Studies on Neurokinin Antagonists. 2. Design and Structure-Activity Relationships of Novel Tripeptide Substance P Antagonists, N^{α}-[N^{α}-(N^{α}-Acetyl-L-threonyl)-N^{1}-formyl-D-tryptophyl]-*N*-methyl-*N*-(phenylmethyl)-L-phenylalaninamide and Its Related Compounds†

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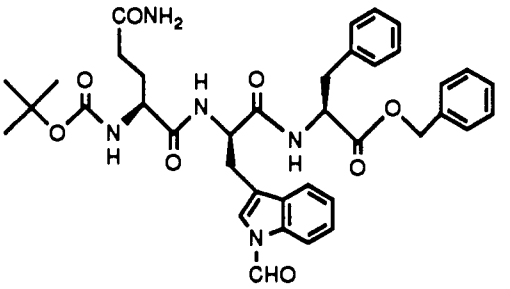
Continuing studies on the chemical modification of the previously reported novel tripeptide SP antagonist, N^{α}-[N^{α}-[N^{α}-(*tert*-butyloxycarbonyl)glutaminyl]-N^{1}-formyl-D-tryptophyl]phenylalanine benzyl ester [Boc-Gln-D-Trp-(CHO)-Phe-OBzl (1)], are described herein. We initially investigated the stability of 1 in guinea pig plasma and liver homogenate to elucidate the most labile part in the structure. It was consequently revealed that the benzyl ester part was easily hydrolyzed to produce the inactive acid analog. Thus we searched for a benzyl ester surrogate that would be more resistant to hydrolytic enzymes. This approach found an isosteric amide structure, *N*-methyl-*N*-(phenylmethyl)amide, suitable in terms of potency and stability. Subsequent modification of the amino terminal into N^{α}-acetyl-L-threonine led to the most potent compound, N^{α}-[N^{α}-(N^{α}-acetyl-L-threonyl)-N^{1}-formyl-D-tryptophyl]-*N*-methyl-*N*-(phenylmethyl)-L-phenylalaninamide [Ac-Thr-D-Trp(CHO)-Phe-NMeBzl (**5a**, FR113680)]. This compound **5a** potently blocked ^{3}H-SP binding to guinea pig lung membranes with IC_{50} of $(5.8 \pm 0.78) \times 10^{-9}$ M. In vitro, **5a** inhibited SP-induced contraction of isolated guinea pig trachea strips with IC_{50} of 2.3×10^{-6} M and caused no contraction when used alone in this preparation up to 3.2×10^{-5} M. In addition **5a** exhibited no effect on the contraction induced by histamine or acetylcholine. Intriguingly, it was demonstrated in vivo that **5a** suppressed the SP-induced bronchoconstriction and airway edema in guinea pigs with ED_{50} of 0.42 mg/kg and 0.66 mg/kg, respectively, when administered intravenously.

Substance P (SP) which belongs to a family of neurokinins[1] is now considered to exert its physiological activities[2] through the SP receptor or NK_1 receptor.[3] As SP is assumed to etiologically participate in various diseases or symptoms, much attention is being denoted to the creation of SP antagonists. In addition to an enormous number of peptide analogs of SP, several nonpeptide SP antagonists, namely quinuclidine,[4] perhydroisoindole,[5] androstanopyrimidobenzimidazole,[6] and imidazoquinoxaline[7] derivatives, have been recently disclosed. On the basis of the proposal by Barnes and Lundberg[8] that SP plays an important role in asthma through a "neurogenic inflammation" mechanism, we were particularly interested in applying a SP antagonist to the remedy of respiratory diseases such as asthma and began the study on discovering a novel SP antagonist.

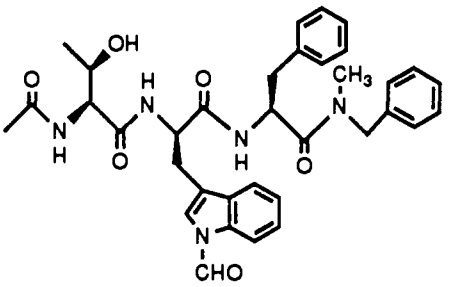
In our preceding papers,[9,10] we reported the discovery of a tripeptide substance P antagonist, N^{α}-[N^{α}-[N^{α}-(*tert*-butyloxycarbonyl)-L-glutaminyl]-N^{1}-formyl-D-tryptophyl]-L-phenylalanine benzyl ester [Boc-Gln-D-Trp-(CHO)-Phe-OBzl (1)] (Chart I) and its structure-activity relationships. This tripeptide antagonist 1 was found through a study to elucidate the essential domain in the known octapeptide antagonist (D-Pro^{4},D-Trp^{7,9,10},Phe^{11})-SP(4-11) (**2**)[11] for the receptor recognition. Although 1 may be classified into a novel class of peptide SP antagonists in terms of its reduced molecular weight and potent antagonistic activity, there still exists some drawbacks which must be ameliorated. Therefore, we continued the chemical modification of 1 in order to overcome its drawbacks.

The lead compound 1 exhibited potent inhibitory activity in the binding assay using guinea pig lung membranes and tritium labelled SP, and antagonized SP-induced contraction of isolated guinea pig trachea strips as

Chart I



Boc-Gln-D-Trp(CHO)-Phe-OBzl
(1)



Ac-Thr-D-Trp(CHO)-Phe-NMeBzl
(5a, FR113680)

† Abbreviations follow IUPAC-IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* 1980, *138*, 9-37. Additional abbreviations used herein are as follows: HPLC, high-performance liquid chromatography; WSCD, 1-ethyl-3-[3-(*N*,*N*-dimethylamino)propyl]carbodiimide; HOBT, 1-hydroxybenzotriazole; DMF, dimethylformamide; IPE, diisopropyl ether; DMSO, dimethyl sulfoxide.

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well. However, in in vivo experiments it did not suppress SP-induced bronchoconstriction in guinea pigs when administered intravenously. The lack of in vivo activity may be ascribed either or both to an insufficiency of the intrinsic activity and to rapid degradation of the molecule within the body. Thus, at the beginning of this study we examined the stability of 1 in plasma and liver homogenate of guinea pigs in order to determine the direction of chemical modification. The results indicated that 1 was unstable in both preparations and the ester part in 1 was easily hydrolyzed to produce the acid analog Boc-Gln-D-Trp(CHO)-Phe-OH, which is completely devoid of the binding affinity.

