Synthesis and Structure−Activity Relationships of 2-Substituted D-Tryptophan-Containing Peptidic Endothelin Receptor Antagonists: Importance of the C-2 Substituent of the D-Tryptophan Residue for Endothelin A and B Receptor Subtype Selectivity

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Continuing studies on modifications of potent cyclic pentapeptide endothelin (ET) receptor antagonists, represented by BQ-123, and potent linear tripeptide derivative ET receptor antagonists, represented by BQ-788, are described herein. The introduction of D-tryptophan analogues with C-2 substituents in these peptidic ET antagonists resulted in potent ET receptor antagonists with various ET_A/ET_B subtype selectivity. Combined ET_A/ET_B receptor antagonists were found in both cyclic pentapeptide and linear tripeptide series with 2-halo- and 2-methyl-D-tryptophans. In contrast, compounds with 2-cyano-D-tryptophan were ET_B receptor-selective antagonists. The C-2 substituent of the D-tryptophanyl residue appeared to be very important for the discrimination of ET_A/ET_B subtype selectivity of the antagonists. The potent ET receptor antagonists with various ET_A/ET_B subtype selectivity synthesized in this study may be useful tools for elucidating the physiological and pathophysiological roles of ET and ET receptors.

Introduction

Endothelin-1 (ET-1), which was first isolated from the culture medium of porcine aortic endothelial cells, is a potent vasoconstrictor consisting of 21 amino acids.[1] ET-1 is some 10-fold more potent than angiotensin II as a vasoconstrictor and has extremely long-lasting pressor effects.[2] Studies including a human genomic analysis have identified two structurally- and functionally-related isopeptides of ET-1 termed ET-2 and ET-3.[3−5] Several studies have characterized two ET receptor subtypes, ET_A and ET_B, in animal and mammalian systems.[6−9] The ET_A receptor is highly specific for ET-1 and ET-2, while the ET_B receptor has almost equal affinity for all three isopeptides. A third ET receptor subtype, ET_C, which is selective for ET-3, was cloned from *Xenopus* dermal melanophores,[10] but this subtype has not been identified yet in mammalian tissues.

ET_A and ET_B receptors are widely distributed not only in vascular but also in nonvascular tissues and possess different functions depending on species and location. ET_A receptors are predominantly found in peripheral tissues, especially in vascular smooth muscle tissues to mediate vasoconstriction, though they are also present in certain regions of the brain.[6,11] In contrast, ET_B receptors have been thought to be exclusively localized to the endothelium and nonvascular tissues such as the liver, kidneys, and brain.[12] Endothelial ET_B receptors are functionally linked to vasodilation, possibly through the release of endothelium-derived relaxing factor.[13] However, it has been confirmed that ET_B receptors are also located in certain vascular and airway smooth muscle tissues, mediating their constriction.[14−21] The ETs possess a number of important biological activities besides potent vasoconstricting activity.[22] Therefore, the identification of potent ET receptor antagonists with various ET_A/ET_B subtype selectivity will be very important for clarifying the physiological and pathophysiological roles of ET and ET receptors.

Various types of ET receptor antagonists have been reported with various ET_A/ET_B receptor subtype selectivity (for a recent review, see ref 23). We previously discovered a selective ET_A receptor antagonist, BQ-123 (**1**),[24−26] which was derived from head-to-tail cyclic pentapeptide leads BE-18257 A and B of microbial origin.[27,28] Subsequent derivation led to the discovery of linear tripeptide ET_A-selective antagonists represented by BQ-485 (**2**).[29,30] Further modification of the linear tripeptide antagonists produced a selective ET_B receptor antagonist, BQ-788 (**3**).[31] In the course of our work on the discovery of the ET_B receptor antagonist, we noticed that the presence of a methoxycarbonyl group on the indole nitrogen of the D-tryptophanyl residue of **3** was very effective for both strong ET_B antagonistic activity and ET_A/ET_B receptor subtype selectivity. We conjectured that further modifications on the indole ring of the D-tryptophanyl residue of the linear tripeptide ET antagonist might produce potent ET receptor antagonists with various ET_A/ET_B receptor subtype selectivity. Of the derivatives comprising D-tryptophan analogues with modifications on the indole ring, potent ET_A/ET_B-nonselective and ET_B-selective receptor antagonists were found in 2-substituted D-tryptophan-containing tripeptide derivatives. For example, the 2-bromo-D-tryptophan-containing linear tripeptide derivative BQ-928 (**4**) is a combined ET_A/ET_B receptor antagonist, while the 2-cyano-D-tryptophan-containing derivative BQ-017 (**5**) is an ET_B-selective antagonist.[32] Since then, we introduced the 2-substituted D-tryptophan analogues into the head-to-tail cyclic pentapeptides that are represented by **1** and found some potent ET receptor antagonists with various ET_A/ET_B receptor subtype selectivity. In this paper, we describe in detail the structure−activity relationships for these

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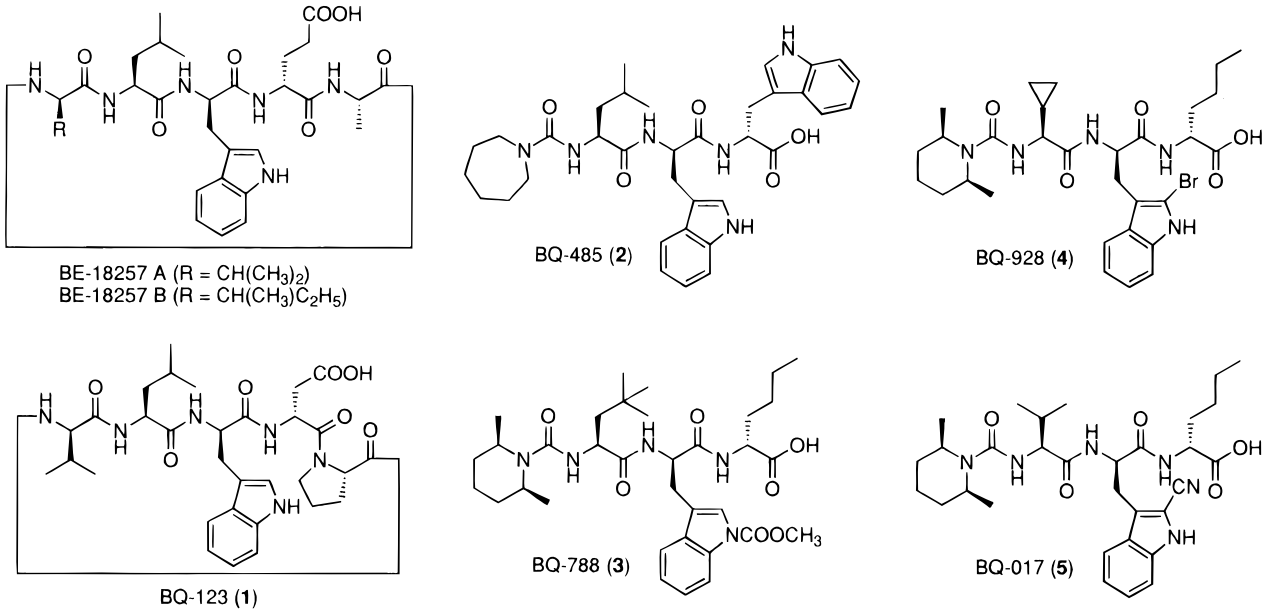
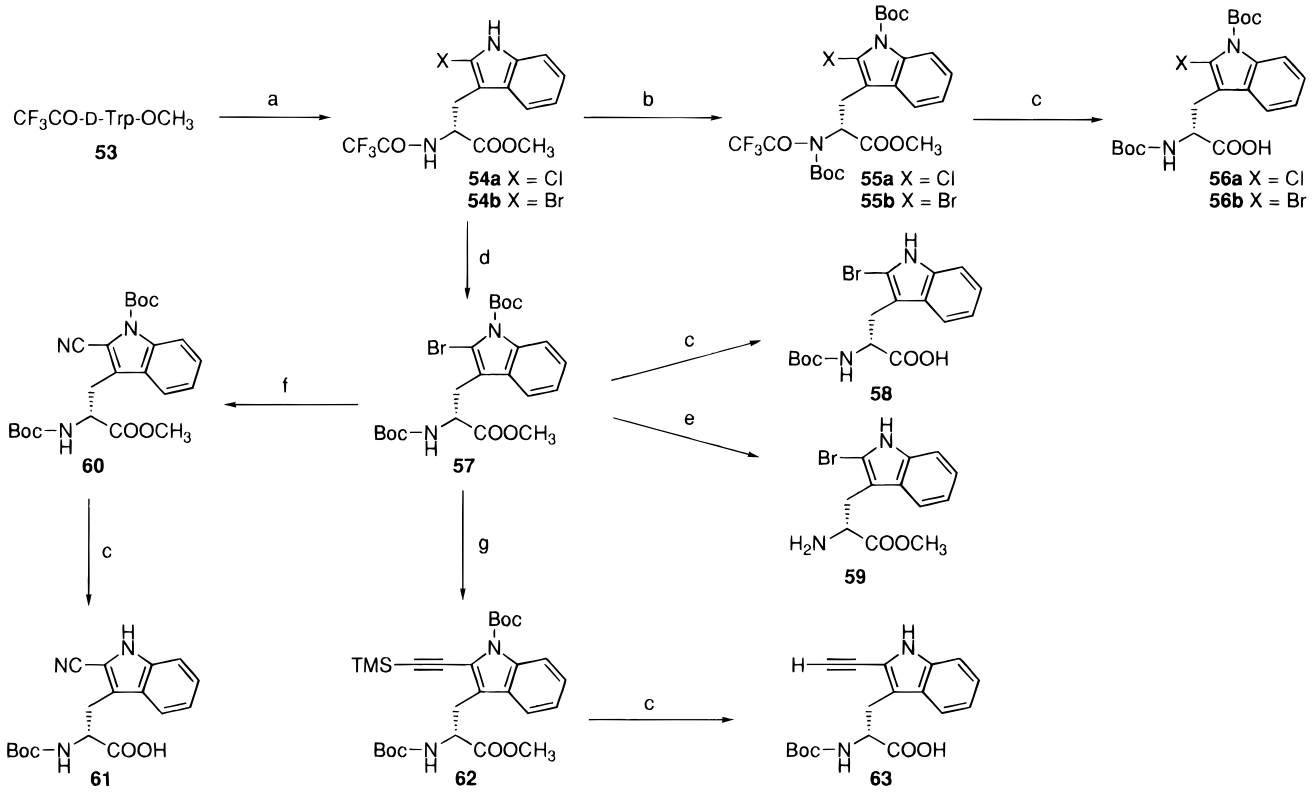
Chart 1



Scheme 1[a]



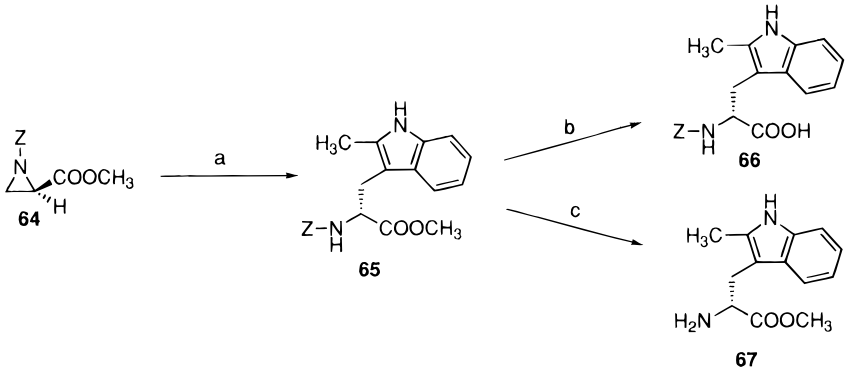
[a] Reagents: (a) see ref 33; (b) Boc_2O, DMAP, CH_3CN; (c) aq NaOH, MeOH; (d) (i) Boc_2O, DMAP, CH_3CN, (ii) 3-(dimethylamino)propylamine; (e) formic acid; (f) CuCN, DMF; (g) (trimethylsilyl)acetylene, $(Ph_3P)Pd$, CuI, diethylamine.

potent ET antagonists with various receptor subtype selectivity and for related analogues.

Chemistry

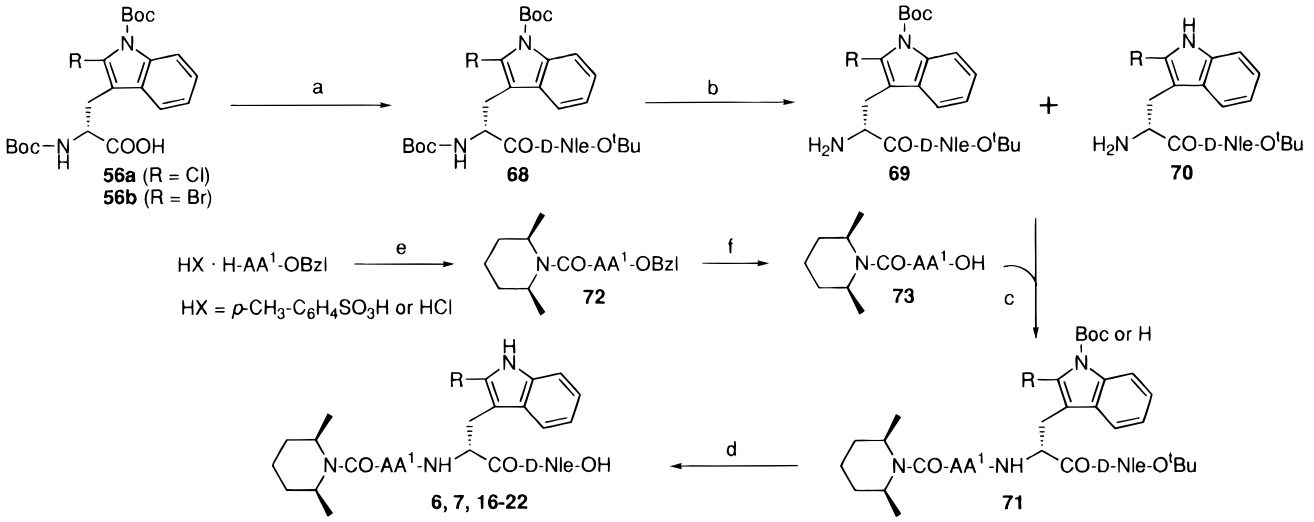
The synthesis of the tripeptide derivatives and the cyclic pentapeptides with 2-substituted D-tryptophans described in this article involves the synthesis of D-tryptophan analogues with C-2 substituents and their subsequent derivation. The D-tryptophan analogues with 2-halo, 2-cyano, and 2-ethynyl substituents were synthesized according to the method given in our previous paper (Scheme 1).[32] The protected 2-halo-D-tryptophans **54a**,**b** were synthesized by radical halogenation of the protected D-tryptophan **53** according to the method reported by Phillips and Cohen.[33] Treatment of **54a**,**b** with an excess amount (3−5 equiv) of di-*tert*-butyldicarbonate (Boc_2O) in CH_3CN in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) afforded the N^{α},N^{in}-bis-Boc-protected compounds **55a**,**b** in yields of 59% and 68%, respectively. Alkaline hydrolysis (2.2 equiv of aqueous NaOH in MeOH) of **55a**,**b** yielded the N^{α},N^{in}-bis-Boc-protected 2-halo-D-tryp-

Scheme 2[a]



[a] Reagents: (a) 2-methylindole, zinc triflate, $CHCl_3$; (b) aq NaOH, MeOH; (c) H_2, Pd/C, MeOH.

Scheme 3. Method A[a]



[a] Reagents: (a) H-D-Nle-O^{t}Bu·HCl, EDCI, HOBT, NMM, CH_2Cl_2; (b) formic acid; (c) EDCI, HOBT, CH_2Cl_2; (d) TFA; (e) *N,N′*-carbonyldiimidazole, Et_3N, THF; (f) H_2, Pd/C, MeOH.

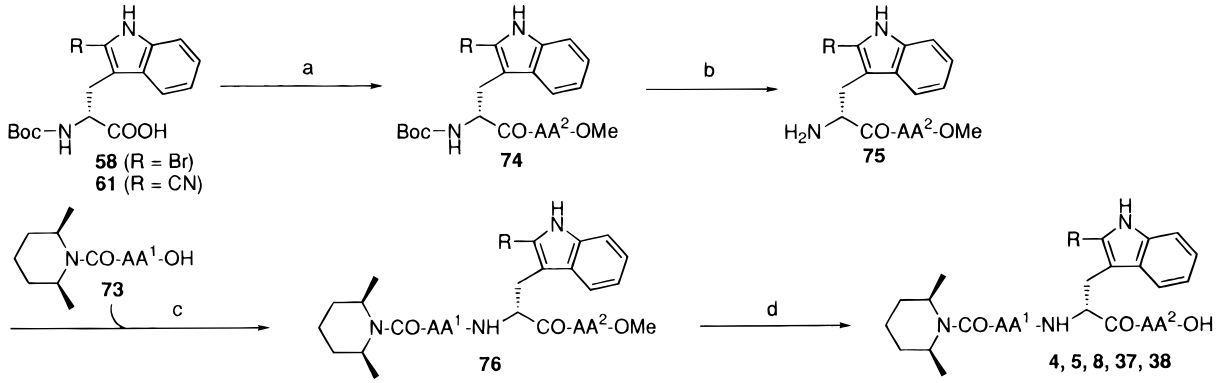
tophans **56a**,**b** in quantitative yields. Alternatively, treatment of **54b** with Boc_2O (3−5 equiv) in CH_3CN followed by 3-(dimethylamino)propylamine in one pot yielded the N^{α},N^{in}-bis-Boc-protected ester **57** in an almost quantitative yield. Alkaline hydrolysis of **57** using an excess of NaOH (3−5 equiv) gave the N^{α}-Boc-protected 2-bromo-D-tryptophan **58** in a quantitative yield. Treatment of **57** with formic acid yielded the amine **59**. Reaction of the protected 2-bromo-D-tryptophan analogue **57** with CuCN (2.5 equiv) in DMF (85 °C, 1−2.5 h) afforded a 2-cyano analogue (**60**) in a yield of 78−99%. Compound **57** also reacted with (trimethylsilyl)acetylene (3 equiv) in the presence of $(Ph_3P)_4Pd$ (10 mol %) and CuI (30 mol %) in diethylamine (40 °C, 2.5−9 h) to yield a 2-(trimethylsilyl)ethynyl analogue (**62**, 57−76%). Treatment of **60** and **62** with an excess of NaOH (4−10 equiv) in MeOH afforded the N^{α}-Boc-protected 2-substituted D-tryptophan analogues **61** and **63** (in a 78−100% yield), respectively.

