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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

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Received May 9, 2002

Diastereoselective synthesis of new ψ[(*E*)-CH=CMe]- and ψ[(*Z*)-CH=CMe]-type alkene dipeptide isosteres corresponding to dipeptides having one *N*-methylamino acid, and application to bioactive peptides, are described. In a key reaction introducing the chiral α-alkyl group of the isosteres, organocopper-mediated alkylation of *syn*-β-methylated γ-mesyloxy-α,β-enoate **26a** afforded *E*- and *Z*-isomers of *anti*-S_N2' products in a solvent-dependent manner. The resulting two isosteres, D-Phe-ψ[(*E*)-CH=CMe]-L-Val **27a** and D-Phe-ψ[(*Z*)-CH=CMe]-L-Val **28b**, which corresponded to *trans*- and *cis*-conformers of D-Phe-L-MeVal, respectively, were utilized in a structure−activity relationship study on cyclic RGD peptides **1** and **2**, in company with a ψ[(*E*)-CH=CH]-type alkene dipeptide isostere, D-Phe-ψ[(*E*)-CH=CH]-L-Val. The cyclic isostere-containing pseudopeptides **3**, **4**, and **40** were synthesized and biological activity against integrin $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ receptors were also evaluated.

Introduction

Integrins are cell membrane receptors consisting of α and β subunits, which participate in cell−cell and cell−matrix adhesive interactions.[1] Among these receptors, $\alpha_V\beta_3$ integrin receptors are 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 (Arg-Gly-Asp) sequence, which is key recognition motif of integrin receptors.[4] In 1991, Kessler et al. reported that a cyclic pentapeptide, cyclo(-Arg-Gly-Asp-D-Phe-Val-) **1**, was a highly active integrin $\alpha_V\beta_3$ antagonist.[5] Their extensive conformational research also revealed that peptide **1** adopted a representative type II′ β/γ structure, in which D-Phe and Gly were at the $i + 1$ positions of β- and γ-turns in dimethyl sulfoxide, respectively. In addition, *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

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10.1021/jo025923m CCC: $22.00
Published on Web 08/01/2002

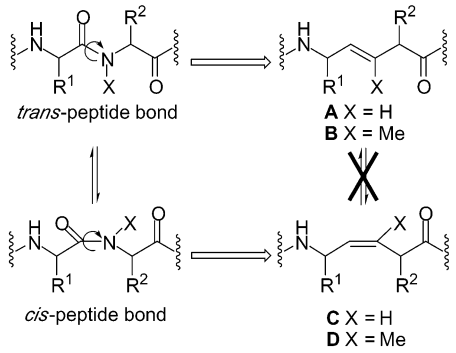
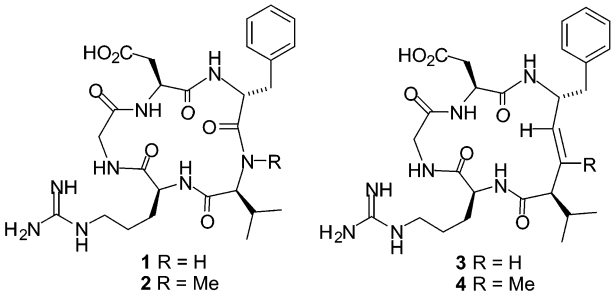


FIGURE 1.

cis−*trans* imide isomerization. This results in greater flexibility, which provides for a higher population of *cis*-peptide bonds ($\omega = 0°$), as compared to usual amidic peptide bonds.[7] It is noteworthy that in water and dimethyl sulfoxide the *N*-methyl group of L-MeVal in the peptide **2** induces a more flexible $\gamma_i/\gamma/\gamma_i$ arrangement, which consists of two inverse γ-turns (γ_i-turns) having Arg and Asp at the $i + 1$ positions and one γ-turn having Gly at the $i + 1$ position.[6] Although the peptide backbone secondary structure was discussed in detail, no suggestion was offered whether the flexible conformation in **2** was derived from rotation of the peptide bond between D-Phe and L-MeVal induced by *N*-methylation or from altered distribution of side chains proximal to the *N*-methyl group with the maintenance of *trans*-amide conformer of D-Phe-L-MeVal ($\omega = 180°$).



Peptidomimetics are valuable tools for conformational investigation of bioactive peptides and proteins, and for development of peptide-leads for pharmaceuticals.[8] Alkene dipeptide isosteres restrict peptide ω-angle rotations to *cis*- or *trans*-conformers (Figure 1). They have been widely utilized for elucidating the importance of amide bond polarity,[9] as surrogates of β-turn substructures[10] and to suppress peptide bond *cis*/*trans*-isomerization,[11] as well as other uses.[12] To characterize bioactive conformations of cyclic RGD peptides **1** and **2**, particularly in D-Phe-L-Val and D-Phe-L-MeVal moieties, we designed two cyclic pseudopeptides **3** and **4**, which contain ψ[(*E*)-CH=CH]- **A** and ψ[(*E*)-CH=CMe]- **B** type (*E*)-alkene dipeptide isosteres (EADIs), respectively. It was assumed that the D-Phe-ψ[(*E*)-CH=CH]-L-Val-type EADI in **3** can be a mimetic of the $i + 1$ and $i + 2$ β-turn sites in **1**. The methylated analogue, D-Phe-ψ[(*E*)-CH=CMe]-L-Val-type EADI, in **4** can be viewed in the same way as an equivalent of the D-Phe-L-MeVal moiety in **2**. In pseudopeptides **3** and **4**, EADI-mediated restriction of ω-angles between D-Phe and L-Val/MeVal may permit rational evaluation of the *N*-methyl group's effect on the conformation of peptide **2**.

To realize the structure−activity relationship objectives outlined above, we required the synthesis of unprecedented alkene dipeptide isosteres **B**, which correspond to dipeptides containing an *N*-methylamino acid such as D-Phe-ψ[(*E*)-CH=CMe]-L-Val-type EADI. A number of synthetic approaches toward ψ[(*E*)-CH=CH]- **A** and ψ[(*Z*)-CH=CH]-type **C** alkene dipeptide isosteres have been reported to date.[11−17] We attempted to syn-

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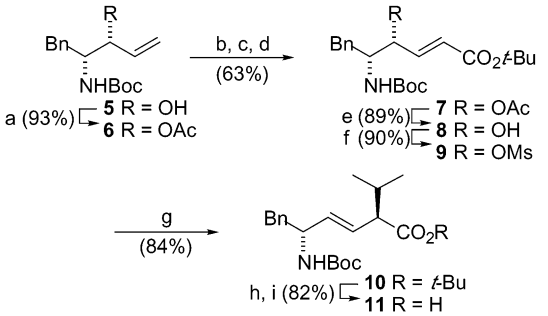
SCHEME 1[a]



[a] Reagents: (a) Ac_2O, pyridine, DMAP; (b) O_3 gas; (c) Me_2S; (d) $(EtO)_2P(O)CH_2CO_2t$-Bu, $(i\text{-}Pr)_2NEt$, LiCl; (e) Na_2CO_3, MeOH; (f) MsCl, pyridine; (g) i-PrCu(CN)MgCl·BF_3; (h) TFA; (i) $(Boc)_2O$, Et_3N.

thesize ψ[(*E*)-CH=CMe]-type EADIs **B** using organocopper-mediated *anti*-S_N2' alkylation of β-methylated α,β-enoates having a leaving group at the γ-position. This was based on well-established stereochemical precedence as in the synthesis of ψ[(*E*)-CH=CH]-type EADIs **A**.[13,18]

