Stereoselective Synthesis of [L-Arg-L/D-3-(2-naphthyl)alanine]-Type (*E*)-Alkene Dipeptide Isosteres and Its Application to the Synthesis and Biological Evaluation of Pseudopeptide Analogues of the CXCR4 Antagonist FC131

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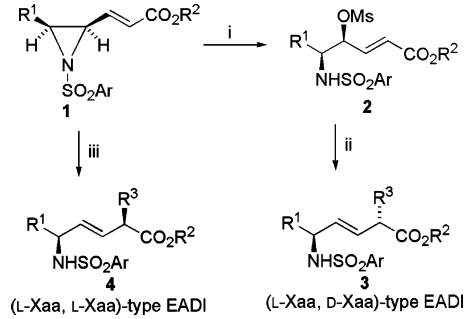
L,L-Type and L,D-type (*E*)-alkene dipeptide isosteres (EADIs) that have unnatural side chains at the α-position were synthesized by the combination of stereoselective aziridinyl ring-opening reactions and organozinc−copper-mediated *anti*-S_N2' reactions toward a single substrate of γ,δ-*cis*-γ,δ-epimino (*E*)-α,β-enoate. The utility of this methodology was demonstrated by the stereoselective synthesis of a set of diastereomeric EADIs of L-Arg-L/D-3-(2-naphthyl)alanine (Nal) that is contained in a small CXCR4 antagonist FC131 [*cyclo*(-D-Tyr-Arg-Arg-Nal-Gly-)]. Furthermore, a (Nal-Gly)-type EADI was synthesized by samarium diiodide (SmI_2)-induced reduction of a γ-acetoxy-α,β-enoate. Several FC131 analogues, in which these EADIs were inserted for reduction of their peptide character, were synthesized with analogues containing reduced amide-type dipeptide isosteres to investigate the importance of these amide bonds for anti-HIV and CXCR4-antagonistic activity.

Introduction

The practical utility of (*E*)-alkene dipeptide isosteres (EADIs) has been intensively investigated in structure−activity relationship (SAR) studies of biologically active peptides toward development of peptide-lead drugs.[1−7] Backbone replacements of amide bonds in peptides by EADIs provide information on the contributions of the corresponding amide bonds on biological activity. We previously established a completely stereocontrolled synthetic process for L,L-type and L,D-type EADIs starting from L-amino acid.[8,9] As shown in Scheme 1, treatment of *N*-aryl-γ,δ-*cis*-γ,δ-epimino (*E*)-α,β-enoates (*cis*-(*E*)-enoates) **1** with methanesulfonic acid (MSA) gives γ-mesyloxy-α,β-enoates **2**, which can be converted into L,D-type EADIs **3** by organocopper-mediated α-alkylation via *anti*-S_N2' reactions, whereas organocopper treatment of *cis*-(*E*)-enoates **1** affords L,L-type EADIs **4**. However, this synthetic procedure has not yet been optimized, because it involves a potential limitation on the introduction of functional groups into the side chain (R^3) at the α-position. In a standard procedure, organocopper reagents, which were prepared by CuCN and RLi or RMgX (X = Cl or Br), are used for α-alkylation.[2,4] In the α-alkylation of the synthesis of (Xaa-L/D-Glu)-type EADIs,[10] organozinc−copper reagents are used, which are prepared from $IZnCH_2CH_2CO_2R$ and CuCN.[11−15] In this study, to demonstrate the general utility of organozinc−copper reagents, a set of EADIs of L-Arg-L/D-3-(2-naphthyl)alanine (Nal) were synthesized as model compounds via the γ,δ-*cis*-γ,δ-epimino (*E*)-α,β-enoate by the combination of MSA-mediated aziridinyl ring-opening reactions and α-alkylation with organozinc−copper reagents, which were prepared from 2-naphthylmethylZnBr and CuCN. The dipeptide sequence, Arg-Nal, is part of the low molecular weight CXCR4 antagonist, FC131, which was recently developed by us (Figure 1).[16]

Scheme 1[a]



[a] R^1, R^2, R^3 = alkyl; Ar = 2,4,6-trimethylphenylsulfonyl (Mts) or Ts. Reagents: (i) MsOH; (ii) $R^3Cu(CN)MgX{\cdot}BF_3$ (X = Cl or Br) or $R^3Cu(CN)Li{\cdot}BF_3$; (iii) $R^3Cu(CN)MgX{\cdot}2LiX$ (X = Cl or Br) or $R^3Cu(CN)Li{\cdot}2LiX$.

CXCR4 is a chemokine receptor, which is involved in cell progression and metastasis of several types of cancer,[17−19] HIV entry,[20] and rheumatoid arthritis.[21,22] Thus, several inhibitors directed against CXCR4 have been developed.[23−27] We previously found a highly potent CXCR4 antagonist, T140, which is a 14-mer

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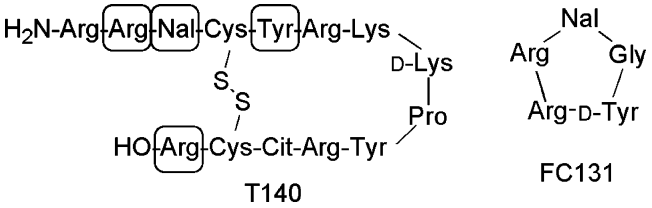


Figure 1. Structures of T140 and its downsized peptide FC131. Circled residues are the indispensable residues of T140 for the expression of strong CXCR4-antagonistic activity. Nal = L-3-(2-naphthyl)alanine, Cit = L-citrulline.

peptide with a disulfide bridge, and we identified four critical residues: Arg^2, Nal^3, Tyr^5, and Arg^{14} (Figure 1).[28−30] Molecular-size reduction of T140 based on the structural requirement led to the discovery of FC131, which has a cyclic pentapeptide template,[31−37] with CXCR4-antagonistic and anti-HIV activity comparable to those of T140.[16] We wish to investigate contributions of each amide bond in FC131 to the biological activity in order to develop pseudopeptides, in which the peptide character is reduced to obtain more druglike structures. For this purpose, EADIs and reduced amide-type dipeptide isosteres (RADIs) of Arg-Nal and Nal-Gly are required, because the amide bonds between Arg^2 and Nal^3 and between Nal^3 and Cys^4 were found to be cleaved by treatment of T140 analogues with rat liver homogenates.[38,39] Thus, (L-Arg-L/D-Nal)-type EADIs were synthesized in the study described here, and a (Nal-Gly)-type EADI was also synthesized by another method using the samarium diiodide (SmI_2)-induced reduction of a γ-acetoxy-α,β-enoate.[40,41] RADIs of Arg-Nal and Nal-Gly were prepared by a standard method of reductive amination. Then, several FC131 analogues, in which the above isosteres were introduced, were synthesized to identify the biological importance of these amide bonds.

Results and Discussion

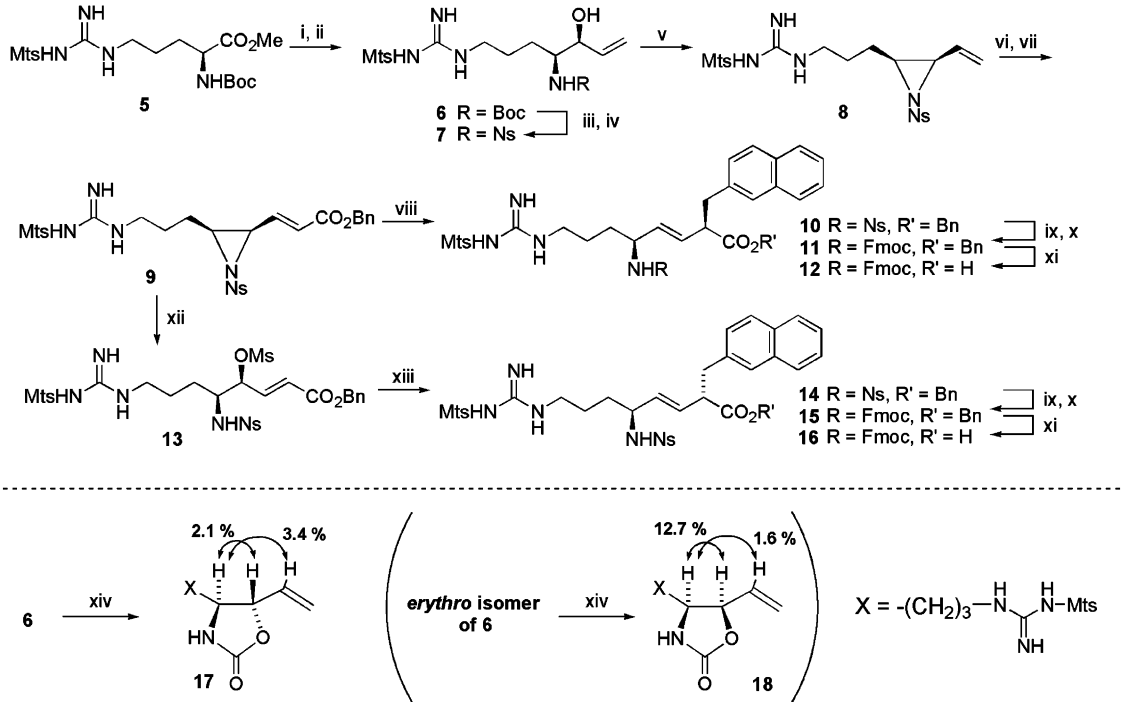
Synthesis of (L-Arg-L/D-Nal)-Type EADIs. (L-Arg-L/D-Nal)-type EADIs were synthesized via the same key intermediate *N*-2-nitrobenzenesulfonyl (Ns)-γ,δ-*cis*-γ,δ-epimino (*E*)-α,β-enoate, **9**, as synthetic model compounds for the investigation of the feasibility of α-alkylation using organozinc−copper reagents as well as precursor dipeptide isosteres used for the synthesis of partial nonpeptide analogues of FC131 (Scheme 2). Boc-Arg(Mts)-OMe (Mts = 2,4,6-trimethylbenzenesulfonyl) **5** was treated successively with diisobutylaluminum hydride (DIBAL-H) and vinylmagnesium chloride (CH_2=CHMgCl) to give exclusively the *threo*-amino alcohol **6** (a separable mixture of allyl alcohol **6**/*erythro*-isomer of **6** = 12:1). N^{α}-Ns protection[42,43] after the cleavage of the N^{α}-Boc group of **6** with HCl/dioxane followed by successive treatments consisting of the Mitsunobu reaction,[44] ozonolysis, and the modified Horner−Wadsworth−Emmons olefination[45] afforded *cis*-(*E*)-enoate **9**. *Anti*-S_N2' reaction of **9** with an organozinc−copper reagent,[11−15] 2-naphthylmethylCu(CN)-ZnBr·2LiCl, afforded an L,L-type EADI **10**, in which a (2*R*)-2-naphthylmethyl side chain was incorporated at the α-position, stereoselectively in 83% yield (diastereoselection > 99:1 from NMR analysis). N^{α}-Fmoc substitution for the N^{α}-Ns group of **10** followed by selective deprotection of the benzyl ester using thioanisole/TFA afforded a desired EADI, Fmoc-L-Arg(Mts)-ψ-[(*E*)-CH=CH]-L-Nal-OH, **12**. Alternatively, exposure of **9** to MSA/$CHCl_3$ afforded exclusively δ-aminated γ-mesyloxy-α,β-enoate **13** by regio- and stereoselective S_N2 ring-opening reaction at the γ-carbon of **9**. Mesylate **13** was successively treated by an organozinc−copper reagent, 2-naphthylmethylCu(CN)ZnBr·BF_3, to afford an L,D-type EADI **14**, in which a (2*S*)-2-naphthylmethyl side chain was incorporated at the α-position, stereoselectively via an *anti*-S_N2'mechanism in 67% yield (diastereoselection > 99:1 from NMR analysis). **14** was similarly converted into another desired EADI, Fmoc-L-Arg(Mts)-ψ[(*E*)-CH=CH]-D-Nal-OH, **16**. As such, α-alkylation of both a *cis*-(*E*)-enoate and its ring-opened product using organozinc−copper reagents was successfully performed in the synthesis of (L-Arg-L/D-Nal)-type EADIs. An *N*-Ns group could be used in this synthetic scheme as an orthogonal *N*-protecting (activating) group instead of an *N*-Mts or *N*-Ts group. Relative configurations of the allyl alcohols (**6** and its erythro isomer) were determined by comparative nuclear Overhauser effect (NOE) measurements of these oxazolidinone derivatives **17** and **18** (Scheme 2).[2] The (*E*)-geometry of the double bond in the synthesized EADIs was assigned based on the coupling constant of the two olefinic protons on ^1H NMR analysis.