The 2-methyl-D-tryptophan analogue was prepared by a method analogous to that described by Sato and Kozikowski (Scheme 2).[34] The *N*-benzyloxycarbonyl-protected aziridine-2-carboxylate **64** prepared from D-serine was reacted with 2-methylindole in the presence of zinc triflate to give the protected-2-methyl-D-tryptophan methyl ester **65** in a 68% yield. Alkaline hydrolysis of **65** yielded the acid **66** in a 93% yield. Catalytic hydrogenation of **65** afforded the amine **67**.

The linear tripeptide derivatives were synthesized by five methods (methods A−E, Scheme 3−7). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole monohydrate (HOBT) were exclusively employed in the coupling reactions. Method A, used for the synthesis starting from N^{α},N^{in}-bis-Boc-protected 2-halo-D-tryptophan **56a** or **56b**, is illustrated in Scheme 3. Compound **56a** or **56b** was coupled with D-norleucine (D-Nle) *tert*-butyl ester to give the dipeptide **68**. N^{α}-Boc deprotection of **68** by formic acid (room temperature, 1.5 h) afforded a mixture of **69** and **70** (55% and 21% for R = Cl, 33% and 47% for R = Br, respectively). Compound **69** as well as **70** was coupled with *N*-*cis*-(2,6-dimethylpiperidino)carbonyl amino acid **73** to give the protected tripeptide *tert*-butyl ester **71**. Treatment of the protected tripeptide **71** with TFA gave the desired compounds **6**, **7**, and **16**−**22**. The *N*-carbamoyl amino acid **73** was prepared from an amino acid benzyl ester *p*-toluenesulfonate or hydrochloride by two steps. Treatment of the amino acid benzyl ester with *N,N′*-carbonyldiimidazole and triethylamine in THF followed by *cis*-2,6-dimethylpiperidine in one pot yielded the urea compound **72** (80−98%). Catalytic hydrogenation of **72** afforded the corresponding acid **73** (94−99%).

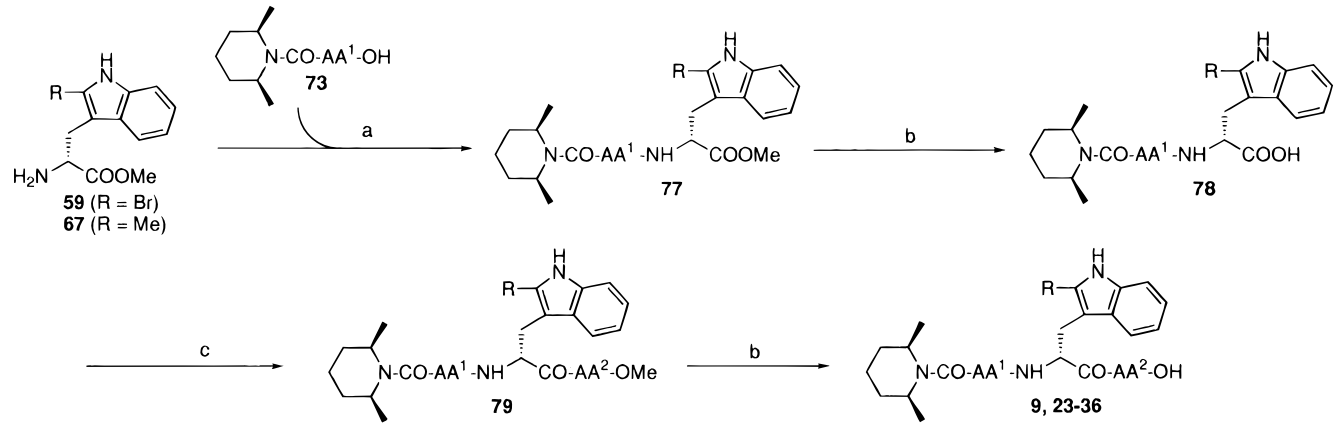
Method B was used for the synthesis starting from N^{α}-Boc-protected 2-bromo- or 2-cyano-D-tryptophan **58** or **61** and is illustrated in Scheme 4. Coupling of the

Scheme 4. Method B[a]



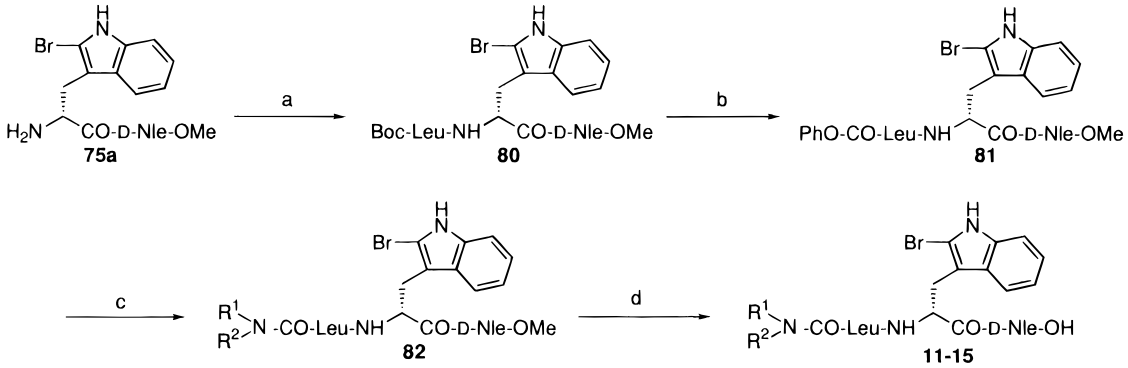
[a] Reagents: (a) H-AA^2-OMe·HCl, EDCI, HOBT, NMM, CH_2Cl_2; (b) formic acid; (c) EDCI, HOBT, CH_2Cl_2; (d) aq NaOH, MeOH.

Scheme 5. Method C[a]



[a] Reagents: (a) EDCI, HOBT, CH_2Cl_2; (b) aq NaOH, MeOH; (c) H-AA^2-OMe·HCl, EDCI, HOBT, NMM, CH_2Cl_2.

Scheme 6. Method D[a]



[a] Reagents: (a) Boc-Leu-OH, EDCI, HOBT, NMM, CH_2Cl_2; (b) (i) formic acid, (ii) phenyl chloroformate, pyridine; (c) R^1R^2NH, Et_3N, $CHCl_3$; (d) aq NaOH, MeOH.

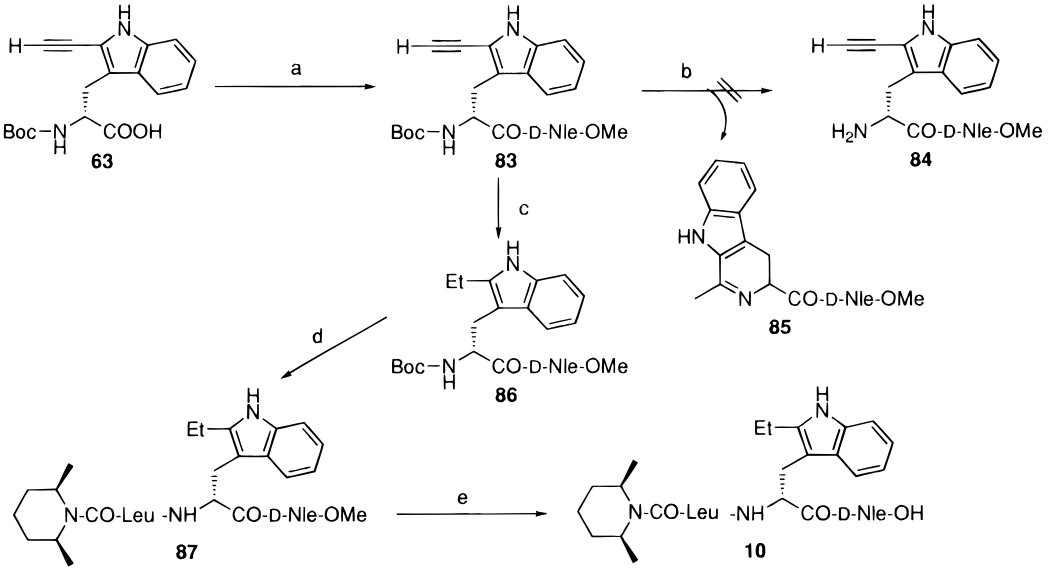
N $^{\alpha}$-Boc-protected D-tryptophan analogue with the C-terminal amino acid methyl ester (AA^2-OMe) gave the dipeptide **74**. The *N* $^{\alpha}$-Boc protecting group in **74** was deprotected by formic acid followed by coupling with the N-terminal component **73** to afford the tripeptide methyl ester **76**. Alkaline hydrolysis of the C-terminal methyl ester gave the target compounds **4**, **5**, **8**, **37**, and **38**.

Method C employed the 2-bromo- or 2-methyl-D-tryptophan methyl ester **59** or **67** as a starting material (Scheme 5). The amino ester was coupled with **73**, which after alkaline hydrolysis afforded the dipeptide **78**. The dipeptide was coupled with the C-terminal amino acid methyl ester (AA^2-OMe) to yield the tripeptide methyl ester **79**. Alkaline hydrolysis of the methyl ester gave the target compounds **9** and **23**−**36**.

Method D, which was used for the synthesis of **11**−**15** with N-terminal urea alteration, involves replacement of a phenoxycarbonyl group with a secondary amine (Scheme 6).[27] The dipeptide methyl ester **75a** was transformed to the N-terminal phenoxycarbonylated tripeptide methyl ester **81**. Reaction of **81** with a secondary amine in the presence of triethylamine yielded the urea **82**. Subsequent alkaline hydrolysis afforded the target compounds.

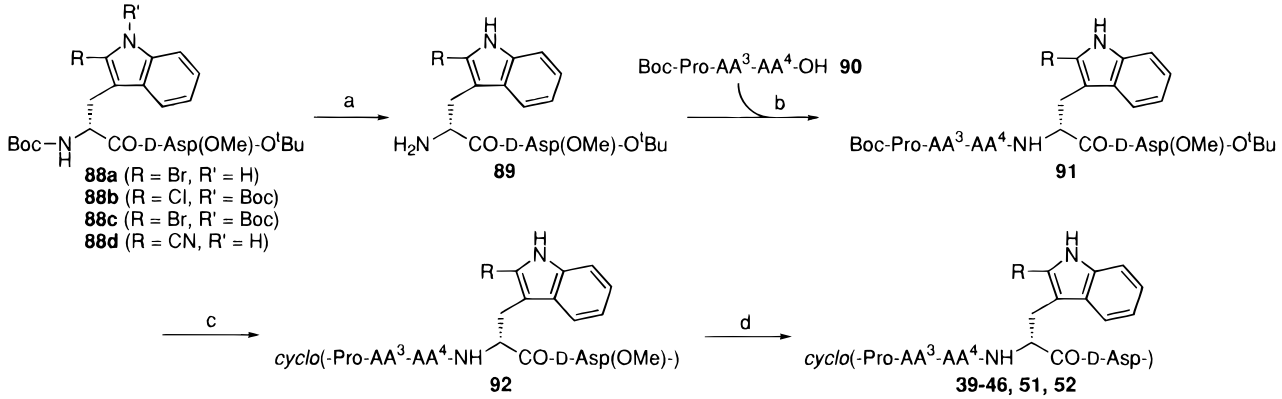
The 2-ethyl-D-tryptophan-containing tripeptide derivative **10** was synthesized by the method illustrated in Scheme 7. We first attempted to prepare a tripeptide derivative with 2-ethynyl-D-tryptophan. However, *N* $^{\alpha}$-Boc deprotection of the dipeptide **83**, derived from **63**, by formic acid did not afford the expected primary amine

Scheme 7. Synthesis of Compound **10**[a]



[a] Reagents: (a) H-D-Nle-OMe·HCl, EDCI, HOBT, NMM, CH_2Cl_2; (b) formic acid; (c) H_2, Pd/$BaSO_4$, MeOH; (d) (i) formic acid, (ii) *cis*-[(2,6-dimethylpiperidino)carbonyl]-Leu-OH (**73a**), EDCI, HOBT, CH_2Cl_2; (e) aq NaOH, MeOH.

Scheme 8. Method F[a]



[a] Reagents: (a) formic acid; (b) EDCI, HOBT, DMF; (c) (i) TFA, (ii) EDCI, HOBT, NMM, DMF; (d) aq NaOH, MeOH.

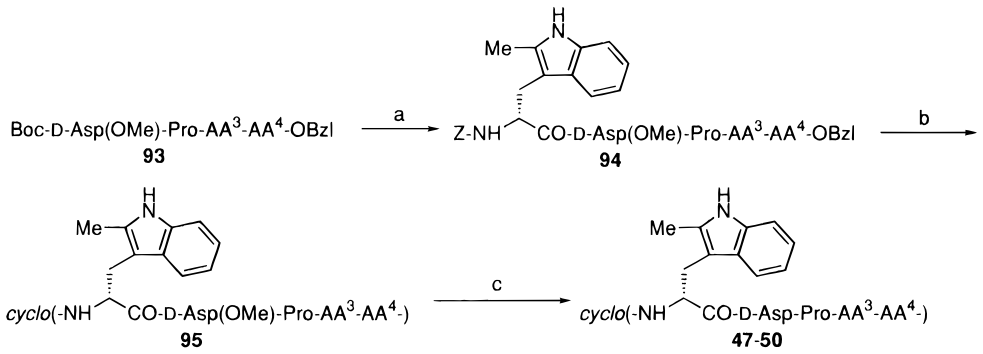
84, and the only isolated compound was a cyclic imine (**85**) with complete racemization at C-α of the tryptophanyl residue (24% yield, as a 1:1 mixture of diastereoisomers). To avoid the intramolecular addition of an α-amino group to the 2-ethynyl group of the indole ring, a stronger acid, TFA, was employed. However, treatment of **83** with TFA produced the same result. We, therefore, gave up preparing a 2-ethynyl analogue and substituted a 2-ethynyl group with a 2-ethyl group. The 2-ethyl derivative **86** was obtained by catalytic hydrogenation of **83**. Deprotection of the N^{α}-Boc group in **86**, followed by coupling and alkaline hydrolysis in the usual manner, yielded compound **10**.

The cyclic pentapeptides were prepared by method F or G. Method F was used for the synthesis of compounds with 2-halo- or 2-cyano-D-tryptophan and is illustrated in Scheme 8. The dipeptides **88a**−**d** were prepared by coupling of N^{α}-Boc (and N^{in}-Boc)-protected 2-halo- or 2-cyano-D-tryptophan with D-Asp(OMe)-O^{t}Bu. Deprotection of the N-terminal (and N^{in}) Boc by formic acid followed by coupling with the N-terminal Boc-protected tripeptide **90** afforded the N-terminal Boc- and C-terminal ^{t}Bu-protected linear pentapeptide **91**. Deprotection of the N-terminal and C-terminal protecting groups by TFA, followed by cyclization by EDCI and HOBT, yielded the D-Asp side chain methyl ester of cyclic pentapeptide **92**. Alkaline hydrolysis of the D-Asp side chain methyl ester afforded the target cyclic pentapeptides **39**−**46**, **51**, and **52**.

Method G was used for the synthesis of cyclic pentapeptides with 2-methyl-D-tryptophan and is illustrated in Scheme 9. The N-terminal Boc-protected linear tetrapeptide benzyl ester **93** was prepared using stepwise elongation method. The Boc group was employed as an amino-protecting group, and deprotection of the Boc was accomplished with TFA or 4 N HCl/1,4-dioxane. EDCI and HOBT were used in coupling reactions. Deprotection of the N-terminal Boc in the tetrapeptide **93**, followed by coupling with N^{α}-Z-protected 2-methyl-D-tryptophan **66**, afforded the N-terminal Z- and C-terminal Bzl-protected linear pentapeptide **94**. Deprotection of the N-terminal- and C-terminal-protecting groups by catalytic hydrogenation, followed by cyclization by EDCI and HOBT, yielded the D-Asp side chain methyl ester of cyclic pentapeptide **95**. Alkaline hydrolysis of the methyl ester afforded the target cyclic pentapeptides **47**−**50**.

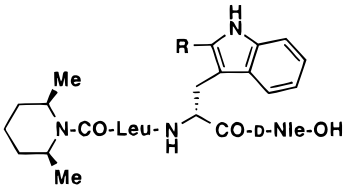
All final compounds were analyzed for homogeneity by HPLC and characterized for structural integrity by

Scheme 9. Method G[a]



[a] Reagents: (a) (i) TFA or 4 N HCl/1,4-dioxane, (ii) Z-D-Trp(2-Me)-OH (**66**), EDCI, HOBT, DMF; (b) (i) H_2, Pd/C, DMF, (ii) EDCI, HOBT, DMF; (c) aq NaOH, MeOH.

