Structure−Activity Relationships of Cyclic Pentapeptide Endothelin A Receptor Antagonists†

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Analogues of the natural product endothelin A (ET_A) receptor antagonists cyclo(-D-Trp^1-D-Glu^2-Ala^3-D-Val^4-Leu^5-) (**1**) and cyclo(-D-Trp^1-D-Glu^2-Ala^3-D-alloIle^4-Leu^5-) (**2**) were prepared and tested for inhibitory activity against [^{125}I]endothelin (ET-1) binding to protein ET_A receptors. The DDLDL chirality sequence of the natural products appeared to be critical for inhibitory activity because conversion of either D-Trp or D-Glu (or both) in **1** to the corresponding L-isomer(s) abolished this property. Systematic modifications at each position of the natural products clarified the structure−activity relationships and led to highly potent and selective ET_A receptor antagonists. Most replacements of D-Trp^1 and Leu^5 with other amino acids caused a significant loss of inhibitory activity. In contrast, replacement of D-Glu^2 with D-Asp^2 enhanced the activity. With regard to the Ala^3 position, all analogues with imino acids, independent of being cyclic or acyclic, showed higher affinities than did the amino acid analogues. In addition, most replacements with amino acids, which had various functional groups in their side chains, did not significantly modify ET_A binding affinity. The D-Val^4/D-alloIle^4 position was very important for inhibitory activity, and a β-position branched D-amino acid or a D-heteroarylglycine was preferable at this position. Among synthesized cyclic pentapeptides, compound **36** (BQ-518) was the most potent ET_A receptor antagonist, with a pA_2 of 8.1 against ET-1-induced vasoconstriction in isolated porcine coronary arteries. This compound also showed the greatest selectivity between ET_A and ET_B receptors (IC_{50} for human ET_A = 1.2 nM, IC_{50} for human ET_B = 55 μM). In contrast, compound **8** (BQ-123) is a highly soluble, potent, and selective ET_A receptor antagonist (pA_2 = 7.4, IC_{50} for human ET_A = 8.3 nM, IC_{50} for human ET_B = 61 μM). The sodium salt of **8** is practically freely soluble in saline. These compounds are useful tools for not only in vitro but also in vivo pharmacological studies.

Introduction

Endothelin (ET-1), which was first isolated from the culture medium of porcine aortic endothelial cells, is a potent vasoconstrictor consisting of 21 amino acids and belongs to a new peptide class.[1] ET-1 is some 10-fold more potent than the vasoconstrictor angiotensin II and has extremely long-lasting pressor effects. Studies including a human genomic analysis have identified two structurally- and functionally-related isopeptides of ET-1 termed ET-2 and ET-3.[2−4] Several studies have demonstrated that there are at least two different ET receptor subtypes: namely, the ET_A receptor, which is highly specific for ET-1 and ET-2, and the ET_B receptor, which has almost equal affinity for all three isopeptides.[5−7] Since these discoveries, evidence has accumulated that anti-ET agents may provide a novel therapy for the treatment of patients with hypertension,[8−10] pulmonary hypertension,[11−13] cerebral vasospasm,[14,15] acute renal failure,[16] asthma,[17] and several other important diseases (for review, see ref 18).

ET_A-selective receptor binding inhibitors **1** and **2** (BE-18257A and B) were isolated from *Streptomyces misakiensis* in our laboratories.[19−21] Their structures were deduced to be novel cyclic pentapeptides with the DDLDL chirality sequence of cyclo(-D-Trp^1-D-Glu^2-Ala^3-D-Val^4-Leu^5-) for 1 and cyclo(-D-Trp^1-D-Glu^2-Ala^3-D-alloIle^4-Leu^5-) for **2**.[22] Compounds **1** and **2** inhibit [^{125}I]ET-1 binding to porcine aortic smooth muscle membranes that are rich in ET_A receptors with IC_{max50} values of 3.0 and 1.4 μM, respectively. In contrast, these compounds barely inhibit [^{125}I]ET-1 binding to porcine cerebellum membranes that contain ET_B receptors exclusively, even at a concentration of 100 μM. Furthermore, compound **2** has been shown to antagonize ET-1-induced vasoconstriction with a pA_2 value of 5.9.[19] Although these natural products are attractive as ET_A-selective antagonists, their moderate potency and poor water solubility[23] (0.21 mg/mL saline as a sodium salt)[24] limit their usefulness as a tool in pharmacological studies. There-

† Abbreviations follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides (*Eur. J. Biochem.* **1984**, *138*, 9−37). Additional abbreviations are defined in the text or as follows: Ama, 2-aminomalonic acid; D-Aad, D-2-aminoadipic acid; D-Sal, D-3-sulfoalanine; D-Hse, D-homoserine; D-NFK, D-*N'*-formylkynurenine; D-Phe, D-penicillamine; D-*tert*-Leu, D-2-amino-3,3-dimethylbutyric acid; D-Cpg, D-2-cyclopentylglycine; D-Phg, D-2-phenylglycine; D-Dpg, D-2-(1,4-cyclohexadienyl)glycine; D-Thg, D-2-(2-thienyl)glycine; MeLeu, *N*-methyl-L-leucine; Cha, L-3-cyclohexylalanine; Aib, α-aminoisobutyric acid; MeAla, *N*-methyl-L-alanine; Sar, sarcosine; Pip, L-pipecolinic acid; Thz, (*R*)-thiazolidine-4-carboxylic acid; Boc, *tert*-butoxycarbonyl; *Z*, benzyloxycarbonyl; Fmoc, fluorenylmethoxycarbonyl; Me, methyl; Et, ethyl; ^{t}Bu, *tert*-butyl; Bzl, benzyl; MBzl, 4-methoxybenzyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Pac, phenacyl; Pfp, pentafluorophenyl; Dhbt, dihydrooxobenzotriazine; AcOH, acetic acid; TosOH, *p*-toluenesulfonic acid; EtOAc, ethyl acetate; TFA, trifluoroacetic acid; DMF, *N,N*-dimethylformamide; DCC, dicyclohexylcarbodiimide; DIPC, diisopropylcarbodiimide; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole monohydrate; DMAP, 4-(dimethylamino)pyridine; TEA, triethylamine; NMM, *N*-methylmorpholine; EDT, 1,2-ethanedithiol; TMSOTf, trimethylsilyl trifluoromethanesulfonate; HPLC, high-performance liquid chromatography; t_R, retention time.

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Table 1. Experimental ^{1}H NMR Data for Compound 1 in DMSO-d_6 Solution[a]

residue	chemical shift (δ, ppm)	^3J(HNCαH) (Hz)	dihedral angle ϕ^b (deg)	$\Delta\delta_{NH}/\Delta T^c$ (ppb/K)
D-Trp	8.75	8.4	−75 to −45, 85−95, 145−155	7.0
D-Glu	7.39	7.3	−85 to −35, 80−90, 155−160	1.3
L-Ala	8.72	8.0	−155 to −145, −95 to −80, 40−80	7.9
D-Val	7.51	9.2	90−105, 135−150	2.3
L-Leu	8.55	6.5	−165 to −155, −85 to −75, 25−45, 75−95	6.0

[a] Spectra were recorded at 298 K and 300 MHz unless otherwise noted. [b] Derived from a Karplus curve (see ref 28). The values consistent with the β,γ-conformation are underlined. [c] Values were measured among 303, 323, and 343 K.

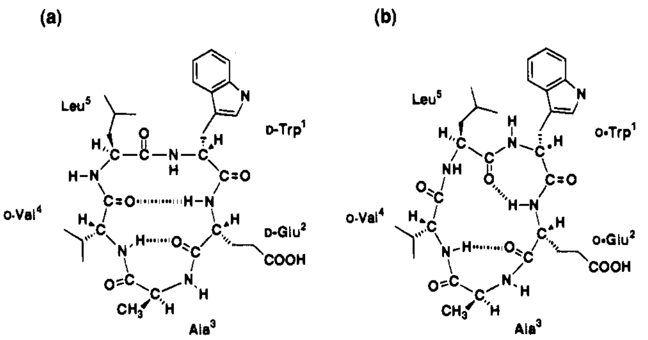
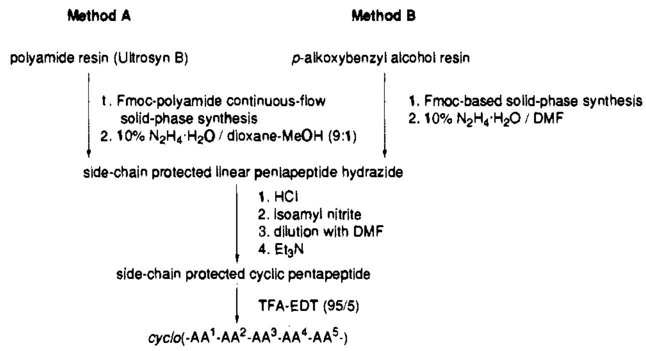


Figure 1. Two possible backbone conformations of compound 1. (a) The β,γ-backbone conformation, which is consistent with both NH shift temperature dependence data and ROESY data. (b) The γ,γ-backbone conformation, which is not consistent with the ROESY data.

Scheme 1. Methods A and B



fore, we attempted modifications of these lead peptides to identify more potent and more soluble ET_A antagonists. A preliminary account of these studies has been reported.[25] In this paper, we describe the experimental details and the additional structure−activity relationships.

Backbone Conformational Analysis

A backbone conformational analysis of **1** was performed using ^{1}H NMR techniques prior to its chemical modification. All of the signals in the spectrum were unambiguously assigned as previously reported.[22] The temperature dependence of each amide proton chemical shift was examined to determine the relative solvent accessibility of the amide hydrogen. The chemical shifts of the amide proton in DMSO-d_6 varied linearly with temperature over a range of 30−70 °C in 20° increments. The temperature coefficients for the amide-NH signals (ppb/K) are listed in Table 1 together with chemical shifts (δ, ppm) for amide protons, vicinal coupling constants ^3J(NHCαH), and all possible values for dihedral angles ϕ (derived from a Karplus curve).[26] The significantly low temperature coefficients for the amide-NH signals of D-Glu^2 and D-Val^4 (1.3 and 2.3, respectively) suggest that these amide protons are involved in intramolecular hydrogen bonding.[27] On the other hand, 2D rotating frame nuclear Overhauser spectroscopy (ROESY) showed close contacts between the D-Trp^1 αNH and the D-Glu^2 αNH, the D-Val^4 αNH and the Ala^3 αCH, the Leu^5 αNH and the D-Val^4 αCH, the Ala^3 αNH and the D-Glu^2 αCH, and the D-Trp^1 αNH and the Leu^5 αCH, as previously described.[22] It has been reported that two basic types of backbone conformation, each with two intramolecular hydrogen bonds, a β,γ- and a γ,γ-backbone conformation, can be distinguished in cyclic pentapeptides.[27] The β,γ-conformation (Figure 1a) is consistent with both NH shift temperature dependence data and ROESY data. In contrast, the γ,γ-conformation (Figure 1b) is not consistent with the ROESY data, although it is consistent with the NH shift temperature dependence data. The dihedral angles ϕ consistent with the β,γ-conformation are underlined in Table 1. The values for the dihedral angles of Leu, D-Trp (i+1 and i+2 of the β-turn, respectively), and Ala (i+1 of the γ-turn) are −85° to −75°, 85−95°, and −95° to −80°, respectively, indicating that compound **1** has a type II β-turn in the D-Val^4-Leu^5-D-Trp^1-D-Glu^2 region and an inverse γ-turn in the D-Glu^2-Ala^3-D-Val^4 region.

Synthesis

The method for preparing most of the cyclic pentapeptides involved formation of precursor side chain-protected linear peptide hydrazides, conversion to acyl azides (by the method of Rudinger),[28] cyclization in a manner analogous to that described by Veber,[29] and deprotection of side chain-protecting groups. The precursor side chain-protected peptide hydrazides were synthesized in a manual synthesizer (Biolynx 4175; Pharmacia LKB Biochrom, Ltd., Cambridge, England) using the Fmoc-polyamide continuous-flow solid-phase methodology[30] (Scheme 1, method A) or synthesized manually by Fmoc-based solid-phase synthesis[31] on a p-alkoxybenzyl alcohol resin[32] (Scheme 1, method B). The first residue was attached to the resin by reaction with an N^{α}-Fmoc-protected amino acid symmetrical anhydride catalyzed by 4-(dimethylamino)pyridine (DMAP). Deprotection of the Fmoc group was accomplished with 20% piperidine in N,N-dimethylformamide (DMF). Subsequent N^{α}-Fmoc-protected (and side chain functional group-protected) amino acid residues were coupled as symmetrical anhydrides (prepared from the protected amino acids and diisopropylcarbodiimide (DIPC) *in situ*), as pentafluorophenyl (Pfp) or dihydrooxobenzotriazine (Dhbt) esters. The final residue was incorporated as an N^{α}-Fmoc-protected (and side chain-protected) amino acid residue, and the N^{α}-Fmoc group was deprotected under standard conditions before cleavage from the resin by hydrazinolysis (10% hydrazine hydrate in dioxane/methanol (9:1) or DMF). The side chain-protected acyl hydrazide was converted to the corresponding acyl azide using isoamyl nitrite at pH

Scheme 2. Synthesis of Compound **10**[a]

TsOH • H-Leu-OBzl
↓ i
Boc-D-Val-Leu-OBzl **66**
↓ ii, iii
Boc-Pro-D-Val-Leu-OBzl **67**
↓ ii, iv
Boc-D-Sal-Pro-D-Val-Leu-OBzl **68**
↓ ii, v
Boc-D-Trp-D-Sal-Pro-D-Val-Leu-OBzl **69**
↓ vi
Boc-D-Trp-D-Sal-Pro-D-Val-Leu-$NHNH_2$ **70**
↓ vii
H-D-Trp-D-Sal-Pro-D-Val-Leu-$NHNH_2$ **71**
↓ viii, ix, x, xi
cyclo(-D-Trp-D-Sal(ONa)-Pro-D-Val-Leu-) **10**

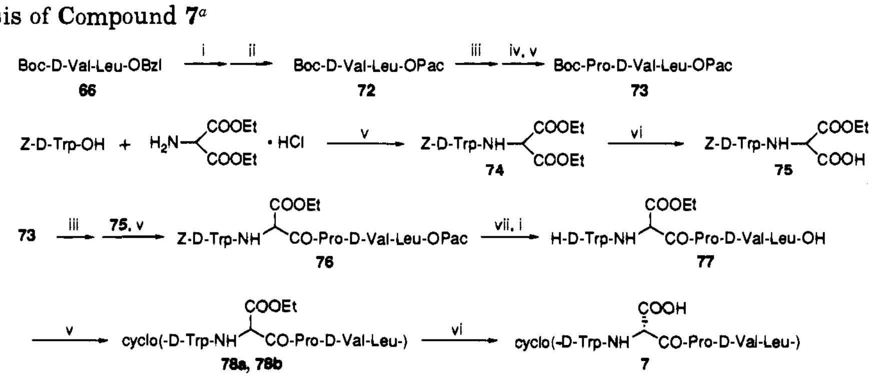
[a]Reagents: (i) EDCI/HOBT/Boc-D-Val-OH; (ii) TFA; (iii) EDCI/HOBT/NMM/Boc-Pro-OH; (iv) EDCI/HOBT/NMM/Boc-D-Sal(ONa)-OH; (v) EDCI/HOBT/NMM/Boc-D-Trp-OH; (vi) hydrazine hydrate/DMF; (vii) TFA−EDT (95:5); (viii) HCl/isoamyl nitrite/−30 to −20 °C; (ix) dilution with DMF/−60 °C; (x) triethyl amine/−60 to −20 °C/overnight; (xi) ion-exchange resin columns (first, amberlite IR-120B H^+-form; second, amberlite IRC-50 Na^+-form).