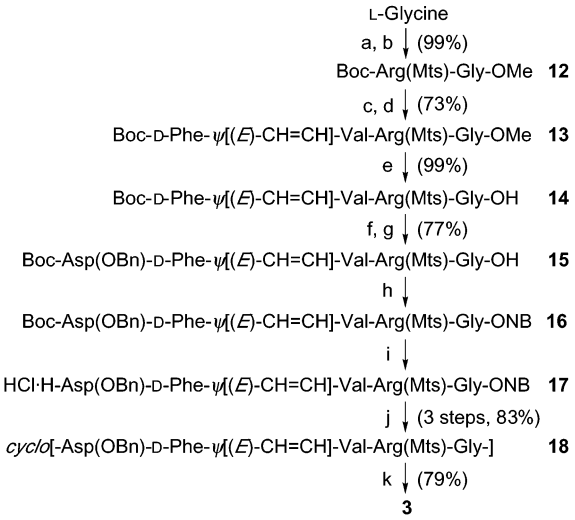
In the current paper, we describe the diastereoselective synthesis of new ψ[(*E*)-CH=CMe]-type alkene dipeptide isosteres **B** as well as unexpected ψ[(*Z*)-CH=CMe]-type ones **D**. We also present an exploration of bioactive conformation of Kessler's cyclic RGD peptides using pseudopeptides containing these isosteres and a ψ[(*E*)-CH=CH]-type isostere **A**.[19]

Results and Discussion

Synthesis of a Cyclic RGD Pseudopeptide Containing D-Phe-ψ[(*E*)-CH=CH]-L-Val-type Alkene Dipeptide Isostere. Initially, we 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 us and others. Here reaction of γ-mesyloxy-α,β-enoates using organocopper reagents gave α-alkylated products regio- and stereoselectively by an *anti*-S_N2' mechanism.[13] A protected D-Phe-ψ[(*E*)-CH=CH]-L-Val-type EADI **11** was synthesized according to established procedures (Scheme 1).

After protection with an acetyl group of the known allyl alcohol **5**,[16b] the resulting acetate **6** was subjected to a sequence of reactions consisting of ozonolysis followed by modified Horner−Wadsworth−Emmons olefination[20] to give γ-acetoxy-α,β-enoate **7** *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 **9**, which is a key substrate for organocopper-mediated alkylation. Treatment of α,β-enoate **9** with i-PrCu(CN)MgCl·BF_3 afforded an α-alkylated product, Boc-D-Phe-ψ[(*E*)-CH=CH]-L-Val-Ot-Bu **10**, as a single diastereomer in 84% yield. Successive TFA treatment and *N*-reprotection of **10** provided *N*-Boc-protected EADI **11**, which was utilized for Boc-based solution-phase peptide synthesis of pseudopeptide **3** (Scheme 2). EADI **11** was converted to protected pseudotetrapeptide **13** by DCC condensation with a HCl-treated sample of Boc-Arg(Mts)-Gly-OMe **12**, 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 **13** was saponified at this stage to give the corresponding acid, Boc-D-Phe-ψ[(*E*)-CH=CH]-Val-Arg(Mts)-Gly-OH **14**. TFA treatment of protected peptide **14** 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 **15**. To facilitate efficient intramolecular peptide bond formation, acid **15** was quantitatively converted to the activated ester, Boc-Asp(OBn)-D-Phe-ψ[(*E*)-CH=CH]-Val-Arg(Mts)-Gly-ONB **16**. 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 **18** was treated with 1 M TMSBr-thioanisole in TFA in the presence of *m*-cresol and 1,2-ethanedithiol[21] to afford expected cyclic peptide **3**, which was fully characterized by ^1H NMR and mass spectra.

SCHEME 2[a,b]



[a] Reagents: (a) $SOCl_2$, MeOH; (b) Boc-Arg(Mts)-OH, DCC, HOBt, $(i\text{-}Pr)_2NEt$; (c) 4 M HCl−dioxane, anisole; (d) **11**, DCC, HOBt, $(i\text{-}Pr)_2NEt$; (e) LiOH; (f) TFA, anisole; (g) Boc-Asp(OBn)-ONB, $(i\text{-}Pr)_2NEt$; (h) DCC, HONB; (i) 4 M HCl−dioxane; (j) *N*-methylmorpholine; (k) 1M TMSBr−thioanisole/TFA, *m*-cresol, 1,2-ethanedithiol. [b]Abbreviations: HOBt = 1-hydroxybenzotriazole; HONB = *N*-hydroxy-5-norbornene-2,3-dicarboximide; Mts = 2,4,6-trimethylphenylsulfonyl.

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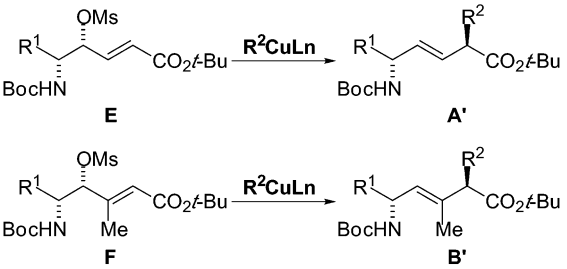
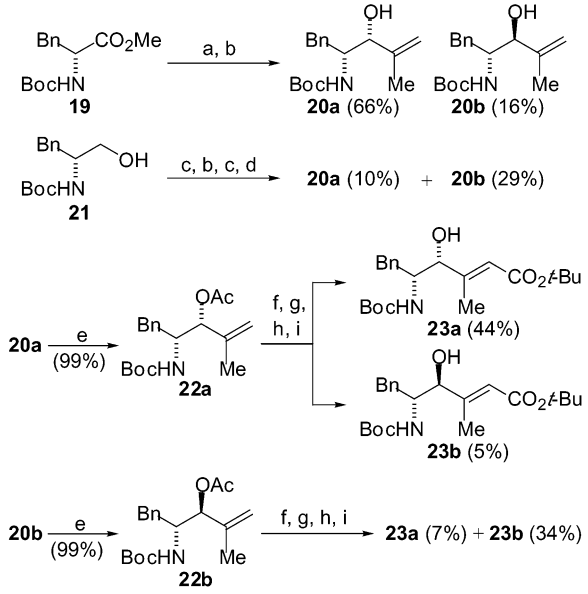
SCHEME 3



Diastereoselective Synthesis of D-Phe-ψ[(*E*)-CH=CMe]-L-Val- and D-Phe-ψ[(*Z*)-CH=CMe]-L-Val-type Alkene Dipeptide Isosteres by Organocopper-Mediated Alkylation. Next, we engaged in the synthesis of a D-Phe-ψ[(*E*)-CH=CMe]-L-Val-type EADI as a D-Phe-L-MeVal dipeptide equivalent. The stereoselective construction of two chiral centers adjacent to a trisubstituted alkene with concomitant control of olefinic geometry were required for the synthesis of ψ[(*E*)-CH=CMe]-type EADIs **B′**.[22] It was assumed that this isostere could be synthesized by organocopper-mediated alkylation of β-methylated γ-mesyloxy-α,β-enoates **F** by essentially the same strategy as that utilized in the synthesis of ψ[(*E*)-CH=CH]-type EADIs **A′** from nonmethylated γ-mesyloxy-α,β-enoates **E**, as shown in Scheme 3.[13] In this synthetic scheme, chirality of the amino group in EADIs **B′** is derived from the amino acid used as starting material, while chirality of the alkyl group at the α-position depends on the γ-mesyloxy group in substrate **F**. Additionally, it was expected that alkylated products should be obtained only as *E*-isomers based on previous investigations.[13−15]

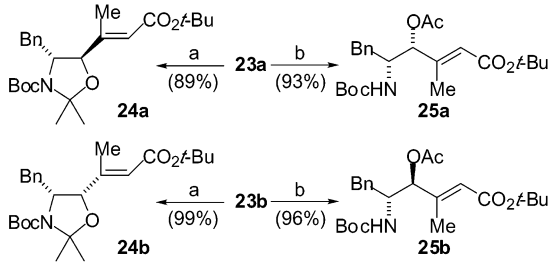
Initially, efficient synthesis of EADIs **B′** precursors, γ-mesyloxy-β-methyl-α,β-enoates **F**, was intensively investigated (Scheme 4). For diastereoselective construction of hydroxy groups corresponding to the chiral γ-mesyloxy groups of **F**, *syn*- and *anti*-amino alcohols **20a**,**b** were synthesized, respectively in selective fashion.[23] Ester **19** was converted predominantly into *syn*-amino alcohol **20a** by reduction with DIBAL-H followed by addition of an isopropenyl group using a Grignard reagent.[24] On the other hand, *anti*-amino alcohol **20b** was obtained preferentially by reduction of the enone with $Zn(BH_4)_2$,[25] which was prepared by Swern oxidation of the *syn*/*anti*-mixture of the allyl alcohols **20a**,**b** derived from **21**. Diastereomerically pure *syn*- and *anti*-amino alcohols **20a**,**b** could be readily separated by flash chromatography over silica gel. After *O*-protection of amino alcohol **20a**, ozonolysis of acetate **22a** followed by reduction using dimethyl sulfide yielded the crude ketone. Wittig reaction using $Ph_3P{=}CHCO_2t$-Bu gave the corresponding γ-acetoxy-β-methyl-α,β-enoates, which were deacetylated to provide two isomeric γ-hydroxy-β-methyl-α,β-enoates.