Table 1. Linear Peptide Derivatives with 2-Substituted D-Tryptophans



compd	R	IC_{50} (μM) ET_A[a]	IC_{50} (μM) ET_B[b]
6	Cl	0.015[c]	0.0056[c]
7	Br	0.0056[d]	0.0025[d]
8	CN	0.47	0.0016
9	CH_3	0.0096	0.013
10	C_2H_5	0.18	0.0050

[a] Binding data in porcine aortic smooth muscle membranes. [b] Binding data in porcine cerebellum membranes. [c] $n = 3$ IC_{50} determinations. [d] $n = 5$ IC_{50} determinations. All other values represent one IC_{50} determination. Variation was generally ±10%.

^{1}H NMR and high-resolution fast atom bombardment (FAB) mass spectrometry.

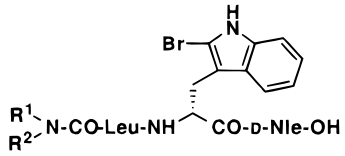
Biological Results and Discussion

The synthesized compounds were first evaluated for their binding affinities for both ET_A and ET_B receptor subtypes. The binding assay for ET_A receptors was performed in porcine aortic smooth muscle membranes, and that for ET_B receptors was in porcine cerebellum membranes, according to the reported method.[27]

Table 1 shows modification at the C-2 position of the D-tryptophanyl residue of the linear tripeptide derivatives. Of these compounds, the analogues with 2-halo-D-tryptophans, **6** and **7**, exhibited potent affinity for both ET_A and ET_B receptors. The 2-cyano-D-tryptophan-containing analogue **8** showed potent affinity for the ET_B receptor; however, its ET_A affinity was 300 times weaker than its ET_B affinity. Analogues with 2-alkyl-D-tryptophans were obscure. The methyl analogue **9** was nonselective, while the ethyl analogue **10** was comparatively ET_B-selective. We then optimized other parts of the compounds to identify potent combined ET_A/ET_B receptor antagonists from the linear tripeptide derivatives with 2-halo- and 2-methyl-D-tryptophans and to find potent ET_B receptor-selective antagonists from the 2-cyano-D-tryptophan-containing linear tripeptide derivatives.

Compounds **11**−**15** represent modifications at the N-terminal urea moiety of the linear tripeptide derivatives (Table 2). All replacements of the *cis*-(2,6-dimethylpiperidino)carbonyl group in **7** with other groups led to a decrease in binding affinity for both ET_A and ET_B receptors. In particular, ET_B affinity was significantly decreased by the replacements. These results suggest that a *cis*-(2,6-dimethylpiperidino)carbonyl group is indispensable for potent affinity for both ET_A and ET_B receptor subtypes. The N-terminal urea moiety was therefore retained during the following modifications of the linear tripeptide series.

Table 2. N-Terminus Urea Modifications

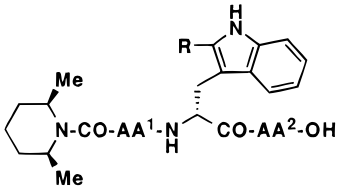


compd	R^1R^2N	IC_{50} (nM) ET_A[a]	IC_{50} (nM) ET_B[b]
7	*cis*-2,6-dimethylpiperidino	0.0056[c]	0.0025[c]
11	1-pyrrolidinyl	0.16	2.7
12	piperidino	0.026	0.11
13	hexahydro-1*H*-azepinyl	0.0091	0.028
14	octahydro-1*H*-azocinyl	0.027	0.026
15	2-methylpiperidino	0.0081	0.034

[a] Binding data in porcine aortic smooth muscle membranes. [b] Binding data in porcine cerebellum membranes. [c] $n = 5$ IC_{50} determinations. All other values represent one IC_{50} determination. Variation was generally ±10%.

Table 3 shows the ET_A and ET_B receptor binding affinities when the N-terminal and/or C-terminal amino acids (AA^1 and AA^2) were substituted in the linear tripeptide derivatives with 2-halo-D-tryptophans. Compounds **16**−**22** represent substitutions at the Leu position in the 2-chloro-D-tryptophan-containing tripeptide derivative **6**. The Nle, 3-cyclopropylalanine (Cpra), and Nva analogues **16**−**18** exhibited almost the same affinity at both ET_A and ET_B receptor subtypes. The substitution of aliphatic amino acids with β-position-branched side chains, such as Val, Ile, and cyclopentylglycine (Cpeg), afforded compounds with almost the same or slightly enhanced ET_B affinity as that of the Leu analogue but led to a 2−3-fold decrease in ET_A affinity. In contrast, the cyclopropylglycine (Cprg) analogue **22** exhibited a 4-fold increase in ET_B affinity together with slightly enhanced ET_A affinity, although Cprg is a β-position-branched amino acid. The same substitution in the 2-bromo-D-tryptophan-containing analogue **7** yielded compound **4** with IC_{50} values of 3.8

Table 3. Linear Tripeptide Derivatives with 2-Halo-D-tryptophans



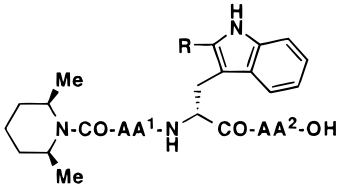
compd	AA1 [a]	R	AA2 [b]	IC50 (μM) ETA [c]	IC50 (μM) ETB [d]
6	Leu	Cl	D-Nle	0.015[e]	0.0056[e]
16	Nle	Cl	D-Nle	0.0090	0.0061
17	Cpra	Cl	D-Nle	0.014	0.0062
18	Nva	Cl	D-Nle	0.020	0.0079
19	Val	Cl	D-Nle	0.033[f]	0.0046[e]
20	Ile	Cl	D-Nle	0.051[f]	0.0046[f]
21	Cpeg	Cl	D-Nle	0.046[f]	0.0027[f]
22	Cprg	Cl	D-Nle	0.0093	0.0013
7	Leu	Br	D-Nle	0.0056[g]	0.0025[g]
4	Cprg	Br	D-Nle	0.0038[h]	0.000 81[h]
23	Leu	Br	D-Aoa	0.11	1.9
24	Leu	Br	D-Leu	0.021	0.014
25	Leu	Br	D-Nva	0.0092[f]	0.0021[f]
26	Leu	Br	D-Alg	0.0098	0.0042
27	Leu	Br	D-Met	0.0022[e]	0.0045[e]
28	Leu	Br	D-Cys(Et)	0.0019[f]	0.0018[f]
29	Leu	Br	D-Cys(Pr)	0.0024	0.0019
30	Leu	Br	D-His	0.0038[f]	0.076[f]
31	Leu	Br	D-Trp	0.0067	1.1

[a] Cpra = 3-cyclopropylalanine, Cpeg = 2-cyclopentylglycine, and Cprg = 2-cyclopropylglycine. [b] Aoa = 2-aminooctanoic acid, Alg = 2-allylglycine, Cys(Et) = *S*-ethylcysteine, and Cys(Pr) = *S*-propylcysteine. [c] Binding data in porcine aortic smooth muscle membranes. [d] Binding data in porcine cerebellum membranes. [e] $n = 3$ IC_{50} determinations. [f] $n = 2$ IC_{50} determinations. [g] $n = 5$ IC_{50} determinations. [h] $n = 4$ IC_{50} determinations. All other values represent one IC_{50} determination. Variation was generally ±10%.

nM for ET_A and 0.81 nM for ET_B. This compound is a representative combined ET_A/ET_B receptor binding inhibitor, in which the affinity for the ET_B receptor is more potent than that for the ET_A receptor. Compounds **23**−**31** represent substitutions at the D-Nle position in the 2-bromo-D-tryptophan-containing tripeptide **7**. Replacement of D-Nle with D-2-aminooctanoic acid (D-Aoa), which has a longer normal alkyl side chain than does D-Nle, resulted in a marked decrease in both ET_A and ET_B affinity. The incorporation of D-Leu also led to a significant decrease in affinity for both receptor subtypes. The D-Nva and D-allylglycine (D-Alg) substitutions did not affect binding affinity at both receptors significantly. The substitution of sulfur-containing amino acids, such as D-Met, *S*-ethyl-D-cysteine (D-Cys(Et)), and *S*-propyl-D-cysteine (D-Cys(Pr)), led to a 2−3-fold increase in ET_A affinity with retention of ET_B affinity. Of these compounds, the D-Cys(Et) analogue **28** exhibited the most potent affinity for both receptor subtypes (ET_A = 1.9 nM and ET_B = 1.8 nM). This compound is another representative combined ET_A/ET_B receptor binding inhibitor among the 2-halo-D-tryptophan-containing tripeptide derivatives. The incorporation of aromatic amino acids such as D-His and D-Trp was tolerated at the ET_A receptor but led a significant decrease in ET_B affinity.

Table 4 shows the ET_A and ET_B receptor binding affinities of linear tripeptide derivatives with 2-methyl- and 2-cyano-D-tryptophans. Compounds **9** and **32**−**36** are derivatives with 2-methyl-D-tryptophan. The replacement of D-Nle with D-Met in **9** did not affect affinity for the ET_A and ET_B receptor subtypes, although the same replacement in the 2-bromo-D-tryptophan derivatives significantly increased ET_A receptor binding affinity (**9** vs **32** and **7** vs **27**). The D-His substitution led to a 3-fold increase in ET_A receptor affinity and an 8-fold decrease in ET_B receptor affinity. With regard to the AA^1 position, the replacement of Leu with Cprg in **9** resulted in a 2-fold increase in ET_A affinity together with a 4.5-fold increase in ET_B affinity. This result parallels findings on the activities of the 2-halo-D-tryptophan series (**6** vs **22** and **7** vs **4**). The subsequent replacement of D-Nle with D-Met in **34** increased ET_A affinity by 6-fold, although the same replacement in **9** did not change the affinity. This compound (**35**) is the most potent combined ET_A/ET_B receptor binding inhibitor among the 2-methyl-D-tryptophan-containing tripeptide derivatives, with IC_{50} values of 1.0 nM for ET_A and 2.9 nM for ET_B. The replacement of D-Nle with D-His in **34** did not increase ET_A receptor binding affinity, although the same replacement in **9** resulted in a 3-fold increase in ET_A affinity. The effects of replacement at the AA^1 and AA^2 positions were not additive in this series.

Table 4. Linear Tripeptide Derivatives with 2-Methyl- and 2-Cyano-D-tryptophans

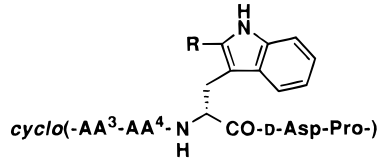


compd	AA1 [a]	R	AA2	IC50 (μM) ETA [b]	IC50 (μM) ETB [c]
9	Leu	CH_3	D-Nle	0.0096	0.013
32	Leu	CH_3	D-Met	0.0080[d]	0.013[d]
33	Leu	CH_3	D-His	0.0028	0.099
34	Cprg	CH_3	D-Nle	0.0057	0.0029
35	Cprg	CH_3	D-Met	0.0010[e]	0.0029[e]
36	Cprg	CH_3	D-His	0.0048	0.17
8	Leu	CN	D-Nle	0.47	0.0016
37	γMeLeu	CN	D-Nle	0.81	0.0071
5	Val	CN	D-Nle	2.0[f]	0.0023[e]
38	Val	CN	D-Nva	3.0[d]	0.0056[d]

[a] Cprg = 2-cyclopropylglycine, γMeLeu = γ-methylleucine, and Cpeg = 2-cyclopentylglycine. [b] Binding data in porcine aortic smooth muscle membranes. [c] Binding data in porcine cerebellum membranes. [d] $n = 2$ IC_{50} determinations. [e] $n = 3$ IC_{50} determinations. [f] $n = 4$ IC_{50} determinations. All other values represent one IC_{50} determination. Variation was generally ±10%.

Compounds **8**, **37**, **5**, and **38** are derivatives with 2-cyano-D-tryptophan. In the course of our work on the discovery of the selective ET_B receptor antagonist **3**, which incorporates 1-(methoxycarbonyl)-D-tryptophan, we observed that the replacement of Leu at AA^1 with γ-methylleucine (γMeLeu) led to enhanced ET_B receptor binding affinity together with decreased ET_A affinity, giving increased ET_B selectivity.[31] We first replaced Leu in **8** with γMeLeu to produce compound **37**. However, this substitution resulted in decreases in both ET_A and ET_B receptor binding affinity and in the ET_A/ET_B selectivity ratio. The structure−activity relationships of the 2-cyano-D-tryptophan-containing tripeptide series seemed to differ from those of the 1-(methoxycarbonyl)-D-tryptophan-containing tripeptide series. Substitution

Table 5. Cyclic Pentapeptides with 2-Substituted D-Tryptophans



compd	R	AA3 [a]	AA4 [b]	IC_{50} (μM) ETA[c]	IC_{50} (μM) ETB[d]
1	H	D-Val	Leu	0.022[e]	18[e]
39	Cl	D-Val	Leu	0.040	0.24
40	Cl	D-*t*-Leu	Leu	0.027	0.053
41	Cl	D-Pen(Me)	Leu	0.10	0.011
42	Br	D-Val	Leu	0.036	0.14
43	Br	D-*t*-Leu	Leu	0.028	0.031
44	Br	D-Pen(Me)	Leu	0.066	0.023
45	Br	D-Cpeg	Leu	0.019	0.087
46	Br	D-Cpeg	Cprg	0.0049[f]	0.015[f]
47	CH_3	D-Val	Leu	0.051	0.87
48	CH_3	D-Cpeg	Leu	0.0027[g]	0.12[f]
49	CH_3	D-Val	Cprg	0.0081[f]	0.27[f]
50	CH_3	D-Cpeg	Cprg	0.0018[f]	0.059[f]
51	CN	D-Val	Leu	2.2	0.17
52	CN	D-Pen(Me)	Leu	23	0.022

[a] *t*-Leu = 2-amino-3,3-dimethylbutyric acid, Pen(Me) = *S*-methylpenicillamine, and Cpeg = 2-cyclopentylglycine. [b] Cprg = 2-cyclopropylglycine. [c] Binding data in porcine aortic smooth muscle membranes. [d] Binding data in porcine cerebellum membranes. [e] Data reported in ref 25. [f] $n = 3$ IC_{50} determinations. [g] $n = 2$ IC_{50} determinations. All other values represent one IC_{50} determination. Variation was generally ±10%.

of Val (**5**) maintained potent binding affinity for the ET_B receptor but decreased ET_A affinity by 4-fold, giving increased ET_B selectivity. The replacement of D-Nle with D-Nva in **5** led to a decrease in both ET_A and ET_B binding affinity. Consequently, compound **5** is the most potent ET_B-selective binding inhibitor among the 2-cyano-D-tryptophan-containing linear tripeptide derivatives, with IC_{50} values of 2.0 μM for ET_A and 2.3 nM for ET_B.

Table 5 shows the ET_A and ET_B receptor binding affinities of cyclic pentapeptide analogues with 2-substituted D-tryptophans. We first replaced D-Trp in **1**, which is a representative ET_A receptor antagonist, with 2-substituted D-tryptophans to produce compounds **39**, **42**, **47**, and **51**. Substitutions of 2-halo- and 2-methyl-D-tryptophans maintained potent binding affinity for the ET_A receptor. In addition, these substitutions led to significant, 20−130-fold, increases in ET_B receptor binding affinity, suggesting that optimization of other residues may produce potent combined ET_A/ET_B receptor antagonists. In contrast, the 2-cyano-D-tryptophan substitution decreased ET_A affinity by 100-fold but led to a 100-fold increase in affinity for the ET_B receptor, yielding a cyclic pentapeptide ET_B receptor-selective binding inhibitor. These results were surprising because a comparatively small structural alteration, introducing 2-halo, 2-methyl, and 2-cyano substituents on the indolyl group of the D-Trp residue in such a large molecule, significantly changed the ET_A/ET_B receptor subtype selectivity of the originally ET_A-selective antagonist.

We then optimized other residues to identify potent combined ET_A/ET_B receptor antagonists and potent ET_B-selective receptor antagonists among cyclic pentapeptides with 2-substituted D-tryptophans. In the course of our structure−activity study on the cyclic pentapeptide ET_A receptor antagonists, we found that the D-Asp-Pro sequence was very important for potent activity.[25,26] We therefore retained this sequence in the following modifications. Compounds **39**−**46** are cyclic pentapeptide analogues with 2-halo-D-tryptophans. The replacement of D-Val in **39** and **42** with D-2-amino-3,3-dimethylbutyric acid (D-*tert*-Leu) increased binding affinity for both receptor subtypes. The introduction of D-*S*-methylpenicillamine (D-Pen(Me)) at the same position in **39** and **42** led to a 6−20-fold increase in ET_B affinity but decreased ET_A binding affinity. The D-2-cyclopentylglycine (D-Cpeg) analogue **45** was slightly more potent than the D-*tert*-Leu analogue **43** with respect to ET_A affinity, although **45** is about 3-fold less potent than **43** in ET_B affinity. Further replacement of Leu with Cprg in **45** led to 4- and 6-fold increases in affinity for ET_A and ET_B receptors, respectively. Compound **46** is one of the representative combined ET_A/ET_B receptor binding inhibitors among the cyclic pentapeptides, with IC_{50} values of 4.9 nM for ET_A and 15 nM for ET_B. Compounds **47**−**50** are 2-methyl-D-tryptophan-containing cyclic pentapeptides. The replacement of D-Val with D-Cpeg resulted in 20- and 7-fold increases in affinity for ET_A and ET_B receptors, respectively. The substitution of Cprg for Leu led to 6- and 3-fold increases in affinity for ET_A and ET_B receptors, respectively. The double substitutions acted additively to produce **50**, with IC_{50} values of 1.8 nM for ET_A and 59 nM for ET_B. The modification of the 2-cyano-D-tryptophan-containing cyclic pentapeptides was assisted by the structure−activity relationships of 2-halo- and 2-methyl-D-tryptophan derivatives. The replacement of D-Val with D-Pen(Me) in **51** afforded **52** with high selectivity for ET_B receptors, as expected ($ET_A = 23$ μM and $ET_B = 22$ nM).