2−3 at −30 to −20 °C. After dilution with precooled DMF, triethylamine (TEA) was added to liberate the free amino (pH 8−9, below −60 °C), which allowed spontaneous cyclization (−20 °C). Deprotection of the side chain-protected cyclic pentapeptides with trifluoroacetic acid (TFA)/1,2-ethanedithiol (EDT) (95:5) yielded the desired cyclic pentapeptides. Usually, the fifth position of the amino acid residues of the cyclic pentapeptides was chosen for the first residues of the solid-phase syntheses. In the case of **38**, however, when Pro^5 was chosen for the first residue of the solid-phase synthesis, rapid loss of D-Val-Pro dipeptide from the resin was observed after Fmoc deprotection of the D-Val residue, presumably by diketopiperazine formation. In this case, the third residue of the cyclic pentapeptide, Ala, was chosen for the first residue of the solid-phase synthesis. In the case of the (*R*)-thiazolidine-4-carboxylic acid (Thz)-containing analogue **64**, the coupling of Fmoc-D-Asp(O^tBu) to the Thz residue was difficult to accomplish by the usual methods (Fmoc-D-Asp(O^tBu)/DIPC/HOBT or Fmoc-D-Asp(O^tBu)-OPfp/HOBT) because of the low nucleophilicity of the α-imino group of the D-Thz residue. For this reason, Fmoc-D-Asp(O^tBu) was coupled as the symmetrical anhydride in the presence of DMAP as a catalyst. The 3-sulfo-L-alanine (Sal)-containing peptide hydrazide for **49** was also prepared in a solid phase. The Sal residue was introduced to the peptide as a tetrabutylammonium salt of Fmoc-Sal according to the reported method.[33]

The D-Sal-containing compound **10** was synthesized in an alternative liquid phase as illustrated in Scheme 2. The N-terminal Boc-protected linear pentapeptide benzyl ester **69** was prepared using a stepwise elongation method. The Boc group was employed as an amino-protecting group. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) and HOBT were used in coupling reactions. Deprotection of the Boc was accomplished with TFA. The linear pentapeptide **69** was treated with hydrazine hydrate in DMF to afford the N-terminal-protected peptide hydrazide **70.** The Boc group was removed by TFA containing 5% EDT. The resulting N-terminal unprotected peptide hydrazide **71** was cyclized by the same procedures as in method A to provide **10** (in 24% total yield), which was isolated as a sodium salt by passage through cation-exchange resin columns.

The synthesis of an aminomalonic acid (Ama)-containing analogue, **7**, is illustrated in Scheme 3. Diethyl aminomalonate was coupled with Z-D-Trp to produce the dipeptide diethyl ester **74** (93%), which was hydrolyzed with 1 equiv of NaOH to give the dipeptide monoethyl ester **75** (88%) as a mixture of two diastereoisomers. The monoethyl ester **75** was coupled with the N-terminal-deprotected tripeptide Pac ester derived from **73** to afford the N- and C-terminal-protected linear pentapeptide **76** (36%) as a mixture of two diastereoisomers, which were inseparable on silica gel chromatography. Deprotection of Pac and Z groups by Zn/AcOH and catalytic hydrogenation, followed by cyclization by EDCI and HOBT, yielded two cyclic pentapeptide ethyl esters **78a,b**, in total yields of 26% and 19%, respectively. Alkaline hydrolysis of **78a** yielded the cyclic pentapeptide **7** in an 87% yield. Alkaline hydrolysis of **78b**, the diastereoisomer of **78a**, also yielded the same cyclic pentapeptide, **7**, in an 80% yield; the corresponding diastereoisomer of **7** was not obtained, perhaps because the α-position of the Ama residue in either of the two ester **78a,b** isomerized under the alkaline hydrolysis conditions. The configuration of the Ama residue in **7** was determined to be "D" by ^1H NMR temperature gradient study. Table 2 lists the temperature coefficients for the amide-NH signals (ppb/K) in **7**. The low values of the Ama and D-Val residues (2.0 and 0.50, respectively) indicate that these amide protons are involved in intramolecular hydrogen bonds. This pattern of intramolecular hydrogen bonds is found in **1**, which has a DDLDL chirality sequence as mentioned

Scheme 3. Synthesis of Compound **7**[a]



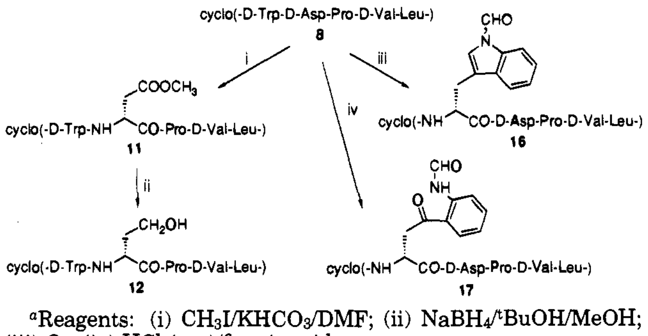
[a]Reagents: (i) H_2/Pd−C; (ii) Cs_2CO_3/phenacyl bromide; (iii) TFA; (iv) Boc-Pro; (v) NMM/EDCI/HOBT; (vi) NaOH (aq); (vii) zinc powder/90% AcOH.

Table 2. Experimental ^{1}H NMR Data for Compound **7** in DMSO-d_6 Solution[a]

residue	chemical shift (δ, ppm)	^3J(HNCαH) (Hz)	$\Delta\delta_{NH}/\Delta T$[b] (ppb/K)
D-Trp	8.99	7.3	5.5
Ama	7.99	8.8	2.0
D-Val	7.50	7.8	0.50
L-Leu	8.69	5.4	5.0

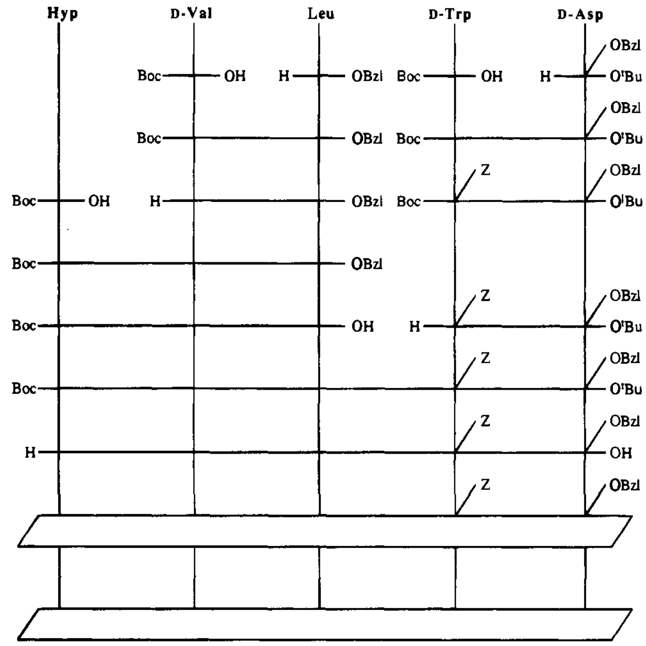
[a] Spectra were recorded at 298 K and 300 MHz unless otherwise noted. [b] Values were measured among 303, 323, and 343 K.

Scheme 4. Syntheses of compounds **11**, **12**, **16**, and **17**[a]



[a]Reagents: (i) $CH_3I/KHCO_3/DMF$; (ii) $NaBH_4/{}^tBuOH/MeOH$; (iii) O_3; (iv) HCl (gas)/formic acid.

Chart 1. Synthesis of Compound **65**

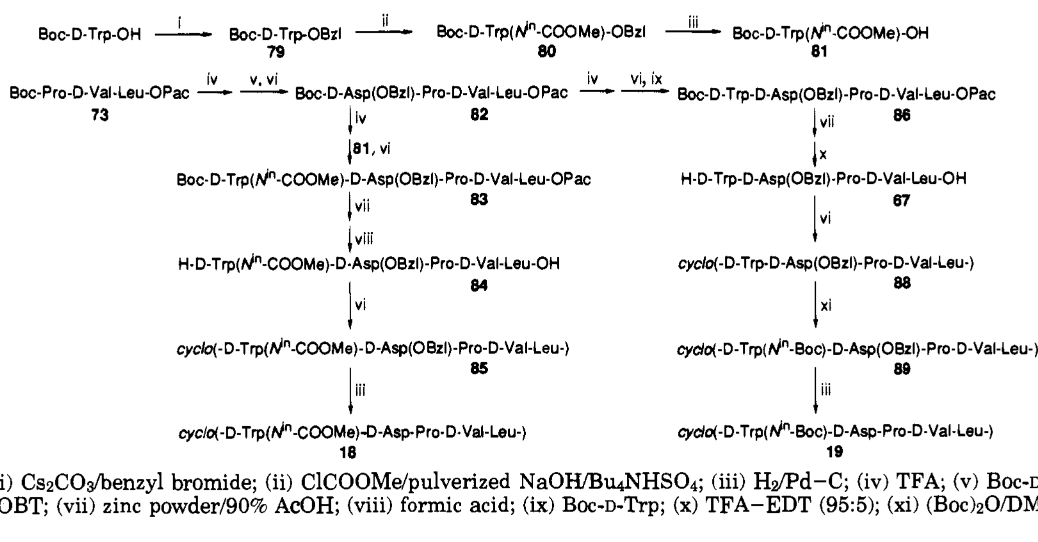


above, but is not found in **3**, which has a DLLDL chirality sequence as discussed later. These findings strongly suggest that **7** has a DDLDL chirality sequence; namely, the configuration of the Ama residue is "D".

Compounds **11**, **12**, **16**, and **17** were derived from compound **8** as illustrated in Scheme 4. Treatment of **8** with iodomethane and $KHCO_3$ in DMF gave the methyl ester analogue **11** in a 58% yield. Reduction of **11** with sodium borohydride in ^{t}BuOH and MeOH[34] afforded the D-homoserine (D-Hse) analogue **12** in a 34% yield. Ozone oxidation[35] of formylation[36] of the indolyl group in **8** afforded the D-*N'*-formylkynurenine (D-NFK) analogue **16** or the D-N^{in}-formyltryptophan analogue **17** in yields of 60% and 51%, respectively.

The synthesis of **18** and **19**, both of which are analogues of N^{in}-modified D-tryptophan, is illustrated in Scheme 5. For the synthesis of the N^{in}-COOMe analogue **18**, the N^{in}-COOMe group was introduced as Boc-D-Trp(N^{in}-COOMe) (**81**). Preparation of **81** was carried out in a manner analogous with that of N^{in}-[(2,2,2-trichloroethoxy)carbonyl]tryptophan.[37] Namely, Boc-D-Trp-OBzl (**79**) prepared from Boc-D-Trp (in a 94% yield) by the cesium salt method[38] was allowed to react with methyl chloroformate in the presence of NaOH and catalytic amounts of tetrabutylammonium hydrogen sulfate to produce **80** (90%), which was subsequently hydrogenated to give **81** in a quantitative yield. Compound **81** was coupled with the N-terminal-deprotected tetrapeptide Pac ester derived from **82** to give the N- and C-terminal-protected linear pentapeptide **83** (84%). Deprotection of the Boc- and Pac-protecting groups of **83** was accomplished by formic acid and Zn/AcOH to give **84** (89%), which was cyclized by EDCI and HOBT to afford the protected cyclic pentapeptide **85** (62%). Catalytic hydrogenation of **85** provided **18** in a 68% yield. In contrast, the N^{in}-Boc analogue **19** was derived from the N^{in}-unmodified D-Trp-containing and side chain-protected cyclic pentapeptides **88**, which is an intermediate for the large-scale synthesis of **8**.[39] Compound **88** was reacted with Boc_2O in the presence of a catalytic amount of DMAP[40] to yield the N^{in}-Boc deriva-

Scheme 5. Syntheses of Compounds **18** and **19**[a]



[a]Reagents: (i) Cs_2CO_3/benzyl bromide; (ii) ClCOOMe/pulverized $NaOH/Bu_4NHSO_4$; (iii) H_2/Pd–C; (iv) TFA; (v) Boc-D-Asp(OBzl); (vi) NMM/EDCI/HOBT; (vii) zinc powder/90% AcOH; (viii) formic acid; (ix) Boc-D-Trp; (x) TFA–EDT (95:5); (xi) $(Boc)_2O/DMAP/CH_3CN$.

Table 3. Physical and Endothelin A Receptor Binding Inhibitory Properties of Cyclo(-AA[1]-AA[2]-AA[3]-AA[4]-Leu[5]-)

compd	AA[1]	AA[2]	AA[3]	AA[4]	IC_{max50} (nM)[a]	method[b]	mp (°C)	FAB-HRMS (MH^+) calcd	FAB-HRMS (MH^+) found	HPLC[c] t_R (min)
1[d]	D-Trp	D-Glu	Ala	D-Val	3000					
2[d]	D-Trp	D-Glu	Ala	D-alloIle	1400					
3	D-Trp	L-Glu	Ala	D-Val	>100 000[e]	A	>270	599.3193	599.3168	21.5
4	L-Trp	D-Glu	Ala	D-Val	>100 000[e]	A	>270	599.3193	599.3189	18.0
5	L-Trp	L-Glu	Ala	D-Val	>100 000[e]	A	>270	599.3193	599.3220	15.8
6	D-Trp	D-Glu	Pro	D-Val	410	A	200 dec	625.3350	625.3334	15.6
7	D-Trp	D-Ama[f]	Pro	D-Val	6700	D	225 dec	597.3036	597.3061	13.2
8	D-Trp	D-Asp	Pro	D-Val	22	A	160 dec	611.3193	611.3206	13.8
9	D-Trp	D-Aad[g]	Pro	D-Val	25 000	A	amorphous	639.3506	639.3531	17.3
10	D-Trp	D-Sal[h]	Pro	D-Val	21	C	>270	669.2682[i]	669.2733[i]	9.6
11	D-Trp	D-Asp(OMe)	Pro	D-Val	390	E	144–150	625.3350	625.3372	21.5
12	D-Trp	D-Hse[j]	Pro	D-Val	1700	F	147–152	597.3401	597.3423	15.3
13	D-Phe	D-Asp	Pro	D-Val	>100 000[e]	A	>270	572.3084	572.3167	20.3
14	D-Tyr	D-Asp	Pro	D-Val	15000	A	180–184	588.3033	588.3055	6.9
15	D-His	D-Asp	Pro	D-Val	>100 000[e]	A	185–189	562.2990	562.3002	4.7
16	D-NFK[k]	D-Asp	Pro	D-Val	40 000	G	amorphous	643.3091	643.3077	11.9
17	D-Trp(N^{in}-CHO)	D-Asp	Pro	D-Val	18	H	170 dec	639.3143	639.3109	14.5
18	D-Trp(N^{in}-COOMe)	D-Asp	Pro	D-Val	29	I	164–173	669.3248	669.3275	30.8
19	D-Trp(N^{in}-Boc)	D-Asp	Pro	D-Val	1500	J	176–177	711.3718	711.3699	18.5[l]
20	D-Trp	D-Asp	Ala	D-Val	110	A	>270	585.3037	585.3057	14.8
21	D-Trp	D-Asp	Ala	D-Thr	4200	A	230 dec	587.2830	587.2834	6.9
22	D-Trp	D-Asp	Ala	D-Leu	440	A	245 dec	599.3193	599.3218	24.5
23	D-Trp	D-Asp	Ser	D-Val	190	A	250 dec	601.2986	601.3000	12.5
24	D-Trp	D-Asp	Ser	Gly	35000	A	245 dec	559.2516	559.2539	6.4
25	D-Trp	D-Asp	Ser	D-Asp	>100 000[m]	A	210 dec	617.2571	617.2593	6.2
26	D-Trp	D-Asp	Pro	D-Ala	260	A	175–180	583.2880	583.2958	8.4
27	D-Trp	D-Asp	Pro	D-Nva	47	A	156 dec	611.3193	611.3193	15.0
28	D-Trp	D-Asp	Pro	D-Nle	120	A	159–165	625.3350	625.3341	25.7
29	D-Trp	D-Asp	Pro	D-alloIle	12	A	190 dec	625.3350	625.3309	21.7
30	D-Trp	D-Asp	Pro	D-Ile	64	A	149–153	625.3350	625.3365	21.4
31	D-Trp	D-Asp	Pro	D-Pen[n]	49	A[o]	185–190	643.2914	643.2894	15.6
32	D-Trp	D-Asp	Pro	D-*tert*-Leu[p]	15	B	212–219	625.3350	625.3370	21.0
33	D-Trp	D-Asp	Pro	D-Cpg[q]	4.0	B	199–203	637.3350	637.3358	26.2
34	D-Trp	D-Asp	Pro	D-Phg[r]	24	A	185–189	645.3036	645.3015	15.6
35	D-Trp	D-Asp	Pro	D-Dpg[s]	19	B	258–261	647.3193	647.3165	21.8
36	D-Trp	D-Asp	Pro	D-Thg[t]	3.0	A	251 dec	651.2601	651.2617	13.9