From these results we first focused on the search for a benzyl ester surrogate, which resists enzymatic degradation and also contributes to augmentation of the antagonistic activity. Having found a suitable structure, *N*-methyl-*N*-(phenylmethyl)amide, we subsequently optimized the amino terminal N^{α}-(*tert*-butyloxycarbonyl)glutaminyl part. These modifications finally led to the most potent compound, N^{α}-[N^{α}-(N^{α}-acetyl-L-threonyl)-N^{1}-formyl-D-tryptophyl]-*N*-methyl-*N*-(phenylmethyl)-L-phenylalaninamide [Ac-Thr-D-Trp(CHO)-Phe-NMeBzl (**5a**, FR113680)] (Chart I). In this report we describe the structure–activity relationships on **5a** and its biological activities.

Chemistry

The Boc-protected tripeptides in the series 3 and 4 were synthesized from Boc-phenylalanine derivatives according to the conventional way which comprises two cycles of deprotection of the Boc group with hydrochloric acid in dioxane and the subsequent coupling process with a Boc-protected amino acid. The WSCD–HOBT method[12] was exclusively applied to the coupling reactions. The starting Boc-phenylalanine derivatives, that is Boc-phenylalanine-*N*-methyl-*N*-(phenylmethyl)amide for **3a** and also for the compounds in the series 4 and Boc-phenylalanine-*N*-(phenylmethyl)amide for **3b**, were produced from Boc-phenylalanine and the corresponding amines by the mixed anhydride method. The starting material for **3c**, 1-(Boc-phenylalanyl)-2-phenylethane, was prepared by Grignard reaction on Boc-phenylalanine 2-pyridyl ester.[13] In the synthesis of **4f**, the histidine moiety was built up using Boc-His(Tos)-OH,[14] followed by selective removal of the tosyl group with pyridine hydrochloride in DMF.[15] The compounds **5a–d** bearing an acetyl at the

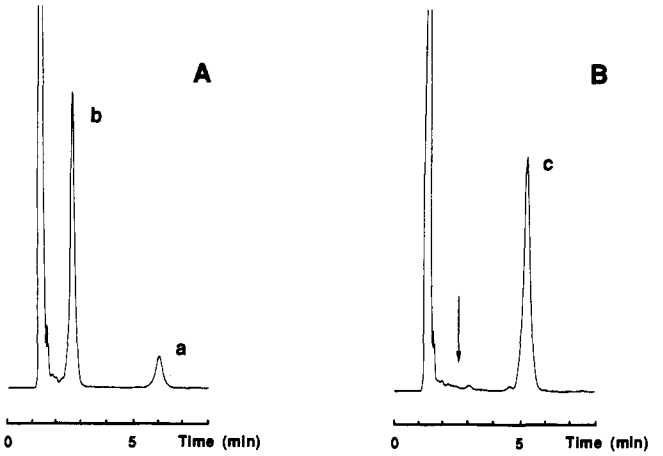

Figure 1. HPLC charts of 1 (A, 10 min) and **3a** (B, 30 min) after incubation in guinea pig liver homogenate. Peaks a and b in chart A are 1 and the metabolite Boc-Gln-D-Trp(CHO)-Phe-OH, respectively. Peak c in chart B is **3a**. The arrow shows no formation of the metabolite. Conditions of the analysis were as follows: column, AM-302 S-5 120A ODS (150 mm × 4 mm) (YMC, Kyoto, Japan); eluent, MeOH–H_2O (3:1) containing 0.1% CF_3CO_2H; detection, UV 254 nm; flow rate, 1.2 mL/min.

Table I. Modification of Benzyl Ester Part of 1

Boc-Gln-D-Trp(CHO)-Phe-R_1

compd	R_1	inhibition of ^{3}H-SP receptor binding (IC_{50}, μM)[a]
1	OCH_2Ph	0.09
3a	$NMeCH_2Ph$	0.048
3b	$NHCH_2Ph$	0.86
3c	CH_2CH_2Ph	0.43

[a] IC_{50} values were determined by a single experiment unless otherwise noted. Each assay was performed in duplicate. The concentration of ^{3}H-SP used in this test was 1 nM.

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amino terminal were produced from the corresponding Boc-protected tripeptides by deprotection of the Boc group with hydrochloric acid in dioxane and subsequent acetylation with acetic anhydride. The following compounds **5e–k** were derived from **5l**, which was prepared by deprotection of the Boc group in **4a**. Namely, the coupling reactions with hydroxyacetic acid and *N,N*-diethyl-β-alanine using the WSCD–HOBT method afforded **5e** and **5h**, respectively. The tetrapeptide analogs **5f** and **5g** were derived through the two-step reactions: coupling with the corresponding Z-protected amino acid and subsequent deprotection of the Z group by catalytic hydrogenation. The compounds **5i–k** were produced by acylation with succinyl anhydride, glutaryl anhydride, and morpholine-1-carbonyl chloride, respectively.

Results

Influence of Guinea Pig Plasma and Liver Homogenate on 1. The stability of the compound **1** in guinea pig plasma and liver homogenate was investigated. In plasma, **1** was fairly stable and the half-life ($T_{1/2}$) for disappearance of **1** was estimated to be 70 min. On the contrary **1** was rapidly degraded in liver homogenate as shown in Figure 1. The $T_{1/2}$ was indicated to be only 2.7 min. The major metabolite in both experiments was the acid analog of **1** [Boc-Gln-D-Trp(CHO)-Phe-OH], which was identified by comparison with an authentic sample by coelution on HPLC. From these results we can conclude that the ester part in **1** was quite sensitive to esterase-like enzymes, and consequently **1** was easily degraded to the corresponding acid analog. We have already demonstrated in the preceding paper[9] that the acid analog of **1**, the major metabolite in this study, is completely devoid of binding affinity. On the basis of these results, one of the reasons why **1** is unable to exhibit in vivo activity seems to be the rapid degradation within the body.