Synthesis of (L-Nal-Gly)-Type EADI. An (L-Nal-Gly)-type EADI was synthesized as shown in Scheme 3. Boc-L-Nal-OMe **19** was treated sequentially with DIBAL-H and CH_2=CHMgCl to give a diastereomixture of allyl alcohol **20**. Acetylation of **20** followed by ozonolysis and the modified Horner−Wadsworth−Emmons olefination afforded a γ-acetoxy-α,β-unsaturated ester **22**. Acetate **22** was reduced with SmI_2−^{t}BuOH to yield an (L-Nal-Gly)-type EADI **23** in 95% yield,[40,41] followed by deprotection of the N^{α}-Boc group and *tert*-butyl ester with TFA and reprotection with an N^{α}-Fmoc group to afford the desired EADI, Fmoc-L-Nal-ψ[(*E*)-CH=CH]-Gly-OH, **24**.

Synthesis of RADIs of Arg-Nal and Nal-Gly. (L-Arg-L-Nal)- and (L-Nal-Gly)-type RADIs were prepared for comparative studies. As shown in Scheme 4, Arg- and Nal-derived Weinreb amides **25** and **29** were treated with DIBAL-H to afford the corresponding aldehyde derivatives. Subsequently, reductive amination of the aldehydes was performed by treatments with carboxy-protected amino acids in the presence of acetic acid and sodium triacetoxy borohydride [$NaBH(OAc)_3$] to afford secondary amines **26** and **30**, respectively.[46] Protection of the *sec*-amino groups with Cbz groups followed by deprotection of the N^{α}-Boc group and *tert*-butyl ester with TFA and reprotection with an N^{α}-Fmoc group afforded the desired RADIs, Fmoc-L-Arg(Mts)-ψ-[CH_2−N(Cbz)]-L-Nal-OH, **28**, and Fmoc-L-Nal-ψ[CH_2−N(Cbz)]-Gly-OH, **32**, respectively.

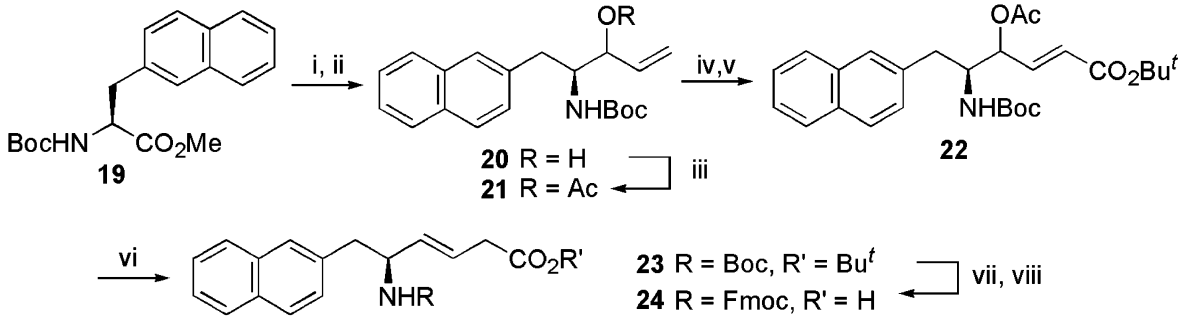
Synthesis of Cyclic Pseudopeptides. The protected peptide chains were constructed on a hydrazino resin **34** by Fmoc-based solid-phase synthesis using ^{t}Bu and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) groups for side-chain protection of D-Tyr and Arg, respectively (Scheme 5). N^{α}-Fmoc-protected dipeptide isosteres, EADIs **12**, **16**, and **24** and RADIs **28** and **32**, were similarly condensed. In the synthesis of cyclic pseudopeptides, two steps of deprotection/cleavage were adopted to prevent guanidino groups of Arg from

Scheme 2[a]



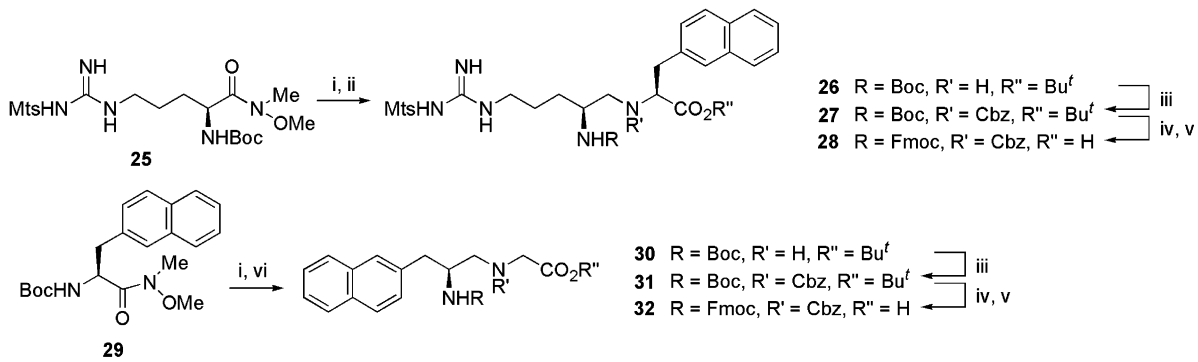
[a] Reagents: (i) DIBAL-H; (ii) CH_2=CHMgCl; (iii) HCl, anisole; (iv) Ns-Cl, pyridine; (v) Ph_3P, DEAD; (vi) O_3, then Me_2S; (vii) $(EtO)_2P(O)CH_2CO_2Bn$, LiCl, DIPEA; (viii) 2-naphthylmethylCu(CN)ZnBr·2LiCl; (ix) PhSH, K_2CO_3; (x) Fmoc-OSu, Et_3N; (xi) thioanisole, TFA; (xii) MsOH; (xiii) 2-naphthylmethylCu(CN)ZnBr·BF_3; (xiv) NaH.

Scheme 3[a]



[a] Reagents: (i) DIBAL-H; (ii) CH_2=CHMgCl; (iii) Ac_2O, DMAP, pyridine; (iv) O_3, then Me_2S; (v) $(EtO)_2P(O)CH_2CO_2{}^tBu$, LiCl, DIPEA; (vi) SmI_2, ^tBuOH; (vii) anisole, TFA; (viii) Fmoc-OSu, Et_3N.

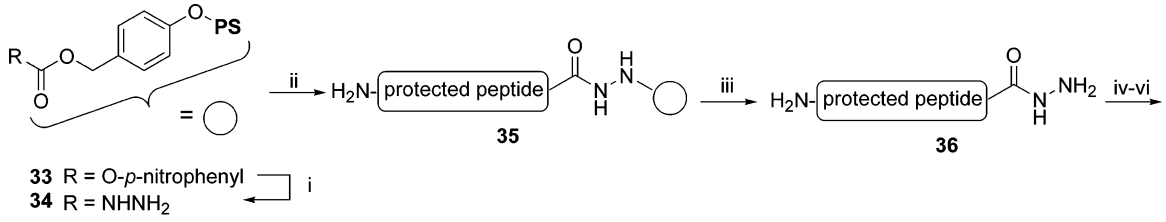
Scheme 4[a]

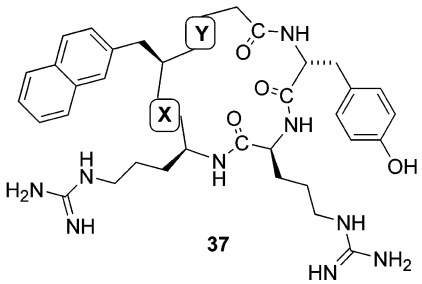


[a] Reagents: (i) DIBAL-H; (ii) H-Nal-O^{t}Bu, AcOH, $NaBH(OAc)_3$; (iii) Cbz-Cl, Et_3N; (iv) anisole, TFA; (v) Fmoc-OSu, Et_3N; (vi) H-Gly-O^{t}Bu, AcOH, $NaBH(OAc)_3$.

participating in cyclizing reaction as follows. After construction of peptide chains, pseudopeptide hydrazides **36** were obtained by cleavage from the resin **35** using 10% TFA/$CHCl_3$ without cleavage of Pbf, Mts, and Cbz groups (first deprotection). Cyclization of linear pseudopeptides by the azide procedure[47] in highly

Scheme 5[a]





Compd.	Sequence (*cyclo*)	X	Y
FC131	(-D-Tyr-Arg-Arg-Nal-Gly-)	-CO-NH-	-CO-NH-
37a	(-D-Tyr-Arg-Arg-Nal-Gly-)	-CH=CH-	-CO-NH-
37b	(-D-Tyr-Arg-Arg-Nal-Gly-)	$-CH_2$-NH-	-CO-NH-
37c	(-D-Tyr-Arg-Arg-Nal-Gly-)	-CO-NH-	-CH=CH-
37d	(-D-Tyr-Arg-Arg-Nal-Gly-)	-CO-NH-	$-CH_2$-NH-
37e	(-D-Tyr-Arg-Arg-D-Nal-Gly-)	-CO-NH-	-CO-NH-
37f	(-D-Tyr-Arg-Arg-D-Nal-Gly-)	-CH=CH-	-CO-NH-

[a] Reagents: (i) $NH_2NH_2 \cdot H_2O$; (ii) Fmoc-based SPPS; (iii) TFA; (iv) HCl, isoamyl nitrite; (v) DIPEA; (vi) 1 M TMSBr−thioanisole/TFA, *m*-cresol, 1,2-ethanedithiol.