[a] Half-maximal inhibition concentrations for the high-affinity sites of porcine aortic smooth muscle membranes. Values represent the average of more than three independent experiments unless otherwise noted. [b] See the Experimental Section for description of general methods. [c] Analytical HPLC was performed on a Capcell Pak C18 column (4.6 × 250 mm, 5-μm particle size; Shiseido Co., Ltd.). Solvent system was $MeOH/H_2O$ (45:55) with 0.1% TFA, and flow rate was 0.85 mL/min unless otherwise noted. [d] Natural product [e] $n = 1$. [f] D-Ama = D-2-aminomalonic acid. [g] D-Aad = D-2-aminoadipic acid. [h] D-Sal = D-3-sulfoalanine. [i] $(M + Na)^+$. [j] D-Hse = D-homoserine. [k] D-NKF = D-N'-formylkynurenine. [l] Solvent system was $MeOH/H_2O$ (70:30) with 0.1% TFA. [m] $n = 2$. [n] D-Pen = D-penicillamine. [o] TMSOTf–thioanisole–EDT/TFA (ref 57) was employed for the final deprotection because the S-MBzl-protecting group of the D-Pen was not cleaved completely by TFA/EDT (95:5). [p] D-*tert*-Leu = D-2-amino-3,3-dimethylbutyric acid. [q] D-Cpg = D-2-cyclopentylglycine. [r] D-Phg = D-2-phenylglycine. [s] D-Dpg = D-2-(1,4-cyclohexadienyl)glycine. [t] D-Thg = D-2-(2-thienyl)glycine.

tive **89** (81%). Deprotection of the D-Asp side chain benzyl by catalytic hydrogenation provided **19** in a 52% yield.

Compound **65** was synthesized by a solution-phase strategy similar to that of the natural product **1** as illustrated in Chart 1.[22] The linear pentapeptide, in which the indolyl group in the D-Trp residue and the β-carboxyl group in the D-Asp residue were protected by benzyloxycarbonyl and benzyl groups, respectively, but in which the hydroxyl group in the Hyp residue was unprotected, was cyclized by EDCI and HOBT to afford the side chain-protected cyclic pentapeptide (74%), which was subsequently hydrogenated to give **65** in a quantitative yield.

Homogeneity of the final products was confirmed by TLC and HPLC, and structural integrity was assessed by ^1H NMR and high-resolution fast atom bombardment (FAB) mass spectrometry.

Biological Results and Discussion

All cyclic pentapeptide analogues prepared were first evaluated for their inhibitory activities against [^{125}I]ET-1 binding to porcine aortic smooth muscle membranes that are rich in ET_A receptors, according to the prepared method.[19] Half-maximal inhibitory concentrations (IC_{max50}s) for the high-affinity sites are regarded as the ET_A receptor binding affinities of the compounds and are listed in Tables 3 and 4.

We first focused on the D-Trp[1] and D-Glu[2] positions in the natural products because we suspected that these two areas mimic the C-terminal Trp of ET-1, which has been reported to be very important for its biological activities.[41,42] Initially, we noted that the side chains of D-Trp[1] and D-Glu[2] in the natural products fit the same pockets in the ET_A receptor as those fitted by the C-terminal L-Trp in native ET. We conjectured that replacement of either D-Trp or D-Glu (or both) in **1** with the corresponding L-isomer(s) might enhance ET_A binding affinity. However, compounds **3–5** showed no affinity for ET_A receptors. The decreased affinities can be explained by changes in the backbone conformation of the peptides, which is supported by ^1H NMR temperature gradient studies. Table 5 lists the temperature coefficients for amide-NH signals in analogues **3–5**, together with chemical shifts and vicinal coupling constants. Temperature coefficients for the amide-NH resonances of L-Glu[2] and D-Val[4] in **3** (4.08 and 8.36, respectively), D-Glu[2] and D-Val[4] in **4** (5.59 and 7.70,

Table 4. Physical and Endothelin A Receptor Binding Inhibitory Properties of Cyclo(-D-Trp^1-D-Asp^2-AA^3-D-Val^4-AA^5-)

compd	AA^3	AA^5	IC_{max50} (nM)[a]	method[b]	mp (°C)	FAB-HRMS (MH$^+$) calcd	FAB-HRMS (MH$^+$) found	HPLC[c] t_R (min)
37	Asp	Leu	180	A	270 dec	629.2935	629.2946	11.8
38	Ala	Pro	8500	A	186 dec	569.2724	569.2737	6.5
39	Pro	MeLeu[d]	35	A	208 dec	625.3350	625.3386	20.2
40	Asp	Ala	2800	A	>270	587.2466	587.2461	5.1
41	Pro	Nva	33	B	175–176	597.3036	597.3052	9.9
42	Pro	Nle	62	A	164–166	611.3193	611.3198	14.4
43	Pro	Ile	230	A	240 dec	611.3193	611.3206	13.6
44	Ala	Cha[e]	5600	A	225 dec	625.3350	625.3358	46.2
45	Gly	Leu	160	A	250 dec	571.2880	571.2917	13.6
46	Thr	Leu	270	A	>270	615.3143	615.3181	14.9
47	Gln	Leu	160	A	>270	642.3252	642.3218	11.4
48	Glu	Leu	140	A	270 dec	643.3091	643.2972	13.2
49	Sal[f]	Leu	140	A	>270	687.2424[g]	687.2468[g]	8.9
50	Orn	Leu	250	A	219 dec	628.3459	628.3448	10.0
51	Lys	Leu	200	A	230 dec	642.3616	642.3522	10.7
52	Arg	Leu	150	A	235 dec	670.3677	670.3700	10.6
53	Leu	Leu	160	B	>270	627.3506	627.3529	27.4
54	Val	Leu	530	B	>270	613.3350	613.3393	20.9
55	Cys	Leu	240	A	245 dec	617.2755	617.2762	17.4
56	Met	Leu	130	A	>270	645.3070	645.3076	22.6
57	Phe	Leu	210	B	>270	661.3350	661.3354	35.7
58	Trp	Leu	150	A	>270	700.3459	700.3422	32.6
59	His	Leu	240	A	235 dec	651.3255	651.3235	10.0
60	Aib[h]	Leu	330	A	169–175	599.3193	599.3179	16.0
61	MeAla[i]	Leu	34	A	230 dec	599.3193	599.3198	13.5
62	Sar[j]	Leu	32	A	175–179	585.3037	585.3066	11.1
63	Pip[k]	Leu	13	B	>270	625.3350	625.3396	16.2
64	Thz[l]	Leu	20	A	205–208	629.2757	629.2749	13.5
65	Hyp	Leu	18	K	188–195	627.3143	627.3159	10.5

[a] Half-maximal inhibition concentrations for the high-affinity sites of porcine aortic smooth muscle membranes. Values represent the average of more than three independent experiments unless otherwise noted. [b] See the Experimental Section for description of general methods. [c] Analytical HPLC was performed on a Capcell Pak C18 column (4.6 × 250 mm, 5-μm particle size; Shiseido Co., Ltd.). Solvent system was MeOH/H_2O (45:55) with 0.1% TFA, and flow rate was 0.85 mL/min unless otherwise noted. [d] MeLeu = *N*-methyl-L-leucine. [e] Cha = 3-cyclohexyl-L-alanine. [f] Sal = 3-sulfo-L-alanine. [g] (M + Na)$^+$. [h] Aib = α-aminoisobutyric acid. [i] MeAla = *N*-methyl-L-alanine. [j] Sar = sarcosine. [k] Pip = L-pipecolinic acid. [l] Thz = (*R*)-thiazolidine-4-carboxylic acid.

Table 5. ^{1}H NMR Data for the Amide-NH Signals of Cyclo(-D-Trp^1-D-Asp^2-AA^3-D-Val^4-Leu^5-) in DMSO-d_6 Solution[a]

compd	AA^3	D-Trp^1 chemical shift δ, ppm	D-Trp^1 ^3J(HNCαH) Hz	D-Asp^2 chemical shift δ, ppm	D-Asp^2 ^3J(HNCαH) Hz	D-Val^4 chemical shift δ, ppm	D-Val^4 ^3J(HNCαH) Hz	Leu^5 chemical shift δ, ppm	Leu^5 ^3J(HNCαH) Hz
20	Ala	8.73	7.8	7.60	7.4	7.37	9.4	8.65	5.6
23	Ser	8.58	8.2	7.69	7.1	7.35	9.3	8.54	6.4
37	Asp	8.76	8.5	7.59	*b*	7.31	*c*	8.66	*b*
45	Gly	8.76	8.5	7.63	7.5	7.37	9.6	8.65	8.1
46	Thr	8.36	8.1	7.78	7.3	7.59	8.6	8.42	6.8
16	Gln	8.71	9.3	7.57	7.6	7.35	9.5	8.63	6.2
48	Glu	8.70	8.5	7.54	6.9	7.73	9.4	8.63	6.3
49	Sal	8.66	8.8	7.55	7.2	7.38	9.4	8.57	6.9
50	Orn	8.68	8.1	7.60	6.6	7.38	9.0	8.60	6.0
51	Lys	8.68	8.7	7.58	7.1	7.36	9.2	8.64	6.3
52	Arg	8.68	8.1	7.58	7.4	7.41	9.4	8.59	5.9
53	Leu	8.69	8.3	7.56	7.1	7.41	9.3	8.60	6.1
54	Val	8.51	8.4	7.56	7.1	7.45	9.3	8.45	6.8
55	Cys	8.69	8.1	7.63	7.4	7.33	9.6	8.62	6.7
56	Met	8.74	8.5	7.55	7.4	7.34	9.3	8.65	7.2
57	Phe	8.67	8.5	7.68	7.3	7.39	9.5	8.58	6.4
58	Trp	8.64	8.6	7.69	7.3	7.44	9.8	8.55	6.3
59	His	8.72	8.7	7.64	7.0	7.34	9.3	8.64	5.3
60	Aib	8.80	9.1	7.44	7.6	7.99	8.4	8.72	6.4
mean ± SD		8.67 ± 0.10	8.5 ± 0.4	7.60 ± 0.07	7.2 ± 0.3	7.44 ± 0.16	9.3 ± 0.3	8.60 ± 0.07	6.4 ± 0.6
n		19	19	19	18	19	18	19	18
8	Pro	8.78	7.9	7.71	8.8	7.49	10.3	8.76	4.3
61	MeAla	8.80	8.0	7.67	7.8	6.82	8.9	8.77	5.1
62	Sar	8.76	*b*	7.69	8.7	6.88	10.1	8.76	5.0
63	Pip	8.81	7.0	7.73	9.0	7.73	9.0	8.82	4.2
64	Thz	8.80	8.3	7.83	9.8	7.01	9.8	8.81	4.7
65	Hyp	8.78	8.1	7.75	9.3	7.40	10.3	8.74	5.1
mean ± SD		8.79 ± 0.02	7.9 ± 0.5	7.73 ± 0.05	8.9 ± 0.6	7.22 ± 0.34	9.7 ± 0.6	8.78 ± 0.03	4.7 ± 0.4
n		6	5	6	6	6	6	6	6

[a] Spectra were recorded at 298 K and 300 MHz. [b] These values could not be identified because of broadening of the signals. [c] This value could not be identified because of overlap with the D-Trp H7 signal.

respectively), and L-Glu^2 and D-Val^4 in **5** (3.45 and 7.82, respectively) are relatively high compared with those of D-Glu^2 and D-Val^4 in **1**. These data suggest that the amide protons of D-(or L-)Glu^2 and D-Val^4 in **3**–**5** are not shielded from solvent and may have an external orientation, whereas the amide protons of D-Glu^2 and D-Val^4 in **1** are involved in intramolecular hydrogen bonding. These findings clearly indicate that analogues **3–5** do not possess the two intramolecular hydrogen bonds found in **1**; namely, these analogues do not adopt the β,γ-backbone conformation in **1**. These chirality conversions cause conformational changes in peptides that are not preferable for the affinity, suggesting that the DDLDL chirality sequence is very important for binding affinity. The chirality sequence was therefore retained throughout the following extensive modifications.

Compounds **6–12** represent modifications at the D-Glu^2 position of the natural products. In these analogues, Pro was incorporated as the third residue because Pro^3 analogues exhibit more potent ET_A binding affinity and higher aqueous solubility than those of the corresponding Ala^3 analogues, as reported in our previous paper.[25] Compounds **6–9** are analogues of acidic D-amino acids that have carboxyl or carboxylalkyl groups as their side chains. In these analogues, ET_A binding affinity was clearly dependent on the length of the alkyl chain between the carboxyl group and the α-carbon of the amino acid residue. Maximum affinity was obtained with the analogue of D-Asp ($n = 1$). The analogue with the shorter length ($n = 0$, the D-Ama analogue) showed decreased affinity, and those with longer lengths ($n = 2$ and 3, the D-Glu and D-Aad analogues, respectively) also showed lower affinities. Compounds **10–12** represent replacements of the carboxyl group of D-Asp^2 in compound **8** with sulfo, methoxycarbonyl, and hydroxymethyl groups, respectively. Replacement with a sulfo group resulted in affinity almost equal to that of **8**. Replacement with a methoxycarbonyl group resulted in an 18-fold decrease in affinity, but the analogue was as potent as the D-Glu analogue and more potent than the D-Ama and D-Aad analogues. The hydroxymethyl analogue was much less potent than the methoxycarbonyl analogue but more potent than the D-Ama and D-Aad analogues. These data imply that the presence of an acidic functional group such as a carboxyl or sulfo group at an appropriate distance from the α-carbon of the amino acid residue is very important for ET_A binding affinity.

Compounds **13–19** represent modifications at the D-Trp^1 position of the natural products. Replacement of D-Trp^1 in **8** with D-Phe, D-Tyr, and D-His resulted in significant decreases in binding affinity. In particular, the D-Phe and D-His analogues exhibited almost no affinity even at a concentration of 100 μM, whereas the D-Tyr analogue exhibited weak affinity. These findings imply that both hydrophobicity and hydrogen-bonding ability are required at this position. N'-Formylkynurenin is known to be an oxidative metabolite of tryptophan and seems to possess both hydrophobicity and hydrogen-bonding ability. However, the D-NFK analogue exhibited lower affinity than did the D-Tyr analogue, suggesting that the ET_A receptor strictly discriminated the indole ring structure of the D-Trp residue. We then examined N^{in}-acyl derivatives of the D-Trp residue (**17–19**). The formyl and methoxycarbonyl derivatives were almost equipotent to **8**, suggesting that the NH is not important for the hydrogen bonding. In contrast, the derivative with a more bulky *tert*-butoxycarbonyl exhibited a 100-fold decrease in affinity.