Modification of Ester Part in 1. Having confirmed that the most labile part in the structure of **1** is the benzyl ester, we sought a surrogate which is more resistant to enzymatic degradation. Thus, we synthesized two amide derivatives **3a** and **3b** and the phenylethylketone derivative **3c**. The carboxy terminal structures of these compounds are supposed to be more stable toward enzymatic hydrolysis than a benzyl ester. As shown in Table I, the SP receptor binding assay disclosed that *N*-benzylamide **3b** and phenylethyl ketone **3c** derivatives were less active than **1**, while *N*-methyl-*N*-(phenylmethyl)amide derivative **3a** exhibited 2-fold more potent activity with IC_{50} of 4.8×10^{-8} M than that of **1** (9.0×10^{-8} M). In addition to the enhanced activity, **3a** was shown to be quite stable in the liver homogenate when compared with **1** (Figure 1). Even after 30 min or longer incubation in this preparation, no significant metabolite was able to be detected. From these results it is likely that a *N*-methyl-*N*-(phenylmethyl)amide structure can be employed as the surrogate of the benzyl ester in **1**.

Therefore, we selected **3a** as the new lead compound for discovering a better SP antagonist. As described in the preceding paper,[9] any modification of the N^1-formyl-D-tryptophan and L-phenylalanine parts in **1** resulted in attenuation of the binding affinity. In accord with these findings, we preserved the D-Trp(CHO)-Phe part in **3a** unchanged as a core structure and mainly focused on the modification of the amino terminal Boc-glutamine part.

Modification of Amino Terminal Boc-Glutamine Part in 3a. We also demonstrated in the preceding paper[9] that the Boc-glutamine moiety in **1** was recognized to be variable, namely the modification to other structures such as Boc-threonine or Boc-serine maintained sufficiently the activity. Accordingly, the influence of the modification of the corresponding part in **3a** was examined using the SP receptor binding assay.

Table II. Modification of Glutamine Part of 3a

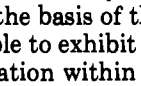
Boc-R_2-D-Trp(CHO)-Phe-NMeBzl

compd	R_2	inhibition of ^{3}H-SP receptor binding (IC_{50}, μM)[a]
4a	Thr	0.06
4b	Ser	0.02
4c	Gln(Me_2)	0.04
4d	Orn(Z)	0.21
4e	Lys(Z)	0.35
4f	His	0.14
4g	Hyp	0.20
4h	isoAsn	0.18
4i	MeThr[b]	0.29

[a] *N*-Methylthreonine. footnote *a* in Table I. [b] *N*-Methylthreonine.

Table III. Modification of Amino Terminal Part

R_3-R_4-D-Trp(CHO)-Phe-NMeBzl

compd	R_3	R_4	inhibition of ^{3}H-SP receptor binding (IC_{50}, μM)[a]
5a	Ac	Thr	0.0058 (n = 3)
5b	Ac	Ser	0.05
5c	Ac	Gln(Me_2)	0.03
5d	Ac	His	0.10
5e	HO-CH_2-CO	Thr	0.02
5f	H_2N-CH_2-CO	Thr	0.23
5g	HCl·H_2N-$(CH_2)_2$-CO	Thr	0.10
5h	HCl·Et_2N-$(CH_2)_2$-CO	Thr	0.02
5i	HO_2C-$(CH_2)_2$-CO	Thr	*b*
5j	HO_2C-$(CH_2)_3$-CO	Thr	*b*
5k		Thr	0.02
5l	HCl·H	Thr	0.60

[a] See footnote *a* in Table I. [b] No inhibition was observed at 0.14 μM.

To search for surrogates of the glutamine part in **3a**, nine compounds having a Boc group fixed were synthesized first. As can be seen in Table II, the compound **4b** which has a serine instead of a glutamine showed 2-fold potent activity in comparison with that of **3a**. The modification to a threonine (**4a**) and a *N,N*-dimethylglutamine (**4c**) was found to be also effective for exhibiting the comparable activity to **3a**. However, the introduction of an ornithine (**4d**) and a lysine (**4e**) both protected with a Z group, a histidine (**4f**), a (2*S*,4*R*)-hydroxyproline (**4g**), an isoasparagine (**4h**), and a *N*-methylthreonine (**4i**) resulted in attenuation of the activity by several times than **3a**.

Next we modified the Boc part of the selected compounds in the series **4**, which exhibited potent activities, into an acetyl group. From the results (Table III) of the four compounds synthesized, this type of modification was most effective in the case of a threonine (**5a**). This compound **5a**, N^{α}-[N^{α}-(N^{α}-acetyl-L-threonyl)-N^1-formyl-D-tryptophyl]-*N*-methyl-*N*-(phenylmethyl)-L-phenylalaninamide [Ac-Thr-D-Trp(CHO)-Phe-NMeBzl, FR113680], inhibited potently the ^{3}H-SP binding to guinea pig lung membranes with IC_{50} of $(5.8 \pm 0.78) \times 10^{-9}$ M, being about 16 times more potent than the lead **1**. The other acetylated compounds bearing a serine (**5b**), a *N,N*-dimethylglutamine (**5c**), and a histidine (**5d**) showed still potent activity but no change in potency when compared to the corresponding Boc analogs.

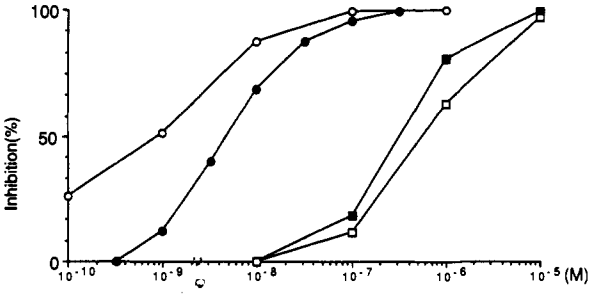


Figure 2. Inhibition of ^{3}H-SP Binding to guinea pig lung membranes. ○, unlabeled substance P; ●, compound **5a** (FR113680); ■, Spantide [(D-Arg^1,D-Trp^{7,8},Leu^{11})-SP]; □, (D-Pro^4,D-Trp^{7,9,10},Phe^{11})-SP (**2**). The IC_{50} values were 7.0×10^{-10}, 5.8×10^{-9}, 3.5×10^{-7}, and 6.0×10^{-7} M, respectively. The concentration of ^{3}H-SP was 1 nM throughout the experiments.