Table 1. Activity and Cytotoxicity of the Synthetic Compounds

compound (no.)	CC_{50}[a] (μM)	EC_{50}[b] (μM)	IC_{50}[c] (μM)
FC131	> 100	0.073	0.0045 ± 0.0018
37a	> 100	2.4	31−100[d]
37b	> 100	> 100	> 100
37c	> 100	2.4	0.18 ± 0.10
37d	> 100	0.98	0.032 ± 0.011
37e	> 100	1.9	0.19 ± 0.071
37f	> 100	9.1	21
T140	> 10	0.035	0.0039 ± 0.0004
AZT	57	0.018	

[a] CC_{50} values are based on the reduction of the viability of mock-infected MT-4 cells. Because the cytotoxicity of T140 was previously evaluated as CC_{50} > 40 μM, further estimation at high concentrations was omitted in this study. [b] EC_{50} values are based on the inhibition of HIV-induced cytopathogenicity in MT-4 cells. [c] IC_{50} values are based on the inhibition of [^{125}I]-SDF-1-binding to CXCR4 transfectants of CHO cells. All data are the mean values for at least three independent experiments. [d] **37a** showed significant antagonistic activity in 100 μM but hardly showed activity in 31 μM.

diluted dimethylformamide (DMF) solution followed by deprotection of Pbf, Mts, and Cbz groups with 1 M TMSBr−thioanisole/TFA (second deprotection) gave the desired cyclic pseudopeptides **37**.

Biological and Conformational Evaluation of Synthetic Cyclic Pseudopeptides. Anti-HIV activity based on the inhibition of HIV-1-induced cytopathogenicity in MT-4 cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[48] CXCR4-antagonistic activity was evaluated by the inhibition of [^{125}I]-SDF-1-binding to CXCR4 transfectants of CHO cells.[49] **37a**, an (L-Arg-L-Nal)-type EADI containing FC131 analogue, showed moderate anti-HIV activity (EC_{50} = 2.4 μM) and CXCR4-antagonistic activity (100 μM > IC_{50} > 31 μM). Introduction of an EADI into the Arg-Nal sequence caused a remarkable decrease in anti-HIV activity (33-fold lower activity). NMR and simulated annealing molecular dynamics (SA-MD) analysis of **37a** showed a pseudopeptide backbone structure with a nearly symmetrical pentagonal shape similar to that of FC131,[16] excluding the difference between the orientation of two protons in the (*E*)-alkene unit of **37a** and that of the carbonyl oxygen/amino proton in the Arg-Nal amide bond of FC131 (Figure 2a).[50] Substitution for the amide bond with the EADI caused an inversion of the olefinic plane (180° rotation of pseudo ψ and ϕ bonds), possibly due to dissolution of the 1,3-pseudo-allylic strain between the carbonyl group of Arg and the side chain of Nal. Introduction of an EADI into the Arg-D-Nal sequence of **37e** (an FC131 epimer, EC_{50} = 1.9 μM, IC_{50} = 190 nM) also caused a significant but moderate decrease in anti-HIV activity (the activity of **37f** is 5-fold lower than that of **37e**). The amide bonds of the Arg-L/D-Nal sequences were necessary for high potency. These results suggested that either a deletion of the hydrogen bond interaction with CXCR4 by the insertion of an EADI or an increase in hydrophobicity might be unsuitable. **37b**, an (L-Arg-L-Nal)-type RADI-containing FC131 analogue, did not show anti-HIV or CXCR4-antagonistic activity up to the concentration of 100 μM, suggesting that the planar nature of the amide bond is critical to maintain the pentagonal global conformation for high anti-HIV activity and that the replacement of the amide bond with RADI causes a conformational change of FC131. **37c**, an (L-Nal-Gly)-type EADI-containing FC131 analogue, showed almost the same anti-HIV activity (EC_{50} = 2.4 μM) as **37a** containing an (L-Arg-L-Nal)-type EADI. NMR and SA-MD analysis of **37c** showed a similar backbone structure with FC131 (Figure 2b).[50] The Nal-Gly amide bond was replaced by the EADI without an inversion of the olefinic plane. **37d**, an (L-Nal-Gly)-type RADI-containing FC131 analogue, exhibited relatively higher anti-HIV and CXCR4-antagonistic activities (EC_{50} = 0.98 μM, IC_{50} = 32 nM) than **37c** (IC_{50} = 180 nM), although these activities were weaker than those of FC131. These results also indicated an importance of the amide bond of the Nal-Gly sequence, as in the case of the Arg-Nal amide bond.

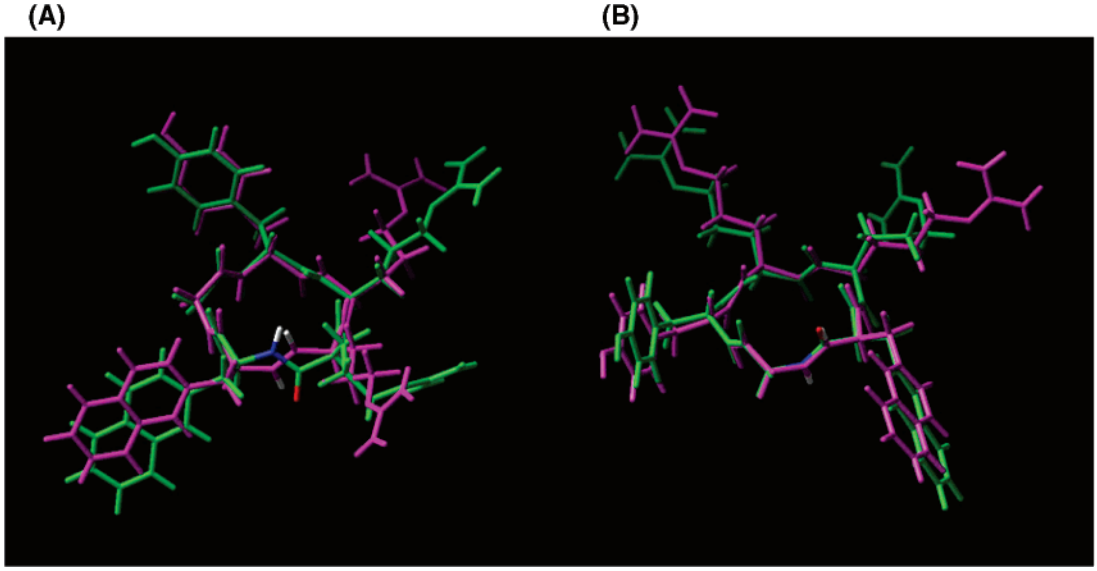


Figure 2. Superimpositions of low-energy structures of FC131 and **37a** (A) or **37c** (B). The FC131 structure is depicted in green, and the **37a** or **37c** structure is depicted in purple.

Conclusion

A set of (L-Arg-L/D-Nal)-type EADIs were synthesized from a single substrate of γ,δ-*cis*-γ,δ-epimino (*E*)-α,β-enoate by combination of MSA-mediated aziridinyl ring-opening reactions and *anti*-S_N2' reactions with organozinc−copper reagents that were prepared from 2-naphthylmethylZnBr and CuCN. Organozinc−copper-mediated α-alkylation reactions are thought to be useful for construction of several side chains at the α-position of EADIs, as organocopper-mediated α-alkylation reactions that have been generally used. Next, EADIs and RADIs of Arg-Nal and Nal-Gly, including the above EADIs, were synthesized and inserted into cyclic pentapeptides, FC131 analogues, to disclose biological importance of each amide bond. The present results will be useful for the development of nonpeptide CXCR4 antagonists derived from FC131. It is also noteworthy that EADI units were introduced into cyclic pentapeptides without any significant conformational changes in the pentagonal backbone structures, except for those of substituted (*E*)-alkene units, suggesting that the planar nature of (*E*)-alkene units caused the conformational maintenance of the backbones excluding the olefinic moiety. SA-MD analysis showed that the parent peptide (FC131) and the EADI-introduced pseudopeptides (**37a** and **37c**) have nearly equal distances between any two β-carbons in all of the side chains. It suggests that these compounds maintain similar dispositions of pharmacophores and that the biological differences between these compounds are derived from the (*E*)-alkene/amide bond units. As such, EADIs become useful tools for investigation of biological contributions of amide bonds.

Experimental Section

General. Melting points are uncorrected. ^{1}H NMR spectra were recorded using a JEOL EX-270, a JEOL AL-400, or a Bruker AM 600 spectrometer at 270, 400, or 600 MHz ^{1}H frequency in $CDCl_3$, respectively. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Nominal (LRMS) and exact mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 or JMS-HX/HX 110A mass spectrometer. Ion-spray (IS)-mass spectrum was obtained with a Sciex API *III*E triple quadrupole mass spectrometer (Toronto, Canada). Optical rotations were measured in $CHCl_3$ or H_2O with a JASCO DIP-360 digital polarimeter (Tokyo, Japan) or a Horiba high-sensitive polarimeter SEPA-200 (Kyoto, Japan). For flash column chromatography, silica gel 60 H (silica gel for thin-layer chromatography, Merck) and Wakogel C-200 (silica gel for column chromatography) were employed. HPLC solvents were H_2O and MeCN, both containing 0.1% (v/v) TFA. For analytical HPLC, a Cosmosil 5C18-AR column (4.6 mm × 250 mm, Nacalai Tesque Inc., Kyoto, Japan) was eluted with a linear gradient of MeCN at a flow rate of 1 mL/min on a Waters model 600 (Nihon Millipore, Ltd., Tokyo, Japan). Preparative HPLC was performed on a Waters Delta Prep 4000 equipped with a Cosmosil 5C18-AR column (20 mm × 250 mm, Nacalai Tesque Inc.) using an isocratic mode of MeCN at a flow rate of 15 mL/min.

Boc-Arg(Mts)-OMe, 5. To a stirred solution of Boc-Arg(Mts)-OH (10.0 g, 21.9 mmol) in DMF (50 mL) at 4 °C were added potassium bicarbonate (4.39 g, 43.8 mmol) and methyl iodide (2.73 mL, 43.8 mmol), and the mixture was stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed successively with saturated citric acid, brine, saturated aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure gave 10.4 g (21.8 mmol, 100%) of methyl ester **5** as a yellow oil.

$[\alpha]^{22}_D$ −4.25 (*c* 0.47, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.42 (9H, s, *tert*-Bu), 1.61 (2H, br m, CH_2), 1.78 (2H, br m, CH_2), 2.59 (3H, s, Ar-*p*-Me), 2.66 (6H, s, Ar-*o*-Me), 3.22 (2H, br m, CH_2), 3.73 (3H, s, OMe), 4.21−4.28 (1H, m, CH), 5.23 (1H, d, J = 8.2 Hz, NH), 6.14 (3H, br, guanidino), 6.89 (2H, s, ArH). *m/z* (FAB-LRMS): 471 (MH^+), 415, 371, 289 (base peak), 119. Found (FAB-HRMS): 471.2268. Calcd for $C_{21}H_{35}O_6N_4S$ (MH^+): 471.2277.

***N-tert*-Butoxy-[2(*S*)-hydroxy-1(*S*)-[3-[[imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]amino]propyl]but-3-enyl]formamide, 6.** To a stirred solution of Boc-Arg(Mts)-OMe, **5** (5.0 g, 10.6 mmol), in toluene/CH_2Cl_2 (1:1 (v/v) 100 mL) was added dropwise a solution of DIBAL-H in toluene (1.0 M, 32 mL, 32 mmol) at −50 °C under argon, and the mixture was stirred at −78 °C for 2 h. To the solution, was added dropwise a vinyl Grignard (CH_2═$CHMgCl$) reagent in THF (13 mL, 32 mmol) at −78 °C, and the mixture was stirred for 6 h with a gradual warming to 0 °C. The reaction was quenched with saturated aqueous citric acid at −78 °C, and organic solvents were concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed successively with saturated aqueous citric acid, satu-

rated aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by flash column chromatography over silica gel with EtOAc/*n*-hexane (2:1) gave a *threo*-allyl alcohol **6** and an *erythro*-allyl alcohol (2*R* isomer of **6**) (12:1), in order of elution (**6**, 1.5 g, 30% yield from **5**).

