptides. In contrast, it is supposed that the carbonyl oxygen of D-Phe^4 in **1** and **2** seems to be unrelated to any significant interactions, since it has little effect on conformation and bioactivity as compared to the amide hydrogen of Val^5.

Utilization of NMR Spectroscopy for Structure Calculations of Isostere-containing Peptides 3-6. Structure calculations of the isostere-containing cyclic peptides **3-6** were carried out by simulated annealing molecular dynamics/energy minimization calculations using dihedral constraints derived from ^{1}H-NMR vicinal coupling constants and
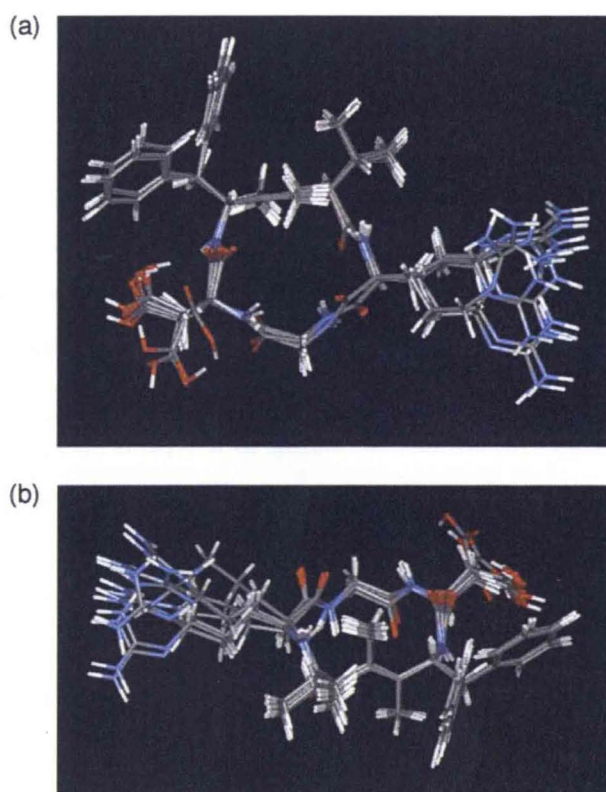
Figure 5. Overlay of ten low-energy structures of peptide **6**. (a) Top view. (b) Sideview.

Table 8. Averaged dihedral angles ϕ and ψ of peptide **6**.

residue	ϕ	ψ
Arg[1]	- 99.6 ± 10.8	+ 95.2 ± 13.0
Gly[2]	+ 116.7 ± 24.4	- 112.9 ± 6.8
Asp[3]	- 103.0 ± 13.9	+ 106.3 ± 5.3
D-Phe[4]	+ 111.6 ± 8.1	- 103.5 ± 2.9
Val[5]	- 92.9 ± 1.6	- 76.4 ± 6.4

NOE distance constraints.[12] Ten superimposed low-energy structures of peptide **6** having the D-Phe-ψ[(*E*)-CMe=CMe]-L-Val-type isostere are depicted in Figure 5 as being representative structures of **3-6**. Peptide **6** was the most potent $\alpha_v\beta_3$ integrin antagonist among the isostere-containing peptides **3-6**. An excellent convergence is seen in the backbone structures. The root mean square deviation (RMSD) value for all backbone heavy atoms of **6** was below 0.22 Å, and total energy values of the refined structures were in the range of 102 - 108 kcal/mol. The olefinic plane in the isostere was peripendicular to the plane of the cyclic peptide, which is an ideal substructural component for a type II' β-turn. In practice, the averaged dihedral angles, the ψ angle of D-Phe[4] (- 103.5°) and the ϕ angle of Val[5] (- 92.9°), were highly
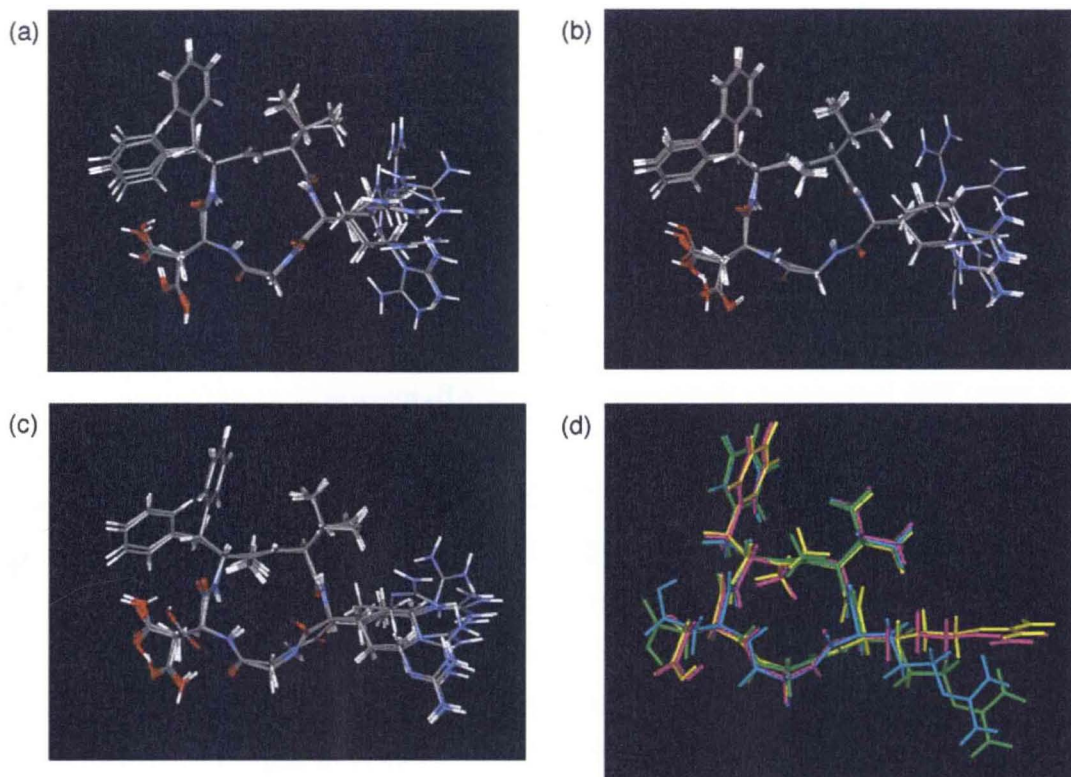
Figure 6. (a) Overlay of ten low-energy structures of **3**. (b) That of **4**. (c) That of **5**. (d) Overlay of the representative low-energy structures of peptides **3-6**. Cyan: **3**; green: **4**; magenta: **5**; yellow: **6**.