Finally, the acetyl group of **5a** was modified into various other acyl groups (Table III). The activity of a hydroxyacetyl (**5e**), a *N*,*N*-diethyl-β-alanine (**5h**), and a morpholine-4-carbonyl (**5k**) was between that of a Boc (**4a**) and an acetyl (**5a**). However, the introduction of a carboxyl group as shown in **5i** and **5j** was found to be deleterious to the receptor binding. The compound having a primary amine such as **5f** and **5g**, and the deacylated compound **5l** resulted in reduced activities in the 10^{-7} M range of IC_{50}.

Influence of Guinea Pig Plasma and Liver Homogenate on 5a. As the binding activity of **5a** was indicated to be most potent among those of the compounds synthesized in this paper, the stability of **5a** in guinea pig plasma and liver homogenate was investigated. The $T_{1/2}$ of **5a** in plasma[16] was estimated to be 75 min, which was comparable to that of the lead **1**. In liver homogenate, however, **5a** was quite stable and remained almost intact after thirty minutes or longer incubation period, in remarkable contrast to the short half-life of **1**.

Biological Activity of 5a. The biological activities of **5a** were examined in vitro and in vivo. The inhibitory activity for the SP receptor binding of **5a** was compared with those of unlabelled SP itself and the two known peptide antagonists, the octapeptide **2** [(D-Pro^4,D-Trp^{7,9,10},Phe^{11})-SP(4-11)] and Spantide[17] (D-Arg^1,D-Trp^{7,9},Leu^{11}-SP). It was concluded from this experiment (Figure 2) that **5a** was able to potently block the binding with 10^{-9} M range of IC_{50}, which was a little inferior to SP itself but markedly superior to the reference peptide antagonists. The result of our detailed study on the receptor selectivity of **5a** will be reported in a seperate paper.[18]

The compound **5a** inhibited the contraction of the isolated guinea pig trachea strips induced by SP (1×10^{-9} M) in a concentration dependent manner with IC_{50} of 2.3×10^{-6} M. On the other hand, Spantide and the octapeptide **2** exhibited no inhibitory action in this experiment at the concentration of 1.0×10^{-8} and 3.2×10^{-6} M, respectively. These two peptides, however, showed contracting activity at the higher concentration referred to above. On the contrary **5a** did not show contracting activity even at the highest concentration (3.2×10^{-5} M) used in our experiments. In addition **5a** indicated no influence on histamine or acethylcholine induced contraction of the isolated guinea pig trachea strips. From these results, **5a** can be considered to be a potent and specific SP antagonist with no agonistic activity.

The in vivo experiments revealed that **5a** was able to inhibit both the bronchoconstriction and airway edema in guinea pigs induced by SP in a dose-dependent fashion when administered intravenouly. The ED_{50} values for the constriction and edema were 0.66 mg/kg and 0.42 mg/kg, respectively. The results of our detailed pharmacological study on **5a** have been reported in a separate paper.[19]

Discussion

We described herein our continuing studies on the chemical modification of tripeptide SP antagonist **1**. The discovery process and the structure–activity relationships of **1** were reported in the preceding papers.[9,10] We also disclosed that **1** was unable to exhibit significant activity in in vivo experiments despite its potent activity to block ^{3}H-SP binding on guinea pig lung membranes. Thus, in order to look into the reason we initially investigated the stability and the metabolites of **1** in guinea pig plasma and liver homogenate. The result revealed that the benzyl ester part, which was indicated to be quite important for the receptor recognition, is the most labile part and easily hydrolyzed to produce the inactive acid analog. The lack of in vivo activity of **1** could be presumably explained by this rapid degradation of the molecule within the body.

On the basis of these results we were convinced that compounds exhibiting in vivo activity could be found, if we used a judicious approach that comprises the search for a surrogate of the benzyl ester part of **1** and the subsequent lead optimization. According to this design concept we modified the ester part first. It was consequently demonstrated that **3a** having a *N*-methyl-*N*-(phenylmethyl)amide structure was the best in terms of potency and stability against hydrolytic enzymes, suggesting that this carboxy terminal structure can be utilized to discover better SP antagonists. Thus, we implemented chemical modification of **3a** focusing mainly on the Boc-Gln part, which might be the variable parts as referred previously in the case of **1**. Finally we reached to the most potent compound **5a**, which carries a *N*-methyl-*N*-(phenylmethyl)amide for the carboxy terminal structure and a N^{α}-acetyl-L-threonine for the amino terminal. However, it has not been elucidated yet what kind of role is played for receptor recognition by each component in the structure of **5a**.

In accord with our expectation, the stability of **5a** against hydrolytic enzymes in a liver homogenate was greatly improved. The compound **5a** potently inhibited the binding of ^{3}H-SP to guinea pig membranes in nanomolar concentration. In vitro, **5a** antagonized the contraction of isolated guinea pig trachea strips induced by SP with 10^{-6} M range of IC_{50} without exhibiting any agonistic activity. However, this antagonistic activity was relatively weak when compared with the potent binding activity. The reason for this observation is not definite at present, but may be explained by the existence of different receptor subtypes in the two

(16) On HPLC analysis a metabolite appeared as a shoulder peak eluting prior to that of **5a** (rt, 4.0 min). This metabolite has not been identified yet but seems to be either the deacylated compound **5l** (rt, 3.56 min) or the deformylated compound (rt, 3.60 min). HPLC conditions were as follows: column, Lichrosorb RP18 (250 mm × 4 mm); eluent, MeOH–H_2O (3:1) containing 0.1% CF_3CO_2H; detection, UV254 nm; flow rate, 1.5 mL/min.

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preparations or by the difference in the experimental conditions used. In in vivo experiments, **5a** inhibited SP-induced bronchoconstriction and airway edema when administered intravenously. This effectiveness can presumably be explained by the fact that **5a** has its antagonistic potency remarkably enhanced and the stability to hydrolytic enzymes increased.