Compound **6**: colorless oil. $[\alpha]^{22}_D$ −15.74 (*c* 0.63, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.40 (9H, s, *tert*-Bu), 1.57 (2H, br m, 2-CH_2), 1.70 (2H, br m, 1-CH_2), 2.26 (3H, s, Ar-*p*-Me), 2.66 (6H, s, Ar-*o*-Me), 3.24 (2H, br m, 3-CH_2), 3.55 (1H, br, 1-H), 4.08 (1H, br, 2-H), 4.97 (1H, d, J = 9.7 Hz, NH), 5.18 (1H, d, J = 10.5 Hz, C*H*H=), 5.28 (1H, d, J = 17.3 Hz, CH*H*=), 5.77−5.89 (1H, m, CH=), 6.20 (3H, br, guanidino), 6.89 (2H, s, ArH). *m/z* (ISMS): 469.5 (MH^+). Found (FAB-HRMS): 469.2490. Calcd for $C_{22}H_{37}O_5N_4S$ (MH^+): 469.2485.

Compound 2*R* isomer of **6**: colorless oil. $[\alpha]^{21}_D$ −4.57 (*c* 2.84, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.41 (9H, s, *tert*-Bu), 1.48 (2H, br m, 2-CH_2), 1.64 (2H, br m, 1-CH_2), 2.30 (3H, s, Ar-*p*-Me), 2.63 (6H, s, Ar-*o*-Me), 3.15 (2H, br m, 3-CH_2), 3.63 (1H, br, 1-H), 4.18 (1H, br, 2-H), 5.12 (1H, br, NH), 5.24 (1H, d, J = 10.5 Hz, CH*H*=), 5.32 (1H, d, J = 17.0 Hz, C*H*H=), 5.74−5.85 (1H, m, CH=), 6.32 (3H, br, guanidino), 6.95 (2H, s, ArH). *m/z* (ISMS): 469.5 (MH^+).

[2(*S*)-Hydroxy-1(*S*)-[3-[[imino[[(2,4,6-trimethylphenyl)-sulfonyl]amino]methyl]amino]propyl]but-3-enyl][(2-nitrophenyl)sulfonyl]amine, 7. To a mixture of allyl alcohol **6** (4.2 g, 9.0 mmol) and anisole (0.97 mL, 9.0 mmol) at 0 °C was added 4 M HCl/dioxane (100 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure. To a stirred solution of the residue in $CHCl_3$ (20 mL) at 0 °C were added 2-nitrobenzenesulfonyl chloride (2.38 g, 10.8 mmol), triethylamine (Et_3N) (5 mL), and pyridine (20 mL), and the mixture was allowed to warm to room temperature, stirred at this temperature for 12 h, and extracted with $CHCl_3$. The extract was washed with saturated aqueous citric acid, saturated aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with $CHCl_3$/MeOH (18:1) gave 3.2 g (5.8 mmol, 65% from **6**) of **7** as a yellow oil.

$[\alpha]^{23}_D$ −57.79 (*c* 1.83, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.61 (4H, br m, 1, 2-CH_2), 2.27 (3H, s, Ar-*p*-Me), 2.64 (6H, s, Ar-*o*-Me), 3.15 (2H, br m, 3-CH_2), 3.50 (1H, br, 1-H), 3.72−3.78 (1H, m, 2-H), 4.72 (1H, d, J = 10.5 Hz, C*H*H=), 5.07 (1H, d, J = 17.0 Hz, CH*H*=), 5.42−5.48 (1H, m, CH=), 5.90 (1H, d, J = 8.1 Hz, NH), 6.26 (3H, br, guanidino), 6.90 (2H, s, ArH), 7.65−7.69 (2H, m, ArH), 7.78−7.82 (1H, m, ArH), 8.04−8.08 (1H, m, ArH). *m/z* (ISMS): 554.5 (MH^+). Found (FAB-HRMS): 554.1735. Calcd for $C_{23}H_{32}O_7N_5S_2$ (MH^+): 554.1743.

3(*S*)-[3-[[Imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]amino]propyl]-1-[(2-nitrophenyl)sulfonyl]-2(*R*)-vinylaziridine, 8. To a stirred solution of allyl alcohol **7** (3.1 g, 5.6 mmol) in dry THF (30 mL) at 0 °C were added triphenylphosphine (1.6 g, 6.2 mmol) and diethyl azodicarbonate (2.4 mL of a 40% solution in toluene, 6.2 mmol), and the reaction mixture was stirred at this temperature for 30 min. The mixture was concentrated under reduced pressure and purified by chromatography over silica gel with EtOAc/*n*-hexane (2:1) to give 2.8 g (5.2 mmol, 93% yield from **7**) of aziridine **8** as a yellow oil.

$[\alpha]^{23}_D$ −10.45 (*c* 2.20, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.48 (4H, br m, 1, 2-CH_2), 2.26 (3H, s, Ar-*p*-Me), 2.65 (6H, s, Ar-*o*-Me), 3.02 (1H, br, 2-H), 3.15 (2H, br m, 3-CH_2), 3.46 (1H, br, 3-H), 5.29 (1H, d, J = 9.7 Hz, C*H*H=), 5.42 (1H, d, J = 17.0 Hz, CH*H*=), 5.45−5.53 (1H, m, CH=), 6.41 (3H, br, guanidino), 6.88 (2H, s, ArH), 7.45−7.50 (2H, m, ArH), 7.54−7.75 (2H, m, ArH). *m/z* (ISMS): 536.5 (MH^+). Found (FAB-HRMS): 536.1630. Calcd for $C_{23}H_{30}O_6N_5S_2$ (MH^+): 536.1638.

Phenylmethyl 3-3(*S*)-[3-[[Imino[[(2, 4, 6-trimethylphenyl)sulfonyl]amino]methyl]amino]propyl]-2(*R*)-[(2-nitrophenyl)sulfonyl-2-aziridinyl]prop-2-enoate, 9. To a solution of aziridine **8** (1.2 g, 2.2 mmol) in CH_2Cl_2 (30 mL) was bubbled O_3 gas at −78 °C until a blue color persisted. To the above solution was added Me_2S (1.7 mL, 22 mmol), and the mixture was stirred for 30 min and then dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue of a crude aldehyde, which was used immediately in the next step without further purification. To a stirred suspension of LiCl (230 mg, 5.4 mmol) in MeCN (5 mL) under argon, were added $(EtO)_2P(O)CH_2CO_2Bn$ (1.5 mL, 5.4 mmol) and $(^iPr)_2NEt$ (DIPEA) (0.94 mL, 5.4 mmol) at 0 °C. After 20 min, the above aldehyde in MeCN (15 mL) was added to the mixture at 0 °C, and the mixture was stirred at this temperature for 8 h. The mixture was concentrated under reduced pressure, and the residue was extracted with EtOAc. The extract was washed successively with saturated aqueous citric acid and H_2O and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with $CHCl_3$/MeOH (40:1) gave the title compound **9** (1.0 g, 1.5 mmol, 67% yield from **8**) as a colorless oil.

$[\alpha]^{23}_D$ −10.1 (*c* 1.49, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.63 (4H, br m, 1, 2-CH_2), 2.17 (3H, s, Ar-*p*-Me), 2.64 (6H, s, Ar-*o*-Me), 3.20 (2H, br m, 3-CH_2), 3.22 (1H, br, 2-H), 3.61 (1H, br, 3-H), 5.15 (2H, s, CH_2), 6.18 (1H, dd, J = 15.7, 0.8 Hz, CH=), 6.22 (3H, br, guanidino), 6.66 (1H, dd, J = 15.5, 6.9 Hz, CH=), 6.88 (2H, s, ArH), 7.34 (5H, s, ArH), 7.56−7.60 (1H, m, ArH), 7.71−7.79 (2H, m, ArH), 8.13−8.17 (1H, m, ArH). *m/z* (ISMS): 670.5 (MH^+). Found (FAB-HRMS): 670.2019. Calcd for $C_{31}H_{36}O_8N_5S_2$ (MH^+): 670.2005.

Phenylmethyl 8-[[Imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]amino]-5(*S*)-[[(2-nitrophenyl)sulfonyl]amino]-2(*R*)-(naphthylmethyl)oct-3-enoate [Ns-L-Arg(Mts)-ψ[(*E*)-CH=CH]-L-Nal-OBn], 10. To a stirred solution of CuCN (219 mg, 2.45 mmol) and LiCl (207 mg, 4.89 mmol) in dry THF (3.0 mL) under argon at −78 °C, was added dropwise 0.5 M (2-naphthylmethyl)zincbromide in THF solution (4.9 mL, 2.45 mmol), and the mixture was stirred at 0 °C for 10 min. A solution of enoate **9** (273 mg, 0.408 mmol) in dry THF (9.0 mL) was added dropwise to the above mixture at −78 °C with stirring, and the stirring was continued for 30 min followed by quenching with 10 mL of a 1:1 saturated aqueous NH_4Cl/28% NH_4OH solution. The mixture was extracted with Et_2O, and the extract was washed with saturated aqueous NH_4Cl and brine and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue, which was purified by chromatography over silica gel with *n*-hexane/EtOAc (1:2) to yield 273 mg (0.337 mmol, 83% yield from **9**) of compound **10** as a yellow oil. $[\alpha]^{33}_D$ −80.9 (*c* 0.61, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.35 (2H, br m, 2-CH_2), 1.55 (2H, br m, 1-CH_2), 2.04 (3H, s, Ar-*p*-Me), 2.26 (6H, s, Ar-*o*-Me), 2.97 (2H, br, CH_2), 2.98 (2H, br m, 3-CH_2), 3.20−3.25 (1H, m, 2-H), 3.90 (1H, br, 5-H), 4.93 (2H, s, CH_2), 5.24 (1H, dd, J = 15.5, 6.9 Hz, CH=), 5.50 (1H, dd, J = 15.4, 8.4 Hz, CH=), 5.67 (1H, d, J = 4.6 Hz, NH), 5.95 (3H, br, guanidino), 6.89 (2H, s, ArH), 7.05−7.28 (7H, m, ArH), 7.42−7.77 (9H, m, ArH), 7.96−8.00 (1H, m, ArH). *m/z* (ISMS): 814.0 (MH^+). Found (FAB-HRMS): 812.2803. Calcd for $C_{42}H_{46}O_8N_5S_2$ (MH^+): 812.2788.