SCHEME 4[a]



[a] Reagents: (a) DIBAL-H; (b) $CH_2{=}CMeMgBr{\cdot}ZnCl_2{\cdot}LiCl$; (c) $(COCl)_2$, DMSO, $(i\text{-}Pr)_2NEt$; (d) $Zn(BH_4)_2$; (e) Ac_2O, pyridine, DMAP; (f) O_3 gas; (g) Me_2S; (h) $Ph_3P{=}CHCO_2t$-Bu; (i) Na_2CO_3, MeOH.

SCHEME 5[a]



[a] Reagents: (a) 2,2-dimethoxypropane, $BF_3{\cdot}Et_2O$; (b) Ac_2O, pyridine, DMAP.

The major product was the expected *syn*-*E*-γ-hydroxy-β-methyl-α,β-enoate **23a**, while the minor isomer proved to be the unexpected *anti*-*E*-γ-hydroxy-β-methyl-α,β-enoate **23b**, which resulted from epimerization at the γ-hydroxy group. The *anti*-isomer of acetate **22b** was converted similarly into a mixture of hydroxy esters **23a**,**b**, which could be purified by flash chromatography to give the respective hydroxy esters as single diastereomers. Relative stereochemistries of the hydroxy groups of **23a**,**b** were established by NOESY spectra of the corresponding acetonides **24a**,**b**, which were prepared by exposure to 2,2-dimethoxypropane (Scheme 5).[26] In the spectrum of **24a**, a cross-peak between the 5-*H* proton of the 1,3-oxazolidine ring and the benzyl protons proved it to be the *trans*-isomer. On the other hand, the corresponding cross-peak in the spectrum of the *cis*-acetonide **24b** was not observed. NOE experiments of acetates **25a**,**b**, which were obtained by acetylation of **23a**,**b**, established *E*-olefinic geometries of the hydroxy esters **23a**,**b**. NOE enhancement of the olefinic proton reso-

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

(23) Hoffman, R. V.; Maslouh, N.; Cervantes-Lee, F. *J. Org. Chem.* **2002**, *67*, 1045.

(24) *Syn*-selective formation of 1,2-amino alcohols by the one-pot reaction, which included reduction with DIBAL-H and alkylation with an alkenyl metal reagent, were previously reported: Tohdo, K.; Hamada, Y.; Shoiri, T. *Tetrahedron Lett.* **1992**, *33*, 2031.

(25) (a) Wasserman, H. H.; Xia, M.; Petersen, A. K.; Jorgensen, M. R.; Curtis, E. A. *Tetrahedron Lett.* **1999**, *40*, 6163. (b) Travins, J. M.; Bursavich, M. G.; Veber, D. F.; Rich, D. H. *Org. Lett.* **2001**, *3*, 2725.

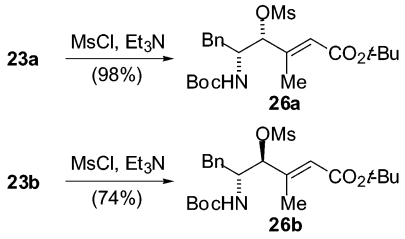
(26) Benedetti, F.; Miertus, S.; Norbedo, S.; Tossi, A.; Zlatoidzky, P. *J. Org. Chem.* **1997**, *62*, 9348.

TABLE 1. Alkylation of a *syn*-γ-Mesyloxy-β-methyl-α,β-unsaturated Ester 26a and the γ-Acetoxy Derivative 25a with Organocyanocuprates

run	substrate	reagent[a]	additive[b]	solvent	condition[d]	product ratio[e] **27a**:**28a**:**27b**+**28b**[f]:**29**	yield[h] (%)
1	**26a**	A	-	THF	A	40:51:—[g]:9	82
2	**26a**	B	-	THF	B	35:64:—[g]:1	94
3	**26a**	B	HMPA	THF	B	28:71:—[g]:1	91
4	**26a**	B	TMEDA	THF	B	27:72:—[g]:1	92
5	**26a**	C	18-crown-6	THF	B	25:73:—[g]:1	86
6	**26a**	A	-	Et_2O	A	36:63:—[g]:1	15[i]
7	**26a**	B	-	Et_2O	B	46:27:26:1	90
8	**26a**	B	HMPA	Et_2O	C	70:27:—[g]:3	93
9	**26a**	B	TMEDA	Et_2O	B	44:25:28:3	81
10	**26a**	C	18-crown-6	Et_2O	B	14:84:—[g]:2	93
11	**26a**	B	THF	Et_2O	B	43:42:12:3	97
12	**26a**	B	-	Et_2O-THF[c]	B	37:62:—[g]:1	87
13	**25a**	B	-	THF	D	21:79:—[g]: —[g]	39[i]
14	**25a**	B	-	Et_2O	D	73:27:—[g]: —[g]	57[i]

[a] All reactions were carried out with 4 mol equiv of reagent, A: *i*-PrCu(CN)MgCl·BF_3; B: *i*-Pr_2Cu(CN)$(MgCl)_2$·BF_3; C: *i*-Pr_2Cu(CN)$(MgCl)_2$. [b] 4 mol equiv. [c] Et_2O:THF = 7:3. [d] A: 0 °C, 0.5 h; B: − 78 °C, 0.5 h; C: − 78 °C, 0.5 h, then 0 °C, 0.5 h; D: − 78 °C, 0.5 h, then 0 °C, 3 h. [e] Product ratios were determined by HPLC and ^{1}H NMR. [f] Containing small amount of an uncharacterized product. [g] Although we cannot conclusively rule out its presence, we failed to isolate the corresponding product. [h] Combined yield. [i] The starting material was recovered (77, 60, and 41% for runs 6, 13, and 14, respectively).

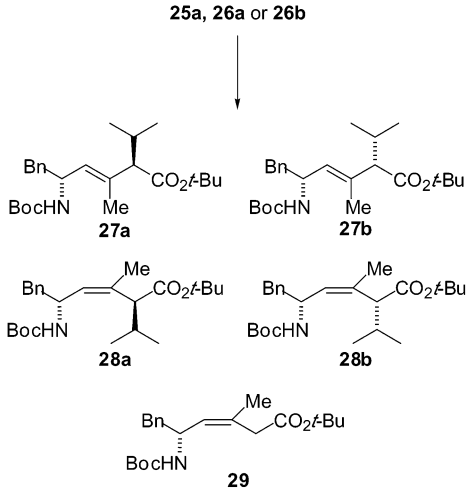
SCHEME 6



nances of **25a**,**b** were observed by irradiation of the 4-*H* proton (10.0% and 9.9%, respectively). Mesylation of hydroxy esters **23a**,**b** gave the expected β-methylated γ-mesyloxy-α,β-enoates **26a**,**b**, respectively, which were used for organocopper-mediated alkylation (Scheme 6).