The novel tripeptide SP antagonist **5a** presented in this paper would be a useful tool for elucidating the physiological role of SP and its involvement in various diseases as well. However, in order to develop this compound in the clinic several points have to be ameliorated in physicochemical and pharmacokinetic features, e.g. aqueous solubility and oral absorbability. The effort to discover a better SP antagonist using **5a** as a new lead is continuing in our laboratories, and the results will be presented in a forthcoming paper.[20]

Experimental Section

Instruments and Materials. Melting points were measured on Mel-Temp (Mitamura Riken Kogyo, Japan), and are uncorrected. Proton NMR spectra were recorded on a 90-MHz spectrometer EM-390 (Varian) or a 200-MHz spectrometer AC-200T (Brucker); chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. Mass spectra (FAB) were recorded on Finnigan MAT TSQ-70. HPLC was performed on 655A system connected with D-2000 data processor (Hitachi, Japan). IR spectra were taken with an IR-408 spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were recorded on a DIP-360 (Nihon Bunkoh, Co. Ltd., Japan) polarimeter. Elemental analyses were performed on a Perkin-Elmer 2400 CHN analyzer. Analytical results were within ±0.4% of the theoretical values unless otherwise noted. Thin-layer chromatography was performed on a precoated silica gel plate Kieselgel $60F_{254}$ (E. Merck, A.G., Darmstadt, Germany). Solvent systems were as follows: A, $CHCl_3$–MeOH–EtOAc (4:1:1); B, $CHCl_3$–MeOH (10:1); C, $CHCl_3$–MeOH (20:1); D, $CHCl_3$–MeOH–AcOH (8:1:1); E, *n*-BuOAc–*n*-BuOH–AcOH–H_2O (80:15:40:24); F, EtOAc–methyl ethyl ketone–HCO_2H-H_2O (5:3:1:1), G, toluene–EtOAc (4:1). Silica gel column chromatography was performed on Kieselgel-60 (230–400 mesh) (E. Merck, A.G., Darmstadt, Germany). Extraction solvents were dried over magnesium sulfate. Solvents used for reactions were dried over 3A molecular sieves. The following amino acid derivatives were commercially available: Boc-Phe-OH, Boc-D-Trp(CHO)-OH, Boc-Gln-OH, Boc-His(Tos)-OH (Peptide Institute, Minoh, Japan), Boc-Hyp-OH, Boc-Orn(Z)-OH, Boc-Lys(Z)-OH, Z-Gly-OH, Z-β-Ala-OH (Kokusan Chemicals Co. Ltd., Tokyo, Japan), and Boc-Thr-OH, Boc-Ser-OH (Eibeiss Co. Ltd., Yokohama, Japan). The amino acid derivatives Boc-Gln(Me_2)-OH,[21] Boc-isoAsn-OH,[22] Boc-MeThr-OH,[23] and Boc-phenylalanine 2-pyridyl ester[24] were synthesized according to the method described in the literature with certain modifications. The reagents WSCD and HOBT were purchased from Eibeiss Co. Ltd. (Yokohama, Japan). The octapeptide 2 was purchased from Bachem Feinchemikalien AG (Switzerland), substance P and Spantide were from Peptide Institute (Minoh, Japan). These materials were used without further purification.

Receptor Binding Assay and Contractile Response of Isolated Guinea Pig Trachea. These in vitro experiments were implemented according to the method described in the preceding paper.[9]

Substance P Induced Airway Constriction in Guinea Pigs. Male Hartley strain guinea pigs weighing 270–395 g were anesthetized by ip injection of pentobarbital (10 mg per animal). The jugular vein and trachea were cannulated and the animals were artificially ventilated (5 mL of air, 60 strokes/min). The pressure in the respirator system, i.e. the insufflation pressure, was measured constantly using a transducer (TP 200T, Nihon Kohden) connected to a polygraph (AP 601G, Nihon Kohden). Substance P (13.5 μg/kg) was injected intravenously every 15 min through the jugular vein cannula to induce airway constriction until a reproducible constriction (control response) was obtained. The test compound was administered intravenously 2 min prior to a further challenge with substance P injection. The resulting constriction was compared with the control constriction.

Substance P Induced Airway Edema in Guinea Pigs. Male Hartley strain guinea pigs weighing 280–375 g were given intravenously a solution containing substance P (1.3 μg/kg) together with Evans blue dye (20 mg/kg) and heparin (200 IU/kg). Animals were stunned 10 min later, bled, and perfused through the pulmonary artery with 50 mL of saline. The trachea and main bronchi were removed, blotted dry, and weighed and were incubated at 37 °C in 0.5 mL of 1 N potassium hydroxide overnight, and then Evans blue dye was extracted by addition of a mixture of 0.6 N phosphoric acid and acetone (5:13, 4.5 mL). After centrifugation at 3000 rpm for 15 min, the concentration of the extractable Evans blue dye in the supernatant was quantified from light absorbance at 620 nm (UV-160, Shimadzu, Japan) by interpolation on standard curve of dye concentrations in the range 0–4.0 μg/mL. The test drug or control vehicle was administered intravenously 2 min prior to the challenge of substance P. Increased amount of leaked Evans blue dye was calculated by subtracting the Eans blue content (11.6 ± 1.6 μg/tissue, $n = 3$) obtained from animals injected with Evans blue dye and heparin solution without substance P.

Influence of Guinea Pig Plasma and Liver Homogenate on Compounds 1, 3a, and 5a. Male Hartley strain guinea pigs weighing 300–400 g were anesthetized with pentobarbital (40 mg/kg, ip) and then sacrificed. Blood was drawn from the abdominal aorta and was added to heparin (300 IU/10 mL of blood). Plasma was obtained by centrifugation at 500*g* for 10 min. Liver was homogenized in 20 volumes of an ice-cooled PBS (–) in a glass homogenizer fitted with a tefron pestle. To the plasma (2.5 mL) or the liver homogenate (2.5 mL) prepared above was added a 25-μL portion of a solution of the test compound (10.3 mg) in DMSO (1 mL). The mixture was incubated at 37 °C with shaking. Sampling time was 0.5, 1, 2, and 5 min for liver homogenate experiment, and 5, 10, 30, 60, and 120 min for plasma. An aliquot (0.1 mL) of the incubated mixture was added to ice-cooled acetnitrile (0.4 mL), and then the mixture was vigorously shaken by the vortex mixer. The supernatant solution was filtered through a membrane filter (0.45 μm, type NS, Millipore) and a 25-μL portion of the filtrate was used for the HPLC analysis. The conditions of the analysis are described in the legend of Figure 1, and the analysis at each sampling time was performed in duplicate.