consistent with the theoretical β-turn values (Table 8). However, the expected β-turn hydrogen bond between the amide hydrogen of Arg[1] and the α-carbonyl oxygen of Asp[3] could not be identified, since the peptide bonds of Asp[3]-D-Phe[4] and Val[5]-Arg[1] were also oriented perpendicular to the cyclic peptide plane. The torsional angles, D-Phe[4] ϕ and Val[5] ψ, were apparently different from those typically associated with a β-turn. This allows the side chains of all residues to exhibit pseudoequatorial conformations as demonstrated in the conformational analysis of the peptide **2**.[2] Additionally, all carbonyl oxygens and γ-methyl groups of the isostere moieties were commonly directed away from side chains of corresponding following residues, most probably to avoid 1,3-allylic strains across the peptide bonds. Similarly, the isostere β-methyl group was oriented upward so as to reduce steric interactions with the D-Phe[4] side chain. The averaged distance between the β-carbons of Arg[1] and Asp[3] of **6**, which provides topological orientation for two significant pharmacophores needed for bioactivity, was 9.0 Å. This distance was slightly longer than observed in **2**, which was previously determined in aqueous solution. These results indicated that the conformation

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of **6** is more similar to that of the most potent peptide **2** having an N-methylvaline, rather than the kinked conformation of **1**, which is based on a type II' β/γ conformation.[13]

In integrin-ligand complexes, ligand **2** was reported to adopt a more distorted conformation as compared with structures in the absence of integrin.[9] Although analysis of the binding mode of **6** was not carried out, analogous conformations in the receptor-free state as well as the presence of common functional groups required for binding interactions, with the exception of the olefinic moiety, could enable an estimation of conformations of **6** in the bound state. This presupposes that **6** can adhere to $\alpha_v\beta_3$ integrin in a conformationally similar manner to **2**.

Calculated low-energy backbone structures of **3-6** are highly similar (Figure 6). Calculations of the pseudopeptides other than **6** also afforded well-converged conformations. Additionally, type II' β- or γ-turn substructures exhibited in **1** were not observed in peptides **3-5**. All backbone structures based on the five α-carbons showed nearly symmetrical pentagonal shapes. In all cases, the olefinic moieties and peptide bonds of **3-6** were found to be vertical to the cyclic peptide plane, although some exhibited slightly differential rotation. From NMR-based conformational studies, the presence of β- and/or γ-methyl groups in the isostere moiety appeared to have no remarkable effect on the global backbone structures of the cyclic peptidomimetics. These results indicated that replacements of the D-Phe^4-Val^5 moiety in **1** by alkene isosteres did not stabilize type II' β-turns in the cyclic pentapeptides, in spite of an increase in biological activity against $\alpha_v\beta_3$ integrin.