It was initially envisioned that regio- and stereoselective alkylation of β-methylated γ-mesyloxy-α,β-enoates **26a**,**b** by organocopper reagents would proceed without significant difficulty based on precedence of the unmethylated analogue **9**. However, obvious differences in reactivity and in stereoselectivity of alkylation products were observed (Scheme 7 and Table 1). Treatment of the *syn*-α,β-enoate **26a** with a "lower-order" cyanocuprate-BF_3 complex, *i*-PrCu(CN)MgCl·BF_3, at −78 °C, which is generally utilized for the synthesis of ψ[(*E*)-CH=CH]-type EADIs **A**,[13] failed to provide product. However, the same reaction proceeded at elevated temperature (0 °C) to yield α-alkylated products **27a** and **28a** with a small amount of a reductive product, Boc-D-Phe-ψ[(*E*)-CH=CMe]-Gly-O*t*-Bu **29** (run 1). Unexpectedly, the major product was an unprecedented *Z*-isomer of an *anti*-S_N2' product, Boc-D-Phe-ψ[(*Z*)-CH=CMe]-D-Val-O*t*-Bu **28a**.[27] This result suggested that β-methylated analogue **26a** has less reactivity toward organocopper reagents than **9**. Employment of a more reactive "higher-order" reagent, *i*-Pr_2Cu(CN)$(MgCl)_2$·BF_3, which tends to reduce aziridinyl substrates,[14b] also provided α-alkylated products **27a** and **28a** with *Z*-selectivity even at −78 °C in excellent combined yields (run 2). It was supposed that *Z*-isomer

SCHEME 7



28a was predominantly formed as a result of preferential reaction from a more favorable conformer **26a-B** in THF (Figure 2).[28] Therefore, introduction of additives such as HMPA, TMEDA, and 18-crown-6 was attempted to improve *E*-selectivity; however, increased *E*-selectivity was not observed (runs 3−5). Although Et_2O has been thought to be an inappropriate solvent for alkylation owing to sluggishness,[29] its use in the present case resulted a remarkable improvement of the *E*-selectivity. Reaction of α,β-enoate **26a** with *i*-PrCu(CN)MgCl·BF_3 in Et_2O proceeded with low efficiency (run 6), while use of *i*-Pr_2Cu(CN)$(MgCl)_2$·BF_3 improved the *E*-selectivity with significant production of *syn*-S_N2' products **27b** and **28b** (run 7). Among additives investigated, HMPA most efficiently decreased production of *syn*-S_N2' products to afford the expected EADI, Boc-D-Phe-ψ[(*E*)-CH=CMe]-L-Val-O*t*-Bu, **27a**, which could be purified by flash

(27) Yang, H.; Sheng, X. C.; Harrington, E. M.; Ackermann, K.; Garcia, A. M.; Lewis, M. D. *J. Org Chem.* **1999**, *64*, 242.

(28) It could be also assumed that the formation of copper−π-allyl complexes as reactive intermediates were involved in the alkylation. In this case, product ratios should depend on stabilities of intermediates.

(29) (a) Ibuka, T.; Tanaka, M.; Nishii, S.; Yamamoto, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4864. (b) Ibuka, T.; Akimoto, N.; Tanaka, M.; Nishii, S.; Yamamoto, Y. *J. Org. Chem.* **1989**, *54*, 4055.

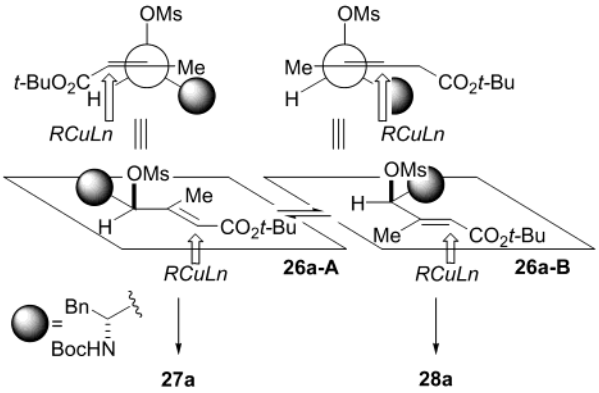


FIGURE 2.

TABLE 2. Alkylation of an *anti*-γ-Mesyloxy-β-methyl-α,β-unsaturated Ester 26b with Organocyanocuprates

run	reagent[a]	solvent	condition[b]	product ratio[c] **27b:28b:27a+28a:29**	yield[e] (%)
1	A	THF	A	27:69:—[d]:4	65
2	B	THF	B	41:54:—[d]:5	87
3	A	Et_2O	A	—	—[f]
4	B	Et_2O	A	7: 41:26:26	68

[a] All reactions were carried out with 4 mol equiv of reagent, A: *i*-PrCu(CN)MgCl·BF_3; B: *i*-Pr_2Cu(CN)$(MgCl)_2$·BF_3. [b] A: − 78 °C, 0.5 h then 0 °C, 3 h; B: − 78 °C, 0.5 h. [c] Product ratios were determined by HPLC and ^{1}H NMR. [d] Although we cannot conclusively rule out its presence, we failed to isolate the corresponding product. [e] Combined yield. [f] The starting material was recovered.

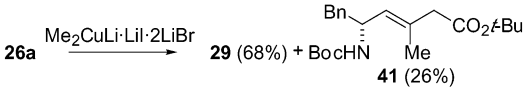
chromatography over silica gel (run 8). In contrast, 18-crown-6 increased *Z*-selectivity of alkylation (run 10). We also examined mixed solvents consisting of THF and Et_2O to quantitatively estimate solvent effects on *E*-selectivity. Alkylation of **26a** with *i*-Pr_2Cu(CN)$(MgCl)_2$·BF_3 in Et_2O in the presence of 4 equiv of THF gave nearly equal amounts of *E*- and *Z*-isomers of *anti*-S_N2' products **27a** and **28a** (run 11). The product ratio of **27a** and **28a** following alkylation in Et_2O−THF (7:3) was identical to that in THF alone (run 12). As such, *Z*-selectivity seemed dependent on addition of cyclic ethers such as THF or 18-crown-6, although the reason was unclear. Alkylation of β-methylated γ-acetoxy-α,β-enoate **25a** with *i*-Pr_2Cu(CN)$(MgCl)_2$·BF_3 in THF or Et_2O afforded α-alkylated products **27a** and **28a** with *Z*- or *E*-selectivity, respectively, in similar solvent-dependent fashion as observed in the reactions of mesylate **26a**. In this case, the overall yields were decreased in comparison with that from **26a**, probably due to the deficient leaving-group ability of the acetoxy group of **25a** (runs 13 and 14).

Organocopper-mediated alkylation of the *anti*-γ-mesyloxy-α,β-enoate **26b**, which was expected to provide Boc-D-Phe-ψ[(*E*)-CH=CMe]-D-Val-O*t*-Bu **27b** by an *anti*-S_N2' mechanism, was also investigated in similar fashion (Scheme 7 and Table 2). Treatment of **26b** with the "lower-order" cyanocuprate-BF_3 complex, *i*-PrCu(CN)-MgCl·BF_3, in THF preferentially yielded a *Z*-isomer of an *anti*-S_N2' product, Boc-D-Phe-ψ[(*Z*)-CH=CMe]-L-Val-O*t*-Bu, **28b** in a moderate yield (run 1). The "higher-order" cyanocuprate-BF_3 complex, *i*-Pr_2Cu(CN)$(MgCl)_2$·BF_3, at −78 °C also provided α-alkylated products **27b** and **28b** with similar *Z*-selectivity (run 2). In sharp contrast to the alkylation of *syn*-isomer **26a**, improvement of *E*-selectivity in the alkylation of *anti*-isomer **26b** was not observed, even following treatment with *i*-Pr_2-Cu(CN)$(MgCl)_2$·BF_3 in Et_2O. This resulted in preferential production of *Z*-isomer **28b** along with *syn*-S_N2'products **27a** and **28a** and a reductive product **29** (run 4). In contrast, alkylation of **26b** with *i*-PrCu(CN)MgCl·BF_3 in Et_2O failed to proceed (run 3). The unexpected *Z*-isomer, Boc-D-Phe-ψ[(*Z*)-CH=CMe]-L-Val-O*t*-Bu, **28b** represents a potential mimetic of the *cis*-peptide bond between D-Phe and L-MeVal (Figure 1, **D**).[11] We therefore utilized the *Z*-isomer in a conformational study on cyclic RGD peptide **2**.