Synthesis of Starting Boc-phenylalanine Derivatives. Boc-phenylalanine-*N*-methyl-*N*-(phenylmethyl)amide (6). A solution of Boc-phenylalanine (54.8 g, 0.206 mol) and *N*-methylmorpholine (20.9 g, 0.206 mol) in CH_2Cl_2 (500 mL) was cooled to –20 °C. To this solution was added isobutyl chloroformate (28.2 g, 0.206 mol) dropwise at this temperature during 7 min. The resulting mixture was stirred for 20 min at the temperature. Then the solution was cooled to –35 °C, and *N*-methylbenzylamine (25.0 g, 0.207 mol) was added. The mixture was stirred for 2 h, while the temperature was gradually raised to 0 °C. The reaction mixture was washed successively with water, sodium hydrogen carbonate solution, 0.5 N hydrochloric acid, and brine. After evaporation the residue obtained was crystallized with IPE–*n*-hexane, filtered, and dried to give 6 (64.9 g, 85.5%): mp 90–91.5 °C; $[\alpha]^{25}_D = +19.99°$ ($c = 1.04$, $CHCl_3$); IR (Nujol) 3380, 1690, 1645 (sh), 1635, 1525 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$)[25] δ 1.39 (minor rotamer, 9 H × 0.25, s), 1.43 (major rotamer,

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9 H × 0.75, s), 2.62 (major rotamer, 3 H × 0.75, s), 2.83 (minor rotamer, 3 H × 0.25, s), 3.0 (2 H, d, J = 7 Hz), 4.11 and 4.37 (minor rotamer, 2 H × 0.25, 2 d, J = 16 Hz), 4.43 and 4.56 (major rotamer, 2 H × 0.75, 2 d, J = 15 Hz), 4.87 (1 H, dt, J = 7, 7 Hz), 5.39 (minor rotamer, 1 H × 0.25, d, J = 7 Hz), 5.44 (major rotamer, 1 H × 0.75, d, J = 7 Hz), 7.0–7.4 (10 H, m); R_f = 0.51 (system G). Anal. ($C_{22}H_{28}N_2O_3$) C, H, N.

Boc-phenylalanine-*N*-(phenylmethyl)amide (7). Prepared similarly as **6** from Boc-phenylalanine and benzylamine: 92.6% yield; mp 131–132 °C (EtOAc–IPE); $[\alpha]^{20}_D$ = +3.93° ($CHCl_3$, c = 1.0) (lit.[28] mp 131–132 °C); IR (Nujol) 3310, 1680, 1660, 1525 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (9 H, s), 3.07 (2 H, d, J = 7 Hz), 4.3 (1 H, m), 4.34 (2 H, d, J = 6 Hz), 5.09 (1 H, d, J = 7 Hz), 6.19 (1 H, t, J = 6 Hz), 7.0–7.3 (10 H, m); R_f = 0.46 (system G). Anal. ($C_{21}H_{26}N_2O_3$) C, H, N.

1-(Boc-phenylalanyl)-2-phenylethane (8). A solution of Boc-phenylalanine 2-pyridyl ester (2.0 g, 5.82 mmol) in THF (30 mL) was cooled to −40 °C. To this solution was added a solution of (phenylethyl)magnesium bromide [prepared from phenylethyl bromide (2.36 g, 12.8 mmol) and magnesium (0.28 g, 11.6 mmol) by a standard method] in THF (15 mL). The reaction mixture was stirred at the temperature for 0.5 h, and then saturated aqueous solution of ammonium chloride (30 mL) was added thereto. After the temperature was raised up to room temperature, the mixture was extracted with ether. The organic layer was washed with brine, dried, and evaporated under reduced pressure. The crude product obtained was recrystallized with EtOH to give **8** (1.17 g, 56.8%): mp 102–103 °C; $[\alpha]^{20}_D$ = +36.86° (c = 1.0, $CHCl_3$); IR (Nujol) 3380, 1718, 1690, 1518 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (9 H, s), 2.6–3.1 (6 H, m), 4.50 (1 H, dt, J = 7, 6 Hz), 5.08 (1 H, d, J = 7 Hz), 7.0–7.3 (10 H, m); R_f = 0.72 (system G). Anal. ($C_{22}H_{27}NO_3$) C, H, N.

Synthesis of Protected Dipeptides. Boc-D-Trp(CHO)-Phe-NMeBzl (9). To a solution of **6** (4.23 g, 11.48 mmol) and anisole (4 mL) in CH_2Cl_2 (15 mL) was added 4 N hydrochloric acid (15 mL) in dioxane under ice-cooling. The resulting mixture was stirred at this temperature for 15 min and at room temperature for 1 h. The solvent was evaporated under reduced pressure, and the residue was crystallized from ether. The crystalline product was filtered and dried to give HCl·H-Phe-NMeBzl (2.99 g, 85.4%): mp 133–135 °C; $[\alpha]^{25}_D$ = +57.78° (c = 1.0, $CHCl_3$). This hydrochloride (9.81 mmol), Boc-D-Trp-(CHO)-OH (3.26 g, 9.81 mmol), and HOBT (1.32 g, 9.81 mmol) were dissolved in DMF (40 mL). To the solution was added WSCD (1.55 g, 10 mmol) under ice-cooling. The reaction mixture was stirred at this temperature for 1 h and at room temperature for 2 h and then concentrated, diluted in water, and extracted with EtOAc. The organic layer was washed successively with sodium hydrogen carbonate solution, water, 0.5 N hydrochloric acid, and brine and evaporated under reduced pressure. The residue obtained was crystallized from EtOAc–IPE (3:4) to give **9** (4.96 g, 86.7%): mp 88–90 °C; $[\alpha]^{25}_D$ = +16.75° (c = 0.79, $CHCl_3$); IR (Nujol) 3300, 1710, 1620, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.41 (9 H, s), 2.70 and 2.85 (3 H, 2s), 2.90 (2 H, d, J = 7 Hz), 3.18 (2 H, d, J = 7 Hz), 4.2–4.7 (3 H, m), 5.0–5.3 (2 H, m), 6.9–7.4 (13 H, m), 7.5–7.7 (2 H, m), 8.3 (1 H, m), 8.8–9.5 (1 H, br s); R_f = 0.88 (system A). Anal. ($C_{34}H_{38}N_4O_5$) C, H, N.

The following two compounds, **10** and **11**, were prepared similarly as **9**.