In conclusion, the author carried out SAR studies on cyclic RGD peptides using novel alkene dipeptide isosteres. Cyclic peptides **5** and **6**, having D-Phe-$\psi[(E)$-CMe=CH]-L-Val- and D-Phe-$\psi[(E)$-CMe=CMe]-L-Val-type isosteres, were designed and synthesized in order to investigate effects of the type II' β/γ arrangement of **1** as well as the role of the N-methyl group of MeVal^5 in **2** on conformation and biological activity. Evaluation of the biological activities of **1-6** against $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin demonstrated that loss of the amide hydrogen of Val^5 in **1** by N-methylation led to a remarkable increase in $\alpha_v\beta_3$ antagonistic activity of **2**, though this was not due to steric factors of the methyl group. Structural analysis showed that γ-methylated isostere moieties would not be expected to serve as β-turn promoters, at least for cyclic pentapeptides. Nevertheless, the conformations of isostere-containing peptides **3-6** appeared to be analogous to the most potent peptide **2** rather than **1**. Structural calculations performed on **3-6** supported conformational results derived from NMR data. Taken together, these results indicate that influences of the N-methyl group on conformation and biological

activity of **2** could be attributed mainly to loss of the amide hydrogen functionality in the D-Phe[4]-MeVal[5] moiety, and not to steric factors such as allylic strain induced by the methyl group.

With advances in genomic science, development of efficient methodologies for rational design of therapeutically relevant agents from natural ligands is an area of increasing importance. As presented herein, alkene isosteres having differential methyl-substitutions could serve as practical tools to derive information concerning pharmacophores and bioactive conformations of bio- and chemoactive peptides and proteins.

Experimental Section

General Methods. ¹H NMR spectra were recorded using a Bruker AC 300 or a Bruker AM 600 spectrometer at 300 or 600 MHz. Chemical shifts of the compounds measured in $CDCl_3$ are reported in parts per million downfield from internal Me_4Si (s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet). Those of the compounds measured in DMSO-d_6 are calibrated to the solvent signal (2.50 ppm). Nominal (LRMS) and exact mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 or JMS-HX/HX 110A mass spectrometer. Optical rotations were measured with a Horiba high-sensitive polarimeter SEPA-200 (Kyoto, Japan). For flash chromatographies, silica gel 60 H (silica gel for thin-layer chromatography, Merck) and Wakogel C-200 (silica gel for column chromatography) were employed. For HPLC separations, a Cosmosil 5C18-ARII analytical (4.6 × 250 mm, flow rate 1 mL/min) column or a Cosmosil 5C18-ARII preparative (20 × 250 mm, flow rate 11 mL/min) column was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA solution (v/v, solvent A) and 0.1% TFA in MeCN (v/v, solvent B) were used for HPLC elution.

(2R,5R,3E)-5-(9-Fluorenylmethoxycarbonyl)amino-2-isopropyl-4-methyl-6-phenylhex-3-enoic acid (Fmoc-D-Phe-ψ[(E)-CMe=CH]-L-Val-OH, 8a)
After treatment of the ester **7a** (108 mg, 0.258 mmol) with TFA (5 mL) for 1.5 h at room temperature, concentration under reduced pressure gave an oily residue. To a stirred solution of the above residue in MeCN-H_2O (2:1, 2.25 mL) were added Et_3N (0.072 mL, 0.517 mmol) and a solution of Fmoc-OSu (91 mg, 0.271 mmol) in MeCN (1.5 mL) at 0 °C. After being stirred for 3 h, the mixture was acidified with 0.1 N HCl and was extracted with EtOAc. The extract was washed with 0.1 N HCl and brine, and dried over $MgSO_4$. Concentration under reduced presssure followed by flash chromatography over silica gel with n-hexane-EtOAc (2:1) gave the title compound **8a** (123 mg, 99% yield) as a colorless oil: $[\alpha]_D^{20}$ - 16.6 (c 0.542, H_2O); ¹H NMR (300 MHz, $CDCl_3$, at 328 K) δ 0.70 (d, J = 6.7 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H), 1.67 (s, 3 H), 1.92 (m, 1 H), 2.77-2.99 (m, 3 H), 4.14 (t, J = 6.6 Hz, 1 H), 4.25-4.41 (m, 3 H), 4.91 (m, 1 H), 5.26 (d, J = 10.1 Hz, 1 H), 7.08 (d, J = 6.9 Hz, 2 H), 7.12-7.30 (m, 5 H), 7.35 (t, J = 7.4 Hz, 2 H), 7.49 (m, 2 H), 7.72 (d, J = 7.5 Hz, 2 H). LRMS (FAB), m/z 484 (MH⁺, base peak), 392, 260, 191, 179, 164, 154, 149, 143, 136, 91, 57, 43. HRMS (FAB), m/z calcd for $C_{31}H_{34}NO_4$ (MH⁺) 484.2488, found: 484.2477.