Regiochemical assignments of alkylated products **27a**,**b** and **28a**,**b** and the reduced product **29** were performed by ^{1}H NMR (NOE experiments, ^{1}H−^{1}H COSY and NOESY spectra). The observed enhancement of the 2-*H* resonance by irradiation of the olefinic proton indicated **27a**,**b** to be *E*-isomers (13.2% and 12.0%). The olefinic resonance following irradiation of the 3-methyl protons indicated that **28a**,**b** and **29** were *Z*-isomers (14.0%, 13.3%, and 3.1%).[30] Stereochemistries of the resulting alkyl groups of **27a**,**b** and **28a**,**b** were established by circular dichroism (CD) measurements based on an empirical method used for determination of α-alkyl group configuration of α-alkyl-β,γ-enoates.[31] Negative Cotton effects around 220 nm in CD spectra of **27a** and **28b** indicated 2*R*-isomers, while positive Cotton effects of **27b** and **28a** indicated 2*S*-isomers. The crystal structure of **28b** also supported these assignments.[32]

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 resulting Boc-D-Phe-ψ[(*E*)-CH=CMe]-L-Val-O*t*-Bu **27a** and Boc-D-Phe-ψ[(*Z*)-CH=CMe]-L-Val-O*t*-Bu **28b**, were utilized for the synthesis of cyclic RGD pseudopeptides (Schemes 8 and 9). 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 **27a** and **28b** were converted to the corresponding Fmoc-amino acids **30** and **35** 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 **34** should be selectively activated by the azide method[33] without modification of a carboxylic acid functionality of Asp (Scheme 8). Accordingly, *p*-nitrophenyl carbonate Wang resin **31** was treated with hydrazine hydrate in DMF to afford a resin possessing a hydrazino linker **32**, which could be easily cleaved by TFA treat-

(30) An *E*-isomer of the reductive products, D-Phe-ψ[(*E*)-CH=CMe]-Gly **41**, resulted from treatment of the mesylate **26a** using Gilman-type reagent, Me_2CuLi·LiI·2LiBr, as shown below.[29]

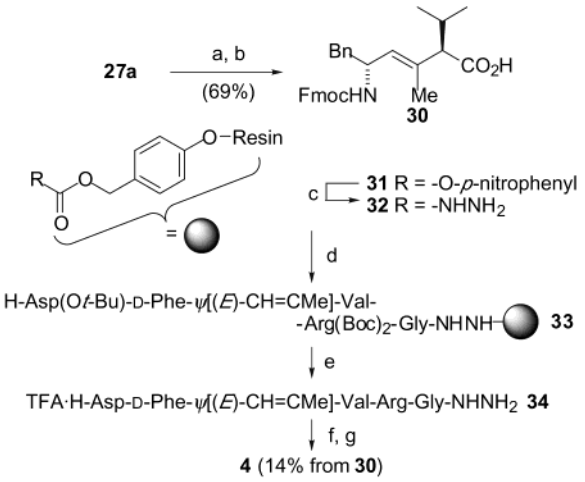


(31) Ibuka, T.; Habashita, H.; Funakoshi, S.; Fujii, N.; Baba, K.; Kozawa, M.; Oguchi, Y.; Uyehara, T.; Yamamoto, Y. *Tetrahedron: Asymmetry* **1990**, *1*, 379.

(32) The crystal structure of **28b** was presented in the preliminary communication.[19]

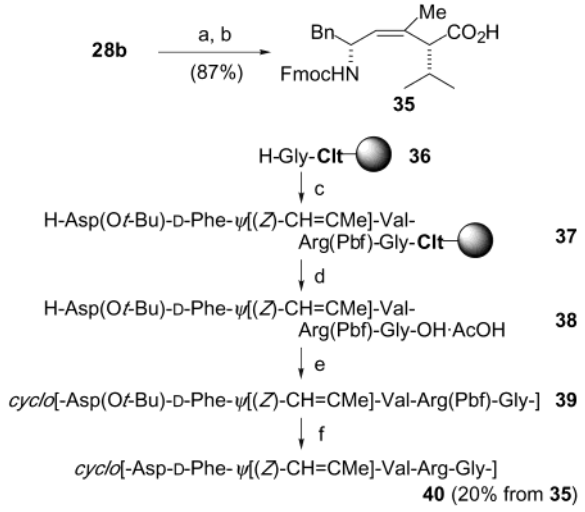
(33) Honzl, J.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1961**, *26*, 2333.

SCHEME 8[a]



[a] Reagents: (a) TFA; (b) Fmoc-OSu, Et_3N; (c) $NH_2NH_2 \cdot H_2O$; (d) Fmoc-based SPPS; (e) TFA; (f) HCl, isoamyl nitrite; (g) $(i\text{-}Pr)_2NEt$.

SCHEME 9[a,b]



[a] Reagents: (a) TFA; (b) Fmoc-OSu, Et_3N; (c) Fmoc-based SPPS; (d) AcOH−TFE−CH_2Cl_2 (1:1:3); (e) DPPA, $NaHCO_3$; (f) 95% TFA-H_2O. [b]Abbreviations: Clt resin = 2-chlorotrityl resin; Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; DPPA = diphenylphosphoryl azide.

ment to yield a peptide hydrazide. Fmoc-amino acid-containing **30** was successively coupled onto the linker using normal Fmoc-based SPPS. Following 95% TFA treatment of the protected peptidyl resin **33**, the resulting peptide hydrazide **34** was cyclized by the azide method in highly diluted DMF solution to yield the cyclic pseudopeptide **4**,[34] which contains a D-Phe-ψ[(*E*)-CH=CMe]-L-Val-type isostere, in 14% yield from **30**.

In contrast, cyclic pseudopeptide **40** was synthesized by well-established cyclic peptide synthesis protocols (Scheme 9). A protected peptide resin **37** was constructed by Fmoc-based SPPS on glycinyl 2-chlorotrityl (Clt) resin **36**, which can provide side chain-protected peptides following mild acidic treatment.[35] Exposing resin **37** to AcOH−TFE−CH_2Cl_2 (1:1:3) provided protected peptide **38**, which was subjected to cyclization using DPPA and $NaHCO_3$ in DMF.[36] Deprotection of protected cyclic peptide **39** using 95% TFA, followed by HPLC purification yielded the expected cyclic pseudopeptide **40** having a D-Phe-ψ[(*Z*)-CH=CMe]-L-Val-type isostere, in 20% yield from the isostere **35**.

TABLE 3. Biological Effects of Cyclic RGD Peptides and Pseudopeptides against Integrin Receptors

	$\alpha_V\beta_3$		$\alpha_{IIb}\beta_3$	
peptide	IC_{50} (nM)	Q[b]	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
40	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).

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 cyclic pseudopeptides **3**, **4** and **40** 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 3). 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 **40**, which contained a ψ[(*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-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

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

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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**.

Conclusion

In conclusion, the synthesis of new ψ[(*E*)-CH=CMe]-type alkene isosteres was accomplished utilizing alkylation of β-methylated γ-mesyloxy-α,β-enoates by organocopper reagents. Alkylation afforded unprecedented *Z*-isomers of *anti*-S_N2' products as well as expected *E*-isomers. These are potential dipeptide mimetics of ω-constrained *cis*- and *trans*-peptide bonds between an amino acid and an *N*-methyl amino acid, respectively. In addition, pseudopeptides **3**, **4**, and **40**, which contain D-Phe-ψ[(*E*)-CH=CH]-L-Val, D-Phe-ψ[(*E*)-CH=CMe]-L-Val, and D-Phe-ψ[(*Z*)-CH=CMe]-L-Val-type isosteres, respectively, were synthesized utilizing three separate routes for conformational analysis of Kessler's cyclic RGD peptides **1** and **2**, and antagonistic activities against integrins were evaluated. The reduced difference in activities against $\alpha_V\beta_3$ integrin between the cyclic pseudopeptides **3** and **4**, as compared to between cyclic peptides **1** and **2**, suggest that the high activity 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_4Si (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.