Compounds **8**, **20**, **23**, **37**, and **45–65** represent modifications at the Ala^3 position of the natural products. Compounds **20**, **23**, **37**, and **45–60** are amino acid analogues, and compounds **8** and **61–65** are imino acid analogues. With regard to the amino acid analogues, it is interesting that replacements at this position do not affect ET_A binding affinity significantly. These analogues exhibited ET_A binding affinities in the 100–300 nM range with a few exceptions. The Gly analogue **45** was only 1.5-fold less potent than the Ala analogue **20**. Incorporation of hydrophilic amino acids that had neutral functional groups such as Ser, Thr, and Gln (**23**, **46**, and **47**), acidic functional groups such as Asp, Glu, and Sal (**37**, **48**, and **49**), or basic functional groups such as Orn, Lys, and Arg (**50–52**) in their side chains afforded compounds with similar affinities. The incorporation of moderately hydrophobic amino acids that had an alkyl group such as Leu (**53**) or aromatic functional groups (Phe, Trp, and His **57–59**) in their side chains did not modify the activity significantly. The analogues with Cys or Met (**55** and **56**) also exhibited similar affinities. These findings imply that the side chain at this position does not bind to the ET_A receptor directly. The Val and α-aminoisobutyric acid (Aib) analogues **54** and **60**, respectively, were the exceptions and exhibited relatively weak affinity compared with the other amino acid analogues. The decreased affinities were presumably due to an undesirable conformational change in the backbone caused by the introduction of a sterically-demanding amino acid such as Val or Aib. In contrast, analogues with cyclic amino acids such as Pro, L-pipecolinic acid (Pip), L-thiaproline ((*R*)-thiazolidine-4-carboxylic acid, Thz), and Hyp (**8** and **63–65**) exhibited about 10-fold higher ET_A binding affinities in the 10–30 nM range, compared with those of the amino acid analogues. Analogues with acyclic amino acids such as *N*-methyl-L-alanine (MeAla) and sarosine (Sar) (**61** and **62**, respectively) also exhibited higher affinities than those of the amino acid analogues but were a little less potent than the cyclic amino acid analogues. The higher affinity produced by incorporation of an imino acid is thought to be associated with a change in the backbone conformation of the cyclic pentapeptides because this moiety did not seem to bind to the ET_A receptor as mentioned above. We thought that a change in backbone conformation should be reflected by a change in the dihedral angle ϕ of each amino acid residue, resulting in a change in the vicinal coupling constant ^3J(NHCαH) in the ^{1}H NMR spectrum. We therefore analyzed the ^{1}H NMR data of analogues with AA^3 modification. Table 6 shows the chemical shifts and vicinal coupling constants ^3J(NHCαH) of the amide-NH signals of the analogues. A significant difference was found between amino acid analogues and imino acid analogues in the vicinal coupling constants ^3J(NHCαH) of the Leu^5 and D-Asp^2 residues but not in those of other residues and chemical shifts of amide-NH signals. This evidence strongly suggests that incorporation of imino acids at the AA^3 position affects the backbone conformation of the cyclic pentapeptides. As discussed above, this series of cyclic pentapeptides is thought to adopt a type II β-turn in the D-Val^4-Leu^5-D-Trp^1-D-Asp^2 region and an inverse γ-turn in the

Table 6. Experimental ^1H NMR Data for Compounds **3**–**5** in DMSO-d_6 Solution[a]

compd	residue	chemical shift (δ, ppm)	^3J(HNCαH) (Hz)	$\Delta\delta_{NH}/\Delta T$[b] (ppb/K)
3	D-Trp	8.29	brs[c]	4.93
	L-Glu	8.30	brs[c]	4.08
	L-Ala	7.26	7.1	0.45
	D-Val	8.31	brs[c]	8.36
	L-Leu	7.80	brs[c]	3.33
4	L-Trp	7.27	7.0	1.18
	D-Glu	8.57	7.9	5.59
	L-Ala	7.75	8.8	1.82
	D-Val	8.42	7.4	7.70
	L-Leu	8.67	7.6	6.03
5	L-Trp	7.43	8.3	1.49
	L-Glu	8.36	6.9	3.45
	L-Ala	7.94	8.3	1.69
	D-Val	8.59	7.3	7.82
	L-Leu	8.49	8.1	7.51

[a] Spectra were recorded at 298 K and 300 MHz unless otherwise noted. [b] Values were measured among 303, 323, and 343 K. [c] Broad signal.

D-Asp^2-AA^3-D-Val^4 region, with two intramolecular hydrogen bonds that are formed between D-Val^4 NH and D-Asp^2 C=O, and D-Asp^2 NH and D-Val^4 C=O. The Leu^5 and D-Asp^2 positions are the $i+1$ and $i+3$ positions of the β-turn, respectively. It has been reported that a typical ϕ value of the $i+1$ position of the amino acid residue in the type II β-turn is −60°.[43] The corresponding vicinal coupling constant ^3J(NHCαH) derived from the Karplus curve[26] is 2.8–4.0 Hz. In the Leu^5 residue, the imino acid analogues exhibited ^3J(NHCαH) values of 4.7 ± 0.4 (mean ± SD, n = 6), while the amino acid analogues exhibited relatively large ^3J(NHCαH) values of 6.4 ± 0.6 (mean ± SD, n = 18). The values of the imino acid analogues were much closer to the typical value than those of the amino acid analogues. This observation implies that imino acid analogues adopt a more constrained type II β-turn than do amino acid analogues. As discussed above, incorporation of imino acids at the AA^3 position, although this position is not involved in the β-turn, generates a more constrained type II β-turn in the D-Val^4-Leu^5-D-Trp^1-D-Asp^2 region, which is thought to bind to the ET_A receptor, resulting in increased ET_A binding affinity.

In the course of our earlier work on analogues of natural products, replacement of D-Val^4 in **1** (or D-alloIle^4 in **2**) with a hydrophilic D-amino acid such as D-Thr or D-Asp was done in order to improve solubility. However, replacement with D-Thr caused a 30-fold decrease in affinity (**20** vs **21**); replacement with D-Asp also resulted in a significant loss of affinity (**23** vs **25**). Furthermore, replacement with Gly resulted in a 200-fold decrease in affinity (**23** vs **24**). These data imply that a hydrophobic amino acid is preferable at this position. Compounds **22** and **26**–**30** represent replacements with hydrophobic D-amino acids having normal or branched alkyl side chains. Among analogues with D-amino acids having normal alkyl side chains (**26**–**28**), the D-Nva analogue exhibited the most potent activity, which was, however, 2-fold less than that of the corresponding D-Val analogue **8**. Compounds **22**, **29**, and **30** represent replacements with D-amino acids having branched butyl side chains. The D-Leu analogue **22** was 4-fold less potent than the corresponding D-Val analogue **20**. The D-alloIle analogue **29** was 2 times as potent as the corresponding D-Val analogue **8**, which is in parallel with the activities of the natural products **1** and **2**. In contrast, the D-Ile analogue **30** was 3-fold less potent than **8**. These data imply that side chain branching on the β-carbon is preferable for the affinity while that on the γ-carbon is not and that the ET_A receptor discriminates the stereochemistry on the β-carbon. We then incorporated D-penicillamine (D-Pen), D-*tert*-Leu, and D-cyclopentylglycine (D-Cpg) at this position. The D-Pen analogue **31** was 2-fold less potent than **8**, which might have been due to the presence of a relatively hydrophilic mercapto group at the β-position. The D-*tert*-Leu analogue **32** was as potent as the corresponding D-alloIle **29**, and the D-Cpg analogue **33** was 3-fold more potent than **29**. We further modified this position and incorporated (hetero)arylglycines. The D-phenylglycine (D-Phg) and D-2-(1,4-cyclohexadienyl)glycine (D-Dpg) analogues **34** and **35** exhibited affinity almost equal to that of the D-Val analogue **8**. The D-2-thienylglycine (D-Thg) analogue **36** was the most potent ET_A binding inhibitor (IC_{max50} = 3.0 nM) among this series of cyclic pentapeptides prepared so far and was 500-fold more potent than the natural product **2**.

Compounds **38**–**44** represent modifications at the Leu^5 position of the natural products. The Pro^5 analogue **38** exhibited 80-fold less potent ET_A binding affinity than the corresponding Leu^5 analogue **20**. In contrast, the analogue with *N*-methyl-L-leucine (MeLeu) (**39**) was slightly less active than the corresponding Leu^5 analogue **8**. These findings imply that the presence of the αNH of the amino acid residue at this position is not necessary but that the side chain is important for binding affinity. We then incorporated various amino acids into this position. The Ala analogue **40** was 16-fold less active than the corresponding Leu analogue **37**. Replacing Leu^5 in **8** with Nva or Nle resulted in a 1.5- or 3-fold decrease in affinity, respectively. Among analogues with amino acids having straight chain alkyl side chains, the Nva analogue was the most potent, although it was less potent than the corresponding Leu analogue. The replacement of Leu with Ile resulted in a 10-fold decrease in affinity, suggesting that side chain branching on the γ-carbon is preferable while that on the β-carbon is not. The analogue with 3-cyclohexyl-L-alanine (Cha) (**44**) was also less active than the corresponding Leu analogue **20**. These results suggest that the ET_A receptor is less tolerant to changes at the Leu^5 position of the natural products.

Selected compounds that exhibited potent binding affinity to the porcine ET_A receptors **8**, **10**, **33**, and **36** were further evaluated; the results are listed in Table 7. These compounds were observed to show only weak affinity to porcine ET_B receptors[44] and thus considered to be highly ET_A-selective binding inhibitors. These compounds were further tested for their antagonistic activities against ET-1-induced vasoconstriction in isolated porcine coronary arteries by the reported method.[45] Compounds **8**, **10**, **33**, and **36** strongly antagonized ET-1-induced vasoconstriction with pA_2 values of 7.4, 7.4, 7.5, and 8.1, respectively. These compounds showed no intrinsic agonist activity even at concentrations of up to 10 μM, indicating that they are potent and selective ET_A receptor antagonists with no agonist activity. Compounds **8**, **10**, **33**, and **36** together with the natural product **2** were further tested with regard to inhibitory effects on [^{125}I]ET-1 binding to human neuroblastoma-derived cell line SK-N-MC membranes, which express

Table 7. Receptor Binding, Antivasoconstriction, and Solubility Data for Selected Cyclic Pentapeptides

compd	receptor binding inhibition (IC_{50}, nM)				antivasoconstriction pA_2	solubility (mg/mL)[g]
	pET_A[a,b]	hET_A[c,d]	pET_B[b,e]	hET_B[d,f]		
2	1400	590	>100 000	>100 000	5.9[h]	0.21
8	22	8.3	18 000	61 000	7.4[i]	>1000
10	21	30	30 000	>100 000	7.4[i]	>10
33	4.0	3.4	4800	21 000	7.5[i]	>100
36	3.0	1.2	19 000	55 000	8.1[i]	4.0

[a] Porcine aortic smooth muscle membranes. [b] Values represent the average of more than three independent experiments. [c] Human neuroblastoma-derived cell line SK-N-MC membranes. [d] Values represent the average of two independent experiments. [e] Porcine cerebellum membranes. [f] Human Girardi heart cell membranes. [g] Measured on sodium salts in saline. [h] Isolated rabbit iliac arteries. [i] Isolated porcine coronary arteries.

ET_A receptors, and human Girardi heart cell membranes, which exclusively possess ET_B receptors.[46,47] Compounds **8**, **10**, **33**, and **36** showed potent affinity to human ET_A receptors as well as to porcine ET_A receptors, whereas the natural product **2** showed moderate affinity in both species. In contrast, the compounds showed only weak affinity to human ET_B receptors. These compounds appeared to be highly ET_A-selective in human receptor systems as well as in porcine receptor systems. We then evaluated the solubility[23] of the sodium salts[24] of compounds **8**, **10**, **33**, and **36** in saline. The sodium salts of these compounds were all much more soluble than those of the natural product **2**; in particular, the sodium salt of **8** showed excellent solubility of >1 g/mL saline.

Conclusions

Backbone conformational analysis of the natural product cyclic pentapeptide **1** using ^1H NMR techniques revealed that this cyclic pentapeptide possesses a type II β-turn in the D-Val^4-Leu^5-D-Trp^1-D-Glu^2 region and an inverse γ-turn in the D-Glu^2-Ala^3-D-Val^4 region with two intramolecular hydrogen bonds. Chirality changes at the D-Trp^1 and/or D-Glu^2 positions of **1** destroyed the β,γ-backbone conformation and abolished ET_A receptor binding affinity. The DDLDL chirality sequence was thus proved to be very important for the activity of this series of compounds.

Systematic modifications of the natural products **1** and **2** clarified the structure–activity relationships at each position of amino acid residues. Most replacements of D-Trp^1 and Leu^5 with other amino acids caused a significant loss of activity, suggesting that the ET_A receptor was less tolerant to changes at these positions. In contrast, replacement of D-Glu^2 with D-Asp^2 enhanced the activity significantly. Replacement of the carboxyl group of D-Asp^2 with a sulfo group was tolerable, while replacements with methoxycarbonyl and hydroxymethyl groups decreased the activity significantly. These results indicate that the existence of an acidic functional group at a specific distance from the main chain of the peptide is important for the strong activity. The Ala^3 position was shown to be widely tolerable. Incorporation of various amino acids with different functional groups in their side chains afforded analogues with similar ET_A binding affinities, suggesting that the side chain at the Ala^3 position does not bind directly to the ET_A receptor. In contrast, the incorporation of imino acids, independent of being cyclic or acyclic, at this position afforded anglogues with increased affinities, compared with those of amino acid analogues. The increased affinity is thought to be associated with the more constrained type II β-turn that is adopted in this series of cyclic pentapeptides. These findings suggest that the amino acid residue at this position can be deleted to generate a linear peptide ET_A receptor antagonist if the β-turn structure is retained. In fact, our linear peptide ET_A receptor antagonists represented by BQ-610 (*N*,*N*-(hexamethylene)carbonyl)-Leu-D-Trp-(CHO)-D-Trp, pA_2 (porcine coronary artery) = 8.2) were generated from this concept.[48] In addition, the introduction of functional groups on the side chain of the AA^3 residue can generate a cyclic pentapeptide ET_A antagonist with a more preferable pharmacokinetic profile, without any loss of activity. The D-Val^4 position is critical for activity, and a lipophilic D-amino acid with a β-position branched alkyl side chain such as D-Val or D-Cpg or a lipophilic D-heteroarylglycine such as D-Thg is preferable.

Of the cyclic pentapeptides synthesized so far, compound **36** (BQ-518) is the most potent ET_A receptor antagonist with the greatest selectivity between ET_A and ET_B receptors. The ET_A/ET_B selectivity ratios in human and porcine receptor systems are 46 000 and 6300, respectively. In contrast, compound **8** (BQ-123) is a potent and highly soluble antagonist with a high ET_A/ET_B selectivity ratio. The sodium salt of **8** is practically freely soluble in saline. Compounds **8** and **36** are useful tools for in vitro and in vivo pharmacological studies of endothelin and endothelin receptors; in particular, compound **8** is now widely utilized as a standard ET_A receptor antagonist.