Table 6. Binding Affinities of Selected Analogues for Human ET_A and ET_B Receptors

compd	IC_{50} (nM) pET_A[a,b]	hET_A[c,d]	pET_B[b,e]	hET_B[d,f]
4	3.8[g]	13	0.81[g]	1.1
28	1.9[h]	6.4	1.8[h]	3.6
35	1.0	3.0	2.9	4.0
46	4.9	8.6	15	29
50	1.8	2.9	59	130
5	2000[g]	2400	2.3	5.0
52	23 000[i]	6200	22	29

[a] Porcine aortic smooth muscle membranes. [b] Values represent the average of three independent experiments unless otherwise noted. [c] Human neuroblastoma-derived cell line SK-N-MC membranes. [d] Values represent the average of two independent experiments unless otherwise noted. Variation was generally ±10%. [e] Porcine cerebellum membranes. [f] Human Girardi heart cell membranes. [g] $n = 4$. [h] $n = 2$. [i] $n = 1$.

It has been suggested that there are notable species differences in the effects of some compounds. For example, Cody et al. reported that significant species differences in the effects of C-terminal hexapeptide analogue ET antagonists were observed between rat and human cloned ET_B receptors.[35] Selected compounds listed in Table 6 were tested for their affinity to human ET_A and ET_B receptors to determine species differences in the effects of these compounds. Binding assays for human receptors were carried out in membranes of the human neuroblastoma-derived cell line SK-N-MC and human Girardi heart cells, expressing ET_A and ET_B

receptors, respectively,[31] using a previously reported method.[26] Compounds **4**, **28**, and **35**, which are linear tripeptide combined ET_A/ET_B receptor binding inhibitors in porcine receptor systems, and **46** and **50**, which are cyclic pentapeptide combined ET_A/ET_B receptor binding inhibitors in porcine receptor systems, have a high affinity for both human ET_A and ET_B receptors. Compounds **5** and **52**, which are ET_B-selective receptor binding inhibitors in porcine receptor systems among linear tripeptides and cyclic pentapeptides, respectively, showed high affinity for human ET_B receptors but low affinity for human ET_A receptors. The differences in IC_{50} values between human and porcine ET_A and ET_B subtype receptors are up to 4-fold. Compounds **4**, **28**, **35**, **46**, and **50** appeared to be combined ET_A/ET_B receptor binding inhibitors, whereas compounds **5** and **52** are ET_B-selective binding inhibitors in human as well as porcine receptor systems.

The combined ET_A/ET_B receptor binding inhibitors **4** and **46** were evaluated for their antagonistic activities against ET-1-induced constriction in isolated porcine coronary arteries, which is mediated by ET_A receptors, and ET_B-selective agonist BQ-3020-induced constriction in isolated rabbit pulmonary arteries, which is mediated by ET_B receptors, by a reported method.[31] These compounds strongly antagonized ET_A-mediated vasoconstriction with pA_2 values of 7.9 and 7.5, respectively, and also antagonized ET_B-mediated vasoconstriction with pA_2 values of 9.0 and 8.2, respectively. It was reported that neither the ET_A- nor ET_B-selective antagonist alone inhibited ET-1-induced constriction in isolated rabbit pulmonary arteries and that the combination of both antagonists inhibited the constriction.[21] Compounds **4** and **46** inhibited the constriction with pA_2 values of 6.4 and 5.7, respectively. The compounds showed no intrinsic agonist activities in these arteries even at concentrations of up to 10 μM, indicating that they are potent combined ET_A/ET_B receptor antagonists with no agonist activities.

The ET_B receptor-selective binding inhibitors **5** and **52** were tested for their inhibitory effects on ET-1-induced $[Ca^{2+}]_i$ increases in human Girardi heart cells, according to a reported method.[31] These compounds potently inhibited the ET-1-induced $[Ca^{2+}]_i$ increase with IC_{50} values of 4.0 and 35 nM, respectively. The compounds alone (each 100 μM) did not increase $[Ca^{2+}]$ in the cells, indicating that they are potent ET_B receptor antagonists with no agonist activities. Compound **5**, which was also evaluated for its antagonistic activity against BQ-3020-induced constriction in isolated rabbit pulmonary arteries, appeared to antagonize this vasoconstriction with a pA_2 value of 8.5, suggesting that **5** is an ET_B receptor antagonist as potent as **3**, a potent and selective ET_B receptor antagonist that we previously found.[31] In addition, **5** is much more soluble than **3** in saline (as sodium salts of both compounds, >110 mg/mL for **5** and 0.30 mg/mL for **3**). This good solubility may make **5** a useful tool for in vivo pharmacological studies.

Conclusions

This work has demonstrated the importance of the C-2 substituent of the D-tryptophanyl residue in both linear tripeptide derivative and cyclic pentapeptide ET antagonists for the discrimination of ET_A/ET_B receptor subtype selectivity. The introduction of 2-halo- and 2-methyl-D-tryptophans to both linear tripeptide derivatives and cyclic pentapeptides produced combined ET_A/ET_B receptor antagonists, while the introduction of 2-cyano-D-tryptophan afforded ET_B-selective antagonists. We previously described a potent ET_A receptor-selective antagonist, BQ-123 (**1**),[25,26] and a potent ET_B receptor-selective antagonist, BQ-788 (**3**),[31] both of which were derived from cyclic pentapeptide leads BE-18257 A and B of microbial origin. Here we have synthesized additional potent ET receptor antagonists with ET_A/ET_B-nonselective subtype selectivity, indicating that the modification of the natural products resulted in potent ET antagonists encompassing all possible ET_A/ET_B receptor subtype selectivity: ET_A-, combined ET_A/ET_B-, and ET_B-selective antagonists.

The effects of the antagonists reported in this paper did not show significant species differences between human and porcine ET receptors. Compound **4** (BQ-928), the representative linear tripeptide ET antagonist of both ET_A and ET_B receptors, showed high affinity for porcine and human ET_A receptors (3.8 and 13 nM, respectively) and ET_B receptors (0.81 and 1.1 nM, respectively). Compound **46** (BQ-238), the representative cyclic pentapeptide combined ET_A/ET_B receptor antagonist, also exhibited potent binding affinity for porcine and human ET_A receptors (4.9 and 8.6 nM, respectively) and ET_B receptors (15 and 29 nM, respectively). The combined ET_A/ET_B receptor antagonistic character of **4** and **46** was confirmed by in vitro contractility studies using porcine coronary arteries and rabbit pulmonary arteries. The representative linear tripeptide derivative ET_B antagonist **5** (BQ-017) showed potent binding affinity for porcine and human ET_B receptors (2.3 and 5.0 nM, respectively), with great ET_A/ET_B subtype selectivity in both receptor systems. Compound **5** antagonized ET_B-selective agonist BQ-3020-induced constriction in isolated rabbit pulmonary arteries as potently as did the previously reported ET_B-selective antagonist BQ-788.[31] Compound **5** is much more soluble than BQ-788 in saline, which may make **5** a useful tool for in vivo pharmacological studies. The potent ET receptor antagonists with various ET_A/ET_B subtype selectivity reported in this paper may be useful tools for the in vitro and in vivo pharmacological study of endothelins and their receptors. The results of pharmacological studies using these potent ET receptor antagonists will be reported elsewhere.

Experimental Section

Abbreviations. Abbreviations follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides; see: *Eur. J. Biochem.* **1984**, *138*, 9−37. Additional abbreviations are defined in the text or as follows: D-Alg, D-2-allylglycine; D-Aoa, D-2-aminooctanoic acid; D-Cpeg, 2-cyclopentyl-D-glycine; Cprg, 2-cyclopropylglycine; D-Cys(Et), *S*-ethyl-D-cysteine; D-Cys(Pr), *S*-propyl-D-cysteine; D-*tert*-Leu, D-2-amino-3,3-dimethylbutyric acid; γMeLeu, γ-methyl-L-leucine; D-Pen(Me), *S*-methyl-D-penicillamine; D-Trp(2-Br), 2-bromo-D-tryptophan; D-Trp(2-Cl), 2-chloro-D-tryptophan; D-Trp(2-CN), 2-cyano-D-tryptophan; D-Trp(2-Et), 2-ethyl-D-tryptophan; D-Trp(2-Me), 2-methyl-D-tryptophan; Boc, *tert*-butoxycarbonyl; Z, benzyloxycarbonyl; Me, methyl; Et, ethyl; ^{t}Bu, *tert*-butyl; Bzl, benzyl; TosOH, *p*-toluenesulfonic acid; AcOH, acetic acid; EtOAc, ethyl acetate; TFA, trifluoroacetic acid; DMF, *N*,*N*-dimethylformamide; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole monohydrate; DMAP, 4-(dimethylami-

no)pyridine; TEA, triethylamine; NMM, *N*-methylmorpholine; HPLC, high-performance liquid chromatography; t_R, retention time.

Instruments and Materials. Melting points were determined on a Yanaco MP-S3 melting point apparatus and are uncorrected. IR spectra were obtained with a Horiba FT-IR FT-200 spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini-200, Gemini-300, or VXR-300 or a JEOL JMN-EX400 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and coupling constants (*J*) are in hertz (Hz). (Note: In the description of the NMR spectra, the designation "brs" used alone indicates a broad signal of undetermined multiplicity.) Fast atom bombardment (FAB) mass spectra (MS) were recorded on a JEOL JMS-DX-300 spectrometer in either a glycerol or 3-nitrobenzyl alcohol matrix using xenon as a target gas. High-resolution mass spectra (HRMS) were determined on the same instrument. Optical rotations were recorded on a Horiba Sepa-200 high-sensitivity polarimeter. HPLC analysis was performed on a Nihon Bunkoh instrument using a YMC-Pack ODS-AQ column (4.6 × 150 mm, 5-μm particle size; YMC Co., Ltd.) or a Capcell Pak C18 column (4.6 × 250 mm, 5-μm particle size; Shiseido Co., Ltd.) at 40 °C. Two different conditions were utilized for the HPLC analysis: (a) 10:90−80:20 CH_3CN with 0.1% TFA:0.1% aqueous TFA, linear gradient over 35 min at 1.0 mL/min (λ = 230 nM) on a YMC-Pack ODS-AQ column, or (b) 45:55 CH_3CN:H_2O with 0.1% H_3PO_4 isocratic system at 1.0 mL/min (λ = 230 nM) on a Capcell Pak C18 column. Column chromatography was carried out on E. Merck silica gel 60 (230−400 mesh) unless otherwise noted. TLC analysis was performed on precoated silica gel glass plates 60 F254 (0.25 mm) purchased from E. Merck in the indicated solvent systems. Components were visualized under UV light by ninhydrin spray and/or phosphomolybdic acid reagent.

The amino acids or amino acid derivatives D-Asp(OMe),[36] D- and L-Cpeg,[37] Cpra,[38] Cprg,[38] *N*$^\alpha$-(trifluoroacetyl)-D-tryptophan methyl ester,[33] *N*$^\alpha$-(trifluoroacetyl)-2-bromo-D-tryptophan methyl ester, [33] and *N*$^\alpha$-(trifluoroacetyl)-2-chloro-D-tryptophan methyl ester[33] were synthesized according to the methods described in the literature, with certain modifications. The following amino acids were commercially available: D-allylglycine (Sigma), D-aminooctanoic acid (Aldrich), D-*tert*-Leu, and γMeLeu (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Other amino acids or amino acid derivatives were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), Kokusan Chemicals Co., Ltd. (Tokyo, Japan), or Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Solvents and other reagents were reagent grade and used without further purification unless otherwise noted.

***N*$^\alpha$,*N*^{in}-Bis(*tert*-butoxycarbonyl)-*N*$^\alpha$-(trifluoroacetyl)-2-chloro-D-tryptophan Methyl Ester (55a).** Boc_2O (0.89 g, 4.1 mmol) and DMAP (20 mg, 0.16 mmol) were added to a solution of *N*$^\alpha$-(trifluoroacetyl)-2-chloro-D-tryptophan methyl ester (**54a**; 283 mg, 0.81 mmol) in dry acetonitrile (5 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was concentrated under reduced pressure, and the residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with EtOAc/hexane (1:5) to afford **55a** (265 mg, 59%) as a colorless oil: TLC R_f (EtOAc:hexane = 1:3) 0.67; FAB-MS *m*/*e* 548, 550 (M^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (9 H, s), 1.67 (9 H, s), 3.54 (1 H, dd, *J* = 4.8, 14.5 Hz), 3.64 (1 H, dd, *J* = 10.5, 14.5 Hz), 3.80 (3 H, s), 5.34 (1 H, dd, *J* = 4.8, 10.5 Hz), 7.23 (1 H, dt, *J* = 1.5, 7.5 Hz), 7.29 (1 H, dt, *J* = 1.5, 7.5 Hz), 7.39 (1 H, dd, *J* = 1.5, 7.5 Hz), 8.05 (1 H, dd, *J* = 1.5, 7.5 Hz).

***N*$^\alpha$,*N*^{in}-Bis(*tert*-butoxycarbonyl)-*N*$^\alpha$-(trifluoroacetyl)-2-bromo-D-tryptophan Methyl Ester (55b).** Compound **54b** (1.00 g, 2.54 mmol) was treated in the same manner as described for the corresponding chloro compound, yielding **55b** (1.02 g, 68%) as a colorless oil: TLC R_f (EtOAc:hexane = 1:5) 0.51; FAB-MS *m*/*e* 592, 594 (M^+); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25 (9 H, s), 1.68 (9 H, s), 3.54 (1 H, dd, *J* = 5.1, 14.8 Hz), 3.65 (1 H, dd, *J* = 10.3, 14.8 Hz), 3.81 (3 H, s), 5.37 (1 H, dd, *J* = 5.1, 10.3 Hz), 7.18−7.43 (3H, m), 8.06 (1 H, dd, *J* = 1.5, 7.5 Hz).

***N*$^\alpha$,*N*^{in}-Bis(*tert*-butoxycarbonyl)-2-chloro-D-tryptophan (56a).** NaOH (1 N) (1.03 mL, 1.03 mmol) was added to a solution of **55a** (255 mg, 0.465 mmol) in MeOH (5 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with water (50 mL), acidified with 10% citric acid, and extracted with EtOAc (30 mL × 3). The combined organic extracts were washed with brine (30 mL) and dried over $MgSO_4$, and the solvent was evaporated to give crude **56a** (206 mg, 104%) as a colorless amorphous solid: TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.46; FAB-MS *m*/*e* 438, 440 (M^+). This material was used without further purification.

***N*$^\alpha$,*N*^{in}-Bis(*tert*-butoxycarbonyl)-2-bromo-D-tryptophan (56b).** Compound **55b** (994 mg, 1.68 mmol) was treated in the same manner as described for the corresponding chloro compound, yielding crude **56b** (820 mg, 101%) as a colorless amorphous solid: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.39; FAB-MS *m*/*e* 482, 484 (M^+).

***N*$^\alpha$,*N*^{in}-Bis(*tert*-butoxycarbonyl)-2-bromo-D-tryptophan Methyl Ester (57).** Boc_2O (15.1 g, 69 mmol) and DMAP (0.28 g, 2.3 mmol) were added to a solution of *N*$^\alpha$-(trifluoroacetyl)-2-bromo-D-tryptophan methyl ester (**54b**; 9.04 g, 23.0 mmol) in dry acetonitrile (50 mL) at 0 °C. After being stirred at 0 °C for 30 min, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was cooled to 0 °C and 3-(dimethylamino)propylamine (4.3 mL, 34.5 mmol) was added. After being stirred at 0 °C for 20 min, the mixture was neutralized with 10% citric acid and concentrated under reduced pressure. The residue was taken up with EtOAc (200 mL), washed successively with 10% citric acid (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL), dried over $MgSO_4$, and evaporated. Trituration of the residue with hexane gave **57** (6.70 g, 58%) as colorless crystals. The mother liquor was purified by column chromatography on silica gel eluted with EtOAc/hexane (1:3) to give **57** (4.72 g, 41%, total 99%): mp 58−62 °C; TLC R_f (EtOAc:hexane = 1:5) 0.33; $[\alpha]^{20}_D$ 5.83° (*c* 1.0, MeOH); FAB-MS *m*/*e* 496, 498 (M^+); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (9 H, s), 1.70 (9 H, s), 3.10−3.40 (2 H, m), 3.68 (3 H, s), 4.52−4.71 (1 H, m), 5.15 (1 H, d, *J* = 7.3 Hz), 7.15−7.40 (2 H, m), 7.50 (1 H, d, *J* = 7.3 Hz), 8.05 (1 H, d, *J* = 7.3 Hz). Anal. Calcd for $C_{22}H_{29}BrN_2O_6$: C, 53.13; H, 5.88; N, 5.63. Found: C, 53.31; H, 5.86; N, 5.56.

***N*$^\alpha$-(*tert*-Butoxycarbonyl)-2-bromo-D-tryptophan (58).** NaOH (4 N) (2.5 mL, 10 mmol) was added to a solution of **57** (995 mg, 2.0 mmol) in MeOH (10 mL) at 0 °C. After being stirred at room temperature overnight, the mixture was concentrated to remove MeOH. The residue was diluted with water (50 mL) and extracted with ether (50 mL). The aqueous layer was acidified with 10% citric acid, and the resulting mixture was extracted with ether (50 mL × 2). The combined organic extracts were washed with brine (25 mL), dried over $MgSO_4$, and evaporated to give crude **58** (806 mg, 105%) as a colorless amorphous solid: TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.33; FAB-MS *m*/*e* 382, 384 (M^+). This material was used without further purification.