(2R,5R,3E)-5-(9-Fluorenylmethoxycarbonyl)amino-2-isopropyl-3,4-dimethyl-6-phenylhex-3-enoic acid (Fmoc-D-Phe-ψ[(E)-CMe=CMe]-L-Val-OH, 8b) By use of a procedure similar to that described for the preparation of the Fmoc-amino acid **8a** from **7a**, the ester **7b** (138 mg, 0.319 mmol) was converted into the title compound **8b** (131 mg, 83% yield) as a colorless oil: $[\alpha]_D^{24}$ - 70.9 (c 1.00, H_2O); ¹H NMR (300 MHz, $CDCl_3$, at 323 K) δ 0.36 (m, 3 H), 0.91 (d, J = 6.4 Hz, 3 H), 1.49 (m, 3 H), 1.71 (d, J = 1.4 Hz, 3 H), 1.96 (m, 1 H), 2.66 (m, 1 H), 2.81 (m, 1 H), 3.98 (m, 1 H), 4.15 (t, J = 6.4 Hz, 1 H), 4.41 (m, 2 H), 4.81 (br, 1 H), 7.06 (br, 2 H), 7.10-7.30 (m, 5 H), 7.31-7.39 (m, 2 H), 7.51 (m, 2 H), 7.72 (m, 2 H). LRMS (FAB), m/z 498 (MH⁺, base peak), 452, 406, 391, 274, 191, 179, 149, 136, 91, 69, 57, 43. HRMS (FAB), m/z calcd for $C_{32}H_{36}NO_4$ (MH⁺) 498.2644, found: 498.2641.

General Procedure for Assembly of the Peptide Chain. Protected peptide-resins were manually constructed by Fmoc-based solid phase peptide synthesis. t-Bu ester for Asp, (Boc)₂ for Arg[1] were employed for side-chain protection. Fmoc-amino acids except for Fmoc-D-Phe-ψ[(E)-CMe=CX]-Val-OH (X = H or Me) were coupled using five equivalents of

reagents [Fmoc-amino acid, N,N'-diisopropylcarbodiimide (DIPCDI), and HOBt·H₂O] to free amino group (or hydrazino group) in DMF for 1.5 h. Fmoc deprotection was performed by 20% piperidine in DMF (2 × 1 min, 1 × 20 min).

H-Asp(Ot-Bu)-D-Phe-ψ[(E)-CMe=CH]-Val-Arg(Boc)₂-Gly-NHNHCO-Wang

Resin (11a). After treatment of p-nitrophenyl carbonate Wang resin **9** (0.93 mmol/g, 161 mg, 0.15 mmol) with NH₂NH₂·H₂O (0.046 mL, 0.75 mmol) in DMF (2 mL) at room temperature for 2 h, Gly and Arg(Boc)₂ residues were coupled by general coupling protocol. Fmoc-D-Phe-ψ[(E)-CMe=CH]-Val-OH **8a** (48.3 mg, 0.100 mmol) was incorporated by twice treatments with DIPCDI (0.018 mL, 0.120 mmol) and HOBt·H₂O (0.015 mg, 0.100 mmol) for 1.5 h each. After protection of the remaining free amino group with Ac₂O-pyridine, Asp(Ot-Bu) residue was coupled by general coupling protocol to provide the title peptide resin **11a**.

H-Asp(Ot-Bu)-D-Phe-ψ[(E)-CMe=CMe]-Val-Arg(Boc)₂-Gly-NHNHCO-Wang

Resin (11b). By use of a procedure similar to that described for the preparation of the resin **11a**, the title resin **11b** was synthesized from p-nitrophenyl carbonate Wang resin **9** (0.15 mmol) and Fmoc-amino acid **8b** (60 mg, 0.121 mmol).

cyclo[-Arg-Gly-Asp-D-Phe-ψ[(E)-CMe=CH]-Val-]·TFA (5).