Phenylmethyl 5(*S*)-[(Fluoren-9-ylmethoxy)carbonylamino]-8-[[imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]amino]-2(*R*)-(2-naphthylmethyl)oct-3-enoate [Fmoc-L-Arg(Mts)-ψ[(*E*)-CH=CH]-L-Nal-OBn], 11. To a stirred solution of enoate **10** (81 mg, 0.10 mmol) in DMSO/MeCN (1:49, 5 mL), were added thiophenol (31 μL, 0.3 mmol) and K_2CO_3 (55 mg, 0.4 mmol) at room temperature, and the mixture was stirred at 50 °C for 1 h. The solution was filtered, and the filtrate was concentrated under reduced pressure. The residue was extracted with EtOAc, washed with brine, and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue, which was dissolved in THF/H_2O (1:1, 50 mL). Fmoc-OSu (33 mg, 0.1 mmol) and Et_3N (27 μL, 0.19 mmol) were added to the above solution at 0 °C. After being stirred for 6 h, the mixture was acidified with 0.1 M HCl and then extracted with EtOAc. The extract was washed with 0.1 M HCl and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with *n*-hexane/EtOAc (1:2) gave the title compound **11** (60 mg, 70.9 μmol, 71% yield from **10**) as a colorless oil.

$[\alpha]^{29}_D$ −11.2 (*c* 0.63, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.26 (4H, br m, 1, 2-CH_2), 2.18 (3H, s, Ar-*p*-Me), 2.63 (6H, s,

Ar-*o*-Me), 2.94 (2H, br, CH_2), 3.19 (2H, br m, 3-CH_2), 3.38 (1H, br, 2-H), 3.96 (1H, br, 5-H), 4.12 (1H, t, J = 5.8 Hz, ArH), 4.35 (2H, t, J = 5.94 Hz, CH_2), 4.82 (1H, br, NH), 5.01 (2H, s, CH_2), 5.24 (1H, dd, J = 15.0, 5.7 Hz, CH=), 5.64 (1H, dd, J = 14.4, 7.8 Hz, CH=), 6.16 (3H, br, guanidino), 6.81 (2H, s, ArH), 7.08−7.28 (8H, m, ArH), 7.33−7.42 (4H, m, ArH), 7.42−7.54 (3H, m, ArH), 7.64−7.74 (5H, m, ArH). *m/z* (ISMS): 850.0 (MH^+). Found (FAB-HRMS): 849.3664. Calcd for $C_{51}H_{53}O_6N_4S_2$ (MH^+): 849.3686.

5(*S*)-[(Fluoren-9-ylmethoxy)carbonylamino]-8-[[imino-[[(2, 4, 6-trimethylphenyl)sulfonyl]amino]methyl]amino]-2(*R*)-(2-naphthylmethyl)oct-3-enoic Acid [Fmoc-L-Arg-(Mts)-ψ[(*E*)-CH=CH]-L-Nal-OH], 12. The enoate **11** (30 mg, 0.037 mmol) was dissolved in TFA (10 mL), and thioanisole (500 μL), *m*-cresol (200 μL), and 1,2-ethanedithiol (100 μL) were added to the solution at 0 °C, and the mixture was stirred for 12 h at room temperature. The mixture was concentrated under reduced pressure and extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with *n*-hexane/EtOAc (1:4) gave the title compound **12** (28 mg, 0.036 mmol, 98% yield from **11**) as a colorless oil.

$[\alpha]^{23}_D$ −15.2 (*c* 0.07, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ: 1.25 (4H, br m, 1, 2-CH_2), 2.20 (3H, s, Ar-*p*-Me), 2.62 (6H, s, Ar-*o*-Me), 2.86 (2H, br, CH_2), 3.05 (2H, br m, 3-CH_2), 3.49 (1H, br, 2-H), 3.68 (1H, br, 5-H), 4.10 (1H, br, ArH), 4.20−4.26 (2H, m, CH_2), 4.93 (1H, br, CH=), 5.34 (1H, br, CH=), 5.38 (1H, br, NH), 5.95 (3H, br, guanidino), 6.84 (2H, s, ArH), 7.20−7.41 (7H, m, ArH), 7.48−7.57 (3H, m, ArH), 7.65−7.77 (5H, m, ArH). *m/z* (ISMS): 760.0 (MH^+). Found (FAB-HRMS): 759.3228. Calcd for $C_{44}H_{47}O_6N_4S_2$ (MH^+): 759.3216.

Phenylmethyl 8-[[Imino[[(2, 4, 6-trimethylphenyl)sulfonyl]amino]methyl]amino]-5(*S*)-[[(2-nitrophenyl)-sulfonyl]amino]-2(*S*)-(naphthylmethyl)oct-3-enoate [Ns-L-Arg(Mts)-ψ[(*E*)-CH=CH]-D-Nal-OBn], 14. To a stirred solution of enoate **9** (500 mg, 0.75 mmol) in $CHCl_3$ (5 mL) was added dropwise MSA (435 μL, 6.7 mmol) at room temperature with stirring, and the stirring was continued for 20 min. The mixture was extracted with EtOAc, and the extract was washed successively with aqueous 5% citric acid, water, aqueous 5% $NaHCO_3$, and water and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue of the crude mesylate **13**, which was used directly in the following step without further purification. To a stirred slurry of CuCN (269 mg, 3.0 mmol) in dry THF (5 mL) under argon at −78 °C was added dropwise (2-naphthylmethyl)zincbromide in THF solution (6.0 mL, 3.0 mmol), and the mixture was stirred at 0 °C for 15 min followed by addition of $BF_3{\cdot}Et_2O$ (369 μL, 3.0 mmol) at −78 °C and then stirred at −78 °C for 15 min. To the mixture at −78 °C with stirring was added by syringe a solution of the crude mesylate **13** in THF (10 mL), and the stirring was continued at −78 °C for 1 h followed by quenching with saturated aqueous NH_4Cl and aqueous 28% NH_4OH (1:1) at 0 °C. The mixture was allowed to warm to room temperature and extracted with Et_2O. The extract was washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by chromatography over silica gel with *n*-hexane/EtOAc (1:2) to give 408 mg (0.50 mmol, 67% from **9**) of protected EADI **14** as a yellow oil. $[\alpha]^{32}_D$ −41.7 (*c* 0.60, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.54 (4H, br m, 1, 2-CH_2), 2.25 (3H, s, Ar-*p*-Me), 2.66 (6H, s, Ar-*o*-Me), 2.86 (2H, br m, 3-CH_2), 2.98−3.06 (2H, m, CH_2), 3.15−3.23 (1H, m, 2-H), 3.78−3.88 (1H, m, 5-H), 4.98 (2H, s, CH_2), 5.09 (1H, dd, J = 15.5, 8.0 Hz, CH=), 5.49 (1H, dd, J = 15.5, 8.2 Hz, CH=), 5.57 (1H, d, J = 8.4 Hz, NH), 6.09 (3H, br, guanidino), 6.89 (2H, s, ArH), 7.10−7.26 (7H, m, ArH), 7.37−7.76 (9H, m, ArH), 7.95−7.98 (1H, m, ArH). *m/z* (ISMS): 814.0 (MH^+). Found (FAB-HRMS): 812.2775. Calcd for $C_{42}H_{46}O_8N_5S_2$ (MH^+): 812.2788.

Phenylmethyl 5(*S*)-[(Fluoren-9-ylmethoxy)carbonyl-amino]-8-[[imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]amino]-2(*S*)-(2-naphthylmethyl)oct-3-enoate [Fmoc-L-Arg(Mts)-ψ[(*E*)-CH=CH]-D-Nal-OBn], 15. By use of a procedure identical with that described for the preparation of **11** from **10**, the enoate **14** (30 mg, 37 μmol) was converted into 28 mg (36 μmol, 98% yield from **14**) of the title compound **15** as a colorless oil.

$[\alpha]^{29}_D$ −1.6 (*c* 0.61, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.14−1.30 (4H, br m, 1, 2-CH_2), 2.17 (3H, s, Ar-*p*-Me), 2.62 (6H, s, Ar-*o*-Me), 2.91 (2H, br m, 3-CH_2), 3.15−3.23 (2H, m, CH_2), 3.40 (1H, br, 2-H), 3.95 (1H, br, 5-H), 4.11 (1H, t, J = 6.5 Hz, ArH), 4.33 (2H, d, J = 5.7 Hz, CH_2), 4.85 (1H, d, J = 6.8 Hz, NH), 5.00 (2H, s, CH_2), 5.25 (1H, dd, J = 16.7, 6.8 Hz, CH=), 5.61 (1H, dd, J = 14.3, 7.6 Hz, CH=), 6.13 (3H, br, guanidino), 6.81 (2H, s, ArH), 7.09−7.27 (8H, m, ArH), 7.32−7.45 (4H, m, ArH), 7.46−7.56 (3H, m, ArH), 7.64−7.73 (5H, m, ArH). *m/z* (ISMS): 850.0 (MH^+). Found (FAB-HRMS): 849.3698. Calcd for $C_{51}H_{53}O_6N_4S_2$ (MH^+): 849.3686.

5(*S*)-[(Fluoren-9-ylmethoxy)carbonylamino]-8-[[imino-[[(2, 4, 6-trimethylphenyl)sulfonyl]amino]methyl]amino]-2(*S*)-(2-naphthylmethyl)oct-3-enoic Acid [Fmoc-L-Arg-(Mts)-ψ[(*E*)-CH=CH]-D-Nal-OH], 16. By use of a procedure identical with that described for the preparation of **12** from **11**, the enoate **15** (153 mg, 0.19 mmol) was converted into 144 mg (0.19 mmol, 99% yield from **15**) of the title compound **16** as a colorless oil.

$[\alpha]^{23}_D$ 10.8 (*c* 0.19, $CHCl_3$). ^1H NMR (270 MHz, $CDCl_3$) δ: 1.24 (4H, br m, 1, 2-CH_2), 2.17 (3H, s, Ar-*p*-Me), 2.70 (6H, s, Ar-*o*-Me), 2.90 (2H, br m, 3-CH_2), 3.07 (2H, m, CH_2), 3.29 (1H, br, 2-H), 3.67 (1H, br, 5-H), 4.06 (1H, t, J = 8.2 Hz, ArH), 4.28 (2H, d, J = 5.9 Hz, CH_2), 5.12 (1H, d, J = 5.9 Hz, NH), 5.26 (1H, dd, J = 15.0, 5.8 Hz, CH=), 5.59 (1H, dd, J = 15.0, 8.0 Hz, CH=), 6.19 (3H, br, guanidino), 6.80 (2H, s, ArH), 7.18−7.40 (7H, m, ArH), 7.46−7.54 (3H, m, ArH), 7.59−7.70 (5H, m, ArH). *m/z* (ISMS): 760.0 (MH^+). Found (FAB-HRMS): 759.3201. Calcd for $C_{44}H_{47}O_6N_4S_2$ (MH^+): 759.3216.

H-D-Tyr(O^{t}Bu)-Arg(Pbf)-Arg(Mts)-ψ[(*E*)-CH=CH]-Nal-Gly-NHNHCO−Wang Resin. *p*-Nitrophenyl carbonate Wang resin **33** (Calbiochem-Novabiochem Japan, Ltd., Tokyo, Japan, 0.93 mmol/g, 323 mg, 0.3 mmol) was treated with $NH_2NH_2{\cdot}H_2O$ (146 μL, 3.0 mmol) in DMF (3 mL) at room temperature for 2 h to give a hydrazide linker **34**. Protected peptide−resins were manually constructed by Fmoc-based solid-phase peptide synthesis. ^{t}Bu for Tyr and Mts or Pbf for Arg were employed for side-chain protection. Fmoc deprotection was achieved by 20% piperidine in DMF (1 min × 2 and 15 min × 1). Fmoc−amino acids including EADIs or RADIs were condensed to free amino groups by treatment with 3 equiv of reagents (Fmoc−amino acid, *N,N′*-diisopropylcarbodiimide (DIPCDI) and $HOBt{\cdot}H_2O$) in DMF for 1.5 h.