Experimental Section

Instruments and Materials. Melting points were determined on a Yanaco MP-S3 melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian VXR-300 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 Capcell Pak C18 (4.6 × 250 mm, 5-μm particle size; Shiseido Co., Ltd.) at 40 °C. The effluent was monitored at 230 nm. The isocratic systems were as follows: flow rate, 0.85 mL/min; and eluent, $MeOH/H_2O$ (45:55) with 0.1% TFA or $MeOH/H_2O$ (70:30) with 0.1% TFA. Column chromatography was carried out on E. Merck silica gel 60 (233–400 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, by Ehrlich reagent and/or by phosphomolybdic acid reagent.

The tetrabutylammonium salt of Fmoc-Sal was prepared from L-cysteic acid (Sigma) according to the reported method.[33] Other N^{α}-Fmoc derivatives of amino acids (bearing appropriate side chain protective groups) were prepared in our laboratory by the method of Lapatsanis *et al.*[49] or purchased from Kokusan Chemicals Co., Ltd. (Tokyo, Japan) or Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Fmoc-Arg(Pmc) Pfp active ester was obtained from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Other N^{α}-Fmoc- (and side chain-) protected amino acid Pfp or Dhbt active esters were obtained from Milligen. The amino acids or amino acid derivatives D-Aad(O^{t}Bu),[50] D-Pen(MBzl),[51] D-Cpg,[52] D-Sal,[53] and Boc-D-Sal[54] were synthesized according to the methods described in the literature with certain modifications. The following amino acids were commercially available: D-Aad, D-*tert*-Leu, and D-Phg (Aldrich); D-alloIle and D-Cys (Sigma); D-Dpg (Nippon Dasei Chemical Co., Ltd., Tokyo, Japan); and D-Thg (Ward Blenkinsop & Co., Ltd., London, England). 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.

Synthetic methods for the final compounds together with their analytical and physical data are outlined in Tables 3 and 4.

Method A. Cyclo(-D-Trp-DAsp-Pro-D-Val-Leu-) (8). (a) **Intermediate Resin Fmoc-Leu-Resin (90).** A functionalized polyamide resin (Ultrosyn B; Pharmacia LKB Biochrom, Ltd., Cambridge, England; 5.04 g, >0.41 mmol of functional hydroxymethyl group) was acylated with the preformed symmetrical anhydride of Fmoc-Leu (2.5 mmol) in the presence of DMAP (61 mg, 0.50 mmol) in DMF for 1 h. The resin was washed with DMF (25 mL × 3), *tert*-amyl alcohol (25 mL × 2), acetic acid (25 mL × 2), *tert*-amyl alcohol (25 mL × 2), DMF (25 mL × 2), and ethyl ether (25 mL × 2) successively and dried to give **90** (5.03 g, 0.093 mmol of Fmoc-Leu/g of resin).

(b) **H-D-Trp-DAsp(O^{t}Bu)-Pro-D-Val-Leu-Resin (91).** The continuous flow column of the Biolynx 4175 manual synthesizer (Pharmacia LKB Biochrom Ltd., Cambridge, England) was charged with Fmoc-Leu-resin (**90**; 1.0 g, 0.093 mmol of Fmoc-Leu). Fmoc-D-Val-OPfp (0.23 mmol, 2.5 equiv), Fmoc-Pro-OPfp (0.23 mmol, 2.5 equiv), Fmoc-D-Asp(^{t}Bu)-OPfp (0.23 mmol, 2.5 equiv), and Fmoc-D-Trp-OPfp (0.23 mmol, 2.5 equiv), together with HOBT (0.23 mmol, 2.5 equiv), were introduced to the continuous-flow system via the loading port of the synthesizer. The synthesis was carried out using the standard protocols of the Biolynx synthesizer. The resin was washed with DMF and deprotected with a 10-min cycle of 20% piperidine in DMF. The deprotection in this and all other cycles was monitored by UV at 304 nm. Deprotection was followed by a 10-min DMF wash. The acylations were performed by recycling a DMF solution of the activated ester of the desired residue and HOBT for 25−45 min. Completion of the acylation reactions was confirmed by the Kaiser test.[55] This was followed by a DMF wash, and the cycle was repeated. After the final cycle, the N-terminus Fmoc group was deprotected with a 10-min cycle of 20% piperidine in DMF. The resin was transferred to a glass filter and washed with DMF (5 mL × 3), *tert*-amyl alcohol (5 mL × 2), acetic acid (5 mL × 2), *tert*-amyl alcohol (5 mL × 2), DMF (5 mL × 3), and ethyl ether (5 mL × 3) successively and dried under reduced pressure to give **91** (1.00 g).

(c) **H-D-Trp-D-Asp(O^{t}Bu)-Pro-D-Val-Leu-NHNH$_2$ (92).** The resin **91** (1.00 g) was treated with 3 mL of 10% hydrazine hydrate in dioxane/MeOH (9:1) for 1 h with occasional shaking. The resin was filtered off and washed three times with 5 mL of dioxane/MeOH (9:1). The combined filtrate and washings were neutralized with dry ice and evaporated. Trituration of the residue with water yielded the peptide hydrazide **92** (26.8 mg, 41%) as a white powder: TLC R_f (CHCl$_3$:MeOH = 10:1) 0.20; FAB-MS *m/e* 699 (M + H)$^+$.

(d) **Cyclo(-D-Trp-D-Asp(O^{t}Bu)-Pro-D-Val-Leu-) (93).** The peptide hydrazide **92** (26.8 mg, 0.038 mmol) dissolved in DMF (1.0 mL) was cooled to −60 °C under an atmosphere of argon followed by addition of 3.1 M HCl/dioxane (49 μL, 0.15 mmol) and isoamyl nitrite (7.6 μL, 0.056 mmol). After 10 min at −30 °C, TLC indicated that formation of the acyl azide was almost complete. The solution was diluted with precooled DMF (11 mL) and TEA (25 μL, 0.15 mmol) Added to pH 8 at −60 °C. The mixture was allowed to stand at −20 °C overnight and concentrated under reduced pressure. Water (4 mL) was added to the residue to precipitate a solid, which was collected by filtration and dried *in vacuo* to give the side chain-protected cyclic pentapeptide **93** (24.0 mg, 94%) as a pale yellow powder: TLC R_f (CHCl$_3$:MeOH = 10:1) 0.44; FAB-MS *m/e* 667 (M + H)$^+$.

(e) **Cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu-) (8).** A solution of the side chain-protected cyclic pentapeptide **93** (19.4 mg, 0.029 mmol) in 2.0 mL of TFA/EDT (95:5) was stirred at 0 °C for 15 min and concentrated under reduced pressure. Ethyl ether and hexane (1:1) were added to the residue to precipitate a pale yellow solid, which was collected by filtration, washed with water and dried *in vacuo* to give **8** (12.0 mg, 68%) as a pale yellow powder: TLC R_f (CHCl$_3$:MeOH:AcOH = 10:1:1) 0.61; [α]^{20}D 50.3° (*c* = 0.31, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.60 (3 H, d, *J* = 6.6 Hz), 0.72 (3 H, d, *J* = 6.6 Hz), 0.82 (3 H, d, *J* = 6.5 Hz), 0.86 (3 H, d, *J* = 6.5 Hz), 0.90−1.10 (1 H, m), 1.10−1.28 (2 H, m), 1.55−1.98 (4 H, m), 2.21−2.32 (1 H, m), 2.34 (1 H, dd, *J* = 3.9, 16.1 Hz), 2.79 (1 H, dd, *J* = 10.2, 16.1 Hz), 2.88 (1 H, dd, *J* = 11.7, 14.4 Hz), 3.10−3.35 (3 H, m), 3.95−4.03 (1 H, m), 4.13 (1 H, dd, *J* = 8.3, 10.3 Hz), 4.22−4.31 (1 H, m), 4.76 (1 H, d, *J* = 7.0 Hz), 4.97 (1 H, dt, *J* = 3.9, 8.8 Hz), 6.95 (1 H, t, *J* = 7.3 Hz), 7.04 (1 H, t, *J* = 7.3 Hz), 7.13 (1 H, d, *J* = 1.7 Hz), 7.31 (1 H, d, *J* = 7.3 Hz), 7.49 (1 H, d, *J* = 10.3 Hz), 7.52 (1 H, d, *J* = 7.3 Hz), 7.71 (1 H, d, *J* = 8.8 Hz), 8.75−8.79 (2 H, m), 10.80 (1 H, d, *J* = 1.7 Hz).

Method B. Cyclo(-D-Trp-D-Asp-Pro-D-*tert*-Leu-Leu-) (32). (a) **Intermediate Resin Fmoc-Leu-AA Resin (94).** To a solution of Fmoc-Leu-OH (4.14 g, 11.7 mmol) in DMF (21 mL) at 0 °C was added *p*-alkoxybenzyl alcohol resin (Kokusan Chemicals Co., Ltd., Tokyo, Japan; 3.00 g, 2.34 mmol of functional hydroxymethyl group) followed by DCC (2.42 g, 11.7 mmol) and then DMAP (146 mg, 1.17 mmol). The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The resin was washed on a glass filter with DMF (25 mL × 2), MeOH (25 mL × 4), MeOH/CH$_2$Cl$_2$ (1:1, 25 mL × 4), and CH$_2$Cl$_2$ (25 mL × 2) successively and dried to give **94** (3.64 g, 0.441 mmol of Fmoc-Leu/g of resin).

(b) **H-D-Asp(O^{t}Bu)-Pro-D-*tert*-Leu-Leu-NHNH$_2$ (96).** Fmoc-Leu-AA resin (**94**; 0.34 g, 0.15 mmol of Fmoc-Leu) was placed in a reaction vessel, and the stepwise solid-phase synthesis was carried out as follows: (1) deprotection, 20% piperidine in DMF (5 min × 3); (2) washing, DMF (1 min × 6); (3) coupling, Fmoc-D-*tert*-Leu-OH (2.5 equiv), DIPC (2.5 equiv), and HOBT (2.5 equiv) in DMF (1.0 mL; 5 h × 1); and (4) washing, DMF (1 min × 4). Fmoc-Pro-OH, Fmoc-D-Asp-(O^{t}Bu)-OH, and Fmoc-D-Trp-OH were consecutively coupled in the same manner. The solvent volume for the deprotection and washing steps was 3.0 mL. The reaction time for the coupling step was 1−14 h, and the completion of the each coupling reaction was checked by the Kaiser test.[55] After the final coupling, the N-terminus Fmoc group was deprotected with 20% piperidine in DMF as described above. The resin was washed with MeOH (3 mL × 3) and CH$_2$Cl$_2$ (3 mL × 3) and then dried *in vacuo*. The resin-bound peptide **95** was added to 10% hydrazine hydrate in DMF (10 mL), and the mixture was stirred for 4 h. The resin was filtered off and washed several times with small amounts of DMF. The combined filtrate and washings were neutralized with dry ice and evaporated. Water (20 mL) was added to the residue, and the suspension was passed through a Sep-Pak C18 cartridge (Waters Chromatography Division, Millipore Corp., MA). The cartridge was washed with water (60 mL) and water/MeOH (10:1, 60 mL) and then eluted with MeOH (60 mL). The eluate was evaporated to yield the peptide hydrazide **96** (59.5 mg, 56%) as a brown amorphous solid: TLC R_f (CH$_2$Cl$_2$:MeOH = 10:1) 0.51; FAB-MS *m/e* 713 (M + H)$^+$.

(c) **Cyclo(-D-Trp-D-Asp(O^{t}Bu)-Pro-D-*tert*-Leu-Leu-) (97).** The peptide hydrazide **96** (57.5 mg, 0.081 mmol) dissolved in DMF (2.0 mL) was cooled to −60 °C under an atmosphere of argon followed by addition of 2.9 M HCl/dioxane (83 μL, 0.24 mmol) and isoamyl nitrite (16 μL, 0.12 mmol). After 1 h at −30 °C, TLC indicated that formation of the acyl azide was almost complete. The solution was diluted with precooled DMF (4.0 mL) and TEA (54 μL, 0.39 mmol) added to pH 8 at −65 °C. The mixture was allowed to stand at −20 °C overnight and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with $MeOH/CH_2Cl_2$ (1:15) to give the side chain-protected cyclic pentapeptide **97** (35.7 mg, 65%) as a white powder: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.55; FAB-MS *m/e* 680 (M^+).

(d) **Cyclo(-D-Trp-D-Asp-Pro-D-*tert*-Leu-Leu-) (32).** A solution of the side chain-protected cyclic pentapeptide **97** (33.1 mg, 0.049 mmol) in 10 mL of TFA/EDT (95:5) was stirred at 0 °C for 1 h and concentrated under reduced pressure. Triuration with ethyl ether and hexane (1:1) gave **32** (28.8 mg, 95%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.68; ^1H NMR (300 MHz, DMSO-d_6) δ 0.61 (3 H, d, *J* = 6.5 Hz), 0.72 (3 H, d, *J* = 6.5 Hz), 0.87 (9 H, s), 0.98−1.24 (3 H, m), 1.60−1.74 (2 H, m), 1.91−1.94 (1 H, m), 2.24−2.29 (1 H, m), 2.33 (1 H, dd, *J* = 3.6, 16.5 Hz), 2.71−2.92 (2 H, m), 3.26−3.37 (2 H, m), 3.45−3.60 (1 H, m), 3.95−4.02 (1 H, m), 4.20 (1 H, d, *J* = 10.2 Hz), 4.19−4.28 (1 H, m), 4.78 (1H, d, *J* = 6.6 Hz), 4.98 (1 H, dt, *J* = 4.2, 9.3 Hz), 6.95 (1 H, t, *J* = 7.5 Hz), 7.04 (1 H, t, *J* = 7.5 Hz), 7.13 (1 H, d, *J* = 2.1 Hz), 7.31 (1 H, d, *J* = 7.5 Hz), 7.51 (1 H, d, *J* = 7.5 Hz), 7.64 (1 H, d, *J* = 10.5 Hz), 7.71 (1 H, d, *J* = 9.3 Hz), 8.76 (1 H, d, *J* = 4.8 Hz), 8.81 (1 H, d, *J* = 7.8 Hz), 10.79 (1 H, brs), 12.28 (1 H, brs).

Method C. Sodium Salt of Cyclo(-D-Trp-D-Sal-Pro-D-Val-Leu-) (10). (a) **Boc-D-Val-Leu-OBzl (66).** To a stirred solution of Leu-OBzl·TosOH (3.94 g, 10.0 mmol), Boc-D-Val (2.17 g, 10.0 mmol), and HOBT (1.53 g, 10.0 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added TEA (1.40 mL, 10.0 mmol) followed by EDCI (2.01 g, 10.5 mmol). After 3 h, 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, dried over $MgSO_4$, and concentrated *in vacuo* to give **66** (4.15 g, 98.6%) as a white solid: TLC R_f (hexane:MeOH = 2:1) 0.57; FAB-MS *m/e* 421 (M + H)$^+$.

(b) **Boc-Pro-D-Val-Leu-OBzl (67).** A solution of **66** (4.15 g, 9.87 mmol) in a mixture of CH_2Cl_2 (40 mL) and TFA (20 mL) was stirred at room temperature for 1.5 h. The volatiles were removed *in vacuo* to give a pale yellow oil. To a solution of the above-mentioned oil and Boc-Pro (2.15 g, 10.0 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added TEA (2.80 mL, 20.0 mmol) to pH 7 followed by HOBT (1.53 g, 10.0 mmol) and then EDCI (2.01 g, 10.5 mmol). The mixture was stirred at 0 °C for 2 h and then at room temperature overnight. The mixture was washed successively with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give a white solid (4.77 g), which was recrystallized from EtOAc/hexane to yield **67** (4.09 g, 80.0%) as colorless crystals: mp 136.5−139.0 °C; TLC R_f (hexane:EtOAc = 1:1) 0.42; FAB-MS *m/e* 518 (M + H)$^+$.