The protected peptide resin **11a** was treated with TFA for 1.5 h at room temperature. Removal of the resin followed by concentration under reduced pressure gave the colorless residue, which was purified by preparative HPLC (linear gradient of B in A, 15 to 20% over 45 min) to provide a peptide hydrazide **12a**. To a stirred solution of **12a** in DMF (12 mL) were added a solution of 4 M HCl in DMF (0.075 mL, 0.300 mmol) and isoamyl nitrite (0.013 mL, 0.100 mmol) at - 40 °C, and the mixture was stirred for 30 min at – 20 °C. After dilution of the mixture with precooled DMF (68 mL), (i-Pr)₂NEt (0.174 mL, 1.00 mmol) was added at - 40 °C, and the mixture was stirred for 24 h at - 20 °C. Concentration under reduced pressure and purification by preparative HPLC (linear gradient of B in A, 20 to 25% over 30 min) to give the cyclic pseudopeptide **5** (13.1 mg, 19% yield from **8a**) as freeze-dried powder: $[\alpha]_D^{20}$ - 59.4 (c 0.656, H₂O); t_R = 33.4 min (linear gradient of B in A, 20 to 40% over 40 min); ¹H NMR (600 MHz, DMSO-d_6) δ 0.46 (d, J = 6.6 Hz, 3 H), 0.66 (d, J = 6.6 Hz, 3 H), 1.32-1.46 (m, 2 H), 1.53 (m, 1 H), 1.58 (s, 3 H), 1.69 (m, 1 H), 1.84 (m, 1 H), 2.40 (dd, J = 16.2, 6.7 Hz, 1 H), 2.56 (t, J = 9.1 Hz, 1 H), 2.66 (dd, J = 16.2, 7.8 Hz, 1 H), 2.74 (dd, J = 13.5, 9.7 Hz, 1 H), 2.84 (dd, J = 13.5, 5.8 Hz, 1 H), 3.07 (m, 2 H), 3.26 (dd, J = 14.4, 4.2 Hz, 1 H), 3.98 (dd, J = 14.4, 6.8 Hz, 1 H), 4.17 (m, 1 H), 4.29 (m, 1 H), 4.55 (m, 1 H), 5.05 (d, J = 9.4 Hz, 1 H), 7.12-7.25 (m, 5 H), 7.36 (d, J = 8.3 Hz, 1 H), 7.46 (m, 1 H), 7.93-7.99 (m, 2 H), 8.11 (d, J = 8.3 Hz, 1 H), 12.28 (br, 1 H). LRMS (FAB), m/z 572 (MH⁺), 185 (base peak), 185, 154, 137, 93. HRMS (FAB), m/z calcd for C₂₈H₄₂N₇O₆ (MH⁺) 572.3197, found: 572.3208.

cyclo[-Arg-Gly-Asp-D-Phe-ψ[(E)-CMe=CMe]-Val-]·TFA (6).

By use of a procedure similar to that described for the preparation of the peptide **5** from the resin **11a**, the resin **11b** was converted into the title peptide **6** (16.9 mg, 20% yield): $[\alpha]_D^{22}$ - 62.6 (c 0.846, H₂O); t_R = 36.3 min (linear gradient of B in A, 20 to 40% over 40 min); ¹H NMR (600 MHz, DMSO-d_6) δ 0.26 (d, J = 6.5 Hz, 3 H), 0.80 (d, J = 6.4 Hz, 3 H), 1.33-1.49 (m, 5 H), 1.71 (s, 3 H), 1.72-1.81 (m, 2 H), 1.86 (m, 1 H), 2.43 (dd, J = 16.5, 6.8 Hz, 1 H), 2.70-2.79 (m, 3 H), 2.84 (dd, J = 13.3, 5.1 Hz, 1 H), 3.07 (m, 2 H), 3.27-3.32 (m, 1 H), 3.86 (m, 1 H), 3.91 (dd, J = 14.4, 6.7 Hz, 1 H), 4.52 (m, 1 H), 4.92 (m, 1 H), 7.10-7.22 (m, 5 H), 7.29 (m, 1 H), 7.50 (br, 1 H), 7.56 (d, J = 6.9 Hz, 1 H), 7.74 (d, J = 7.4 Hz, 1 H), 8.54 (d, J = 8.2 Hz, 1 H), 12.30 (br,

Table 9. Schedule for the molecular dynamic calculation of peptides **3-6**.

Phase	1	2	3	4	5
Time steps (fs)	1	1	1	1	1
Simulation time (ps)	28	10	7	25	15
Initial temperature (K)	1000	1000	650	475	300
Final temperature (K)	1000	650	475	300	300
Force constants for the distance restraints (kcal·mol^{-1}·Å^{-1})	25	50	75	100	100
Force constants for the dihedral angle restraints (kcal·mol^{-1}·rad^{-2})	25	50	75	100	100

1 H). LRMS (FAB), m/z 586 (MH$^+$), 154 (base peak), 93, 91, 87, 70. HRMS (FAB), m/z calcd for $C_{29}H_{44}N_7O_6$ (MH$^+$) 586.3353, found: 586.3368.

Integrin-binding Assays. Compounds were evaluated for their inhibitory activities in $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$-ELISA (enzyme linked immunosorbent assay). $\alpha_v\beta_3$ was purified from human placenta, using RGDSPK-sepharose CL-4B affinity chromatography, followed by mono Q ion exchange chromatography, according to Pytela's protocol.[15] $\alpha_{IIb}\beta_3$ was purified from human platelet by RGDSPK-sepharose CL-4B as well.[15] $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ binding assays were performed according to the modified method of Kouns et al.[16] EIA plates were coated with $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$, and blocked with bovine serum albumin. In each reaction, a test sample in the reaction mixture (20 mM Tris-HCl, 150 mM NaCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, pH 7.4, 0.100 mL) including vitronectin or fibrinogen, was added to the receptor-coated plate and incubated for 4 h at 25 °C. Thereafter the ligand binding was measured using anti-vitronectin rabbit antibody and peroxidase-conjugated anti-rabbit IgG antibody for $\alpha_v\beta_3$, or peroxidase-conjugated anti-fibrinogen antibody for $\alpha_{IIb}\beta_3$, and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) as the substrate of peroxidase. The IC$_{50}$ values were determined from measurement of absorbance at 415 nm.