***cyclo*(-D-Tyr-Arg-Arg-ψ[(*E*)-CH=CH]-Nal-Gly-)·2TFA (37a).** The protected **37a** resin (34 mg, 0.025 mmol) was treated with TFA (0.5 mL) in $CHCl_3$ (4.5 mL) at room temperature for 2 h, and the mixture was filtered. Concentration of the filtrate under reduced pressure gave a crude hydrazide (H-D-Tyr-Arg(Pbf)-Arg(Mts)-ψ[(*E*)-CH=CH]-Nal-Gly-$NHNH_2$) as a colorless powder. To a stirred solution of the hydrazide in DMF (1 mL) were added a solution of 4 M HCl in DMF (16.6 μL, 75 μmol) and isoamyl nitrite (40 μL, 0.20 mmol) at −30 °C. After being stirred at −10 °C for 20 min, the mixture was diluted with precooled DMF (50 mL). To the above solution was added DIPEA (191 μL, 1.1 mmol) at −30 °C, and the mixture was stirred for 48 h at −20 °C. Concentration under reduced pressure gave a yellow oil (crude *cyclo*(-D-Tyr-Arg(Pbf)-Arg(Mts)-ψ[(*E*)-CH=CH]-Nal-Gly-)). To the protected cyclic peptide were added *m*-cresol (0.4 mL, 3.6 mmol), 1, 2-ethanedithiol (160 μL, 1.9 mmol), thioanisole (1.0 mL, 8.5 mmol), TFA (10 mL), and bromotrimethylsilane (1.2 mL, 9.1 mmol) at 0 °C, and the stirring was continued at room temperature for 12 h. Concentration under reduced pressure and purification by preparative HPLC gave the cyclic pseudopeptide **37a** (8.5 mg, 36% yield from protected **37a** resin) as a freeze-dried powder.

$[\alpha]^{27}_D$ −53.3 (*c* 0.24, H_2O). t_R = 28.6 min (linear gradient of MeCN in H_2O, 10 to 40% over 30 min). *m/z* (ISMS): 714.0 (MH^+). Found (FAB-HRMS): 713.3879. Calcd for $C_{37}H_{49}O_5N_{10}$ (MH^+): 713.3887.

***cyclo*(-D-Tyr-Arg-Arg-ψ[(*E*)-CH=CH]-D-Nal-Gly-)·2TFA (37f).** By use of a procedure identical with that described

for the preparation of **37a**, the protected **37f** (34 mg, 0.025 mmol) was converted into 9.8 mg (10.5 μmol, 42%) of the title compound **37f**, as a freeze-dried powder.

$[\alpha]^{26}_D$ −32.0 (*c* 0.13, H_2O). t_R = 28.9 min (linear gradient of MeCN in H_2O, 10 to 40% over 30 min). *m/z* (ISMS): 714.0 (MH^+). Found (FAB-HRMS): 713.3903. Calcd for $C_{37}H_{49}O_5N_{10}$ (MH^+): 713.3887.

(*tert*-Butoxy)-*N*-[2(*R*,*S*)-hydroxy-1(*S*)-(2-naphthylmethyl)but-3-enyl]formamide, 20. To a stirred solution of Boc-Nal-OMe **19** (4.0 g, 12.2 mmol) in CH_2Cl_2 (100 mL) was added dropwise a solution of DIBAL-H in toluene (1.0 M, 24.4 mL, 24.4 mmol) at −78 °C under argon, and the mixture was stirred at −78 °C for 2 h. To the solution was added dropwise a vinyl Grignard (CH_2=CHMgCl) reagent in THF (12.6 mL, 36.6 mmol) at −78 °C, and the mixture was stirred for 6 h with warming to 0 °C. The reaction was quenched with saturated aqueous citric acid at −78 °C, and organic solvents were concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed successively with saturated aqueous citric acid, saturated aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (3:1) gave a mixture of *threo*- and *erythro*-allyl alcohols **20** (1.4 g, 35% yield from **19**) as a colorless oil. The mixture of diastereoisomer was used in the following step without further purification.

^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.24−1.36 (9H, br, *tert*-Bu), 2.90−2.94 (1H, br, 2-H), 3.00−3.03 (2H, br, CH_2), 3.86−3.94 (1H, br, 1-H), 4.95−5.01 (1H, br, NH), 5.13−5.17 (1H, m, C*H*H=), 5.22−5.29 (1H, m, CH*H*=), 5.82−5.94 (1H, m, CH=), 7.38−7.43 (3H, m, ArH), 7.68−7.80 (4H, m, ArH). *m/z* (ISMS): 328.5 (MH^+). Found (FAB-HRMS): 328.1921. Calcd for $C_{20}H_{26}O_3N$ (MH^+): 328.1913.

1(*S*)-[1-[(*tert*-Butoxy)carbonylamino]-2-(naphthyl)ethyl]prop-2(*R*, *S*)-enyl Acetate, 21. To a stirred solution of allyl alcohol **20** (8.5 g, 26.0 mmol) in $CHCl_3$ (10 mL), were added acetic anhydride (11.0 mL, 117 mmol) and pyridine (18.9 mL, 234 mmol) at 4 °C, and the mixture was stirred at room temperature for 6 h. The mixture was concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed with aqueous 5% $NaHCO_3$, aqueous 1 M HCl, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (2:1) gave acetates **21** (5.7 g, 59% yield from **20**) as a colorless oil.

^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.29−1.37 (9H, br, *tert*-Bu), 2.06−2.08 (3H, br, Me), 2.80−2.84 (1H, br, 2-H), 2.89−2.95 (2H, br, CH_2), 4.14−4.20 (1H, br, 1-H), 4.74−4.78 (1H, br, NH), 5.20−5.24 (1H, br m, C*H*H=), 5.25−5.30 (1H, br m, CH*H*=), 5.74−5.80 (1H, m, CH=), 7.30−7.42 (3H, m, ArH), 7.60−7.76 (4H, m, ArH). *m/z* (ISMS): 370.5 (MH^+). Found (FAB-HRMS): 370.2016. Calcd for $C_{22}H_{28}O_4N$ (MH^+): 370.2018.

***tert*-Butyl 4(*R*,*S*)-Acetoxy-5(*S*)-[(*tert*-butoxy)carbonylamino]-6-(2-naphthyl)hex-2-enoate, 22.** To a solution of acetate **21** (5.7 g, 15.4 mmol) in CH_2Cl_2 (40 mL) was bubbled O_3 gas at −78 °C until a blue color persisted. To the above solution, was added Me_2S (11 mL, 154 mmol), and the mixture was stirred for 30 min. The mixture was dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue of a crude aldehyde, which was used immediately in the next step without further purification. To a stirred suspension of LiCl (1.57 g, 37 mmol) in MeCN (10 mL) under argon were added $(EtO)_2P(O)CH_2CO_2{}^t$Bu (8.7 mL, 37 mmol) and DIPEA (6.4 mL, 37 mmol) at 0 °C. After 20 min, the above aldehyde in MeCN (20 mL) was added to the above mixture at 0 °C, and the mixture was stirred at this temperature for 8 h. The mixture was concentrated under reduced pressure, and the residue was extracted with EtOAc. The extract was washed successively with saturated aqueous citric acid and H_2O and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (1:2) gave enoates **22** (2.1 g, 29% yield from **21**) as a white amorphous semisolid.

Found: C, 68.97; H, 7.60; N, 2.92. $C_{27}H_{35}O_6N$ Calcd: C, 69.06; H, 7.51; N, 2.98. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.34−1.38 (9H, br, *tert*-Bu), 1.43−1.47 (9H, br, *tert*-Bu), 2.13−2.17 (3H, br, Me), 2.91−2.99 (2H, br, CH_2), 4.22−4.32 (1H, br, 5-H), 4.71−4.77 (1H, br, 4-H), 5.42−5.46 (1H, br, NH), 5.79−5.99 (1H, m, CH=), 6.70−6.83 (1H, m, CH=), 7.43−7.49 (3H, m, ArH), 7.77−7.82 (4H, m, Ar). *m/z* (FAB-LRMS): 468 [$(M-H)^-$], 305, 199, 153, 151, and 46 (base peak). Found (FAB-HRMS): 468.2375. Calcd for $C_{27}H_{34}O_6N$ [$(M-H)^-$]: 468.2386.

***tert*-Butyl 5(*S*)-[(*tert*-Butoxy)carbonylamino]-6-(2-naphthyl)hex-3-enoate (Boc-L-Nal=Gly-O^{t}Bu), 23.** To a stirred slurry of Sm (900 mg, 6.0 mmol) in dry THF (20 mL) under argon at room temperature was added a solution of CH_2I_2 (322 μL, 4.0 mmol) in dry THF (20 mL), and the slurry was stirred at room temperature for 2 h until a dark green color persisted. To a stirred solution of enoate **22** (600 mg, 1.3 mmol) in dry THF (16 mL) in the other vessel were added *tert*-BuOH (8 mL, excess) and the above SmI_2 solution (38 mL, 3.8 mmol) under argon at room temperature, and the mixture was stirred for 1 h. The reaction was then quenched with saturated aqueous NH_4Cl (10 mL) at 4 °C, and the mixture was extracted with Et_2O (20 mL). The extract was washed with saturated aqueous NH_4Cl and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (1:4) gave the enoate **23** (530 mg, 95% yield from **22**) as white crystals.

Mp: 80−82 °C (from *n*-hexane). Found: C, 73.17; H, 8.17; N, 3.39. $C_{25}H_{33}O_4N$ Calcd: C, 72.96; H, 8.08; N, 3.40. $[\alpha]^{25}_D$ 11.00 (*c* 1.09, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.37 (9H, s, *tert*-Bu), 1.41 (9H, s, *tert*-Bu), 2.93 (2H, d, J = 6.4 Hz, 6-CH_2), 2.98 (2H, d, J = 6.8 Hz, 2-CH_2), 4.48−4.59 (1H, br, NH), 5.43 (1H, t, J = 11.2 Hz, 5-H), 5.55 (1H, dd, J = 15.6, 5.6 Hz, CH=), 5.61−5.69 (1H, br, CH=), 7.31−7.46 (3H, m, ArH), 7.60−7.62 (1H, br, ArH), 7.74−7.78 (3H, m, ArH). *m/z* (ISMS): 412.0 (MH^+). Found (FAB-HRMS): 412.2491. Calcd for $C_{25}H_{34}O_4N$ (MH^+): 412.5418.

5(*S*)-[(Fluoren-9-ylmethoxy)carbonylamino]-6-(2-naphthyl)hex-3-enoic Acid (Fmoc-L-Nal=Gly-OH), 24. The enoate **23** (1.79 g, 4.35 mmol) was dissolved in TFA (30 mL), anisole (472 μL, 4.35 mmol) was added to the solution at 4 °C, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and dissolved in THF and H_2O (1:1 (v/v) 20 mL). To the stirred solution were added Fmoc-OSu (1.47 g, 4.35 mmol) and Et_3N (10 mL, 71.7 mmol) at 4 °C, and the mixture was stirred at room temperature for 8 h. The mixture was acidified with aqueous 1 M HCl and was extracted with EtOAc. The extract was washed with aqueous 0.1 M HCl and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (3:1) gave the enoic acid **24** (1.61 g, 78% yield from **23**) as white crystals.