Boc-D-Trp(CHO)-Phe-NHBzl (10). Prepared from **7** and Boc-D-Trp(CHO)-OH: 88.3% yield; mp 190–191 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = −17.75° (c = 1.11, DMF); IR (Nujol) 3310, 1700, 1685, 1640, 1550, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.27 (9 H, s), 2.6–3.1 (4 H, m), 4.1–4.8 (2 H, m), 4.35 (2 H, d, J = 6 Hz), 6.92 (1 H, d, J = 9 Hz), 7.0–7.8 (14 H, m), 8.2 (1 H, br s), 8.47 (2 H, d, J = 9 Hz), 9.4 (1 H, br s); R_f = 0.95 (system D). Anal. ($C_{33}H_{36}N_4O_5$) C, H, N.

Boc-D-Trp(CHO)-Phe-CH_2CH_2Ph (11). Prepared from **8** and Boc-D-Trp(CHO)-OH: 73.8% yield; mp 171–172 °C (EtOH); $[\alpha]^{25}_D$ = −38.6° (c = 1.03, DMF); IR (Nujol) 3350, 1720, 1660, 1520 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.13 (9 H, s), 2.5–3.2 (8 H, m), 4.2 (1 H, m), 4.5 (1 H, m), 6.7–7.7 (5 H, m), 7.10 (10 H, s), 8.1 (1 H, m), 8.58 (1 H, d, J = 9 Hz), 9.3 (1 H, br s); R_f = 0.80 (system C). Anal. ($C_{34}H_{37}N_3O_5$) C, H, N.

Synthesis of Tripeptides Having a Boc at Amino Terminal. Boc-Thr-D-Trp(CHO)-Phe-NMeBzl (4a). To a solution of **9** (15.3 g, 26.3 mmol) in CH_2Cl_2 (150 mL) was added 4 N hydrochloric acid (150 mL) in dioxane under ice-cooling. The solution was stirred at this temperature for 10 min and then at room temperature for 1 h. The reaction mixture was concentrated and the residue obtained was triturated with IPE to form precipitates. The precipitates, which were identified as HCl·H-D-Trp(CHO)-Phe-NMeBzl, were collected by filtration. This hydrochloride (13.4 g, 25.8 mmol), Boc-Thr-OH (5.66 g, 25.8 mmol), and HOBT (3.48 g, 25.8 mmol) were dissolved in DMF (160 mL). To the solution was added WSCD (4.4 g, 28.4 mmol) under ice-cooling. The reaction mixture was stirred at this temperature for 1.5 h and at room temperature for 2 h. The solvent was evaporated under reduced pressure. The residue obtained was diluted with water and extracted with ethyl acetate. The organic layer was washed successively with sodium hydrogen carbonate solution, water, 0.5 N hydrochloric acid, and brine and evaporated under reduced pressure. The residue obtained was crystallized from a mixed solvent of EtOAc and IPE (1:1) to give **4a** (16.7 g, 94.7%): mp 108–111 °C dec; $[\alpha]^{25}_D$ = +39.03° (c = 1.14, $CHCl_3$); IR (Nujol) 3360, 3220, 1718, 1690, 1668, 1650, 1626, 1560, 1530 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.81 (3 H, br s), 1.34 (9 H, s), 2.79 and 2.87 (3 H, 2 s), 2.6–3.1 (4 H, m), 3.6–3.9 (2 H, m), 4.3–4.75 (4 H, m), 4.9–5.1 (1 H, m), 6.33 (1 H, d, J = 7 Hz), 7.0–7.7 (14 H, m), 7.9–8.2 (2 H, m), 8.65 and 8.75 (1 H, 2 d, J = 8 Hz), 9.13 and 9.61 (1 H, 2 s); R_f = 0.66 (system C). Anal. ($C_{38}H_{45}N_5O_7$) C, H, N.

The following tripeptides were prepared similarly as **4a**.

Boc-Gln-D-Trp(CHO)-Phe-NMeBzl (3a). Prepared from **9** and Boc-Gln-OH: 63.8% yield; mp 197–199 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +11.70° (c = 1.06, DMF); IR (Nujol) 3440, 3350 (sh), 3300, 3240 (sh), 1715, 1690, 1665, 1650, 1635, 1550, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.5–2.2 (4 H, m), 2.6–3.2 (4 H, m), 2.79 and 2.87 (3 H, 2 s), 3.7–4.2 (1 H, m), 4.2–5.3 (4 H, m), 6.7 (2 H, br s), 7.0–7.6 (14 H, m), 7.6–7.9 (1 H, m), 7.9–8.4 (2 H, m), 8.7 (1 H, m), 9.3 (1 H, br s); R_f = 0.63 (system B). Anal. ($C_{39}H_{46}N_6O_7$) C, H, N.

Boc-Gln-D-Trp(CHO)-Phe-NHBzl (3b). Prepared from **10** and Boc-Gln-OH: 77.8% yield; mp 206 °C (EtOH–H_2O) dec; $[\alpha]^{25}_D$ = −6.65° (c = 1.08, DMF); IR (Nujol) 3300, 1705, 1690, 1660, 1640, 1545 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30 (9 H, s), 1.5–2.2 (4 H, m), 2.6–3.1 (4 H, m), 3.7–4.2 (1 H, m), 4.31 (2 H, d, J = 6 Hz), 4.5–4.9 (2 H, m), 6.6–6.9 (2 H, m), 7.1–7.8 (15 H, m), 7.8–8.3 (2 H, m), 8.4–8.7 (2 H, m), 9.3 (1 H, br s); R_f = 0.63 (system D). Anal. ($C_{38}H_{44}N_6O_7 \cdot 0.33H_2O$) C, H, N.

Boc-Gln-D-Trp(CHO)-Phe-CH_2CH_2Ph (3c). Prepared from **11** and Boc-Gln-OH: 65.7%; mp 193 °C (EtOH) dec; $[\alpha]^{25}_D$ = −7.85° (c = 1.07, DMF); IR (Nujol) 3330, 1710, 1690, 1655, 1640, 1525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.31 (9 H, s), 1.4–2.1 (4 H, m), 2.5–3.3 (8 H, m), 3.7–4.1 (1 H, m), 4.3–4.8 (2 H, m), 6.6–6.9 (2 H, m), 7.0–7.8 (5 H, m), 7.18 (10 H, s), 7.8–8.3 (2 H, m), 8.3–8.7 (1 H, m), 9.25 (1 H, br s); R_f = 0.78 (system D). Anal. ($C_{39}H_{45}N_5O_7$) C, H, N.