2-Bromo-D-tryptophan Methyl Ester (59). **57** (1.20 g, 2.41 mmol) was dissolved in formic acid (30 mL), and the mixture was stirred at room temperature for 8 h. The mixture was concentrated under reduced pressure. The residue was taken up with EtOAc, washed with saturated $NaHCO_3$, dried over $MgSO_4$, and evaporated to give crude **59** (762 mg, 106%) as a pale yellow amorphous solid: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.16. This material was used without further purification.

***N*$^\alpha$,*N*^{in}-Bis(*tert*-butoxycarbonyl)-2-cyano-D-tryptophan Methyl Ester (60).** A mixture of *N*$^\alpha$,*N*^{in}-bis(*tert*-butoxycarbonyl)-2-bromo-D-tryptophan methyl ester (**57**; 500 mg, 1.01 mmol) and CuCN (225 mg, 2.51 mmol) in dry DMF (1.0 mL) was stirred at 85 °C in an argon atmosphere for 1 h. After being cooled to room temperature, the reaction mixture was diluted with EtOAc (80 mL), washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, and evaporated. The residue was purified by column chromatog-

raphy on silica gel eluted with EtOAc to give **60** (442 mg, 99%) as a pale yellow amorphous solid: TLC R_f (EtOAc:hexane = 1:5) 0.30; $[\alpha]^{20}_D$ 4.26° (*c* 0.99, MeOH); FAB-HRMS calcd for $C_{23}H_{29}N_3O_6$ $(M + H)^+$ 444.2135, found 444.2136; IR (KBr, cm^{-1}) 2224 (CN), 1722 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (9 H, s), 1.71 (9 H, s), 3.34 (1 H, dd, *J* = 5.9, 14.2 Hz), 3.49 (1 H, dd, *J* = 5.9, 14.2 Hz), 3.79 (3 H, s), 4.67−4.74 (1 H, m), 5.20 (1 H, d, *J* = 7.5 Hz, α-N*H*), 7.34 (1 H, t, *J* = 7.8 Hz), 7.50 (1 H, t, *J* = 7.8 Hz), 7.69 (1 H, d, *J* = 7.8 Hz), 8.21 (1 H, d, *J* = 7.8 Hz).

***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-2-cyano-D-tryptophan (61).** NaOH (4 N) (2.0 mL, 8.0 mmol) was added to a solution of **60** (420 mg, 0.947 mmol) in MeOH (6 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 32 h. The mixture was concentrated under reduced pressure to remove MeOH. The residue was acidified with 10% citric acid, and the mixture was extracted with $CHCl_3$ (30 mL × 3). The combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated to give **61** (243 mg, 78%) as a pale yellow amorphous solid: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.50; FAB-MS *m*/*e* 330 $(M + H)^+$. This material was used without further purification.

***N*$^{\alpha}$,*N*^{in}-Bis(*tert*-butoxycarbonyl)-2-[2-(trimethylsilyl)-ethynyl]-D-tryptophan Methyl Ester (62).** (Trimethylsilyl)-acetylene (590 mg, 6.0 mmol) was added to a mixture of *N*$^{\alpha}$,*N*^{in}-bis(*tert*-butoxycarbonyl)-2-bromo-D-tryptophan methyl ester (**57**; 994 mg, 2.00 mmol), CuI (115 mg, 0.60 mmol), and $(Ph_3P)_4Pd$ (232 mg, 0.20 mmol) in dry diethylamine (10 mL) in an argon atmosphere. The mixture was stirred at 40 °C for 9 h and then cooled. Ethyl ether (60 mL) was added to the reaction mixture, and the resulting suspension was filtered. The filtrate was evaporated. The residue was dissolved in ethyl ether (60 mL), washed with 10% citric acid (60 mL), saturated $NaHCO_3$ (60 mL), and brine (60 mL), dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluted with EtOAc/hexane (1:10) to give **62** (782 mg, 76%) as a pale yellow amorphous solid: TLC R_f (EtOAc:hexane = 1:5) 0.49; $[\alpha]^{20}_D$ 22.4° (*c* 0.85, MeOH); FAB-HRMS calcd for $C_{27}H_{38}N_2O_6Si$ (M^+) 514.2499, found 514.2522; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.32 (9 H, s), 1.38 (9 H, s), 1.70 (9 H, s), 3.12−3.37 (2 H, m), 3.69 (3 H, s), 4.43−4.62 (1 H, m), 5.32 (1 H, d, *J* = 8.1 Hz), 7.27 (1 H, dt, *J* = 1.4, 7.8 Hz), 7.37 (1 H, dt, *J* = 1.4, 7.8 Hz), 7.55 (1 H, dd, *J* = 1.4, 7.8 Hz), 8.19 (1 H, dd, *J* = 1.4, 7.8 Hz).

***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-2-ethynyl-D-tryptophan (63).** NaOH (4 N) (5.0 mL, 20 mmol) was added to a solution of **62** (400 mg, 0.78 mmol) in MeOH (10 mL) at 0 °C under an argon atmosphere. After 1 h, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was concentrated under reduced pressure to remove MeOH. The residue was acidified with 10% citric acid and extracted with EtOAc (30 mL × 3). The combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated to give **63** (280 mg, 110%) as a pale yellow oil: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.33; FAB-MS *m*/*e* 329 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (9 H, s), 3.20−3.55 (2 H, m), 3.46 (1 H, s), 4.50−4.70 (1 H, m), 5.11 (1 H, brs), 7.14 (1 H, dt, *J* = 2.0, 7.9 Hz), 7.20−7.38 (2 H, m), 7.60 (1 H, dd, *J* = 2.0, 7.9 Hz), 8.19 (1 H, s). This material was used without further purification.

***N*$^{\alpha}$-(Benzyloxycarbonyl)-2-methyl-D-tryptophan Methyl Ester (65).** A mixture of methyl (2*R*)-1-(benzyloxycarbonyl)-2-aziridinecarboxylate (**64**; 540 mg, 2.30 mmol), 2-methylindole (544 mg, 4.15 mmol), and zinc triflate (1.67 g, 4.60 mmol) in dry $CHCl_3$ was placed in a pressure bottle and stirred at 78 °C for 19 h. After being cooled to room temperature, the mixture was partitioned between $CHCl_3$ (20 mL) and water (50 mL). The aqueous layer was extracted twice with $CHCl_3$ (20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with $CHCl_3$/MeOH (50:1) to afford **65** (571 mg, 68%) as off-white crystals: mp 140−141 °C; TLC R_f (EtOAc:hexane = 1:3) 0.15; $[\alpha]^{20}_D$ −59.2° (*c* 1.0, $CHCl_3$); FAB-HRMS calcd for $C_{21}H_{22}N_2O_4$ (M^+) 366.1580, found 366.1585; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.31 (3 H, s), 3.25 (2 H, d, *J* = 5.3 Hz), 3.65 (3 H, s), 4.63−4.70 (1 H, m), 5.06 and 5.12 (each 1 H, ABq, *J* = 12.3 Hz), 5.29 (1 H, d, *J* = 8.4 Hz), 7.03 (1 H, t, *J* = 7.3 Hz), 7.10 (1 H, t, *J* = 7.3 Hz), 7.24 (1 H, d, *J* = 7.3 Hz), 7.28−7.38 (5 H, m), 7.40 (1 H, d, *J* = 7.3 Hz), 7.81 (1 H, s).

***N*$^{\alpha}$-(Benzyloxycarbonyl)-2-methyl-D-tryptophan (66).** NaOH (1 N) (17.2 mL, 17.2 mmol) was added to a solution of **65** (2.10 g, 5.73 mmol) in 1,4-dioxane (10 mL) at 0 °C. After being stirred at 0 °C for 30 min, the mixture was allowed to warm to room temperature and stirred for 4.5 h. The mixture was concentrated under reduced pressure. The residue was diluted with water (150 mL) and acidified with 10% citric acid, and the resulting mixture was extracted with EtOAc (80 mL × 3). The combined organic extracts were dried over $MgSO_4$ and evaporated to give **66** (1.92 g, 95%) as an off-white solid: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.50; FAB-MS *m*/*e* 353 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.28 (3 H, s), 3.48 (2 H, d, *J* = 7.1 Hz), 4.65−4.71 (1 H, m), 5.06 and 5.12 (each 1 H, ABq, *J* = 12.4 Hz), 5.27 (1 H, d, *J* = 8.2 Hz), 7.02 (1 H, t, *J* = 7.4 Hz), 7.10 (1 H, t, *J* = 7.4 Hz), 7.24 (1 H, d, *J* = 7.4 Hz), 7.27−7.38 (5 H, m), 7.46 (1 H, d, *J* = 7.4 Hz), 7.83 (1 H, brs).

2-Methyl-D-tryptophan Methyl Ester (67). A mixture of **65** (133 mg, 0.363 mmol) and 10% Pd/C (60 mg) in MeOH (5 mL) was stirred in a hydrogen atmosphere for 8 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give **67** (96 mg, 114%) as a pale yellow amorphous solid: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.22; FAB-MS *m*/*e* 233 $(M + H)^+$. This material was used without further purification.

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-OBzl (72a).** Triethylamine (1.53 mL, 11 mmol) was added to a suspension of H-Leu-OBzl·TosOH (3.94 g, 10 mmol) and *N*,*N*′-carbonyldiimidazole (1.78 g, 11 mmol) in dry THF (20 mL) at 0 °C under a nitrogen atmosphere over a period of 10 min. After a 30 min stirring at 0 °C, *cis*-2,6-dimethylpiperidine (1.48 mL, 11 mmol) was added to the mixture. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched by the addition of water (100 mL), and the resulting mixture was extracted with EtOAc (50 mL × 3). The combined organic extracts were washed with brine (50 mL), dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluted with EtOAc/hexane (1:2) to give *cis*-[(2,6-dimethylpiperidino)carbonyl]-Leu-OBzl (2.88 g, 80%) as a white solid: TLC R_f (EtOAc:hexane = 1:2) 0.49; FAB-MS *m*/*e* 361 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (3 H, d, *J* = 6.2 Hz), 0.94 (3 H, d, *J* = 6.2 Hz), 1.19 (3 H, d, *J* = 7.0 Hz), 1.24 (3 H, d, *J* = 7.0 Hz), 1.40−1.82 (9 H, m), 4.04−4.32 (2 H, m), 4.53−4.69 (1 H, m), 4.78 (1 H, d, *J* = 7.9 Hz), 5.10 and 5.20 (each 1 H, ABq, *J* = 12.3 Hz), 7.30−7.40 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-OBzl (72b).** This compound was prepared from H-Cprg-OBzl·HCl in 91% yield according to the procedure for the synthesis of **72a**. **72b:** white solid; TLC R_f (EtOAc:hexane = 1:3) 0.25; FAB-MS *m*/*e* 345 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.31−0.64 (4 H, m), 0.99−1.15 (1 H, m), 1.20 (3 H, d, *J* = 7.3 Hz), 1.24 (3 H, d, *J* = 7.3 Hz), 1.40−1.89 (6 H, m), 4.02−4.31 (3 H, m), 4.96 (1 H, d, *J* = 7.2 Hz), 5.12 and 5.28 (each 1 H, ABq, *J* = 12.4 Hz), 7.29−7.43 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Nle-OBzl (72c).** This compound was prepared from H-Nle-OBzl·HCl in 98% yield according to the procedure for the synthesis of **72a**. **72c:** white solid; TLC R_f (EtOAc:hexane = 1:2) 0.32; FAB-MS *m*/*e* 361 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.85 (3 H, t, *J* = 6.7 Hz), 1.20 (3 H, d, *J* = 7.0 Hz), 1.24 (3 H, d, *J* = 7.0 Hz), 1.20−1.95 (12 H, m), 4.06−4.32 (2 H, m), 4.53−4.66 (1 H, m), 4.90 (1 H, d, *J* = 7.5 Hz), 5.10 and 5.24 (each 1 H, ABq, *J* = 12.3 Hz), 7.30−7.40 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cpra-OBzl (72d).** This compound was prepared from H-Cpra-OBzl·HCl in 96% yield according to the procedure for the synthesis of **72a**. **72d:** colorless oil; TLC R_f (EtOAc:hexane = 1:3) 0.29; FAB-MS *m*/*e* 359 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ −0.96−0.16 (2 H, m), 0.34−0.54 (2 H, m), 0.57−0.78 (1 H, m), 1.23 (3 H, d, *J* = 6.9 Hz), 1.26 (3 H, d, *J* = 6.9 Hz), 1.42−1.90 (9 H, m), 4.10−

4.34 (2 H, m), 4.61−4.75 (1 H, m), 5.15 and 5.23 (each 1 H, ABq, J = 12.4 Hz), 7.30−7.41 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Nva-OBzl (72e).** This compound was prepared from H-Nva-OBzl·TosOH in 98% yield according to the procedure for the synthesis of **72a**. **72e:** colorless oil; TLC R_f (EtOAc:hexane = 1:3) 0.37; FAB-MS m/e 347 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.91 (3 H, t, J = 7.2 Hz), 1.20 (3 H, d, J = 7.7 Hz), 1.23 (3 H, d, J = 7.7 Hz), 1.00−1.93 (10 H, m), 4.04−4.33 (2 H, m), 4.52−4.68 (1 H, m), 4.90 (1 H, d, J = 7.5 Hz), 5.11 and 5.22 (each 1 H, ABq, J = 12.3 Hz), 7.20−7.44 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Val-OBzl (72f).** This compound was prepared from H-Val-OBzl·TosOH in 89% yield according to the procedure for the synthesis of **72a**. **72f:** colorless oil; TLC R_f (EtOAc:hexane = 1:2) 0.48; FAB-MS m/e 347 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (3 H, d, J = 6.9 Hz), 0.94 (3 H, d, J = 6.9 Hz), 1.21 (3 H, d, J = 7.0 Hz), 1.24 (3 H, d, J = 7.0 Hz), 1.43−1.70 (6 H, m), 2.10−2.28 (1 H, m), 4.10−4.33 (2 H, m), 4.58 (1 H, dd, J = 4.6, 8.3 Hz), 4.94 (1 H, d, J = 8.2 Hz), 5.11 and 5.23 (each 1 H, ABq, J = 12.3 Hz), 7.30−7.44 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Ile-OBzl (72g).** This compound was prepared from H-Ile-OBzl·TosOH in 98% yield according to the procedure for the synthesis of **72a**. **72g:** colorless oil; TLC R_f (EtOAc:hexane = 1:2) 0.48; FAB-MS m/e 361 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (3 H, t, J = 7.3 Hz), 0.90 (3 H, d, J = 7.6 Hz), 1.20 (3 H, d, J = 7.1 Hz), 1.24 (3 H, d, J = 7.1 Hz), 1.30−2.00 (9 H, m), 4.07−4.31 (2 H, m), 4.61 (1 H, dd, J = 4.6, 8.2 Hz), 4.95 (1 H, d, J = 8.0 Hz), 5.09 and 5.23 (each 1 H, ABq, J = 12.2 Hz), 7.30−7.40 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cpeg-OBzl (72h).** This compound was prepared from H-Cpeg-OBzl·HCl in 94% yield according to the procedure for the synthesis of **72a**. **72h:** colorless oil; TLC R_f (EtOAc:hexane = 1:3) 0.42; FAB-MS m/e 347 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.20 (3 H, d, J = 7.1 Hz), 1.23 (3 H, d, J = 7.1 Hz), 1.40−2.00 (14 H, m), 2.15−2.31 (1 H, m), 4.10−4.30 (2 H, m), 4.54 (1 H, dd, J = 7.1, 8.1 Hz), 4.90 (1 H, d, J = 8.1 Hz), 5.10 and 5.22 (each 1 H, ABq, J = 12.2 Hz), 7.29−7.50 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-γMeLeu-OBzl (72i).** This compound was prepared from H-γMeLeu-OBzl·TosOH in 91% yield according to the procedure for the synthesis of **72a**. **72i:** colorless crystals; TLC R_f (EtOAc:hexane = 1:3) 0.33; FAB-MS m/e 375 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (9 H, s), 1.19 (3 H, d, J = 7.1 Hz), 1.23 (3 H, d, J = 7.1 Hz), 1.40−1.80 (8 H, m), 4.10−4.23 (2 H, m), 4.58−4.70 (2 H, m), 5.10 and 5.19 (each 1 H, ABq, J = 12.5 Hz), 7.30−7.36 (5 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-OH (73a).** A mixture of *cis*-[(2,6-dimethylpiperidino)carbonyl]-Leu-OBzl (2.86 g, 7.93 mmol) and 10% Pd/C (0.15 g) in MeOH (30 mL) was stirred under an atmospheric pressure of hydrogen for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give **73a** (2.12 g, 99%) as a white amorphous solid: TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.51; FAB-MS m/e 271 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.94 (3 H, d, J = 6.4 Hz), 0.98 (3 H, d, J = 6.4 Hz), 1.22 (3 H, d, J = 6.8 Hz), 1.26 (3 H, d, J = 6.8 Hz), 1.43−1.92 (9 H, m), 4.04−4.39 (3 H, m), 4.64−4.79 (1 H, m).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-OH (73b).** This compound was prepared from **72b** in 94% yield according to the procedure for the synthesis of **73a**. **73b:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH:AcOH = 50:1:1) 0.19; FAB-MS m/e 255 $(M + H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.19−0.54 (4 H, m), 1.00−1.18 (1 H, m), 1.07 (3 H, d, J = 7.0 Hz), 1.11 (3 H, d, J = 7.0 Hz), 1.33−1.80 (6 H, m), 3.44 (1 H, dd, J = 7.3, 8.8 Hz), 4.06−4.25 (2 H, m), 6.30 (1 H, d, J = 7.3 Hz).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Nle-OH (73c).** This compound was prepared from **72c** in 94% yield according to the procedure for the synthesis of **73a**. **73c:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH = 10:1) 0.36; FAB-MS m/e 271 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (3 H, t, J = 7.1 Hz), 1.21 (3 H, d, J = 7.1 Hz), 1.24 (3 H, d, J = 7.1 Hz), 1.27−1.98 (12 H, m), 4.11−4.33 (3 H, m), 5.22 (1 H, d, J = 6.6 Hz).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cpra-OH (73d).** This compound was prepared from **72d** in 91% yield according to the procedure for the synthesis of **73a**. **73d:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH:AcOH = 50:1:1) 0.31; FAB-MS m/e 269 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.07−0.16 (2 H, m), 0.41−0.51 (2 H, m), 0.71−0.84 (1 H, m), 1.22 (3 H, d, J = 7.2 Hz), 1.25 (3 H, d, J = 7.2 Hz), 1.30−1.90 (8 H, m), 4.15−4.27 (2 H, m), 4.31−4.40 (1 H, m), 5.42 (1 H, d, J = 6.6 Hz).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Nva-OH (73e).** This compound was prepared from **72e** in 94% yield according to the procedure for the synthesis of **73a**. **73e:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH = 10:1) 0.44; FAB-MS m/e 257 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (3 H, t, J = 7.0 Hz), 1.21 (3 H, d, J = 7.0 Hz), 1.24 (3 H, d, J = 7.0 Hz), 1.29−1.96 (10 H, m), 4.11−4.35 (3 H, m), 5.16 (1 H, d, J = 6.6 Hz).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Val-OH (73f).** This compound was prepared from **72f** in 97% yield according to the procedure for the synthesis of **73a**. **73f:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.38; FAB-MS m/e 257 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.97 (3 H, d, J = 6.8 Hz), 1.02 (3 H, d, J = 6.8 Hz), 1.23 (3 H, d, J = 7.0 Hz), 1.26 (3 H, d, J = 7.0 Hz), 1.43−1.87 (6 H, m), 2.17−2.39 (1 H, m), 4.09−4.29 (2 H, m), 4.32 (1 H, dd, J = 5.7, 7.8 Hz), 5.03 (1 H, d, J = 7.8 Hz).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Ile-OH (73g).** This compound was prepared from **72g** in 94% yield according to the procedure for the synthesis of **73a**. **73g:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH = 10:1) 0.42; FAB-MS m/e 271 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (3 H, t, J = 7.3 Hz), 0.96 (3 H, d, J = 6.9 Hz), 1.21 (3 H, d, J = 7.2 Hz), 1.24 (3 H, d, J = 7.2 Hz), 1.29−2.02 (9 H, m), 4.12−4.30 (2 H, m), 4.27 (1 H, dd, J = 5.3, 7.4 Hz), 5.24 (1 H, d, J = 7.4 Hz).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cpeg-OH (73h).** This compound was prepared from **72h** in 97% yield according to the procedure for the synthesis of **73a**. **73h:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.48; FAB-MS m/e 283 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (3 H, d, J = 7.4 Hz), 1.23 (3 H, d, J = 7.4 Hz), 1.27−1.89 (14 H, m), 2.23−2.41 (1 H, m), 4.12−4.28 (2 H, m), 4.24 (1 H, t, J = 7.3 Hz), 5.23 (1 H, d, J = 7.3 Hz).