Mp: 134−136 °C (from *n*-hexane). $[\alpha]^{23}_D$ −2.75 (*c* 0.73, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.96 (2H, br, 6-CH_2), 3.03 (2H, br, 2-CH_2), 4.14 (1H, t, J = 6.6 Hz, ArH), 4.32 (1H, dd, J = 14.9, 7.2 Hz, CH=), 4.38 (1H, m, CH=) 4.54 (1H, d, J = 7.6 Hz, CH_2), 4.81 (1H, br, 5-H), 5.62 (1H, br, NH), 7.18−7.28 (2H, m, ArH), 7.30−7.52 (5H, m, ArH), 7.57−7.63 (2H, m, ArH), 7.70−7.79 (6H, m, ArH). *m/z* (ISMS): 478.0 (MH^+). Found (FAB-HRMS): 478.2016. Calcd for $C_{31}H_{28}O_4N$ (MH^+): 478.2018.

H-D-Tyr(O^{t}Bu)-Arg(Pbf)-Arg(Pbf)-Nal-ψ[(*E*)-CH=CH]-Gly-NHNHCO−Wang Resin. On the hydrazide resin, were coupled successively Fmoc-D-Tyr(O^{t}Bu)-OH, Fmoc-Nal-ψ[(*E*)-CH=CH]-Gly-OH, and Fmoc-Arg(Pbf)-OH by use of the procedure identical with that described for the preparation of H-D-Tyr(O^{t}Bu)-Arg(Pbf)-Arg(Mts)-ψ[(*E*)-CH=CH]-Nal-Gly-NHNHCO−Wang resin to afford the protected **37c** resin.

***cyclo*(-D-Tyr-Arg-Arg-Nal-ψ[(*E*)-CH=CH]-Gly-)·2TFA (37c).** By use of a procedure identical with that described for the preparation of **37a,** the protected **37c** resin (173 mg, 0.13 mmol) was converted into 7.0 mg (7.4 μmol, 5.9%) of the title compound **37c**, as a freeze-dried powder.

$[\alpha]^{21}_D$ −43.1 (*c* 0.33, H_2O). t_R = 24.3 min (linear gradient of MeCN in H_2O, 10 to 40% over 30 min). *m/z* (ISMS): 713.0

(MH$^+$). Found (FAB-HRMS): 713.3911. Calcd for $C_{37}H_{49}O_5N_{10}$ (MH$^+$): 713.3887.

***tert*-Butyl 2(*S*)-[[2(*S*)-[(*tert*-butoxy)carbonyamino]-5-[[imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]-amino]pentyl]amino]-3-(2-naphthyl) Propanoate [Boc-L-Arg(Mts)-ψ[CH$_2$−NH]-L-Nal-O^{t}Bu], 26.** To a stirred solution of **25** (5.0 g, 10 mmol) in toluene/CH_2Cl_2 (1:1 (v/v), 50 mL) was added dropwise a solution of DIBAL-H in toluene (1.0 M, 60 mL, 60 mmol) at −50 °C under argon, and the mixture was stirred for 4 h at −78 °C. The reaction was quenched with saturated aqueous citric acid at −78 °C, and the organic solvents were concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed successively with saturated aqueous citric acid and brine and dried over $MgSO_4$. Concentration under reduced pressure gave a crude aldehyde (Boc-Arg(Mts)-H), which was used in the following step without further purification. To the stirred solution of Boc-Arg(Mts)-H in $ClCH_2CH_2Cl$/DMF (1:6 (v/v), 100 mL), were added H-L-Nal-O^{t}Bu (5.4 g, 20 mmol) and AcOH (1.1 mL, 20 mmol) at 4 °C and stirred for 10 min. $NaBH(OAc)_3$ (6.4 g, 30 mmol) was added to the above mixture at 4 °C and stirred for 8 h with warming to room temperature. The mixture was concentrated under reduced pressure, and the residue was extracted with $CHCl_3$. The extract was washed with aqueous 5% $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue, which was purified by chromatography over silica gel with $CHCl_3$/MeOH (39:1) to yield 3.4 g (4.9 mmol, 49% yield from **25**) of compound **26** as a yellow oil.

[α]$^{23}_D$ 4.08 (*c* 1.96, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.24 (9H, s, *tert*-Bu), 1.32 (9H, s, *tert*-Bu), 1.55 (2H, br m, 4-CH_2), 1.74 (2H, br m, 3-CH_2), 2.25 (3H, s, Ar-*p*-Me), 2.67 (6H, s, Ar-*o*-Me), 3.08 (2H, br m, 5-CH_2), 3.20 (2H, br, 3-CH_2), 3.34 (2H, br, 1-CH_2), 3.62 (1H, br, 2-H), 3.82 (1H, br, 2-H), 3.99 (1H, br, NH), 5.95 (1H, br, NH), 6.61 (3H, br, guanidino), 6.87 (2H, s, Ar-*m*-H), 7.36−7.44 (3H, m, ArH), 7.67−7.75 (4H, m, ArH). *m/z* (ISMS): 697.0 (MH$^+$). Found (FAB-HRMS): 696.3812. Calcd for $C_{37}H_{54}O_6N_5S$ (MH$^+$): 696.3795.

***tert*-Butyl 2(*S*)-[*N*-[2(*S*)-[(*tert*-Butoxy)carbonylamino]-5-[[imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]-amino]pentyl](phenylmethoxy)carbonylamino]-3-(2-naphthyl)propanoate [Boc-Arg(Mts)-ψ[CH$_2$−N(Cbz)]-Nal-O^{t}Bu], 27.** To a stirred solution of propanoate **26** (1.4 g, 2.0 mmol) in DMF (100 mL) at 4 °C, were added Cbz-Cl (0.69 g, 4.0 mmol) and Et_3N (560 μL, 4.0 mmol) and stirred at room temperature for 8 h. The mixture was concentrated under reduced pressure and extracted with EtOAc. The extract was washed with saturated aqueous citric acid, saturated aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (1:1) gave the title compound **27** (1.6 g, 77% yield from **26**) as a yellow oil.

[α]$^{23}_D$ −36.44 (*c* 0.67, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.39 (9H, s, *tert*-Bu), 1.42 (9H, s, *tert*-Bu), 1.47 (2H, s, 4-CH_2), 1.64 (2H, br, 3-CH_2), 2.42 (3H, s, Ar-*p*-Me), 2.65 (6H, s, Ar-*o*-Me), 2.98 (2H, br, 5-CH_2), 3.27 (2H, br, 3-CH_2), 3.31 (2H, br, 1-CH_2), 3.40 (1H, br, 2-H), 4.24 (1H, br, 2-H), 5.08 (2H, s, CH_2), 5.90 (1H, br, NH), 6.13 (3H, br, guanidino), 6.86 (2H, s, Ar-*m*-H), 7.26 (5H, s, ArH), 7.31−7.46 (3H, m, ArH), 7.54−7.75 (4H, m, ArH). *m/z* (ISMS): 831.5 (MH$^+$). Found (FAB-HRMS): 830.4153. Calcd for $C_{45}H_{60}O_8N_5S$ (MH$^+$): 830.4163.

2(*S*)-[*N*-[2(*S*)-[(Fluoren-9-ylmethoxy)carbonylamino]-5-[[imino[[(2,4,6-trimethylphenyl)sulfonyl]amino]methyl]-amino]pentyl](phenylmethoxy)carbonylamino]-3-(2-naphthyl)propanoic Acid [Fmoc-Arg(Mts)-ψ[CH$_2$−N(Cbz)]-Nal-OH], 28. The propanoate **27** (1.3 g, 1.57 mmol) was dissolved in TFA (30 mL), anisole (170 μL, 1.57 mmol) was added to the solution at 4 °C, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and dissolved in THF and H_2O (1:1 (v/v) 100 mL). To the stirred solution were added Fmoc-OSu (530 mg, 1.57 mmol) and Et_3N (10 mL, 71.7 mmol) at 4 °C, and the mixture was stirred at room temperature for 8 h. The mixture was acidified with aqueous 1 M HCl and extracted with EtOAc. The extract was washed with aqueous 0.1 M HCl and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (4:1) gave the propanoic acid **28** (1.39 g, 99% yield from **27**) as white crystals.

Mp: 156−158 °C (from *n*-hexane). [α]$^{24}_D$ −8.17 (*c* 1.96, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$) δ: 1.85 (2H, br, 4-CH_2), 1.93 (2H, br, 3-CH_2), 2.11 (3H, s, Ar-*p*-Me), 2.47 (6H, s, Ar-*o*-Me), 3.03 (2H, br, 5-CH_2), 3.22 (2H, br, 3-H), 3.37 (2H, br, 1-CH_2), 4.08 (1H, br, ArH), 4.15 (2H, br, CH_2), 4.30 (1H, br, 2-H), 5.16 (1H, br, NH), 6.36 (3H, br, guanidino), 6.83 (2H, s, Ar-*m*-H) 7.25 (5H, s, ArH), 7.29−7.40 (6H, m, ArH), 7.50−7.83 (9H, m, ArH). *m/z* (ISMS): 897.0 (MH$^+$). Found (FAB-HRMS): 896.3693. Calcd for $C_{51}H_{54}O_8N_5S$ (MH$^+$): 896.3710.

H-D-Tyr(O^{t}Bu)-Arg(Pbf)-Arg(Mts)-ψ[CH$_2$−NH]-Nal-Gly-NHNHCO−Wang Resin. On the hydrazide resin were coupled successively Fmoc-Gly-OH, Fmoc-Arg-ψ[CH_2−N(Cbz)]-Nal-OH, Fmoc-Arg(Pbf)-OH, and Fmoc-D-Tyr(O^{t}Bu)-OH by use of a procedure identical with that described for the preparation of H-D-Tyr(O^{t}Bu)-Arg(Pbf)-Arg(Mts)-ψ[(*E*)-CH=CH]-Nal-Gly-NHNHCO−Wang resin to afford the protected **37b** resin.

***cyclo*(-D-Tyr-Arg-Arg-ψ[CH$_2$−NH]-Nal-Gly-)·3TFA (37b).** By use of a procedure identical with that described for the preparation of **37a**, the protected **37b** (200 mg, 0.15 mmol) was converted into 0.6 mg (0.57 μmol, 0.86%) of the title compound **37b**, as a freeze-dried powder.

[α]$^{21}_D$ −22.6 (*c* 0.27, H_2O). t_R = 19.3 min (linear gradient of MeCN in H_2O, 10 to 40% over 30 min). *m/z* (ISMS): 717.0 (MH$^+$). Found (FAB-HRMS): 716.4016. Calcd for $C_{36}H_{49}O_5N_{11}$ (MH$^+$): 716.3996.