(c) **Boc-D-Trp-D-Sal-Pro-D-Val-Leu-$NHNH_2$ (70).** A solution of **67** (259 mg, 0.50 mmol) in TFA (5 mL) was stirred at room temperature for 15 min. The mixture was evaporated *in vacuo* to give a pale yellow oil. To a solution of the above-mentioned oil and Boc-D-Sal(ONa) (153 mg, 0.53 mmol) in DMF (2.0 mL) at 0 °C was added TEA (150 μL, 1.08 mmol) to pH 7 followed by HOBT (80 mg, 0.52 mmol) and EDCI (101 mg, 0.53 mmol). The mixture was allowed to warm to room temperature and stirred overnight. The mixture was evaporated to give crude product of **68**, which was utilized directly in the next reaction. The residue was dissolved in TFA (5 mL) and the mixture was stirred at room temperature for 30 min. The mixture was evaporated *in vacuo*, and the residue was dissolved in DMF (5 mL). To the solution at 0 °C were added Boc-D-Trp (168 mg, 0.55 mmol) and TEA (140 μL) to pH 7 followed by HOBT (84 mg, 0.55 mmol) and EDCI (105 mg, 0.55 mmol). The mixture was allowed to warm to room temperature and stirred overnight. The mixture was evaporated *in vacuo*. The residue was taken up in water (20 mL) and extracted with CH_2Cl_2 (10 mL × 5). The combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated *in vacuo* to give 535 mg of crude product of **52** as a pale yellow oil. An aliquot (322 mg) of the crude **69** obtained above was dissolved in 10% hydrazine hydrate in DMF (1 mL), and the mixture was stirred at room temperature for 22 h and concentrated *in vacuo*. The residue was purified by reverse-phase chromatography (nacalai tesque, cosmosil 75 C18-OPN, 30 × 2.5 cm, elution with 3:7 and then 1:1 MeOH/water) to give **70** (149 mg, 62%) as a colorless solid: TLC R_f (BuOH:AcOH:H_2O = 4:1:1) 0.60; FAB-MS *m/e* 823 (M + 2Na−H)$^+$.

(d) **H-D-Trp-D-Sal-Pro-D-Val-Leu-$NHNH_2$ (71).** A solution of 70 (149 mg, 0.19 mmol) in 20 mL of TFA/EDT (95:5) was stirred at 0 °C for 15 min and evaporated *in vacuo*. Trituration of the residue with ethyl ether gave a crude product (162 mg), which was purified by reverse-phase chromatography (nacalai tesque, cosmosil 75 C18-OPN, 30 × 2.5 cm, elution with 1:4 and then 2:3 MeOH/water) to give **71** (103 mg, 82%) as a pale yellow solid: TLC R_f (BuOH:AcOH:H_2O = 4:1:1) 0.48; FAB-MS *m/e* 701 (M + Na)$^+$.

(e) **Sodium Salt of Cyclo(-D-Trp-D-Sal-Pro-D-Val-Leu-) (10).** To a solution of the peptide hydrazide **71** (103 mg, 0.15 mmol) in DMF (1.0 mL) at −60 °C was added 3.1 M HCl/dioxane (120 μL, 0.37 mmol). The mixture was allowed to warm to −30 °C, and isoamyl nitrite (40 μL, 0.30 mmol) was added. After being stirred at −30 °C for 40 min, the mixture was again cooled to −60 °C and diluted with precooled DMF (40 mL) and then TEA (75 μL, 0.54 mmol) added to pH 7.5. The mixture was allowed to stand at −20 °C overnight and concentrated under reduced pressure. The residue was purified by reverse-phase chromatography (nacalai tesque, cosmosil 75 C18-OPN, 30 × 2.5 cm, elution with 3:7 and then 2:3 MeOH/water) to give the TEA salt of **10**, which was dissolved in water and passed through ion-exchange resin columns (first, amberlite IR-120B H^+-form; second, amberlite IRC-50 Na^+-form). The columns were washed with water, and fractions that contained pure materials were combined and evaporated. The residue was reprecipitated with EtOH and ethyl ether to give a sodium salt of **10** (60 mg, 59%) as a pale yellow powder: TLC R_f (BuOH:ACOH:MeOH = 4:1:1) 0.66; $[\alpha]^{20}_D$ 60.5° (*c* = 0.35, MeOH); ^1H NMR (300 MHz, DMSO-d_6) δ 0.62 (3 H, d, *J* = 6.3 Hz), 0.71 (3 H, d, *J* = 6.3 Hz), 0.81 (3 H, d, *J* = 6.6 Hz), 0.83 (3 H, d, *J* = 6.6 Hz), 0.95−1.10 (1 H, m), 1.16−1.22 (2 H, m), 1.55−1.93 (4 H, m), 2.19−2.27 (1 H, m), 2.58 (1 H, dd, *J* = 2.7, 12.2 Hz), 2.92 (1 H, dd, *J* = 11.6, 14.3 Hz), 3.15−3.45 (3 H, m), 3.63−3.71 (1 H, m), 4.05−4.30 (3 H, m), 4.62 (1 H, d, *J* = 6.6 Hz), 4.93−5.03 (1 H, m), 6.95 (1 H, t, *J* = 7.5 Hz), 7.03 (1 H, t, *J* = 7.5 Hz), 7.13 (1 H, d, *J* = 1.7 Hz), 7.20 (1 H, d, *J* = 8.3 Hz), 7.30 (1 H, d, *J* = 7.5 Hz), 7.53 (1 H, d, *J* = 7.5 Hz), 8.10 (1 H, d, *J* = 9.0 Hz), 8.57 (1 H, d, *J* = 7.1 Hz), 8.69 (1 H, d, *J* = 8.3 Hz), 10.77 (1 H, d, *J* = 1.7 Hz).

Method D. Cyclo(-D-Trp-D-Ama-Pro-D-Val-Leu-) (7). (a) **Boc-D-Val-Leu-OPac (72).** Compound **66** (90.0 g, 0.214 mol) was hydrogenated in MeOH (750 mL) over 10% Pd−C (4.8 g) under an atmospheric pressure of hydrogen. The catalyst was removed by filtration after the uptake of hydrogen ceased, and the filtrate was concentrated to produce the corresponding carboxylic acid (70.7 g, 100%). The crude carboxylic acid (70.7 g) was dissolved in MeOH (400 mL), and a solution of Cs_2CO_3 (34.86 g, 0.107 mol) in water (120 mL) was added. The mixture was evaporated *in vacuo*, and the residue was dissolved in DMF (600 mL). To the solution was added phenyacyl bromide (42.6 g, 0.214 mol), and the mixture was stirred at room temperature for 30 min. The mixture was then filtered to remove $CsBr_2$, and the filtrate was evaporated. The mixture was dissolved in EtOAc (500 mL), washed successively with water, saturated $NaHCO_3$, and water, dried over $MgSO_4$, and concentrated *in vacuo*. Recrystallization of the residue from EtOAc/hexane gave **72** (90.48 g, 94.4%) as colorless crystals: TLC R_f ($CHCl_3$:MeOH = 9:1) 0.76; FAB-MS *m/e* 449 (M + H)$^+$.

(b) **Boc-Pro-D-Val-Leu-OPac (73).** A mixture of **72** (90.48 g, 0.202 mol) in TFA (180 mL) was stirred at 0 °C for 1.5 h and evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 (700 mL), and Boc-Pro (49.7 g, 0.231 mol) was added. To the mixture at 0 °C was added NMM (125 mL, 1.14 mol) to pH 7 followed by HOBT (32.5 g, 0.212 mol) and EDCI (40.7 g, 0.212 mol). The mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was washed successively with saturated $NaHCO_3$, 5% $KHSO_4$, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give **73** (110.2 g, 100%) as a white 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 = 9:1) 0.65; FAB-MS *m/e* 546 $(M + H)^+$.

(c) Z-D-Trp-NHCH(COOEt)$_2$ (74). EDCI (633 mg, 3.3 mmol) was added to a solution of Z-D-Trp (1.02 g, 3.0 mmol), diethyl aminomalonate hydrochloride (698 mg, 3.3 mmol), TEA (0.46 mL, 3.3 mmol), and HOBT (505 mg, 3.3 mmol) in CH_2Cl_2 at 0 °C. After 2 h of stirring, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated $NaHCO_3$, 10% citric acid, and brine, dried over $MgSO_4$, and evaporated *in vacuo*. Recrystallization of the residue from CH_2Cl_2/hexane gave **74** (1.38 g, 93%) as colorless crystals: mp 68–70 °C; TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.72; FAB-MS *m/e* 496 $(M + H)^+$.

(d) Z-D-Trp-DL-Ama(OEt)-OH (75). To a stirred solution of **74** (496 mg, 1.0 mmol) in EtOH (3.0 mL) and acetone (2.0 mL) at 0 °C was added 2 N NaOH (0.50 mL, 1.0 mmol). After 1 h, the mixture was allowed to warm to room temperature and stirred for a further 1 h. The mixture was evaporated to remove organic solvents. The residue was taken up in water (30 mL) and extracted with ethyl ether (20 mL × 2) to remove unreacted diester. The aqueous layer was acidified with 6 N HCl and extracted with CH_2Cl_2 (20 mL × 3). The combined CH_2Cl_2 extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluted with MeOH/CH_2Cl (1:3) to give **75** (412 mg, 88%) as a mixture of two diastereoisomers: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.32; FAB-MS *m/e* 468 $(M + H)^+$.

(e) Z-D-Trp-DL-Ama(OEt)-Pro-D-Val-Leu-OPac (76). A mixture of **73** (375 mg, 0.69 mmol) in CH_2Cl_2 (2 mL) and TFA (2 mL) was stirred at room temperature for 1 h and evaporated *in vacuo* to give a pale yellow oil. To the amine TFA salt prepared above and **75** (376 mg, 0.80 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added TEA (0.25 mL, 1.79 mmol) to pH 7 followed by HOBT (115 mg, 0.75 mmol) and EDCI (145 mg, 0.76 mmol). After 2 h, the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with saturated $NaHCO_3$, 1 N HCl, and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60, size B) eluted with EtOAc/hexane (3:1) to yield **76** (222 mg, 36%): TLC R_f ($CHCl_3$:MeOH = 30:1) 0.15; FAB-MS *m/e* 895 $(M + H)^+$.

(f) Cyclo(-D-Trp-D- or -L-Ama(OEt)-Pro-D-Val-Leu-) (78a,b). Zinc powder (0.80 g, 12 mmol) was added to a stirred solution of **76** (218 mg, 0.24 mmol) in 90% AcOH at 0 °C. After 2 h, the reaction mixture was allowed to warm to room temperature and stirred for a further 1 h. The reaction mixture was filtered and concentrated under reduced pressure. The residue was taken up with 1 N HCl (60 mL) and extracted with CH_2Cl_2 (30 mL × 3). The combined organic extracts were dried over $MgSO_4$ and evaporated to give a colorless amorphous solid. The benzyloxycarbonyl-protecting group was removed by hydrogenation (10% Pd–C, H_2, 10 mL of MeOH) to produce **77** (144 mg, 96%) as a colorless amorphous solid. A solution of **77** (135 mg, 0.21 mmol) in DMF (20 mL) was added dropwise to a stirred solution of HOBT (49 mg, 0.32 mmol) and EDCI (61 mg, 0.32 mmol) in DMF (20 mL) at room temperature over a period of 4 h. After being stirred at room temperature overnight, the mixture was concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (30 mL) and saturated $NaHCO_3$ (30 mL). The aqueous layer was extracted with CH_2Cl_2 (15 mL × 2), and the combined organic extracts 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, size A) eluted with MeOH in CH_2Cl_2 (1:50 → 1:30) to yield **78a** (33 mg, 26%) and **78b** (26 mg, 19%). **78a**: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.51; FAB-MS *m/e* 625 $(M + H)^+$; ^1H NMR (300 MHz, CD_3OD) δ 0.57 (3 H, d, J = 6.5 Hz), 0.64 (3 H, d, J = 6.5 Hz), 0.89 (3 H, d, J = 6.5 Hz), 0.92 (3 H, d, J = 6.5 Hz), 1.10–2.42 (8 H, m), 1.30 (3 H, t, J = 7.3 Hz), 2.97–3.70 (4 H, m), 4.00 (1 H, dd, J = 6.4, 9.5 Hz), 4.09 (1 H, d, J = 8.1 Hz), 4.27 (2 H, q, J = 7.3 Hz), 4.68 (1 H, dd, J = 3.7, 11.7 Hz), 4.75–4.90 (1 H, m), 5.50 (1 H, s), 6.98 (1 H, t, J = 7.8 Hz), 7.07 (1 H, t, J = 7.8 Hz), 7.12 (1 H, s), 7.31 (1 H, d, J = 7.8 Hz), 7.58 (1 H, d, J = 7.8 Hz).

78b: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1) 0.36; FAB-MS *m/e* 625 $(M + H)^+$; ^1H NMR (300 MHz, CD_3OD) δ 0.80–2.40 (23 H, m), 2.90–3.70 (4 H, m), 4.00–4.50 (5 H, m), 6.90–7.70 (5 H, m).

(g) Cyclo(-D-Trp-D-Ama-Pro-D-Val-Leu-) (7). To a stirred solution of **78a** (19 mg, 0.031 mmol) in EtOH (0.30 mL) at 0 °C was added 2 N NaOH (20 μL, 0.04 mmol). After 1 h, 1 N HCl (40 μL, 0.04 mmol) was added and the mixture was concentrated under reduced pressure. Trituration of the residue with water gave **7** (16 mg, 87%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 5:1:1) 0.49; ^1H NMR (300 MHz, DMSO-d_6) δ 0.61 (3 H, d, J = 6.5 Hz), 0.74 (3 H, d, J = 6.5 Hz), 0.81 (3 H, d, J = 7.2 Hz), 0.84 (3 H, d, J = 7.2 Hz), 0.90–1.30 (3 H, m), 1.40–2.00 (4 H, m), 2.20–2.30 (1 H, m), 2.90 (1 H, dd, J = 12.1, 15.5 Hz), 3.05–3.50 (3 H, m), 4.00–4.30 (3 H, m), 4.77 (1 H, d, J = 6.9 Hz), 5.25 (1 H, d, J = 9.1 Hz), 6.95 (1 H, t, J = 7.4 Hz), 7.04 (1 H, t, J = 7.4 Hz), 7.17 (1 H, d, J = 2.2 Hz), 7.31 (1 H, d, J = 7.4 Hz), 7.50 (1 H, d, J = 7.4 Hz), 8.00 (1 H, d, J = 8.8 Hz), 8.68 (1 H, d, J = 5.4 Hz), 8.98 (1 H, d, J = 7.6 Hz), 10.79 (1 H, d, J = 2.2 Hz).

Alkaline hydrolysis of **78b** gave the same product as that obtained above. To a suspension of **78b** (20 mg, 0.032 mmol) in MeOH (2.0 mL) at 0 °C was added 2 N NaOH (20 μL, 0.04 mmol). After being stirred at 0 °C for 2 h, the mixture was allowed to warm to room temperature and stirred overnight. To the mixture was added 1 N HCl (40 μL, 0.04 mmol), and the resulting mixture was diluted with water (5 mL). The mixture was passed through a Sep-Pak C18 cartridge (Waters Chromatography Division, Millipore Corp., MA), and the cartridge was washed with water. The product was eluted with MeOH, and the eluate was concentrated under reduced pressure to give **7** (15 mg, 80%).