***cis*-[(2,6-Dimethylpiperidino)carbonyl]-γMeLeu-OH (73i).** This compound was prepared from **72i** in 99% yield according to the procedure for the synthesis of **73a**. **73i:** white amorphous solid; TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.33; FAB-MS m/e 285 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (9 H, s), 1.23 (3 H, d, J = 6.9 Hz), 1.25 (3 H, d, J = 6.9 Hz), 1.42−1.88 (6 H, m), 1.47 (1 H, dd, J = 8.7, 14.6 Hz), 2.14 (1 H, dd, J = 3.4, 14.6 Hz), 4.07−4.25 (2 H, m), 4.28−4.35 (1 H, m), 4.62 (1 H, d, J = 7.6 Hz).

Method A. *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-D-Trp(2-Cl)-D-Nle-OH (6). (a) Boc-D-Trp(1-Boc,2-Cl)-D-Nle-O^{t}Bu (68a). EDCI (107 mg, 0.56 mmol) was added to a mixture of **56a** (206 mg, 0.465 mmol), D-Nle-O^{t}Bu·HCl (125 mg, 0.56 mmol), HOBT (86 mg, 0.56 mmol), and NMM (62 μL, 0.56 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After being stirred at 0 °C for 1 h, the mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with 10 mL each of saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluted with EtOAc/hexane (1:5) to give **68a** (235 mg, 83%) as a white amorphous solid: TLC R_f (EtOAc:hexane = 1:3) 0.53; FAB-MS m/e 608, 610 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (3 H, t, J = 7.1 Hz), 1.41 (9 H, s), 1.55 (9 H, s), 1.69 (9 H, s), 1.01−1.83 (6 H, m), 3.21 (2 H, d, J = 6.3 Hz), 4.28−4.50 (2 H, m), 5.20 (1 H, brs), 6.34 (1 H, d, J = 7.3 Hz), 7.20−7.35 (2 H, m), 7.57 (1 H, d, J = 7.6 Hz), 8.04 (1 H, d, J = 7.6 Hz).

(b) H-D-Trp(1-Boc,2-Cl)-D-Nle-O^{t}Bu (69a) and H-D-Trp-(2-Cl)-D-Nle-O^{t}Bu (70a). 68a (252 mg, 0.415 mmol) was dissolved in formic acid (10 mL). After being stirred at room

temperature for 1.5 h, the mixture was concentrated under reduced pressure. The residue was taken up with EtOAc (50 mL), which was washed with saturated $NaHCO_3$ (50 mL × 2) and brine (50 mL), dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC ($CHCl_3$:MeOH = 30:1) to give **69a** (116 mg, 55%) and **70a** (35 mg, 21%). **69a**: TLC R_f ($CHCl_3$:MeOH = 30:1) 0.53; FAB-MS *m/e* 508, 510 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3 H, t, J = 6.9 Hz), 1.46 (9 H, s), 1.55 (9 H, s), 1.12−1.82 (6 H, m), 2.95 (1 H, dd, J = 9.6, 14.5 Hz), 3.38 (1 H, dd, J = 3.9, 14.5 Hz), 3.75 (1 H, dd, J = 3.9, 9.6 Hz), 4.38−4.49 (1 H, m), 7.20−7.34 (2 H, m), 7.59 (1 H, dd, J = 1.5, 7.2 Hz), 8.06 (1 H, dd, J = 1.5, 7.2 Hz), 7.80 (1 H, d, J = 8.4 Hz).

70a: TLC R_f ($CHCl_3$:MeOH = 30:1) 0.22; FAB-MS *m/e* 408, 410 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3 H, t, J = 7.1 Hz), 1.46 (9 H, s), 1.14−1.83 (6 H, m), 2.93 (1 H, dd, J = 9.3, 14.7 Hz), 3.36 (1 H, dd, J = 4.0, 14.7 Hz), 3.75 (1 H, dd, J = 4.0, 9.3 Hz), 4.41−4.51 (1 H, m), 7.13 (1 H, dt, J = 1.6, 7.5 Hz), 7.19 (1 H, dt, J = 1.6, 7.5 Hz), 7.28 (1 H, dd, J = 1.6, 7.5 Hz), 7.61 (1 H, dd, J = 1.6, 7.5 Hz), 7.77 (1 H, d, J = 9.5 Hz), 8.11 (1 H, s).

(c) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-D-Trp-(1-Boc,2-Cl)-D-Nle-O^{t}Bu (71a). EDCI (17 mg, 0.091 mmol) was added to a mixture of **73a** (25 mg, 0.091 mmol), **69a** (31 mg, 0.061 mmol), and HOBT (14 mg, 0.091 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h and then at room temperature for 4 h. The mixture was diluted with CH_2Cl_2 (30 mL), washed with 20 mL each of saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and evaporated to give a crude product of **71a** (51.3 mg, 111%). This material showed a single spot on TLC and was used in the next reaction without further purification: TLC R_f (EtOAc:hexane = 1:2) 0.30; FAB-MS *m/e* 760, 762 $(M + H)^+$.

(d) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-D-Trp-(2-Cl)-D-Nle-OH (6). 71a (49.8 mg, 0.059 mmol) was dissolved in TFA (1 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$:MeOH:AcOH = 30:1:1) followed by trituration with water (10 mL) to give **6** (16.7 mg, 47%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.29; ^1H NMR (300 MHz, $CDCl_3$) δ 0.79 (3 H, t, J = 7.2 Hz), 0.86 (3 H, d, J = 4.9 Hz), 0.88 (3 H, d, J = 4.9 Hz), 1.15 (3 H, d, J = 6.9 Hz), 1.16 (3 H, d, J = 6.9 Hz), 0.93−1.87 (15 H, m), 3.21 (1 H, dd, J = 6.3, 14.4 Hz), 3.38 (1 H, dd, J = 6.3, 14.4 Hz), 3.91−4.07 (2 H, m), 4.07−4.20 (1 H, m), 4.20−4.34 (1 H, m), 4.52−4.73 (1 H, m), 4.82−5.02 (1 H, m), 6.52−6.70 (1 H, m), 7.11 (1 H, t, J = 7.1 Hz), 7.17 (1 H, t, J = 7.1 Hz), 7.26 (1 H, d, J = 7.1 Hz), 7.59 (1 H, d, J = 7.1 Hz), 7.31−7.49 (1 H, m), 8.63 (1 H, brs).

Method B. *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp(2-Br)-D-Nle-OH (4). (a) Boc-D-Trp(2-Br)-D-Nle-OMe (74a). EDCI (1.39 g, 7.24 mmol) was added to a mixture of **58** (2.31 g, 6.03 mmol), D-Nle-OMe·HCl (1.20 g, 6.63 mmol), HOBT (1.11 g, 7.24 mmol), and NMM (0.73 mL, 6.63 mmol) in CH_2Cl_2 (20 mL) at 0 °C. After being stirred at 0 °C for 0.5 h, the mixture was allowed to warm to room temperature and stirred for 1.5 h. The mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluted with EtOAc/hexane (1:3) to give **74a** (2.62 g, 85%) as a white solid: TLC R_f (EtOAc:hexane = 1:3) 0.18; FAB-MS *m/e* 510, 512 $(M + H)^+$.

(b) H-D-Trp(2-Br)-D-Nle-OMe (75a). 74a (2.08 g, 4.08 mmol) was dissolved in formic acid (30 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure, and the residue was taken up with EtOAc (60 mL), which was washed with saturated $NaHCO_3$ (40 mL × 2) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give **75a** (1.60 g, 97%) as a white amorphous solid. This material showed a single spot on TLC and was used in the next reaction without further purification: TLC R_f ($CHCl_3$:MeOH = 30:1) 0.31; FAB-MS *m/e* 410, 412 $(M + H)^+$.

(c) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp-(2-Br)-D-Nle-OMe (76a). EDCI (490 mg, 2.55 mmol) was added to a mixture of **75a** (960 mg, 2.34 mmol), **73b** (540 mg, 2.12 mmol), and HOBT (390 mg, 2.55 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After being stirred at 0 °C for 0.5 h and at room temperature for 1 h, the mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel eluted with EtOAc/hexane (1:2) to give **76a** (1.25 g, 91%) as a white amorphous solid: TLC R_f (EtOAc:hexane = 1:2) 0.34; FAB-MS *m/e* 646, 648 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.21−0.35 (2 H, m), 0.40−0.52 (2 H, m), 0.79 (3 H, t, J = 7.0 Hz), 1.00−1.80 (13 H, m), 1.18 (3 H, d, J = 6.9 Hz), 1.18 (3 H, d, J = 6.9 Hz), 3.24 (1 H, dd, J = 6.7, 14.6 Hz), 3.36 (1 H, dd, J = 10.0, 14.6 Hz), 3.30−3.44 (1 H, m), 3.63 (3 H, s), 4.00−4.22 (2 H, m), 4.35−4.46 (1 H, m), 4.73−4.84 (1 H, m), 5.04 (1 H, d, J = 5.6 Hz), 6.37 (1 H, d, J = 8.6 Hz), 7.05 (1 H, d, J = 9.6 Hz), 7.09 (1 H, dt, J = 1.3, 7.1 Hz), 7.16 (1 H, dt, J = 1.3, 7.1 Hz), 7.27 (1 H, dd, J = 1.3, 7.1 Hz), 7.64 (1 H, dd, J = 1.3, 7.1 Hz), 8.35 (1 H, s).

(d) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp-(2-Br)-D-Nle-OH (4). NaOH (1 N) (2.93 mL, 2.93 mmol) was added to a solution of **76a** (1.13 g, 1.74 mmol) in MeOH (6 mL) at 0 °C. After 10 min, the mixture was allowed to warm to room temperature and stirred for 3.5 h. The mixture was diluted with water (15 mL) and concentrated to remove MeOH. The resulting aqueous solution was acidified with 1 N HCl at 0 °C to form a precipitate. The precipitate was collected by filtration, washed with a small amount of water, and dried to give **4** (1.02 g, 92%) as a white crystalline powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.27; ^1H NMR (400 MHz, $CDCl_3$) δ −0.13−0.23 (4 H, m), 0.84 (3 H, t, J = 7.1 Hz), 0.75−0.92 (1 H, m), 1.03 (3 H, d, J = 7.1 Hz), 1.05 (3 H, d, J = 7.1 Hz), 1.18−1.78 (12 H, m), 2.87 (1 H, dd, J = 10.3, 14.7 Hz), 3.12−3.55 (2 H, m), 4.04−4.20 (3 H, m), 4.55−4.65 (1 H, m), 6.21 (1 H, d, J = 6.4 Hz), 6.96 (1 H, t, J = 7.7 Hz), 7.04 (1 H, t, J = 7.7 Hz), 7.22 (1 H, d, J = 7.7 Hz), 7.64 (1 H, d, J = 7.7 Hz), 7.99 (1 H, d, J = 9.3 Hz), 8.09 (1 H, d, J = 7.3 Hz), 11.56 (1 H, s).

Method C. *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp(2-Me)-D-His-OH (36). (a) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp(2-Me)-OMe (77a). EDCI (92 mg, 0.48 mmol) was added to a mixture of **67** (93 mg, 0.40 mmol), **73b** (101 mg, 0.40 mmol), and HOBT (73 mg, 0.48 mmol) in DMF (5 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was partitioned between EtOAc (50 mL) and water (50 mL). The organic layer was washed with 50 mL each of 10% citric acid, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with EtOAc/hexane (1:1) to afford **77a** (112 mg, 60%): TLC R_f (EtOAc:hexane = 1:2) 0.30; FAB-MS *m/e* 469 $(M + H)^+$.

(b) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp-(2-Me)-OH (78a). NaOH (1 N) (0.5 mL, 0.5 mmol) was added to a solution of **77a** (112 mg, 0.24 mmol) in MeOH (2 mL) at 0 °C. The mixture was stirred at 0 °C for 2.5 h and at room temperature for 2 h; 10% citric acid (30 mL) was added to the mixture, which was extracted with EtOAc (15 mL × 3). The combined organic extracts were washed with brine (15 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give **78a** (76 mg, 70%). This material showed a single spot on TLC and was used in the next reaction without further purification: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.60; FAB-MS *m/e* 455 $(M + H)^+$.

(c) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp-(2-Me)-D-His-OMe (79a). EDCI (15.8 mg, 0.082 mmol) was added to a mixture of **78a** (24.9 mg, 0.055 mmol), D-His-OMe·2HCl (19.9 mg, 0.082 mmol), HOBT (12.6 mg, 0.082 mmol), and NMM (23 μL, 0.16 mmol) in DMF (1.0 mL) at 0 °C. After being stirred at 0 °C for 3 h, the mixture was allowed to warm to room temperature and stirred for 6 h. The mixture was partitioned between EtOAc (50 mL) and saturated $NaHCO_3$ (50 mL). The organic layer was dried over $MgSO_4$ and

concentrated. The residue was purified by preparative TLC ($CHCl_3$:MeOH = 10:1) to give **79a** (9.9 mg, 30%) as a white amorphous solid: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.38; FAB-MS *m/e* 606 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.23−0.33 (1 H, m), 0.44−0.70 (3 H, m), 1.13 (3 H, d, J = 7.1 Hz), 1.18 (3 H, d, J = 7.1 Hz), 1.07−1.28 (1 H, m), 1.38−1.82 (6 H, m), 2.11 (3 H, s), 2.89 (1 H, dd, J = 10.6, 14.7 Hz), 2.85−2.98 (1 H, m), 3.14 (1 H, dd, J = 6.6, 14.7 Hz), 3.23 (1 H, dd, J = 4.0, 14.7 Hz), 3.45 (1 H, dd, J = 3.9, 14.7 Hz), 3.69 (3 H, s), 3.93−4.12 (2 H, m), 4.38−4.50 (1 H, m), 4.62−4.73 (1 H, m), 5.03 (1 H, d, J = 5.2 Hz), 6.23 (1 H, d, J = 7.5 Hz), 6.79 (1 H, s), 7.03−7.19 (3 H, m), 7.23−7.40 (1 H, m), 7.33 (1 H, d, J = 7.4 Hz), 7.49 (1 H, d, J = 7.4 Hz), 8.24 (1 H, s).