(3*R*,4*R*)-*O*-Acetyl-4-[*N*-(*tert*-butoxycarbonyl)amino]-5-phenylpent-1-en-3-ol (6). To a stirred solution of the alcohol **5**[16b] (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_2O (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 **6** (13.8 g, 93% yield) as colorless crystals: mp 60−63 °C (*n*-hexane/Et_2O = 5:1); $[\alpha]^{24}_D$ +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 (4*R*,5*R*,2*E*)-4-Acetoxy-5-[*N*-(*tert*-butoxycarbonyl)amino]-6-phenylhex-2-enoate (7).** To a solution of the acetate **6** (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_2S (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 $(EtO)_2P(O)CH_2CO_2t$-Bu (12.0 mL, 50.5 mmol) and $(i\text{-Pr})_2$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 **7** (5.38 g, 63% yield) as colorless crystals: mp 101−105 °C (*n*-hexane/Et_2O = 3:1); $[\alpha]^{29}_D$ +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 (4*R*,5*R*,2*E*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-4-hydroxy-6-phenylhex-2-enoate (8).** Powdered Na_2CO_3 (5.44 g, 51.3 mmol) was added to a solution of the ester **7** (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 **8** (4.35 g, 89% yield) as colorless crystals: mp 91−95 °C (*n*-hexane/Et_2O = 3:1); $[\alpha]^{30}_D$ +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-[*N*-(*tert*-Butoxycarbonyl)amino]-4-methylsulfonyloxy-6-phenylhex-2-enoate (9).** To a stirred mixture of the alcohol **8** (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 **9** (4.75 g, 90% yield) as colorless crystals: mp 132−135 °C (*n*-hexane/Et_2O = 3:1); $[\alpha]^{27}_D$ +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-[*N*-(*tert*-Butoxycarbonyl)amino]-2-isopropyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*E*)-CH=CH]-Val-O*t*-Bu, 10).** 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. $BF_3\cdot Et_2O$ (5.28 mL, 41.7 mmol) was added to the above mixture at −78 °C. After 5 min, a solution of the mesylate **9** (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 **10** (3.56 g, 84% yield) as colorless crystals: mp 78−81 °C (*n*-hexane/Et_2O = 10:1); $[\alpha]^{26}_D$ −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-[*N*-(*tert*-Butoxycarbonyl)amino]-2-isopropyl-6-phenylhex-3-enoic acid (Boc-D-Phe-ψ[(*E*)-CH=CH]-Val-OH, 11). The ester **10** (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 **11** (379 mg, 82% yield) as a colorless oil: $[\alpha]^{20}_D$ −28.5 (*c* 1.54, $CHCl_3$); ^{1}H 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 (12). 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 $(i\text{-Pr})_2$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 **12** (26.1 g, 99% yield) as a colorless amorphous semisolid: $[\alpha]^{24}_D$ −8.80 (*c* 1.02, $CHCl_3$); ^{1}H 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 (13). To the protected dipeptide ester **12** (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 $(i\text{-Pr})_2$NEt (0.618 mL, 3.54 mmol), Boc-D-Phe-ψ[(*E*)-CH=CH]-Val-OH **11** (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 **13** (527 mg, 73% yield) as a colorless powder: mp 151−154 °C; $[\alpha]^{22}_D$ −29.5 (*c* 1.01, $CHCl_3$); ^{1}H 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 (14). To a stirred solution of the protected tetrapeptide ester **13** (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 **14** (461 mg, 99% yield) as a colorless amorphous semisolid: $[\alpha]^{18}_D$ −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 (15). 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 **14** (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 **15** (466 mg, 77% yield) as a colorless powder: $[\alpha]^{21}_D$ −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-] (18). To a stirred mixture of the protected pseudopentapeptide **15** (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 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 **18** (145 mg, 83% yield) as colorless crystals: mp 291 °C (decomp, Et_2O); $[\alpha]^{18}_D$ −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 **18** (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, $[\alpha]^{24}_D$ −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.

General Procedure for *Syn*-Selective Synthesis of *N*-Boc-Protected 4-Aminoalk-1-en-3-ols: Synthesis of (3*R*,4*R*)-4-[*N*-(*tert*-Butoxycarbonyl)amino]-2-methyl-5-phenylpent-1-en-3-ol (20a) and Its (3*S*,4*R*)-Isomer (20b). To a stirred solution of Boc-D-Phe-OMe **19** (25.0 g, 89.5 mmol) in toluene−CH_2Cl_2 (4:1, 125 mL) was added dropwise a solution of DIBAL-H in toluene (1.0 M, 179 mL, 179 mmol) at −78 °C under argon, and the mixture was stirred for 1 h at this temperature. In another flask, to a stirred solution of $ZnCl_2$ (36.6 g, 268 mmol) and LiCl (11.4 g, 268 mmol) in dry THF (90 mL) was added dropwise a solution of CH_2=CMeMgBr in THF (0.5 M, 537 mL, 268 mmol) at 0 °C under argon, and the mixture was stirred for 1 h. The resulting solution of CH_2=CMeMgBr·$ZnCl_2$·LiCl in THF was added dropwise to the above substrate mixture at −78 °C, and the mixture was stirred for 3 h with warming to 0 °C. The mixture was quenched with saturated citric acid at −78 °C, and the whole was concentrated under reduced pressure. The residue was extracted with EtOAc, and 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 (10:1) gave a *syn*-allyl alcohol **20a** (17.3 g, 66% yield) and an *anti*-allyl alcohol **20b** (4.21 g, 16% yield), in order of elution.

Compound **20a**: colorless crystals; mp 56−58 °C (*n*-hexane/Et_2O = 3:1); $[\alpha]^{21}_D$ +38.5 (*c* 0.337, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9 H), 1.68 (s, 3 H), 2.44 (m, 1 H), 2.91 (d, J = 7.3 Hz, 2 H), 3.90 (m, 1 H), 3.94 (m, 1 H), 4.78 (d, J = 7.5 Hz, 1 H), 4.92 (m, 1 H), 5.02 (m, 1 H), 7.17−7.33 (m, 5 H). Anal. Calcd for $C_{17}H_{25}NO_3$: C, 70.07; H, 8.65; N, 4.81. Found: C, 70.32; H, 8.87; N, 4.64.

Compound **20b**: colorless crystals; mp 134−137 °C (*n*-hexane/Et_2O = 3:1); $[\alpha]^{25}_D$ +47.6 (*c* 0.713, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 9 H), 1.81 (s, 3 H), 2.35−2.52 (br, 1 H), 2.62−2.77 (m, 1 H), 2.89 (dd, J = 14.2, 3.8 Hz, 1 H), 3.97 (m, 1 H), 4.18 (m, 1 H), 4.64 (s, 1 H), 4.98 (m, 1 H), 5.07 (m, 1 H), 7.17−7.33 (m, 5 H). Anal. Calcd for $C_{17}H_{25}NO_3$: requires C, 70.07; H, 8.65; N, 4.81. Found: C, 69.77; H, 8.56; N, 4.68.