NMR Spectroscopy. The peptide sample was dissolved in DMSO-d_6 at concentration of 5 mM. ^{1}H NMR spectra of the peptides were recorded at 300 K using a Bruker AM 600 spectrometer at 600 MHz ^{1}H frequency. The chemical shifts were referenced to the residual DMSO (2.50 ppm). The assignments of the proton resonances were completely achieved by use of ^{1}H-^{1}H COSY spectra (see Supporting Information). $^3J(H^N,H^\alpha)$ coupling constants were measured from one-dimensional spectra. The mixing time for the NOESY experiments was set at 200, 300 and 400 ms. NOESY spectra were composed of 2048 real points in the F2 dimension and 512 real points, which were zero-filled to 1024 points in the F1 dimension, with 32 scans per t1 increment. The cross-peak intensities were evaluated by relative build-up rates of the cross-peaks. For the examination of the temperature dependence of the amide protons, the spectra of all peptides were also recorded at the every 10 K in the range of 300-340 K.

Calculation of Structures. The structure calculations were performed on a Silicon Graphics Origin 2000 workstation with the NMR-refine program within the Insight II/Discover package using the consistent valence force field (CVFF). The prochiralities of two γ-methyl protons of Val^5 were assigned based on the $^3J(H^\alpha,H^\beta)$ and the different NOE

intensities in the NOESY spectra. On the other hand, The pseudoatoms were defined for the methylene protons of Arg[1], Asp[3] and D-Phe[4], prochiralities of which were not identified by ^{1}H-NMR data. The restraints, in which the Gly[2] α-methylene participated, were defined for the separate protons without definition of the prochiralities. The dihedral ϕ angle constraints were calculated based on the Karplus equation: $^{3}J(\text{H}^{\text{N}},\text{H}^{\alpha}) = 6.7\cos^{2}(\theta - 60) - 1.3\cos(\theta - 60) + 1.5$.[17] Lower and upper angle errors were set to 15°. The NOESY spectra with a mixing time of 200 ms were used for the estimation of the distance restraints between protons. The NOE intensities were classified into three categories (strong, medium and weak) based on the number of contour lines in the cross-peaks to define the upper-limit distance restraints (2.7, 3.5 and 5.0 Å, respectively). The upper-limit restraints were increased by 1.0 Å for the involved pseudoatoms. Lower bounds between nonbonded atoms were set to their van der Walls radii (1.8 Å). These restraints were included with force constants of 25 - 100 kcal·mol^{-1}·Å^{-2} for the distances and of 25 - 100 kcal·mol^{-1}·rad^{-2} for the dihedral angles. The 50 initial structures generated by the NMR refine program randomly were subjected to the simulated annealing calculations. Detailed protocols for the calculation are found in Table 9. The final minimization stage was achieved until the maximum derivative became less than 0.01 kcal·mol^{-1}·Å^{-2} by the steepest descents and conjugate gradients methods. The families of the preferred conformations were selected from the structures with energies not higher than 8 kcal/mol compared with the lowest energy.

References and Footnotes

1   Aumailley, M.; Gurrath, M.; Muller, G.; Calvete, J.; Timpl, R.; Kessler, H. *FEBS Lett.* **1991**, *291*, 50.

2   Dechantsreiter, M. A.; Planker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033.

3   (a) Gardner, R. R.; Liang G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 3280. (b) Gardner, R. R.; Liang G.-B.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 1806.

4   Wipf, P.; Henninger, T. C.; Geib, S. J. *J. Org. Chem.* **1998**, *63*, 6088.

5   Hoffman, R. W. *Chem. Rev.* **1989**, *89*, 1841.

6   Oishi, S.; Kamano, T.; Niida, A.; Odagaki, Y.; Hamanaka, N.; Yamamoto, M.; Ajito, K.; Tamamura, H.; Otaka, A.; Fujii, N. *J. Org. Chem.* **2002**, *67*, 6162.

7   (a) Oishi, S.; Niida, A.; Kamano, T.; Odagaki, Y.; Tamamura, H.; Otaka, A.; Hamanaka, N.; Fujii, N. *Org. Lett.* **2002**, *4*, 1055. (b) Oishi, S.; Niida, A.; Kamano, T.; Odagaki, Y.; Hamanaka, H.; Yamamoto, M.; Ajito, K.; Tamamura, H.; Otaka, A.; Fujii, N. *J. Chem. Soc., Perkin Trans. 1*, **2002**, 1786.