(d) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Cprg-D-Trp-(2-Me)-D-His-OH (36). NaOH (1 N) (80 μL, 0.080 mmol) was added to a solution of **79a** (10.0 mg, 0.016 mmol) in MeOH (0.30 mL) at 0 °C. After being stirred at 0 °C for 30 min, the mixture was allowed to warm to room temperature and stirred for 3.5 h. The mixture was cooled to 0 °C, and 1 N HCl (80 μL, 0.080 mmol) was added. The resulting mixture was concentrated under reduced pressure. The residue was dissolved in water (3 mL), and the solution was passed through a Sep-Pak C18 cartridge (Waters Chromatography Division, Millipore Corp., MA). The cartridge was washed with water (5 mL) and then eluted with MeOH (5 mL). The eluate was concentrated under reduced pressure to give **36** (9.2 mg, 94%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.17; ^1H NMR (300 MHz, $CDCl_3$) δ 0.00−0.28 (4 H, m), 0.80−0.95 (1 H, m), 1.06 (3 H, d, J = 6.6 Hz), 1.07 (3 H, d, J = 6.6 Hz), 1.31−1.77 (6 H, m), 2.30 (3 H, s), 2.70−3.60 (5 H, m), 4.06−4.21 (2 H, m), 4.30−4.53 (2 H, m), 6.18 (1 H, d, J = 6.3 Hz), 6.80 (1 H, s), 6.86 (1 H, t, J = 7.5 Hz), 6.94 (1 H, t, J = 7.5 Hz), 7.17 (1 H, d, J = 7.5 Hz), 7.51 (1 H, d, J = 7.5 Hz), 7.53 (1 H, s), 8.02 (1 H, d, J = 8.6 Hz), 8.27 (1 H, d, J = 7.0 Hz), 10.62 (1 H, s).

Method D. [(Octahydro-1*H*-azocinyl)carbonyl]-Leu-D-Trp(2-Br)-D-Nle-OH (14). (a) Boc-Leu-D-Trp(2-Br)-D-Nle-OMe (80). EDCI (214 mg, 1.12 mmol) was added to a mixture of Boc-Leu-OH·H_2O (278 mg, 1.12 mmol), **75a** hydrochloride (415 mg, 0.93 mmol), HOBT (171 mg, 1.12 mmol), and NMM (0.12 mL, 1.12 mmol) in CH_2Cl_2 (15 mL) at 0 °C. After being stirred at 0 °C for 1.5 h and at room temperature for 2.5 h, the mixture was diluted with CH_2Cl_2 (30 mL). The mixture was washed with saturated $NaHCO_3$ (20 mL × 3), 10% citric acid (20 mL × 2), and brine (20 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$/MeOH (20:1) to give **80** (564 mg, 97%) as a white amorphous solid: TLC R_f ($CHCl_3$:MeOH = 20:1) 0.28; FAB-MS *m/e* 623, 625 $(M + H)^+$.

(b) (Phenoxycarbonyl)-Leu-D-Trp(2-Br)-D-Nle-OMe (81). **80** (530 mg, 0.85 mmol) was dissolved in formic acid (20 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated, and the residue was taken up with EtOAc (50 mL). The mixture was washed with saturated $NaHCO_3$ (40 mL × 2) and brine (40 mL) and dried over $MgSO_4$. The solvent was evaporated to give the corresponding amine (434 mg, 98%): TLC R_f ($CHCl_3$:MeOH = 10:1) 0.50; FAB-MS *m/e* 523, 525 $(M + H)^+$.

The above-mentioned amine (422 mg, 0.806 mmol) was dissolved in pyridine (3.0 mL), and phenyl chloroformate (0.20 mL, 1.61 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 1.5 h, and the reaction was quenched by the addition of 1 drop of water. The mixture was concentrated under reduced pressure, and the residue was partitioned between EtOAc (40 mL) and water (40 mL). The aqueous layer was extracted with EtOAc (20 mL × 2), and the combined organic layers were washed with 10% citric acid (40 mL), saturated $NaHCO_3$ (40 mL), and brine (40 mL) and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by column chromatography on silica gel eluted with $CHCl_3$/MeOH (50:1) to give **81** (472 mg, 91%) as a white solid: mp 193−195 °C; TLC R_f ($CHCl_3$:MeOH = 30:1) 0.25; FAB-MS *m/e* 643, 645 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.77 (3 H, t, J = 7.1 Hz), 0.89 (3 H, d, J = 6.0 Hz), 0.90 (3 H, d, J = 6.0 Hz), 0.80−1.70 (9 H, m), 3.14 (1 H, dd, J = 7.7, 14.6 Hz), 3.31 (1 H, dd, J = 6.6, 14.6 Hz), 3.64 (3 H, s), 4.13−4.19 (1 H, m), 4.36−4.43 (1 H, m), 4.69−4.77 (1 H, m), 5.47 (1 H, d, J = 8.1 Hz), 6.32 (1 H, d, J = 7.6 Hz), 6.58 (1 H, d, J = 7.9 Hz), 7.07−7.22 (5 H, m), 7.28 (1 H, d, J = 7.5 Hz), 7.32 (2 H, t, J = 7.5 Hz), 7.65 (1 H, d, J = 7.6 Hz), 8.15 (1 H, s).

(c) [(Octahydro-1*H*-azocinyl)carbonyl]-Leu-D-Trp(2-Br)-D-Nle-OH (14). A mixture of **81** (38 mg, 0.059 mmol), heptamethyleneimine (75 μL, 0.59 mmol), and TEA (118 μL, 0.85 mmol) in $CHCl_3$ (1.6 mL) and THF (1.0 mL) was stirred at 50 °C for 4.5 h. The mixture was diluted with EtOAc (30 mL), washed with 20 mL each of 1 N HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by preparative TLC (EtOAc:hexane = 3:2) to give the methyl ester **82a** (33 mg, 85%) as a colorless oil: TLC R_f (EtOAc:hexane = 3:2) 0.40; FAB-MS *m/e* 662, 664 $(M + H)^+$.

A mixture of the methyl ester **82a** (28 mg, 0.042 mmol) and 2 N NaOH (0.20 mL, 0.40 mmol) in MeOH (0.8 mL) was stirred at 0 °C for 1 h and at room temperature for 3 h. The mixture was concentrated under reduced pressure; 1 N HCl (0.8 mL) was added to the residue at 0 °C to form a precipitate. The precipitate was collected by filtration, washed with a small amount of water, and dried to give **14** (13.1 mg, 49%) as a white powder: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.33; ^1H NMR (300 MHz, $CDCl_3$) δ 0.68 (3 H, d, J = 7.3 Hz), 0.71 (3 H, d, J = 7.3 Hz), 0.82 (3 H, t, J = 6.7 Hz), 1.01−1.68 (19 H, m), 2.87 (1 H, dd, J = 9.4, 14.5 Hz), 3.00 (1 H, dd, J = 4.4, 14.5 Hz), 3.14−3.47 (4 H, m), 3.80−3.86 (1 H, m), 4.03−4.09 (1 H, m), 4.43−4.52 (1 H, m), 5.88 (1 H, d, J = 7.8 Hz), 6.94 (1 H, t, J = 7.4 Hz), 7.03 (1 H, t, J = 7.4 Hz), 7.21 (1 H, d, J = 7.4 Hz), 7.59 (1 H, d, J = 7.1 Hz), 7.63 (1 H, d, J = 7.4 Hz), 7.97 (1 H, d, J = 8.5 Hz), 11.58 (1 H, s).

Method E. *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-D-Trp(2-Et)-D-Nle-OH (10). (a) Boc-D-Trp(2-ethynyl)-D-Nle-OMe (83). EDCI (224 mg, 1.17 mmol) was added to a mixture of **63** (280 mg, 0.78 mmol), H-D-Nle-OMe·HCl (156 mg, 0.86 mmol), HOBT (179 mg, 0.17 mmol), and NMM (94 μL, 0.86 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After being stirred at 0 °C for 1 h and at room temperature for 30 min, the mixture was diluted with EtOAc (60 mL), washed with 60 mL each of saturated $NaHCO_3$, 10% citric acid, and brine, and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with EtOAc/hexane (1:2) to give **83** (303 mg, 86%) as a pale yellow oil: TLC R_f (EtOAc:hexane = 1:2) 0.34; FAB-MS *m/e* 456 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.80 (3 H, t, J = 7.1 Hz), 0.92−1.11 (2 H, m), 1.11−1.23 (2 H, m), 1.41 (9 H, s), 1.33−1.73 (2 H, m), 3.21−3.43 (2 H, m), 3.50 (1 H, s), 3.65 (3 H, s), 4.39−4.52 (2 H, m), 5.24−5.37 (1 H, m), 6.30 (1 H, d, J = 7.3 Hz), 7.13 (1 H, dt, J = 1.3, 7.8 Hz), 7.24 (1 H, dt, J = 1.3, 7.8 Hz), 7.29 (1 H, dd, J = 1.3, 7.8 Hz), 7.66 (1 H, dd, J = 1.3, 7.8 Hz), 8.17 (1 H, brs).

(b) Deprotection of the Boc Group in 83. 83 (115 mg, 0.252 mmol) was dissolved in formic acid (5 mL), and the mixture was stirred at room temperature for 1.5 h. The mixture was concentrated under reduced pressure, and the residue was partitioned between EtOAc and saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by preparative TLC ($CHCl_3$:MeOH = 25:1) to give the cyclic imine **85** (21 mg, 24%) as a 1:1 mixture of two diastereoisomers presumably due to a racemization at the C-α position of the tryptophanyl residue: TLC R_f ($CHCl_3$:MeOH = 20:1) 0.42; FAB-MS *m/e* 356 $(M + H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90 and 0.92 (3 H, t, J = 7.0 Hz), 1.27−1.45 (4 H, m), 1.71−2.00 (2 H, m), 2.43 (3 H, s), 2.72 and 2.77 (1 H, dd, J = 4.1, 16.5 Hz), 3.52 and 3.55 (1 H, dd, J = 7.6, 16.5 Hz), 3.76 and 3.78 (3 H, s), 4.13−4.29 (1 H, m), 4.67−4.77 (1 H, m), 7.17 (1 H, t, J = 7.4 Hz), 7.30 (1 H, t, J = 7.4 Hz), 7.39 (1 H, d, J = 7.4 Hz), 7.62 (1 H, t, J = 7.4 Hz), 8.21 (1H, brs), 8.29 and 8.32 (1 H, d, J = 7.8 Hz).

(c) Boc-D-Trp(2-Et)-D-Nle-OMe (86). A mixture of **83** (180 mg, 0.395 mmol) and 5% Pd/$BaSO_4$ (50 mg) in MeOH (4 mL) was stirred in a hydrogen atmosphere for 3.5 h. The catalyst was removed by filtration, and the solvent was evaporated to give **86** (166 mg, 91%) as a pale yellow oil: TLC R_f (EtOAc:hexane = 1:2) 0.37; FAB-MS *m/e* 459 (M^+); ^1H NMR (300 MHz, $CDCl_3$) δ 0.81 (3 H, t, J = 7.0 Hz), 0.94−1.25 (4 H, m),

1.29 (3 H, t, J = 7.7 Hz), 1.43 (9 H, s), 1.36−1.73 (2 H, m), 2.79 (2 H, q, J = 7.7 Hz), 3.08 (1 H, dd, J = 8.6, 14.8 Hz), 3.27 (1 H, dd, J = 2.7, 14.8 Hz), 3.58 (3 H, s), 4.29−4.42 (2 H, m), 5.15−5.30 (1 H, m), 6.08 (1 H, d, J = 7.3 Hz), 7.06 (1 H, dt, J = 1.5, 7.3 Hz), 7.11 (1 H, dt, J = 1.5, 7.3 Hz), 7.28 (1 H, dd, J = 1.5, 7.3 Hz), 7.54 (1 H, dd, J = 1.5, 7.3 Hz), 7.88 (1 H, brs).

(d) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-D-Trp-(2-Et)-D-Nle-OMe (87). A mixture of **86** (160 mg, 0.348 mmol) in formic acid (5 mL) was stirred at room temperature for 1.5 h. The mixture was concentrated, and the resulting residue was taken up with EtOAc, which was washed with saturated $NaHCO_3$ and dried over $MgSO_4$. The solvent was evaporated to give the corresponding primary amine (123 mg, 98%) as a pale yellow oil: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.49; FAB-MS m/e 360 $(M + H)^+$.

The primary amine (120 mg, 0.334 mmol) was dissolved in CH_2Cl_2 (5 mL). **73a** (100 mg, 0.367 mmol), HOBT (62 mg, 0.40 mmol), and EDCI (78 mg, 0.40 mmol) were added to the mixture at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc, washed with saturated $NaHCO_3$, 10% citric acid, and brine, and dried over $MgSO_4$. The solvent was evaporated. The residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with EtOAc/hexane (2:1) to give **87** (143 mg, 64%) as a pale yellow amorphous solid: TLC R_f (EtOAc:hexane = 2:1) 0.42; FAB-MS m/e 612 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.75−0.90 (9 H, m), 1.16 (3 H, d, J = 7.1 Hz), 1.17 (3 H, d, J = 7.1 Hz), 1.27 (3 H, t, J = 7.8 Hz), 1.02−1.82 (15 H, m), 2.70−2.91 (2 H, m), 3.15 (1 H, dd, J = 7.5, 14.5 Hz), 3.38 (1 H, dd, J = 5.9, 14.5 Hz), 3.60 (3 H, s), 3.91−4.20 (3 H, m), 4.29−4.40 (1 H, m), 4.67−4.80 (2 H, m), 6.44 (1 H, d, J = 8.5 Hz), 6.92 (1 H, d, J = 7.5 Hz), 7.05 (1 H, dt, J = 1.2, 7.6 Hz), 7.11 (1 H, dt, J = 1.2, 7.6 Hz), 7.21 (1 H, dd, J = 1.2, 7.6 Hz), 7.53 (1 H, dd, J = 1.2, 7.6 Hz), 7.90 (1 H, brs).

(e) *cis*-[(2,6-Dimethylpiperidino)carbonyl]-Leu-D-Trp-(2-Et)-D-Nle-OH (10). NaOH (4 N) (1 mL) was added to a solution of **87** (43.4 mg, 0.071 mmol) in MeOH (2 mL) at 0 °C, and the resulting mixture was stirred at room temperature for 2 h. MeOH was removed by evaporation, and 1 N HCl was added to the aqueous residue at 0 °C. The precipitate formed was collected by filtration, washed with a small amount of water and dried to give **10** (36 mg, 85%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.39; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.71 (6 H, d, J = 6.8 Hz), 0.83 (3 H, t, J = 6.7 Hz), 1.03 (3 H, d, J = 6.3 Hz), 1.05 (3 H, d, J = 6.3 Hz), 1.19 (3 H, t, J = 7.6 Hz), 0.95−1.76 (15 H, m), 2.59−2.89 (3 H, m), 3.18 (1 H, dd, J = 4.8, 14.6 Hz), 3.94−4.20 (4 H, m), 4.40−4.48 (1 H, m), 6.07 (1 H, d, J = 7.3 Hz), 6.87 (1 H, t, J = 7.4 Hz), 6.94 (1 H, t, J = 7.4 Hz), 7.18 (1 H, d, J = 7.4 Hz), 7.53 (1 H, d, J = 7.4 Hz), 7.89 (1 H, brs), 7.98 (1 H, d, J = 9.8 Hz), 10.64 (1 H, s).

Boc-D-Trp(2-Br)-D-Asp(OMe)-O^{t}Bu (88a). A mixture of **58** (766 mg, 2.00 mmol), H-D-Asp(OMe)-O^{t}Bu·HCl (480 mg, 2.00 mmol), NMM (0.30 mL, 2.20 mmol), HOBT (337 mg, 2.20 mmol), and EDCI (422 mg, 2.20 mmol) in CH_2Cl_2 (20 mL) was stirred at 0 °C for 1 h and at room temperature for 14 h. The mixture was washed with saturated $NaHCO_3$ (20 mL), 10% citric acid (20 mL), and brine (20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (2:1) to give **88a** (1.09 g, 96%) as a white amorphous solid: FAB-MS m/e 568, 570 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (9 H, s), 1.41 (9 H, s), 2.74 (1 H, dd, J = 5.0, 16.6 Hz), 2.87 (1 H, dd, J = 5.3, 16.6 Hz), 3.10−3.28 (2 H, m), 3.60 (3 H, s), 4.35−4.60 (2 H, m), 5.15 (1 H, brs), 6.67 (1 H, d, J = 7.2 Hz), 7.03−7.32 (3 H, m), 7.57 (1 H, d, J = 7.3 Hz), 8.11 (1 H, s).

Boc-D-Trp(1-Boc,2-Cl)-D-Asp(OMe)-O^{t}Bu (88b). This compound was prepared from **56a** and H-D-Asp(OMe)-O^{t}Bu·HCl in 85% yield according to the procedure for the synthesis of **88a**. **88b:** colorless oil; FAB-MS m/e 624, 626 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (9 H, s), 1.41 (9 H, s), 1.69 (9 H, s), 2.74 (1 H, dd, J = 3.9, 16.4 Hz), 2.89 (1 H, dd, J = 4.1, 16.4 Hz), 3.21 (2 H, d, J = 6.8 Hz), 3.62 (3 H, s), 4.33−4.52 (2 H, m), 5.18 (1 H, brs), 6.70 (1 H, d, J = 7.2 Hz), 7.18−7.33 (2 H, m), 7.55 (1 H, dd, J = 1.7, 7.0 Hz), 8.05 (1 H, dd, J = 1.7, 7.0 Hz).

Boc-D-Trp(1-Boc,2-Br)-D-Asp(OMe)-O^{t}Bu (88c). This compound was prepared from **56b** and H-D-Asp(OMe)-O^{t}Bu·HCl in 94% yield according to the procedure for the synthesis of **88a**. **88c:** colorless oil; FAB-MS m/e 668, 670 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (9 H, s), 1.40 (9 H, s), 1.70 (9 H, s), 2.76 (1 H, dd, J = 4.9, 16.8 Hz), 2.88 (1 H, dd, J = 4.1, 16.8 Hz), 3.22 (2 H, d, J = 6.8 Hz), 3.62 (3 H, s), 4.38−4.51 (2 H, m), 5.21 (1 H, d, J = 5.9 Hz), 6.66 (1 H, d, J = 7.1 Hz), 7.18−7.33 (2 H, m), 7.57 (1 H, d, J = 7.5 Hz), 8.05 (1 H, d, J = 7.5 Hz).