Method E. Cyclo(-D-Trp-D-Asp(OMe)-Pro-D-Val-Leu-) (11). To a solution of **8** (122 mg, 0.20 mmol) in DMF (0.5 mL) was added pulverized $KHCO_3$ (40 mg, 0.40 mmol) followed by iodomethane (20 μL, 0.32 mmol), and the mixture was stirred at room temperature for 4 h. Water (10 mL) was added, and the mixture was extracted with EtOAc (10 mL × 3). The organic extracts were washed successively with water (10 mL), 5% Na_2SO_3 (10 mL), water (10 mL), and brine (10 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60, size A) eluted with MeOH/$CHCl_3$ (1:100) to give 11 (72.9 mg, 58%) as a colorless powder: TLC R_f ($CHCl_3$:MeOH = 30:1) 0.33; ^1H NMR (300 MHz, $CDCl_3$) δ 0.72 (3 H, d, J = 6.6 Hz), 0.75 (3 H, d, J = 6.6 Hz), 0.85 (3 H, d, J = 6.6 Hz), 0.92 (3 H, d, J = 6.6 Hz), 1.15–2.22 (7 H, m), 2.37 (1 H, dd, J = 4.0, 16.5 Hz), 2.37–2.50 (1 H, m), 2.85 (1 H, dd, J = 9.7, 16.5 Hz), 3.28 (1 H, dd, J = 4.9, 14.6 Hz), 3.3–3.55 (2 H, m), 3.44 (1 H, dd, J = 6.7, 14.6 Hz), 3.67 (3 H, s), 3.73 (1 H, q, J = 7.4 Hz), 3.90 (1 H, t, J = 9.8 Hz), 4.74 (1 H, dt, J = 4.9, 6.7 Hz), 4.79 (1 H, d, J = 8.2 Hz), 5.15 (1 H, dt, J = 4.0, 9.7 Hz), 6.12 (1 H, d, J = 7.4 Hz), 6.59 (1 H, d, J = 4.9 Hz), 7.05 (1 H, d, J = 1.6 Hz), 7.11 (1 H, d, J = 9.7 Hz), 7.11 (1 H, t, J = 7.7 Hz), 7.22 (1 H, t, J = 7.7 Hz), 7.39 (1 H, d, J = 7.7 Hz), 7.58 (1 H, d, J = 7.7 Hz), 7.71 (1 H, d, J = 9.8 Hz), 8.18 (1 H, d, J = 1.6 Hz).

Method F. Cyclo(-D-Trp-D-Hse-Pro-D-Val-Leu-) (12). MeOH (0.15 mL) was added dropwise to a stirred mixture of 11 (117 mg, 0.19 mmol) and $NaBH_4$ (36 mg, 0.94 mmol) in ^{t}BuOH (0.75 mL) at 55 °C over a period of 30 min. After 3 h, excess $NaBH_4$ was quenched with 1 N NCl and the mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc and 1 N HCl. The organic layer was washed with 1 N HCl 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, size A) eluted with MeOH in CH_2Cl_2 (1:40) to produce **12** (39 mg, 34%) as a colorless powder: TLC R_f ($CHCl_3$:MeOH = 30:1) 0.22 ^1H NMR (300 MHz, DMSO-d_6) δ 0.60 (3 H, d, J = 6.3 Hz), 0.71 (3 H, d, J = 6.3 Hz), 0.82 (3 H, d, J = 6.5 Hz), 0.86 (3 H, d, J = Hz), 0.90–2.10 (9 H, m), 2.03–2.30 (1 H, m), 2.87 (1 H, dd, J = 11.8, 14.5 Hz), 3.04–3.50 (5 H, m), 3.99 (1 H, q, J = 5.9 Hz), 4.11 (1 H, dd, J = 8.1, 10.1 Hz), 4.24 (1 H, ddd, J = 3.1, 8.2, 11.8 Hz), 4.42 (1 H, t, J = 5.2 Hz), 4.72–4.83 (1 H, m), 4.75 (1 H, d, J = 7.0 Hz), 6.95 (1 H, t, J = 7.4 Hz), 7.04 (1 H, t, J = 7.4 Hz), 7.12 (1 H, d, J = 1.8 Hz), 7.31 (1 H, d, J = 7.4 Hz), 7.52 (1 H, d, J = 7.4 Hz), 7.62 (1 H, d, J = 8.8 Hz), 7.63 (1 H, d, J = 10.1 Hz), 8.70 (1 H, d, J = 5.9 Hz), 8.80 (1 H, d, J = 8.2 Hz), 10.79 (1 H, d, J = 1.8 Hz).

Method G. Cyclo(-D-NFK-D-Asp-Pro-D-Val-Leu) (16). Ozone (1–2%, generated from oxygen) was bubbled into a solution of **8** (10 mg, 0.016 mmol) and pyrogallol (4.1 mg, 0.33 mmol) in formic acid (3.3 mL) at room temperature for 30 min. The mixture was concentrated under reduced pressure, and the residue was dissolved in water. The aqueous solution was passed through a Sep-Pak C18 cartridge (Waters Chromatography Division, Millipore Corp., MA), and the cartridge was washed with H_2O and H_2O/MeOH (9:1). The product was eluted with MeOH, and the fractions which contained pure material were combined and evaporated *in vacuo* to give **16** (6.3 mg, 60%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 20:1:1) 0.26; ^1H NMR (300 MHz, DMSO-d_6%) δ 0.76 (3 H, d, J = 6.2 Hz), 0.79 (3 H, d, J = 6.2 Hz), 0.81 (3 H, d, J = 6.6 Hz), 0.87 (3 H, d, J = 6.6 Hz), 1.20–2.00 (7 H, m) 2.15–2.34 (1 H, m), 2.40 (1 H, dd, J = 3.9, 16.2 Hz), 2.82 (1 H, dd, J = 10.5, 16.2 Hz), 3.10–3.50 (3 H, m), 3.60 (1 H, dd, J = 9.7, 17.8 Hz), 3.88–4.00 (1 H, m), 4.09–4.23 (1 H, m), 4.57–4.70 (1 H, m), 4.83–4.98 (1 H, m), 4.77 (1 H, d, J = 7.6 Hz), 7.24 (1 H, t, J = 7.5 Hz), 7.49 (1 H, d, J = 10.3 Hz), 7.60 (1 H, t, J = 7.5 Hz), 7.71 (1 H, d, J = 9.0 Hz), 8.03 (1 H, d, J = 7.5 Hz), 8.39 (1 H, d, J = 7.3 Hz), 8.44 + 11.00 (1 H, brs), 8.78 (1 H, d, J = 7.5 Hz), 8.83 (1 H, d, J = 5.1 Hz).

Method H. Cyclo(-D-Trp(CHO)-D-Asp-Pro-D-Val-Leu-) (17). Dry HCl gas was bubbled into a stirred solution of **8** (3.0 mg, 0.0049 mmol) in formic acid (0.50 mL) at room temperature for 15 min. After 50 min, the mixture was concentrated under reduced pressure. Trituration of the residue with water gave **17** (1.6 mg, 51%) as a pale yellow powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.74; ^1H NMR (300 MHz, DMSO-d_6) δ 0.52–0.60 (3 H, m), 0.60–0.70 (3 H, m), 0.83 (3 H, d, J = 6.9 Hz), 0.86 (3 H, d, J = 6.9 Hz), 1.11–1.31 (3 H, m), 1.55–1.83 (3 H, m), 1.89–1.98 (1 H, m), 2.22–2.31 (1 H, m), 2.33–2.40 (1 H, m), 2.71–3.30 (5 H, m), 3.89–4.03 (1 H, m), 4.14 (1 H, dd, J = 8.1, 9.9 Hz), 4.38–4.48 (1 H, m), 4.77 (1 H, d, J = 7.1 Hz), 4.92–5.02 (1 H, m), 6.91–7.60 (4 H, m), 7.63 (1 H, d, J = 8.1 Hz), 7.79 (1 H, d, J = 8.4 Hz), 7.92–8.29 (1 H, m), 8.75–9.00 (2 H, m), 9.25 + 9.64 (1 H, brs × 2).

Method I. Cyclo(-D-Trp(COOMe)-D-Asp-Pro-D-Val-Leu-) (18). (a) Boc-D-Trp(COOMe)-OH (81). Boc-D-Trp (42.77 g, 0.141 mmol) was converted to its benzyl ester **79** by the cesium salt method described by Wang *et al.*[38] (42.22 g, 94%). Methyl chloroformate (6.0 mL, 78 mmol) was added at 0 °C to a mixture of **79** (12.19 g, 30.9 mmol), pulverized NaOH (5.1 g, 130 mmol), and tetra-*n*-butylammonium hydrogen sulfate (0.30 g, 0.88 mmol) in CH_2Cl_2 (60 mL). After being stirred at 0 °C for 4 h, the mixture was washed with water (60 mL × 3), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from CH_2Cl_2/hexane to give **80** as colorless crystals (12.57 g, 90%). Compound **80** (500 mg, 1.1 mmol) was hydrogenated in 8 mL each of MeOH and THF over 10% Pd–C (60 mg) under an atmospheric pressure of hydrogen. The catalyst was removed by filtration, and the filtrate was evaporated to yield **81** (399 mg, 100%): TLC R_f ($CHCl_3$:MeOH:AcOH = 100:10:1) 0.44; FAB-MS *m/e* 363 $(M + H)^+$.

(b) Boc-D-Asp(OBzl)-Pro-D-Val-Leu-OPac (82). A solution of **73** (110 g, 0.202 mmol) in TFA (180 mL) was stirred at 0 °C for 40 min and evaporated *in vacuo* to produce a pale yellow oil. To the product obtained above in CH_2Cl_2 (1.0 L) at 0 °C were added Boc-D-Asp(OBzl) (75.0 g, 0.232 mmol) and NMM (226 mL, 2.06 mol) to pH 7 followed by HOBT (32.5 g, 0.212 mol) and EDCI (44.7 g, 0.233 mol). The mixture was allowed to warm to room temperature and stirred for 2.5 h. The mixture was washed with saturated $NaHCO_3$, 5% $KHSO_4$, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under reduced pressure to yield **82** (152 g, 100%). This material showed a single spot on TLC and was used in the next reaction without further purification: TLC R_f ($CHCl_3$:MeOH = 9:1) 0.69; FAB-MS *m/e* 751 $(M + H)^+$.

(c) Cyclo(-D-Trp(COOMe)-D-Asp-Pro-D-Val-Leu-) (18). A solution of **82** (700 mg, 0.93 mmol) in TFA (6 mL) was stirred at room temperature for 1.5 h and evaporated *in vacuo*. To the product obtained above in CH_2Cl_2 (15 mL) at 0 °C were added **65** (372 mg, 1.03 mmol) and NMM (0.50 mL, 4.5 mmol) to pH 7 followed by HOBT (139 mg, 1.03 mmol) and EDCI (196 mg, 1.03 mmol). After 30 min, the mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was diluted with CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ (100 mL), 10% citric acid (100 mL), water (100 mL), and brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with MeOH in CH_2Cl_2 (1:30) to produce **83** (776 mg, 84%). The Pac protective group was removed by zinc powder (2.54 g, 39 mmol) in 90% AcOH (23 mL), and the Boc protective group was removed by formic acid (20 mL, room temperature, 2 h) to yield **84** (537 mg, 89%). A solution of **84** (535 mg, 0.69 mmol) in DMF (20 mL) was added to a stirred solution of EDCI (197 mg, 1.03 mmol) and HOBT (140 mg, 1.03 mmol) in DMF (50 mL) at room temperature over a period of 3.5 h. The resulting mixture was stirred overnight and concentrated under reduced pressure. The residue was taken up with $CHCl_3$ (150 mL), washed with saturated $NaHCO_3$ (100 mL), 10% citric acid (100 mL), and brine (100 mL), 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 MeOH in $CHCl_3$ (1:100 → 1:30) to afford **85** (323 mg, 62%). The benzyl protective group of **85** (100 mg, 0.13 mmol) was removed by hydrogenation (10% Pd–C, H_2, 3 mL of MeOH). The crude product was purified by reverse-phase chromatography (E. Merck, Lobar, Lichroprep, RP-18, size B) eluted with H_2O/MeOH (1:2) to yield **18** (60 mg, 68%) as a colorless powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 100:10:1) 0.46; ^1H NMR (300 MHz, DMSO-d_6) δ 0.57 (3 H, d, J = 6.3 Hz), 0.66 (3 H, d, J = 6.3 Hz), 0.82 (3 H, d, J = 6.9 Hz), 0.85 (3 H, d, J = 6.9 Hz), 0.80–1.00 (1 H, m), 1.10–1.32 (2 H, m), 1.57–1.83 (3 H, m), 1.85–1.98 (1 H, m), 2.20–2.32 (1 H, m), 2.40 (1 H, dd, J = 3.2, 16.1 Hz), 2.69–2.82 (1 H, m), 2.90 (1 H, dd, J = 12.3, 15.0 Hz), 3.03–3.20 (2 H, m), 3.20–3.50 (1 H, m), 3.87–4.00 (1 H, m), 3.96 (3 H, s), 4.14 (1 H, t, J = 8.8 Hz), 4.32–4.42 (1 H, m), 4.77 (1 H, d, J = 7.1 Hz), 4.95 (1 H, dt, J = 2.6, 8.8 Hz), 7.26 (1 H, t, J = 7.5 Hz), 7.43–7.58 (2 H, m), 7.56 (1 H, t, J = 7.5 Hz), 7.59 (1 H, d, J = 7.5 Hz), 7.78 (1 H, d, J = 8.8 Hz), 8.06 (1 H, d, J = 7.5 Hz), 8.79 (1 H, d, J = 4.3 Hz), 8.92 (1 H, d, J = 8.0 Hz).

Method J. Cyclo(-D-Trp(Boc)-D-Asp-Pro-D-Val-Leu-) (19). (a) Cyclo(-D-Trp-D-Asp(OBzl)-Pro-D-Val-Leu-) (88). The Boc protective group of **82** (152 g, 0.202 mol) was removed by TFA (200 mL), room temperature, 1.5 h), and the product was dissolved in CH_2Cl_2 (1.0 L). To the solution of 0 °C were added Boc-D-Trp (66 g, 0.22 mol) and NMM (89 mL, 0.81 mol) to pH 7 followed by HOBT (37 g, 0.24 mol) and EDCI (46 g, 0.24 mol). After being stirred at 4 °C overnight, the mixture was washed with saturated $NaHCO_3$, 5% $KHSO_4$, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with MeOH/EtOAc/CH_2Cl_2 (1:1:19) to produce **86** (122.3 g, 65%). The Pac protective group was removed by zinc powder (134 g, 2.05 mol) in 90% AcOH (1.0 L), and the Boc protective group was removed by formic acid (500 mL, room temperature, 4 h) to afford **87** (77.7 g, 83%). Cyclization of **87** by EDCI/HOBT in DMF was performed as described previously for **85**, and purification by column chromatography on silica gel eluted with MeOH in CH_2Cl_2 (1:50 → 1:30) yielded **88** (68.8 g, 92%): TLC R_f ($CHCl_3$:MeOH = 9:1) 0.54; FAB-MS *m/e* 701 $(M + H)^+$.