8   Honzl, J.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1961**, *26*, 2333.

9   Xiong, J.-P.; Stehle, T.; Zhang, R.; Joachimiak, A.; Frech, M.; Goodman, S. L.; Arnaout, M. A. *Science* **2002**, *296*, 151.

10 (a) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461. (b) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374.

11 (a) Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 512. (b) Coles, M., Sowemimo, V., Scanlon, D., Munro, S. L., Craik, D. J. *J. Med. Chem.* **1993**, *36*, 2658. (c) Mierke, D. F.; Kurz, M.; Kessler, H. *J. Am. Chem. Soc.* **1994**, *116*, 1042. (d) Kessler, H.; Gemmecker, G.; Haupt, A.; Klein, M.; Wagner, K.; Will, M. *Tetrahedron* **1996**, *44*, 745. (e) Melacini, G.; Zhu, Q.; Ösapay, G.; Goodman, M. *J. Med. Chem.* **1997**, *40*, 2252. (f) Mattern, R.-H.; Tran, T.-A.; Goodman, M. *J. Med. Chem.* **1998**, *41*, 2686. (g) Nikiforovich, G. V.; Kövér, K. E.; Zhang, W.-J.; Marshall, G. R. *J. Am. Chem. Soc.* **2000**, *122*, 3262. (h) Yokokawa, F.; Sameshima, H.; In, Y.; Minoura, K.; Ishida, T.; Shioiri, T. *Tetrahedron* **2002**, *58*, 8127.

12 (a) Miyamoto, K.; Nakagawa, T.; Kuroda, Y. *J. Pept. Res.* **2001**, *58*, 193. (b) Miyamoto, K.; Nakagawa, T.; Kuroda, Y. *Biopolymers* **2001**, *59*, 380, and references cited therein.

13 In the recent review,[14] it was noticed that the ligand backbone conformations of **2** are similar between in the crystalline complex with $\alpha_v\beta_3$ integrin and in the ligand solution. In addition, the shorter distance between Arg^1 and Asp^3 C^α atoms of **1** was also observed in comparison with that of **2**.

14 Gottschalk, K.-E.; Kessler, H. *Angew. Chem. Int. Ed.* **2002**, *41*, 3767.

15 Pytela, R.; Pierschbacher, M. D.; Argraves, S.; Suzuki, S.; Rouslahti, E. *Methods Enzymol.* **1987**, *144*, 475.

16 Kouns, W. C.; Kirchhofer, D.; Hadvary, P.; Edenhofer, A.; Weller, T.; Pfenninger, G.; Baumgartner, H. R.; Jennings, L. K.; Steiner, B. *Blood* **1992**, *80*, 2539.

17 Ludvigsen, S.; Andersen, K. V.; Poulsen, F. M. *J. Mol. Biol.* **1991**, *217*, 731.

Chapter 3. Conclusions

1. Two diastereisomeric L-Xaa-ψ[(E)-CH=CH]-L-Glu- and L-Xaa-ψ[(E)-CH=CH]-D-Glu-type EADIs were stereoselectively synthesized from a common key substrate. When *cis*-aziridine derivatives were used as key substrates, direct α-alkylation provided (L,L)-type EADIs, while the same reaction using *syn*-γ-chloro-α,β-enoates, obtained by pre-treatment of the aziridines with HCl/1,4-dioxane, gave (L,D)-type stereoisomers. For the key reaction, utilization of organozinc-copper complexes having carboxylate functionality provides side chains corresponding to glutamic acid residues at the α-position.

2. New ψ[(E)-CMe=CH]- and ψ[(E)-CMe=CMe]-type EADIs, which potentially represent superior β-turn promoters, were synthesized via regio- and stereoselective alkylation of 1,3-oxazolidin-2-one derivatives using organocopper reagents. In some cases, alkylations concomitantly afforded Z-congeners of the *anti*-S_N2' products. Plausible mechanisms leading to reaction selectivities are fully discussed based on conformational aspects of the substrates or potential copper-π-allyl intermediates in the transition state. This synthetic approach is also applicable to synthesis of usual ψ[(E)-CH=CH]-type EADIs.

3. It is found that the ψ[(E)-CMe=CH]- and ψ[(E)-CMe=CMe]-type EADIs as well as ψ[(E/Z)-CH=CMe]-type isosteres are amenable to usual protocols for Fmoc-based solid-phase peptide synthesis. In addition, cyclization of peptide hydrazides containing Arg and Asp residues by azide methodology enabled to efficiently prepare cyclic RGD peptidomimetics without the use of side chain protecting groups.