Boc-D-Trp(2-CN)-D-Asp(OMe)-O^{t}Bu (88d). This compound was prepared from **61** and H-D-Asp(OMe)-O^{t}Bu·HCl in 52% yield according to the procedure for the synthesis of **88a**. **88d:** white amorphous solid; FAB-MS m/e 515 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (9 H, s), 1.45 (9 H, s), 2.80 (1 H, dd, J = 4.9, 16.9 Hz), 2.93 (1 H, dd, J = 4.3, 16.9 Hz), 3.27−3.50 (2 H, m), 3.64 (3 H, s), 4.48−4.70 (2 H, m), 5.29 (1 H, d, J = 6.2 Hz), 6.81 (1 H, d, J = 6.8 Hz), 7.18 (1 H, t, J = 8.0 Hz), 7.24−7.38 (2 H, m), 7.74 (1 H, d, J = 8.0 Hz), 8.73 (1 H, s).

Boc-Pro-D-Cpeg-Cprg-OH (90a). EDCI (138 mg, 0.72 mmol) was added to a mixture of Boc-D-Cpeg-OH (145 mg, 0.60 mmol), H-Cprg-OBzl·HCl (160 mg, 0.66 mmol), HOBT (110 mg, 0.72 mmol), and NMM (0.10 mL, 0.72 mmol) in DMF (3 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was partitioned between EtOAc (30 mL) and water (20 mL), and the organic layer was washed with 20 mL each of 10% citric acid, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated. Crystallization of the residue from EtOAc−hexane gave Boc-D-Cpeg-Cprg-OBzl (221 mg, 86%) as colorless crystals.

Boc-D-Cpeg-Cprg-OBzl (220 mg, 0.51 mmol) was dissolved in 4 N HCl/EtOAc (10 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure to give H-D-Cpeg-Cprg-OBzl·HCl (189 mg, 100%) as a white amorphous solid.

EDCI (173 mg, 0.90 mmol) was added to a mixture of H-D-Cpeg-Cprg-OBzl·HCl (276 mg, 0.75 mmol), Boc-Pro-OH (194 mg, 0.90 mmol), HOBT (138 mg, 0.90 mmol), and NMM (124 μL, 0.90 mmol) in DMF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The mixture was partitioned between EtOAc (50 mL) and water (50 mL), and the organic layer was washed with 50 mL each of 10% citric acid, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. The solvent was evaporated to give Boc-Pro-D-Cpeg-Cprg-OBzl (399 mg, 100%). Boc-Pro-D-Cpeg-Cprg-OBzl (399 mg, 0.756 mmol) was hydrogenated over 10% Pd/C (50 mg) in MeOH (10 mL) under an atmospheric pressure of hydrogen to give **90a** (332 mg, 100%) as a white amorphous solid: FAB-MS m/e 438 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.43−0.65 (4 H, m), 1.09−1.22 (1 H, m), 1.28−1.92 (10 H, m), 1.44 (9 H, s), 1.98−2.33 (3 H, m), 3.29−3.60 (2 H, m), 4.08 (1 H, t, J = 7.7 Hz), 4.21−4.37 (1 H, m), 4.73 (1 H, t, J = 9.6 Hz), 7.14−7.50 (2 H, brs).

Boc-Pro-D-Val-Leu-OH (90b): white amorphous solid; FAB-MS m/e 428 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81−1.00 (12 H, m), 1.43 (9 H, s), 1.51−2.24 (8 H, m), 3.25−3.53 (2 H, m), 4.20−4.34 (1 H, m), 4.49−4.68 (2 H, m), 7.15−7.60 (2 H, brs).

Boc-Pro-D-*tert*-Leu-Leu-OH (90c): white amorphous solid; FAB-MS m/e 442 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88−1.08 (6 H, m), 1.02 (9 H, s), 1.45 (9 H, s), 1.57−2.22 (7 H, m), 3.28−3.60 (2 H, m), 4.20−4.38 (1 H, m), 4.48−4.64 (1 H, m), 4.81 (1 H, d, J = 9.7 Hz), 7.05−7.42 (2 H, brs).

Boc-Pro-D-Cpeg-Leu-OH (90d): white amorphous solid; FAB-MS m/e 454 $(M + H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85−2.38 (15 H, m), 1.44 (9 H, s), 3.25−3.53 (2 H, m), 4.20−4.30 (1 H, m), 4.49−4.65 (2 H, m), 7.05−7.35 (2 H, brs).

Boc-Pro-D-Pen(Me)-Leu-OH (90e). Iodomethane (0.68 mL, 11 mmol) was added to a mixture of D-penicillamine (1.49 g, 10 mmol) and 1 N NaOH (11 mL, 11 mmol) in EtOH (10 mL) at 0 °C. The mixture was allowed to warm to room

temperature and stirred overnight. Di-*tert*-butyldicarbonate (2.53 mL, 11 mmol) and 1 N NaOH (11 mL, 11 mmol) were added to the mixture, and the resulting mixture was stirred at room temperature for 3 h. The mixture was concentrated to remove EtOH, and the resulting aqueous solution was extracted with ethyl ether (10 mL). The aqueous layer was acidified with 10% citric acid and extracted with EtOAc (10 mL × 3). The combined organic extracts were washed with brine (10 mL) and dried over $MgSO_4$, and the solvent was evaporated to give Boc-D-Pen(Me)-OH (2.47 g, 94%) as a colorless oil.

NMM (0.66 mL, 6.0 mmol), HOBT (0.92 g, 6.0 mmol), and EDCI (1.15 g, 6.0 mmol) were added to a mixture of Boc-Pen-(Me)-OH (1.32 g, 5.0 mmol) and H-Leu-OMe·HCl (1.09 g, 6.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was washed successively with saturated $NaHCO_3$, 10% citric acid, and brine and dried over $MgSO_4$. The solvent was evaporated to give Boc-D-Pen(Me)-Leu-OMe (1.75 g, 90%) as a white amorphous solid.

Boc-D-Pen(Me)-Leu-OMe (1.75 g, 4.48 mmol) was dissolved in 4 N HCl/1,4-dioxane (20 mL). After being stirred for 30 min, the mixture was concentrated under reduced pressure. Trituration of the residue with ethyl ether gave H-D-Pen(Me)-Leu-OMe·HCl (1.387 g, 95%) as white crystals.

NMM (0.24 mL, 2.2 mmol), HOBT (337 mg, 2.2 mmol), and EDCI (422 mg, 2.2 mmol) were added to a mixture of H-D-Pen(Me)-Leu-OMe·HCl (654 mg, 2.0 mmol) and Boc-Pro-OH (474 mg, 2.2 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and concentrated to give Boc-Pro-D-Pen(Me)-Leu-OMe (0.946 g, 97%) as a white amorphous solid.

Boc-Pro-D-Pen(Me)-Leu-OMe (0.943 g, 1.93 mmol) was dissolved in MeOH (2.5 mL), and 1 N NaOH (2.5 mL, 2.5 mmol) was added to the mixture at 0 °C. After 30 min, the mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was concentrated under reduced pressure to remove MeOH, and the resulting aqueous solution was extracted with ethyl ether. The aqueous layer was acidified with 10% citric acid and extracted with EtOAc (10 mL × 3). The combined organic extracts were washed with brine and dried over $MgSO_4$, and the solvent was evaporated to give **90e** (0.92 g, 100%) as a white amorphous solid: FAB-MS *m/e* 474 $(M + H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (3 H, d, J = 6.0 Hz), 0.97 (3 H, d, J = 6.0 Hz), 1.35 (3 H, s), 1.38 (3 H, s), 1.45 (9 H, s), 1.56−2.22 (7 H, m), 2.09 (3 H, s), 3.28−3.60 (2 H, m), 4.22−4.39 (1 H, m), 4.48−4.63 (1 H, m), 4.93−5.12 (1 H, m), 7.30−7.60 (2 H, brs).

Method F. *cyclo*(-D-Trp(2-Br)-D-Asp-Pro-D-Cpeg-Cprg-) (46). 88a (1.09 g, 1.92 mmol) was dissolved in formic acid (20 mL). After being stirred for 1.5 h, the mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc (50 mL) and saturated $NaHCO_3$ (50 mL), and the organic layer was dried over $MgSO_4$. The solvent was evaporated, and the residue was dissolved in ethyl ether (50 mL); 4 N HCl/1,4-dioxane (0.5 mL) was added to the solution at 0 °C, and the volatiles were evaporated to give a hydrochloride of the corresponding primary amine **89a** (0.826 g, 85%) as a white hygroscopic powder.

An aliquot (420 mg, 0.83 mmol) of the hydrochloride of **89a** and Boc-Pro-D-Cpeg-Cprg-OH (**90a**; 332 mg, 0.76 mmol) were dissolved in DMF (10 mL). NMM (114 μL, 0.83 mmol), HOBT (127 mg, 0.83 mmol), and EDCI (159 mg, 0.83 mmol) were added to the mixture at 0 °C, and the resulting mixture was allowed to warm to room temperature and stirred overnight. The mixture was partitioned between EtOAc (50 mL) and water (50 mL), and the organic layer was washed with 50 mL each of 10% citric acid, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. The solvent was evaporated. The residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with $CHCl_3$/MeOH (50:1) to give a protected linear pentapeptide, **91a** (619 mg, 92%), as a pale yellow amorphous solid.

91a (599 mg, 0.68 mmol) was dissolved in TFA (10 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure to give H-Pro-D-Cpeg-Cprg-D-Trp(2-Br)-D-Asp(OMe)-OH as a brown amorphous solid (535 mg, 92%).

The above-mentioned linear pentapeptide (511 mg, 0.60 mmol) was dissolved in DMF (40 mL). NMM (83 μL, 0.60 mmol), HOBT (174 mg, 0.91 mmol), and EDCI (174 mg, 0.91 mmol) were added to the mixture at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The mixture was concentrated, and the residue was taken up with water (50 mL), which was extracted with CH_2Cl_2 (30 mL × 3). The combined organic extracts were washed with 10% citric acid, saturated $NaHCO_3$, and brine and dried over $MgSO_4$, and the solvent was evaporated. The residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with EtOAc/hexane (5:1) to give a protected cyclic pentapeptide, **92a** (151 mg, 35%), as a pale yellow powder.

92a (148 mg, 0.21 mmol) was dissolved in MeOH (5 mL), and 1 N NaOH (1.0 mL, 1.0 mmol) was added at 0 °C. After being stirred at 0 °C for 1 h, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was concentrated under reduced pressure to remove MeOH, and 1 N HCl was added to the resulting aqueous residue at 0 °C to adjust the pH to 2−3. The precipitate was collected by filtration and dried to give **46** (132 mg, 91%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.37; ^{1}H NMR (400 MHz, DMSO-d_6) δ −0.13−0.03 (3 H, m), 0.30−0.40 (1 H, m), 0.76−0.88 (1 H, m), 1.19−1.70 (9 H, m), 1.70−1.87 (1 H, m), 1.87−2.00 (2 H, m), 2.19−2.30 (1 H, m), 2.32 (1 H, dd, J = 3.9, 16.1 Hz), 2.76 (1 H, dd, J = 10.7, 16.1 Hz), 3.01 (1 H, dd, J = 9.8, 14.7 Hz), 3.08−3.51 (4 H, m), 4.24 (1 H, t, J = 9.8 Hz), 4.30−4.40 (1 H, m), 4.73 (1 H, d, J = 7.3 Hz), 4.90−5.01 (1 H, m), 6.97 (1 H, t, J = 7.6 Hz), 7.06 (1 H, t, J = 7.6 Hz), 7.25 (1 H, d, J = 7.6 Hz), 7.48 (1 H, d, J = 9.8 Hz), 7.68 (1 H, d, J = 7.6 Hz), 7.84 (1 H, d, J = 9.3 Hz), 8.46 (1 H, d, J = 8.8 Hz), 9.07 (1 H, d, J = 4.9 Hz), 11.64 (1 H, s), 12.30 (1 H, brs).

Method G. *cyclo*(-D-Trp(2-Me)-D-Asp-Pro-D-Cpeg-Leu-) (48). (a) Boc-D-Asp(OMe)-Pro-D-Cpeg-Leu-OBzl (93a). EDCI (620 mg, 3.24 mmol) was added to a mixture of H-Leu-OBzl·TosOH (935 mg, 2.38 mmol), Boc-D-Cpeg-OH (540 mg, 2.16 mmol), NMM (0.26 mL, 2.38 mmol), and HOBT (496 mg, 3.24 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and at room temperature for 3 h. The mixture was washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (3:1) to give Boc-D-Cpeg-Leu-OBzl (0.86 g, 89%) as a white solid.

Boc-D-Cpeg-Leu-OBzl (100 mg, 0.224 mmol) was dissolved in 4 N HCl/1,4-dioxane (5 mL), and the solution was stirred at room temperature for 1 h. The solvent was evaporated, and the residue was dissolved in DMF (3 mL). Boc-Pro-OH (53 mg, 0.25 mmol), NMM (25 μL, 0.22 mmol), HOBT (41 mg, 0.27 mmol), and EDCI (52 mg, 0.27 mmol) were added to the solution at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with EtOAc (50 mL), washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (2:1) to give Boc-Pro-D-Cpeg-Leu-OBzl (107 mg, 88%).

The Boc protecting group in Boc-Pro-D-Cpeg-Leu-OBzl (103 mg, 0.19 mmol) was removed by 4 N HCl/1,4-dioxane (room temperature, 1.5 h, 100%). The corresponding primary amine hydrochloride and Boc-D-Asp(OMe)-OH (71 mg, 0.28 mmol) were dissolved in DMF (3 mL). NMM (21 μL, 0.19 mmol), HOBT (44 mg, 0.28 mmol), and EDCI (55 mg, 0.28 mmol) were added to the solution at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with EtOAc (50 mL), washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on silica gel eluted with hexane/EtOAc (1:2)

to give **93a** (127 mg, 100%) as a pale yellow amorphous solid: TLC R_f (hexane:EtOAc = 1:2) 0.38; FAB-MS *m/e* 673 (M + H)$^+$.

(b) Z-D-Trp(2-Me)-D-Asp(OMe)-Pro-D-Cpeg-Leu-OBzl (94a). The Boc protecting group in **93a** (125 mg, 0.19 mmol) was removed by 4 N HCl/1,4-dioxane (room temperature, 1.5 h, 115 mg, 100%). An aliquot (65 mg, 0.095 mmol) of the corresponding primary amine hydrochloride and Z-D-Trp(2-Me)-OH (**66**; 37 mg, 0.10 mmol) were dissolved in DMF (3 mL). NMM (23 μL, 0.21 mmol), HOBT (22 mg, 0.14 mmol), and EDCI (27 mg, 0.14 mmol) were added to the solution at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with EtOAc (50 mL), washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and concentrated. The residue was purified by preparative TLC (hexane/EtOAc = 1:4) to give **94a** (62 mg, 72%) as a pale yellow amorphous solid: TLC R_f (hexane:EtOAc = 1:5) 0.35; FAB-MS *m/e* 907 (M + H)$^+$.

(c) *cyclo*(-D-Trp(2-Me)-D-Asp-Pro-D-Cpeg-Leu-) (48). A mixture of **94a** (60 mg, 0.066 mmol) and 10% Pd/C (40 mg) in DMF (3 mL) was stirred under an atmospheric pressure of hydrogen for 1.5 h. The catalyst was removed by filtration and washed with DMF (10 mL). EDCI (19 mg, 0.099 mmol) and HOBT (15 mg, 0.099 mmol) were added to the combined filtrate and washings at 0 °C. The resulting mixture was stirred at 0 °C for 1 h and at room temperature overnight. The mixture was concentrated, and the residue was taken up with EtOAc (30 mL), which was washed with saturated $NaHCO_3$, 1 N HCl, and brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by preparative TLC (hexane/EtOAc = 1:5) to give *cyclo*(-D-Trp-(2-Me)-D-Asp(OMe)-Pro-D-Cpeg-Leu-) (**95a**; 19 mg, 43%).

95a (15.5 mg, 0.023 mmol) was dissolved in MeOH (1 mL), and 1 N NaOH (0.5 mL, 0.5 mmol) was added at 0 °C. After being stirred at 0 °C for 4 h, the mixture was concentrated under reduced pressure to remove MeOH; 1 N HCl was added to the residue. The precipitate was collected by filtration and dried to give **48** (12.7 mg, 84%) as a white powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 30:1:1) 0.35; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.61 (3 H, d, J = 6.0 Hz), 0.69 (3 H, d, J = 6.0 Hz), 0.98−2.02 (15 H, m), 2.17−2.50 (2 H, m), 2.32 (3 H, s), 2.80 (1 H, dd, J = 10.5, 16.0 Hz), 2.88 (1 H, dd, J = 10.6, 14.7 Hz), 3.05−3.50 (3 H, m), 3.92−4.05 (1 H, m), 4.19 (1 H, t, J = 10.0 Hz), 4.27−4.39 (1 H, m), 4.73 (1 H, d, J = 7.1 Hz), 4.91−5.03 (1 H, m), 6.87 (1 H, t, J = 7.6 Hz), 6.94 (1 H, t, J = 7.6 Hz), 7.18 (1 H, d, J = 7.6 Hz), 7.48 (1 H, d, J = 7.6 Hz), 7.51 (1 H, d, J = 10.0 Hz), 7.76 (1 H, d, J = 8.7 Hz), 8.67 (1 H, d, J = 9.0 Hz), 8.78 (1 H, d, J = 5.0 Hz), 10.67 (1 H, s).

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Supporting Information Available: List of synthetic methods, melting points, high-resolution FAB mass spectra, and HPLC data of all final compounds and ^{1}H NMR data of target compounds not reported in the text (15 pages). Ordering information is given on any current masthead page.

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