General Procedure for *Anti*-Selective Synthesis of *N*-Boc-Protected 4-Aminoalk-1-en-3-ols. To a stirred solution of oxalyl dichloride (28.4 mL, 326 mmol) in CH_2Cl_2 (350 mL) was added dropwise DMSO (46.3 mL, 653 mmol) in CH_2Cl_2 (50 mL) at −78 °C under argon. After 10 min, a solution of Boc-D-phenylalaninol **21** (41.0 g, 163 mmol) in CH_2Cl_2 (100 mL) was added to the above mixture at −78 °C, and the mixture was stirred for 1 h at this temperature. (i-Pr)$_2$NEt (227 mL, 1306 mmol) was added to the mixture at −78 °C, and the mixture was stirred for 15 min with warming to 0 °C. Saturated NH_4Cl was added to the mixture, and the whole was extracted with Et_2O. The extract was washed successively with 5% citric acid, water, 5% $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure gave an aldehyde. To a stirred solution of CH_2=CMeMgBr·$ZnCl_2$·LiCl in THF (1216 mL, 408 mmol) was added the above aldehyde in dry THF (100 mL) dropwise at −78 °C under argon, and the mixture was stirred for 3 h with warming to 0 °C. The mixture was quenched with saturated citric acid at −78 °C followed by concentration under reduced pressure. The residue was extracted with EtOAc, and the extract was washed 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*-hexanes−EtOAc (3:1) gave the diastereomixture of allyl alcohols (30.2 g). Next, the allyl alcohols were converted into the corresponding enone by the same procedure with that of the above Swern oxidation. To a stirred solution of the enone in dry Et_2O (150 mL) was added $Zn(BH_4)_2$ in Et_2O (0.25 M, 1000 mL, 248 mmol) at −78 °C under argon, and the stirring was continued for 1 h with warming to 0 °C. The mixture was quenched with saturated citric acid at −78 °C. The same purification described for that of stereoselective synthesis of *syn*-allyl alcohol yielded a *syn*-allyl alcohol **20a** (4.59 g, 10% yield) and *anti*-allyl alcohol **20b** (14.2 g, 29% yield), in order of elution.

***tert*-Butyl (4*R*,5*R*,2*E*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-4-hydroxy-3-methyl-6-phenylhex-2-enoate (23a) and Its (4*S*,5*R*,2*E*)-Isomer (23b).** By use of a procedure similar to that described for the successive treatment of the acetate **6** with O_3 gas and Me_2S, the acetate **22a** (5.77 g, 17.3 mmol) was converted into the corresponding ketone. To the stirred solution of the ketone in $CHCl_3$ (20 mL) was added Ph_3P=$CHCO_2t$-Bu (17.4 g, 51.9 mmol), and the mixture was gently refluxed for 2 d. The mixture was concentrated under reduced pressure, and purified by flash chromatography over silica gel to give a yellow oil (4.29 g). To a solution of the above oil in dry MeOH (10 mL) was added powdered Na_2CO_3 (4.20 g, 39.5 mmol) at room temperature, and the mixture was stirred for 3 h. The mixture was filtered, and the filtrate was extracted with Et_2O. 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

(7:1) gave the title compound **23a** (2.99 g, 44% yield) and **23b** (336 mg, 4.9% yield), in order of elution.

Compound **23a**: colorless powder; mp 45−46 °C; $[\alpha]^{22}_D$ +45.8 (*c* 2.42, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 1.38 (s, 9 H), 1.45 (s, 9 H), 2.00 (m, 3 H), 2.95 (m, 2 H), 3.93 (m, 2 H), 4.79 (d, J = 9.6 Hz, 1 H), 5.89 (d, J = 0.9 Hz, 1 H), 7.20−7.34 (m, 5 H); LRMS (FAB), *m*/*z* 392 (MH^+, base peak), 336, 292, 280, 262, 236, 220, 218, 201, 164, 144, 120, 116, 99, 91, 57, 41, 29; HRMS (FAB), *m*/*z* calcd for $C_{22}H_{34}NO_5$ (MH^+) 392.2437, found: 392.2451.

Compound **23b**: colorless powder; mp 80−82 °C; $[\alpha]^{25}_D$ +2.51 (*c* 0.796, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 9 H), 1.48 (s, 9 H), 2.14 (d, J = 1.0 Hz, 3 H), 2.73 (m, 1 H), 2.82 (dd, J = 14.3, 4.2 Hz, 1 H), 4.01 (m, 1 H), 4.27 (m, 1 H), 4.69 (d, J = 8.1 Hz, 1 H), 5.95 (m, 1 H) 7.14−7.31 (m, 5 H). Anal. Calcd for $C_{22}H_{33}NO_5$: C, 67.49; H, 8.50; N, 3.58. Found: C, 67.43; H, 8.27; N, 3.48.

***tert*-Butyl (2*E*)-3-[(4*R*,5*R*)-4-Benzyl-*N*-(*tert*-butoxycarbonyl)-2,2-dimethyl-1,3-oxazolidin-5-yl]but-2-enoate (24a).** To a stirred solution of the hydroxy ester **23a** (90.8 mg, 0.231 mmol) and 2,2-dimethoxypropane (0.0855 mL, 0.695 mmol) in CH_2Cl_2 (1.0 mL) was added dropwise BF_3·Et_2O (0.00171 mL, 0.0139 mmol) at 0 °C, and the mixture was stirred overnight with warming to room temperature. Saturated $NaHCO_3$ (1 mL) was added to the mixture at 0 °C, and the whole was extracted with Et_2O. 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 (3:1) gave the title compound **24a** (89.2 mg, 89% yield) as a colorless powder: mp 109−110 °C (*n*-hexane); $[\alpha]^{27}_D$ +26.9 (*c* 0.852, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 3 H), 1.46 (s, 9 H), 1.55 (s, 9 H), 1.62 (s, 3 H), 1.85 (s, 3 H), 3.01 (m, 1 H), 3.18 (dd, J = 13.2, 2.8 Hz, 1 H), 4.05 (m, 1 H), 4.31 (dd, J = 5.6, 0.6 Hz, 1 H), 5,71 (m, 1 H), 7.19−7.33 (m, 5 H). Anal. Calcd for $C_{25}H_{37}NO_5$: C, 69.58; H, 8.64; N, 3.25. Found: C, 69.44; H, 8.64; N, 3.28.

***tert*-Butyl (4*R*,5*R*,2*E*)-4-Acetoxy-5-[*N*-(*tert*-butoxycarbonyl)amino]-3-methyl-6-phenylhex-2-enoate (25a).** To a stirred solution of the hydroxy ester **23a** (1.77 g, 4.52 mmol) in $CHCl_3$ (5 mL), pyridine (7.31 mL, 90.4 mmol), and DMAP (55 mg, 0.450 mmol) was added Ac_2O (4.26 mL, 45.2 mmol) at 0 °C, and the stirring was continued overnight with warming to room temperature. The mixture was poured into ice-cooled water, 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 **25a** (1.83 g, 93% yield) as colorless crystals: mp 67−68 °C (*n*-hexane/Et_2O = 3:1); $[\alpha]^{23}_D$ +65.3 (*c* 1.20, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at 323 K) δ 1.36 (s, 9 H), 1.44 (s, 9 H), 2.05 (d, J = 1.1 Hz, 3 H), 2.13 (s, 3 H), 2.77 (m, 2 H), 4.24 (m, 1 H), 4.56 (m, 1 H), 5.04 (m, 1 H), 5.61 (m, 1 H), 7.12−7.32 (m, 5 H). Anal. Calcd for $C_{24}H_{35}NO_6$: C, 66.49; H, 8.14; N, 3.23. Found: C, 66.19; H, 8.26; N, 3.08.

General Procedure for Synthesis of ψ[(*E*)-CH=CMe]-type Alkene Dipeptide Isosteres via Organocopper-Mediated Alkylation: Synthesis of *tert*-Butyl (2*R*,5*R*,3*E*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-2-isopropyl-3-methyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*E*)-CH=CMe]-L-Val-O*t*-Bu, 27a), Its (2*S*,5*R*,3*Z*)-Isomer (Boc-D-Phe-ψ[(*Z*)-CH=CMe]-D-Val-O*t*-Bu, 28a) and *tert*-Butyl (5*R*,3*Z*)-5-[*N*-(*tert*-Butoxycarbonyl)amino]-3-methyl-6-phenylhex-3-enoate (Boc-D-Phe-ψ[(*Z*)-CH=CMe]-Gly-O*t*-Bu, 29). To a stirred suspension of CuCN (75.0 mg, 0.837 mmol) in dry Et_2O (1 mL)−HMPA (0.146 mL, 0.837 mmol) was added dropwise a solution of *i*-PrMgCl in Et_2O (0.87 M, 1.93 mL, 1.68 mmol) at −78 °C under argon, and the mixture was stirred for 15 min at 0 °C. BF_3·Et_2O (0.106 mL, 0.837 mmol) was added to the above mixture, and the mixture was stirred for 3 min. A solution of the mesylate **26a** (100 mg, 0.212 mmol) in dry Et_2O (1.5 mL) was added dropwise to the above reagent at −78 °C, and the stirring was continued for 30 min at −78 °C and for 30 min at 0 °C. The mixture was quenched with saturated NH_4Cl−28% NH_4OH (1:1, 2 mL), and the whole was extracted with Et_2O. 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 (40:1) gave the mixture of the title compounds **27a**, **28a**, and **29** (83.4 mg, 93% yield). The product ratio was determined by RP-HPLC and ^1H NMR analyses (**27a**:**28a**:**29** = 70:27:3).