4. D-Phe-ψ[(E/Z)-CH=CMe]-L-Val-type isosteres were utilized to evaluate peptide bond *cis-trans* isomerization in the D-Phe-L-MeVal dipeptide moiety of Kessler's cyclic RGD peptide. Increased conformational flexibility and enhanced $\alpha_v\beta_3$ integrin antagonism as compared to the D-Phe-L-Val-type cyclic peptide induced by the *N*-methylation, are unlikely to be attributable to isomerization. As such, conformational restriction induced by a set of two ψ[(E/Z)-CH=CMe]-type isosteres, presents a novel chemical methodology to define roles of ω-angle rotations.

5. Comparative bioevaluation of cyclic RGD peptidomimetics containing D-Phe-ψ[(E)-CX=CX]-L-Val-type EADIs demonstrated that effects of the *N*-methyl group of the

MeVal residue (X = H or Me) are derived from the lack of a Val amide hydrogen rather than from steric interactions. On the other hand, the presence of a carbonyl oxygen may contribute to cyclic peptide conformations that are more appropriate for interaction with $\alpha_v\beta_3$ integrin. Thus, combination of EADIs that include a multiple styles of methyl-substituting groups allows evaluation of the effects of *N*-methyl groups and carbonyl oxygens in peptide bonds. These often have influences on conformations and bioactivities through $A^{1,2}$- and $A^{1,3}$-strain.

6.   Based on intensive structural analysis by ^{1}H-NMR and molecular dynamics calculations, it was found that all EADI-containing peptides adopted essentially identical conformations, at least in the case of cyclic RGD peptides. Additionally, these peptides exhibited conformations that were similar to the most potent D-Phe-L-MeVal dipeptide-containing $\alpha_v\beta_3$ integrin antagonist, rather than to the D-Phe-L-Val dipeptide-containing antagonist.

7.   It was found that γ-methylated isosteres utilizing $\psi[(E)$-CMe=CH]- and $\psi[(E)$-CMe=CMe]-type EADIs are inappropriate promoters, at least for representative type II' β-turn substructures in the cyclic RGD pentapeptide. This is in contrast to models suggested by Wipf et al. and Gellman et al.

Taken together, the author has achieved the synthesis of novel highly functionalized (E)-alkene dipeptide isosteres from chiral amino acids using organocopper-mediated regio- and stereoselective alkylations. The author also utilized these isosteres for SAR studies on cyclic RGD peptides. These investigations may facilitate SAR studies of a variety of bioactive peptides, and thereby advance drug discovery efforts in the field of medicinal chemistry.

Acknowledgments

List of Publications

This study was published in the following papers.

1. Stereoselective synthesis of a set of two functionalized (E)-alkene dipeptide isosteres of L-amino acid-L-Glu and L-amino acid-D-Glu.

   Shinya Oishi, Hirokazu Tamamura, Masaki Yamashita, Yoshihiko Odagaki, Nobuyuki Hamanaka, Akira Otaka and Nobutaka Fujii

   J. Chem. Soc., Perkin Trans. 1, **2001**, 2445-2451.

2. Diastereoselective synthesis of ψ[(E)-CMe=CH]- and ψ[(E)-CMe=CMe]-type dipeptide isosteres based on organocopper-mediated *anti*-S_N2' reaction.

   Shinya Oishi, Ayumu Niida, Takae Kamano, Yoshihiko Odagaki, Hirokazu Tamamura, Akira Otaka, Nobuyuki Hamanaka and Nobutaka Fujii

   Org. Lett., **2002**, *4*, 1055-1058.

3. Regio- and stereoselective ring-opening of chiral 1,3-oxazolidin-2-one derivatives by organocopper reagents provides novel access to di-, tri- and tetra-substituted alkene dipeptide isosteres.

   Shinya Oishi, Ayumu Niida, Takae Kamano, Yoshihiko Odagaki, Nobuyuki Hamanaka, Mikio Yamamoto, Keiichi Ajito, Hirokazu Tamamura, Akira Otaka and Nobutaka Fujii

   J. Chem. Soc., Perkin Trans. 1, **2002**, 1786-1793.

4. Diastereoselective synthesis of new ψ[(E)-CH=CMe]- and ψ[(Z)-CH=CMe]-type alkene dipeptide isosteres by organocopper reagents and application to conformationally restricted cyclic RGD peptidomimetics.

   Shinya Oishi, Takae Kamano, Ayumu Niida, Yoshihiko Odagaki, Nobuyuki Hamanaka, Mikio Yamamoto, Keiichi Ajito, Hirokazu Tamamura, Akira Otaka and Nobutaka Fujii

   J. Org. Chem., **2002**, *67*, 6162-6173.

5. Utilization of novel tri- and tetrasubstituted alkene dipeptide mimetics in conformational studies of cyclic peptides

   Shinya Oishi, Kazuhide Miyamoto, Ayumu Niida, Takae Kamano, Mikio Yamamoto, Keiichi Ajito, Hirokazu Tamamura, Akira Otaka, Yoshihiro Kuroda and Nobutaka Fujii

   J. Am. Chem. Soc,, *submitted.*