(b) Cyclo(-D-Trp(Boc)-D-Asp-Pro-D-Val-Leu-) (19). A mixture of **88** (123 mg, 0.175 mmol), DMAP (2.1 mg, 0.018 mmol), and Boc_2O (38.2 mg, 0.175 mmol) in CH_3CN (5 mL) was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was taken up with EtOAc, washed with diluted HCl, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with MeOH in $CHCl_3$ (1:30) to yield **89** (114 mg, 81%). Compound **89** (32.4 mg, 0.040 mmol) was hydrogenated in MeOH (5 mL) over 10% Pd–C under an atmospheric pressure of hydrogen. The catalyst was filtered off after the reaction was completed, and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluted with MeOH in $CHCl_3$ (1:9) to give **19** (15 mg, 52%): TLC R_f ($CHCl_3$:MeOH = 9:1) 0.13; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.60 (3 H, d, J = 6.6 Hz), 0.72 (3 H, d, J = 6.6 Hz), 0.82 (3 H, d, J = 6.5 Hz), 0.86 (3 H, d, J = 6.5 Hz), 0.90–1.10 (1 H, m), 1.10–1.28 (2 H, m), 1.55–1.72 (2 H, m), 1.60 (9 H, s), 1.72–1.87 (1 H, m), 1.87–1.98 (1 H, m), 2.21–2.32 (1 H, m), 2.33 (1H, dd, J = 3.9, 16.1 Hz), 2.79 (1 H, dd, J = 10.2, 16.1 Hz), 2.88 (1 H, dd, J = 11.7, 14.4 Hz), 3.10–3.35 (3 H, m), 3.88–3.98 (1 H, m), 4.13 (1 H, dd, J = 8.3, 10.3 Hz), 4.22–4.31 (1 H, m), 4.76 (1 H, d, J = 7.3 Hz), 4.97 (1 H, ddd, J = 3.9, 8.8, 10.2 Hz), 7.23 (1 H, t, J = 7.0 Hz), 7.30 (1 H, t, J = 7.0 Hz), 7.48 (1 H, d, J = 10.0 Hz), 7.50 (1 H, s), 7.57 (1 H, d, J = 7.0 Hz), 7.78 (1 H, d, J = 8.8 Hz), 8.01 (1 H, d, J = 8.1 Hz), 8.80 (1 H, d, J = 5.4 Hz), 8.90 (1 H, d, J = 8.3 Hz).

Method K. Cyclo(-D-Trp-D-Asp-Hyp-D-Val-Leu-) (65). (a) **Boc-D-Trp-D-Asp(OBzl)-O^{t}Bu (98).** EDCI (540 mg, 2.82 mmol) was added to a solution of Boc-D-Trp (767 mg, 2.52 mmol), D-Asp(OBzl)-O^{t}Bu (prepared by the method of C. C. Yang and R. B. Merrifield;[56] 701 mg, 2.51 mmol), and HOBT (429 mg, 2.80 mmol) in CH_2Cl_2 (5 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 2 h. The mixture was partitioned between CH_2Cl_2 (30 mL) and saturated NaHCO33 (3 mL). The aqueous layer was extracted with CH_2Cl_2 (30 mL × 2), and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with EtOAc to give **98** (1.43 g, 100%) as a white amorphous solid: TLC R_f (CH_2Cl_2:MeOH = 20:1) 0.67; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (9 H, s), 1.41 (9 H, s), 2.80 (1 H, dd, J = 4.9, 17.0 Hz), 2.92 (1 H, dd, J = 4.3, 17.0 Hz), 3.16–3.37 (2 H, m), 4.39–4.51 (1 H, m), 4.55–4.65 (1 H, m), 4.98 + 5.06 (each 1 H, ABq, J = 12.4 Hz), 6.79 (1 H, d, J = 7.4 Hz), 7.03 (1 H, d, J = 2.3 Hz), 7.10 (1 H, dt, J = 1.3, 7.6 Hz), 7.17 (1 H, dt, J = 1.3, 7.6 Hz), 7.25–7.38 (6 H, m), 7.61 (1 H, dd, J = 1.3, 7.6 Hz), 7.96 (1 H, brs).

(b) **Boc-D-Trp(Z)-D-Asp(OBzl)-O^{t}Bu (99).** Benzyl chloroformate (0.54 mL, 3.78 mmol) was added to a suspension of **98** (1.40 g, 2.47 mmol), tetra-n-butylammonium hydrogen sulfate (10 mg, 0.025 mmol), and pulverized NaOH (163 mg, 3.79 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The mixture was stirred at 0 °C for 2 h and then at room temperature for 1 h. After the addition of water (20 mL), the mixture was extracted with CH_2Cl_2 (30 mL × 3). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (E. Merck, Lobar, Lichroprep, Si 60) eluted with hexane/EtOAc (3:1) to give **99** (1.39 g, 80%) as a white amorphous solid: TLC R_f (hexane: EtOAc = 2:1) 0.65; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (9 H, s), 1.39 (9 H, s), 2.76, (1 H, dd, J = 4.6, 17.0 Hz), 2.92 (1 H, dd, J = 4.6, 17.0 Hz), 3.15 (2 H, d, J = 4.9 Hz), 4.37–4.49 (1 H, m), 4.54–4.61 (1 H, m), 4.97 + 5.07 (each 1 H, ABq, J = 12.2 Hz), 5.42 (2 H, s), 6.77 (1 H, d, J = 7.8 Hz), 7.20–7.60 (15 H, m), 8.15 (1 H, brs).

(c) **H-D-Trp(Z)-D-Asp(OBzl)-O^{t}Bu (100).** Compound **99** (350 mg, 0.50 mmol) was added to ice-cooled TFA (2.5 mL), and the mixture was stirred at 0 °C for 10 min. The mixture was concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (20 mL) and saturated NaHCO33 (4 mL). The aqueous layer was extracted with CH_2Cl_2 (20 mL × 2), and the combined organic extracts were washed with brine (3 mL), dried over $MgSO_4$, and evaporated to give **100** (289 mg, 96%) as a colorless oil: TLC R_f ($CHCl_3$:MeOH = 30:1) 0.44; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (9 H, s), 2.70–2.80 (2 H, m), 2.94 (1 H, dd, J = 4.7, 16.9 Hz), 3.26 (1 H, dd, J = 3.7, 14.5 Hz), 3.71 (1 H, dd, J = 3.7, 9.1 Hz), 4.67–4.75 (1 H, m), 5.02 + 5.11 (each 1 H, ABq, J = 12.2 Hz), 5.44 (2 H, s), 7.23–7.50 (13 H, m), 7.60 (1 H, d, J = 7.3 Hz), 8.07 (1 H, d, J = 8.0 Hz), 8.17 (1 H, d, J = 7.3 Hz).

(d) **Boc-Hyp-D-Val-Leu-OBzl (101).** A solution of Boc-D-Val-Leu-OBzl (210 mg, 0.50 mmol) in TFA (1.0 mL) was stirred at 0 °C for 1 h and then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ (4 mL), dried over $MgSO_4$, and evaporated to give a colorless oil (H-D-Val-Leu-OBzl; 157 mg, 98%), which was dissolved in DMF (2.5 mL). Boc-Hyp (119 mg, 0.51 mmol) and HOBT (75 mg, 0.49 mmol) were added to the solution, and then EDCI (103 mg, 0.54 mmol) was added at 0 °C. The mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was concentrated *in vacuo*. The residue was taken up with EtOAc (50 mL), washed with 10% citric acid (5 mL) and saturated $NaHCO_3$ (5 mL), dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluted with EtOAc/$CHCl_3$ (1:1) to give **101** (231 mg, 88%) as a white powder: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.43; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84–1.02 (12 H, m), 1.44 (9 H, s), 1.52–1.72 (3 H, m), 1.92–2.50 (3 H, m), 3.49 (1 H, d, J = 5.4 Hz), 3.43–3.62 (2 H, m), 4.37 (1 H, t, J = 7.6 Hz), 4.38–4.52 (2 H, m), 4.58–4.70 (1 H, m), 5.12 + 5.17 (each 1 H, ABq, J = 12.2 Hz), 6.83 (1H, brs), 6.98 (1 H, brs), 7.28–7.42 (5 H, m).

(e) **Boc-Hyp-D-Val-Leu-D-Trp(Z)-D-Asp(OBzl)-O^{t}Bu (103).** Compound **101** (229 mg, 0.43 mmol) was hydrogenated in a mixture of THF (4.4 mL) and MeOH (2.2 mL) over 10% Pd–C (23 mg) under an atmospheric pressure of hydrogen for 3 h. The catalyst was filtered off, and the filtrate was evaporated to give Boc-Hyp-D-Val-Leu-OH (**102**; 191 mg, 100%) as a white powder. EDCI (89 mg, 0.46 mmol) was added to a mixture of **102** (187 mg, 0.42 mmol), **100** (252 mg, 0.42 mmol), and HOBT (65 mg, 0.42 mmol) in DMF (2.1 mL) at 0 °C. The mixture was allowed to warm to room temperature, stirred for 14 h, and then concentrated *in vacuo*. The residue was taken up with EtOAc (50 mL), washed with 10% citric acid (5 mL) and saturated $NaHCO_3$ (5 mL), dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$/EtOAc (1:1) to give **103** (369 mg, 86%) as a white powder: TLC R_f (CHCl33:MeOH = 10:1) 0.59; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.75–0.96 (12 H, m), 1.30–1.80 (3 H, m), 1.35 (9 H, s), 1.36 (9 H, s), 1.98–2.43 (3 H, m), 2.80 (1 H, dd, J = 5.1, 16.6 Hz), 2.89 (1 H, dd, J = 5.6, 16.6 Hz), 3.13–3.30 (2 H, m), 3.33–3.45 (1 H, m), 3.53 (1 H, dd, J = 4.4, 11.5 Hz), 4.21–4.45 (4 H, m), 4.61–4.77 (2 H, m), 5.04 + 5.10 (each 1 H, ABq, J = 12.2 Hz), 5.40 + 5.45 (each 1 H, ABq, J = 12.2 Hz), 7.03 (1 H, brs), 7.15–7.62 (17 H, m), 8.14 (1 H, brs).

(f) **Cyclo(-D-Trp(Z)-D-Asp(OBzl)-Hyp-D-Val-Leu-) (104).** Compound **103** (364 mg, 0.36 mmol) was dissolved in TFA (3.6 mL), and the solution was left at room temperature for 1 h. The mixture was concentrated *in vacuo*, and the residue was triturated with water (10 mL) to give a white powder (320 mg). An aliquot (315 mg) of the powder obtained above was dissolved in DMF (16 mL), and the solution was neutralized with NMM (35 μL, 0.32 mmol). The solution was added to a mixture of EDCI (68 mg, 0.32 mmol) and HOBT (49 mg, 0.32 mmol) in DMF (16 mL) over a period of 80 min. After being stirred at room temperature for 11 h, the mixture was concentrated *in vacuo*. The residue was taken up with EtOAc (50 mL), washed with 10% citric acic (5 mL) and saturated $NaHCO_3$ (5 mL), dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel eluted with CH_2Cl_3/EtOAc (1:2) to give **104** (218 mg, 74%) as a white powder: TLC R_f ($CHCl_3$:MeOH = 10:1) 0.63; FAB-MS m/e 851 (M + H)$^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.61 (3 H, d, J = 6.2 Hz), 0.66 (3 H, d, J = 6.2 Hz), 0.86 (3H, d, J = 6.2 Hz), 0.95 (3 H, d, J = 6.2 Hz), 1.00–1.15 (1 H, m), 1.25–1.40 (1 H, m), 1.45–1.60 (1 H, m), 1.75–1.95 (2 H, m), 2.25 (1 H, d, J = 4.6 Hz), 2.45 (1 H, dd, J = 4.7, 16.4 Hz), 2.75–2.84 (1 H, m), 2.91 (1 H, dd, J = 9.5, 16.4 Hz), 3.19 (1 H, dd, J = 7.9, 15.0 Hz), 3.29 (1 H, dd, J = 4.4, 15.0 Hz), 3.38–3.49 (2 H, m), 3.68–3.76 (1 H, m), 3.86 (1 H, t, J = 9.5 Hz), 4.55–4.63 (1 H, m), 4.19–4.28 (1 H, m), 4.90 (1 H, dd, J = 3.6, 8.6 Hz), 4.99 + 5.22 (each 1 H, ABq, J = 12.6 Hz), 5.16–5.23 (1 H, m), 5.39 +

5.44 (each 1 H, ABq, $J = 11.9$ Hz), 6.06 (1 H, d, $J = 8.1$ Hz), 6.56 (1 H, d, $J = 4.6$ Hz), 7.20−7.50 (14 H, m), 7.54 (1 H, d, $J = 7.6$ Hz), 7.64 (1 H, d, $J = 10.1$ Hz), 8.17 (1 H, brs).

(g) Cyclo-(-D-Trp-D-Asp-Hyp-D-Val-Leu-) (65). A mixture of **104** (15.3 mg, 0.018 mmol) and 10% Pd−C (3.1 mg) in DMF (0.54 mL) was stirred under an atmospheric pressure of hydrogen for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. 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 and then eluted with MeOH. The eluate was evaporated to give **65** (11.5 mg, 100%) as a white powder: TLC R_f ($CHCl_3$:MeOH:AcOH = 10:1:1) 0.25; FAB-MS *m/e* 627 $(M + H)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 0.60 (3 H, d, $J = 6.3$ Hz), 0.72 (3 H, d, $J = 6.4$ Hz), 0.81 (3 H, d, $J = 6.9$ Hz), 0.86 (3 H, d, $J = 6.9$ Hz), 0.90−1.30 (3 H, m), 1.55−1.75 (3 H, m), 2.25−2.40 (1 H, m), 2.77 (1 H, dd, $J = 9.8$, 15.9 Hz), 2.87 (1 H, dd, $J = 11.7$, 14.6 Hz), 3.10−3.50 (3 H, m), 3.95−4.05 (1 H, m), 4.10 (1 H, dd, $J = 8.6$, 10.1 Hz), 4.20−4.30 (2 H, m), 4.82 (1 H, dd, $J = 1.8$, 8.1 Hz), 4.95−5.05 (1 H, m), 5.10−5.30 (1 H, brs), 6.95 (1 H, t, $J = 7.4$ Hz), 7.04 (1 H, t, $J = 7.4$ Hz), 7.13 (1 H, d, $J = 1.8$ Hz), 7.31 (1 H, d, $J = 7.4$ Hz), 7.40 (1 H, d, $J = 10.3$ Hz), 7.51 (1 H, d, $J = 7.4$ Hz), 7.75 (1 H, d, $J = 9.3$ Hz), 8.74 (1 H, d, $J = 5.1$ Hz), 8.78 (1 H, d, $J = 8.1$ Hz), 10.80 (1 H, d, $J = 1.8$ Hz).

Endothelin Receptor Binding Assay Protocol. The binding assay protocol using porcine aortic smooth muscle and cerebellum membranes has been reported previously.[19] The affinities for human ET_A and ET_B receptors were assessed using human neuroblastoma-derived SK-N-MC cell and Girardi heart cell membranes, respectively. These human cell lines have been reported to possess only ET_A and ET_B receptors, respectively.[46] Binding experiments on $[^{125}I]$ET-1 to membranes prepared from SK-N-MC or Girardi heart cells were performed by adding 12 pM $[^{125}I]$ET-1 to membranes in the absence or presence of increasing concentrations of test compounds in 50 mM Tris/HCl buffer, pH 7.4, containing 0.1 mM phenylmethanesulfonyl fluoride, 1 μM pepstatin, 2 μM leupeptin, 1 mM 1,10-phenanthroline, 1 mM EDTA, 10 μM $CaCl_2$, 10 μM $MgCl_2$, and 0.3% BSA. After incubation (4 h, 25 °C), membrane-bound ligands were separated by GF/C filters using Brandel's cell harvester and washed with cold 5 mM Hepes/Tris buffer containing 0.3% BSA. The filters were counted by a γ-counter. The nonspecific binding was defined by adding 0.2 μM ET-1 to the assay mixture.

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Supporting Information Available: ^1H NMR data of target cyclic pentapeptides not reported in the text (12 pages). Ordering information is given on any current masthead page.

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