Compound **27a**: colorless powder; $[\alpha]^{21}_D$ −58.8 (*c* 0.629, $CHCl_3$); $\Delta\epsilon$ = −11.04 (228 nm, isooctane); t_R = 56.6 min (65% B in A); ^1H NMR (300 MHz, $CDCl_3$) δ 0.59 (d, J = 6.6 Hz, 3 H), 0.88 (d, J = 6.4 Hz, 3 H), 1.40 (s, 9 H), 1.41 (s, 9 H), 1.55 (d, J = 1.3 Hz, 3 H), 1.97 (m, 1 H), 2.35 (d, J = 10.7 Hz, 1 H), 2.67 (dd, J = 13.2, 7.8 Hz, 1 H), 2.92 (dd, J = 13.1, 4.7 Hz, 1 H), 4.45 (m, 1 H), 4.56 (m, 1 H), 5.15 (m, 1 H), 7.14−7.27 (m, 5 H). LRMS (FAB), *m*/*z* 418 (MH^+, base peak), 326, 306, 260, 245, 214, 199, 170, 164, 143, 120, 91. HRMS (FAB), *m*/*z* calcd for $C_{25}H_{40}NO_4$ (MH^+) 418.2957, found: 418.2951.

Compound **28a**: colorless oil; $[\alpha]^{25}_D$ +135.9 (*c* 0.103, $CHCl_3$); $\Delta\epsilon$ = +27.40 (226 nm, isooctane); t_R = 62.7 min (65% B in A); ^1H NMR (300 MHz, $CDCl_3$) δ 0.43 (d, J = 6.7 Hz, 3 H), 0.88 (d, J = 6.4 Hz, 3 H), 1.40 (s, 9 H), 1.43 (s, 9 H), 1.63 (d, J = 1.3 Hz, 3 H), 2.00 (m, 1 H), 2.74 (dd, J = 13.1, 7.3 Hz, 1 H), 2.92 (d, J = 10.9 Hz, 1 H), 3.02 (m, 1 H), 4.38−4.62 (m, 2 H), 5.23 (m, 1 H), 7.13−7.38 (m, 5 H); LRMS (FAB), *m*/*z* 418 (MH^+, base peak), 362, 326, 306, 270, 260, 250, 244, 214, 199, 170, 164, 143, 120, 111, 91, 57; HRMS (FAB), *m*/*z* calcd for $C_{25}H_{40}NO_4$ (MH^+) 418.2957, found: 418.2950.

Compound **29**: colorless oil; $[\alpha]^{24}_D$ −81.63 (*c* 0.049, $CHCl_3$); t_R = 49.3 min (65% B in A); ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 9 H), 1.43 (s, 9 H), 1.75 (d, J = 1.4 Hz, 3 H), 2.86 (m, 3 H), 3.04 (dd, J = 14.9, 0.7 Hz, 1 H), 4.49 (m, 2 H), 5.19 (m, 1 H), 7.17−7.31 (m, 5 H); LRMS (FAB), *m*/*z* 376 (MH^+, base peak), 264, 228, 203, 172, 143, 128, 57; HRMS (FAB), *m*/*z* calcd for $C_{22}H_{34}NO_4$ (MH^+), 376.2488, found: 376.2485.

(2*R*,5*R*,3*E*)-5-[*N*-(9-Fluorenylmethoxycarbonyl)amino]-2-isopropyl-3-methyl-6-phenylhex-3-enoic Acid (Fmoc-D-Phe-ψ[(*E*)-CH=CMe]-L-Val-OH, 30). The ester **27a** (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 **30** (198 mg, 69% yield) as a colorless oil: $[\alpha]^{22}_D$ −34.0 (*c* 1.29, $CHCl_3$); ^1H 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 **33** or **37**, 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_2O] to free amino group (or hydrazino group) in DMF for 1.5 h.

H-Asp(O*t*-Bu)-D-Phe-ψ[(*E*)-CH=CMe]-Val-Arg(Boc)$_2$-Gly-NHNHCO-Wang Resin (33). *p*-Nitrophenyl carbonate Wang resin (0.93 mmol/g, 161 mg, 0.15 mmol) was treated with $NH_2NH_2 \cdot H_2O$ (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]-Val-OH **30** (51.0 mg, 0.105 mmol) was incorporated by twice treatments with DIPCDI (0.019 mL, 0.126 mmol) and $HOBt \cdot H_2O$ (0.016 mg, 0.105 mmol) for 1.5 h each. After capping of free amino groups with Ac_2O-pyridine, Asp(O*t*-Bu) residue was coupled by general coupling protocol to afford the title protected peptide resin **33**.

Cyclo(-Arg-Gly-Asp-D-Phe-ψ[(*E*)-CH=CMe]-Val-)·TFA (4). The protected peptide resin **33** 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 **34** as a colorless powder. To a stirred solution of **34** 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**[34] (10.3 mg, 14% yield from **30**) as freeze-dried powder: $[\alpha]^{21}_D$ −36.8 (*c* 0.516, H_2O); 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 $C_{28}H_{42}N_7O_6$ (MH^+) 572.3197, found: 572.3185.

Synthesis of H-Asp(O*t*-Bu)-D-Phe-ψ[(*Z*)-CH=CMe]-Val-Arg(Pbf)-Gly-Clt Resin (37). 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 **35** (55.0 mg, 0.113 mmol) was incorporated by twice treatments with DIPCDI (0.021 mL, 0.136 mmol) and $HOBt \cdot H_2O$ (0.017 mg, 0.113 mmol) for 1.5 h each. After capping of free amino groups with Ac_2O-pyridine, Asp(O*t*-Bu) residue was coupled by general coupling protocol to afford the title protected peptide resin **37**.

Cyclo(-Arg-Gly-Asp-D-Phe-ψ[(*Z*)-CH=CMe]-Val-)·TFA (40). The protected peptide resin **37** was subjected to AcOH/TFE/CH_2CH_2 (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 **38** as a colorless powder. To a stirred suspension of **38** and $NaHCO_3$ (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 $CHCl_3$−MeOH (9:1) to remove inorganic salts. The resulting cyclic protected peptide **39** 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 **40** (15.7 mg, 20% yield from **35**) as freeze-dried powder: $[\alpha]^{22}_D$ −5.61 (*c* 0.712, H_2O); t_R = 30.6 min (linear gradient of B in A, 20 to 35% over 30 min); ^{1}H 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.[37] $\alpha_{IIb}\beta_3$ was purified from human platelet by RGDSPK-sepharose CL-4B as well.[37] $\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$ binding assays were performed according to the modified method of Kouns et al.[38] 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.

Acknowledgment. We thank Dr. Terrence R. Burke, Jr., NCI, NIH, for reading manuscript and valuable discussions. This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and the Japan Health Science Foundation. S.O. is grateful for Research Fellowships of the JSPS for Young Scientists.

Supporting Information Available: Experimental procedures and characterization data for **22a,b**, **24b**, **25b**, **26a,b**, **27b**, **28b**, **35**, and **41**; ^{1}H NMR spectra for all new compounds. This material are available free of charge via the Internet at http://pubs.acs.org.

JO025923M

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