***tert*-Butyl 2(*S*)-[[2-[(*tert*-Butoxy)carbonylamino]-3-(2-naphthyl)propyl]amino]acetate [Boc-L-Nal-ψ[CH$_2$−NH]-Gly-O^{t}Bu], 30.** To a stirred solution of Boc-Nal-NMe(OMe) **29** (5.5 g, 15 mmol) in toluene/CH_2Cl_2 (1:1 (v/v) 50 mL) was added dropwise a solution of DIBAL-H in toluene (1.0 M, 62 mL, 62 mmol) at −78 °C under argon, and the mixture was stirred at −78 °C for 4 h. The reaction was quenched with saturated aqueous citric acid at −78 °C, and organic solvents were concentrated under reduced pressure. The residue was extracted with EtOAc, and the extract was washed successively with saturated aqueous citric acid and brine and dried over $MgSO_4$. Concentration under reduced pressure gave a crude aldehyde (Boc-Nal-H), which was used in the following step without further purification. To the stirred solution of Boc-Nal-H in $ClCH_2CH_2Cl$/DMF (1:6 (v/v), 200 mL), was added H-Gly-O^{t}Bu·AcOH (5.8 g, 31 mmol) at 4 °C and stirred for 10 min. $NaBH(OAc)_3$ (9.8 g, 46 mmol) was added to the above mixture at 4 °C and stirred for 8 h with warming to room temperature. The mixture was concentrated under reduced pressure, and the residue was extracted with $CHCl_3$. The extract was washed with aqueous 5% $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue, which was purified by chromatography over silica gel with $CHCl_3$ to yield 3.2 g (7.7 mmol, 50% yield from **29**) of compound **30** as a yellow oil.

[α]$^{22}_D$ −0.67 (*c* 4.47, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (9H, s, *tert*-Bu), 1.47 (9H, s, *tert*-Bu), 2.87 (2H, br, CH_2), 3.02 (2H, br, 3-CH_2), 3.85−3.94 (2H, m, 1-CH_2), 4.09−4.21 (1H, m, 2-H), 5.44 (1H, br, NH), 6.45 (1H, br, NH), 7.29−7.36 (2H, m, Ar−H), 7.41−7.48 (2H, m, ArH), 7.60−7.62 (1H, m, ArH), 7.72−7.83 (2H, m, ArH). *m/z* (ISMS): 415.5 (MH$^+$). Found (FAB-HRMS): 415.2594. Calcd for $C_{24}H_{35}O_4N_2$ (MH$^+$): 415.2597.

***tert*-Butyl 2(*S*)-[*N*-[2-[(*tert*-Butoxy)carbonylamino]-3-(2-naphthyl)propyl](phenylmethoxy)carbonylamino]acetate [Boc-L-Nal-ψ[CH$_2$−N(Cbz)]-Gly-O^{t}Bu], 31.** To a stirred solution of acetate **30** (5.0 g, 12.1 mmol) in DMF (100 mL) at 4 °C were added Cbz-Cl (20.6 g, 121 mmol) and DIPEA (21.7 mL, 121 mmol), and the mixture was stirred at room temperature for 8 h. The mixture was concentrated under reduced pressure and extracted with EtOAc. The solution was washed with saturated aqueous citric acid, saturated aqueous $NaHCO_3$, and brine and dried over $MgSO_4$. Concentration under

reduced pressure followed by chromatography over silica gel with EtOAc/*n*-hexane (1:2) gave the title compound **31** (4.0 g, 60% yield from **30**) as white crystals.

Mp: 107−109 °C (from *n*-hexane). Found: C, 70.01; H, 7.42 N, 4.98. Calcd for $C_{32}H_{40}O_6N_2$: C, 70.05; H, 7.35; N, 5.11. $[\alpha]^{23}_D$ −14.73 (*c* 0.48, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (9H, s, *tert*-Bu), 1.35 (9H, s, *tert*-Bu), 2.94 (2H, br, CH_2), 3.37 (2H, br, 3-CH_2), 3.88 (2H, m, 1-CH_2), 4.05 (1H, m, 2-H), 4.94 (1H, br, NH), 5.13 (2H, s, CH_2), 7.28−7.33 (5H, br, ArH), 7.35−7.47 (3H, m, ArH), 7.74−7.81 (4H, m, ArH). *m/z* (ISMS): 549.5 (MH^+). Found (FAB-HRMS): 549.2953. Calcd for $C_{32}H_{41}O_6N_2$ (MH^+): 549.2965.

2(*S*)-[*N*-[2-[(Fluoren-9-ylmethoxy)carbonylamino]-3-(2-naphthyl)propyl](phenylmethoxy)carbonylamino]-acetic Acid [Fmoc-L-Nal-ψ[CH_2−N(Cbz)]-Gly-OH], 32. The acetate **31** (4.0 g, 7.29 mmol) was dissolved in TFA (30 mL), anisole (792 μL, 7.29 mmol) was added to the solution at 4 °C, and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and dissolved in THF and H_2O (1:1 (v/v) 100 mL). To the stirred solution, were added Fmoc-OSu (2.46 g, 7.29 mmol) and Et_3N (10 mL, 71.7 mmol) at 4 °C, and the mixture was stirred at room temperature for 8 h. The mixture was acidified with aqueous 1 M HCl and was extracted with EtOAc. The extract was washed with aqueous 0.1 M HCl and brine and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with $CHCl_3$/MeOH (39:1) gave the title compound **32** (4.20 g, 94% yield from **31**) as a yellow oil.

$[\alpha]^{23}_D$ −5.01 (*c* 4.79, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.99 (2H, d, J = 6.4 Hz, 3-CH_2), 3.41 (2H, s, CH_2), 4.00 (2H, br, 1-CH_2), 4.08 (1H, t, J = 6.6 Hz, Ar−H), 4.24 (1H, t, J = 6.2 Hz, 2-H), 4.27 (2H, br, CH_2), 5.05 (2H, s, CH_2), 5.53 (1H, d, J = 8.0 Hz, NH), 7.18 (5H, s, Ar−H) 7.30−7.50 (8H, m, ArH), 7.61−7.73 (7H, m, ArH). *m/z* (ISMS): 615.0 (MH^+). Found (FAB-HRMS): 615.2509. Calcd for $C_{38}H_{35}O_6N_2$ (MH^+): 615.2495.

H-D-Tyr(O^{t}Bu)-Arg(Pbf)-Arg(Pbf)-Nal-ψ[CH_2−N(Cbz)]-Gly-NHNHCO−Wang Resin. On the hydrazide resin, were coupled successively Fmoc-D-Tyr(O^{t}Bu)-OH, Fmoc-Nal-ψ[CH_2−N(Cbz)]-Gly-OH, and Fmoc-Arg(Pbf)-OH by use of a procedure identical with that described for the preparation of H-D-Tyr-(O^{t}Bu)-Arg(Pbf)-Arg(Mts)-ψ[(*E*)-CH=CH]-Nal-Gly-NHNHCO−Wang resin to afford the protected **37d** resin.

***cyclo*(-D-Tyr-Arg-Arg-Nal-ψ[CH_2−NH]-Gly-)·3TFA (37d).** By use of a procedure identical with that described for the preparation of **37a**, the protected **37d** (173 mg, 0.13 mmol) was converted into 7.0 mg (7.4 μmol, 5.9%) of the title compound **37d**, as a freeze-dried powder.

$[\alpha]^{19}_D$ −58.0 (*c* 0.69, H_2O). t_R = 21.0 min (linear gradient of MeCN in H_2O, 10 to 40% over 30 min). *m/z* (ISMS): 717.0 (MH^+). Found (FAB-HRMS): 716.4003. Calcd for $C_{36}H_{49}O_5N_{11}$ (MH^+): 716.3996.

Cell Culture. Human T-cell lines, MT-4, and MOLT-4 cells were grown in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin.

Virus. A strain of X4-HIV-1, HIV-1$_{IIIB}$, was used for the anti-HIV assay. This virus was obtained from the culture supernatant of HIV-1 persistently infected MOLT-4/HIV-1$_{IIIB}$ cells and stored at −80 °C until used.

Anti-HIV-1 Assay. Anti-HIV-1 activity was determined based on the protection against HIV-1-induced cytopathogenicity in MT-4 cells. Various concentrations of test compounds were added to HIV-1-infected MT-4 cells at a multiplicity of infection (MOI) of 0.01 and placed in wells of a flat-bottomed microtiter tray (1.5×10^4 cells/well). After 5 days incubation at 37 °C in a CO_2 incubator, the number of viable cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (EC_{50}).[48] Cytotoxicity of compounds was determined based on the viability of mock-infected cells using the MTT method (CC_{50}). 3′-Azido-3′-dideoxythymidine (AZT) was tested as a control.

[^{125}I]-SDF-1 Binding and Displacement. Stable CHO cell transfectants expressing CXCR4 variants were prepared as described previously.[49] CHO transfectants were harvested by treatment with trypsin/EDTA, allowed to recover in complete growth medium (MEM-α, 100 μg/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, 10% (v/v)) for 4−5 h, and then washed in cold binding buffer (PBS containing 2 mg/mL BSA). For ligand binding, the cells were resuspended in binding buffer at 1×10^7 cells/mL, and 100 μL aliquots were incubated with 0.1 nM of [^{125}I]-SDF-1 (PerkinElmer Life Sciences) for 2 h on ice under constant agitation. Free and bound radioactivities were separated by centrifugation of the cells through an oil cushion, and bound radioactivity was measured with a gamma counter (Cobra, Packard, Downers Grove, IL). Inhibitory activity of FC131 analogues was determined based on the inhibition of [^{125}I]-SDF-1-binding to CXCR4 transfectants (IC_{50}).

NMR Spectroscopy (37a and 37c). The peptide sample was dissolved in DMSO-d_6 at a concentration of 5 mM. ^{1}H NMR spectra of the peptides were recorded at 300 K. The assignments of the proton resonances were achieved by use of ^{1}H−^{1}H COSY spectra. $^3J(H^N, H^\alpha)$ coupling constants were measured from one-dimensional spectra. The mixing time for the nuclear Overhauser spectroscopy (NOESY) experiments was set at 400 ms. NOESY spectra were composed of 512 real points in the F2 dimension and 256 real points, which were zero-filled to 256 points in the F1 dimension, with 144 scans per t1 increment. The cross-peak intensities were evaluated by relative buildup rates of the cross peaks. Temperature dependence of the chemical shifts of all of the amide bonds was investigated in **37a** and **37c**. The only temperature coefficient for the NH of Arg^5 was small, but NOE was not observed between the D-Tyr^3 C$^\alpha$H and the Arg^5 NH in both **37a** and **37c**. Thus, no hydrogen bond restraints were used in the simulated annealing calculations.

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).[51] Pseudoatoms were defined for the methylene protons of Nal^1, D-Tyr^3, Arg^4, and Arg^5, 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: $^3J(H^N,H^\alpha) = 6.7\cos^2(\theta - 60°) - 1.3\cos(\theta - 60°) + 1.5$.[52] Lower and upper angle errors were set to 15°. The NOESY spectrum with a mixing time of 400 ms was 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 Waals radii (1.8 Å). These distance and dihedral angle restraints were included with force constants of 25−100 kcal/mol·Å^2 and 25−100 kcal/mol·rad^2, respectively. The 50 initial structures generated by the NMR refine program randomly were subjected to the simulated annealing calculations. The final minimization stage was achieved until the maximum derivative became less than 0.01 kcal/mol·Å^2 by the steepest descents and conjugate gradients methods.

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Supporting Information Available: HPLC charts for synthetic compounds of **37a**, **37b**, **37c**, **37d**, and **37f**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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