Boc-Ser-D-Trp(CHO)-Phe-NMeBzl (4b). Prepared from **9** and Boc-Ser-OH: 60.5% (amorphous solid); $[\alpha]^{25}_D$ = +11.12° (c = 1.0, DMF); IR (Nujol) 3300, 1710, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.34 (9 H, s), 2.5–3.1 (4 H, m), 2.77 and 2.91 (3 H, 2 s), 3.42 (2 H, m), 3.9 (1 H, m), 4.2–5.1 (5 H, m), 6.51 (1 H, d, J = 7 Hz), 6.9–7.7 (14 H, m), 7.8–8.2 (2 H, m), 8.64 (1 H, m), 9.15 (1 H, br s); R_f = 0.60 (system B). Anal. ($C_{37}H_{43}N_5O_7 \cdot 0.17$-$n$-$C_6H_{14}$) H, N; C: calcd, 66.78; found, 65.84.

Boc-Gln(Me_2)-D-Trp(CHO)-Phe-NMeBzl (4c). Prepared from **9** and Boc-Gln(Me_2)-OH: 87.0% (amorphous solid); $[\alpha]^{25}_D$ = +11.43° (c = 1.11, DMF); IR (Nujol) 3300, 1710, 1635, 1525 (sh), 1510 (sh), 1490 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.3–2.1 (4 H, m), 2.6–3.2 (4 H, m), 2.69 (3 H, s), 2.77 (3 H, s), 2.82 and 2.91 (3 H, 2 s), 3.8–4.1 (1 H, m), 4.2–5.2 (4 H, m), 6.77 (1 H, d, J = 6 Hz), 7.0–7.7 (13 H, m), 7.7–7.9 (1 H, m), 7.9–8.3 (2 H,

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m), 8.5–8.9 (1 H, m), 9.3 (1 H, br s); R_f = 0.46 (system C). Anal. ($C_{41}H_{50}N_6O_7$) C, H; N: calcd, 11.37; found, 10.92.

Boc-Orn(Z)-D-Trp(CHO)-Phe-NMeBzl (4d). Prepared from 9 and Boc-Orn(Z)-OH: 86.9% yield (amorphous solid); $[\alpha]^{25}_D$ = +11.27° (c = 1.0, DMF); IR (Nujol) 3330, 3300, 1710, 1695, 1645, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.9–1.5 (4 H, m), 1.33 (9 H, s), 2.5–3.1 (6 H, m), 2.77 and 2.85 (3 H, 2 s), 3.7–4.0 (1 H, m), 4.1–5.1 (4 H, m), 4.97 (2 H, s), 6.63 (1 H, d, J = 7 Hz), 6.9–7.5 (19 H, m), 7.5–7.8 (1 H, m), 7.8–8.3 (2 H, s), 8.5–8.8 (1 H, m), 9.2 (1 H, br s); R_f = 0.62 (system B). Anal. ($C_{47}H_{54}N_6O_8$) C, H, N.

Boc-Lys(Z)-D-Trp(CHO)-Phe-NMeBzl (4e). Prepared from 9 and Boc-Lys(Z)-OH: 81.7% yield (amorphous solid); $[\alpha]^{25}_D$ = +6.82° (c = 1.08, DMF); IR (Nujol) 3300, 1710, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.8–1.5 (4 H, m), 1.30 (9 H, s), 2.5–3.1 (6 H, m), 2.77 and 2.85 (3 H, 2 s), 3.6–4.0 (1 H, m), 4.2–5.0 (4 H, m), 4.97 (2 H, s), 6.6 (1 H, m), 6.9–7.5 (19 H, m), 7.5–7.8 (1 H, m), 7.8–8.3 (2 H, m), 8.5–8.9 (1 H, m), 9.3 (1 H, br s); R_f = 0.47 (system B). Anal. ($C_{48}H_{56}N_6O_8 \cdot 0.5H_2O$) C, H, N.

Boc-Hyp-D-Trp(CHO)-Phe-NMeBzl (4g). Prepared from 9 and Boc-Hyp-OH: 87.3% yield (amorphous solid); $[\alpha]^{25}_D$ = +3.94° (c = 1.04, DMF); IR (Nujol) 3300, 1710, 1690, 1670, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.13, 1.20, and 1.33 (9 H, 3 s), 1.6–2.1 (2 H, m), 2.6–3.0 (7 H, m), 3.23 (2 H, m), 3.9–4.2 (2 H, m), 4.3–5.1 (5 H, m), 6.9–7.5 (13 H, m), 7.65 (1 H, m), 7.9–8.3 (2 H, m), 8.8 (1 H, m), 9.3 (1 H, br s); R_f = 0.63 (system A). Anal. ($C_{39}H_{45}N_5O_7 \cdot H_2O$) C, H, N.

Boc-isoAsn-D-Trp(CHO)-Phe-NMeBzl (4h). Prepared from 9 and Boc-isoAsn-OH: 84.8% yield; mp 213–216 °C (DMF–H_2O); $[\alpha]^{25}_D$ = +15.72° (c = 0.97, DMF); IR (Nujol) 3400, 3300, 3230, 1715, 1670, 1640, 1525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30 (9 H, s), 2.3–2.5 (2 H, m), 2.6–3.2 (4 H, m), 2.76 and 2.83 (3 H, 2 s), 4.0–5.1 (5 H, m), 6.6–7.7 (17 H, m), 7.8–8.3 (2 H, m), 8.4–8.8 (1 H, m), 9.3 (1 H, br s); R_f = 0.73 (system D). Anal. ($C_{38}H_{44}N_6O_7$) C, H, N.

Boc-MeThr-D-Trp(CHO)-Phe-NMeBzl (4i). Prepared from 9 and Boc-MeThr-OH: 69.5% yield (amorphous solid); $[\alpha]^{25}_D$ = −9.63° (c = 1.03, DMF); IR (Nujol) 3420, 3300, 1710, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.6–1.0 (3 H, m), 1.35 (9 H, s), 2.6–3.1 (4 H, m), 2.73 (3 H, s), 2.78 and 2.85 (3 H, 2 s), 3.6–5.2 (7 H, m), 6.9–7.8 (14 H, m), 7.8–8.2 (2 H, m), 8.65 (1 H, m), 9.2 (1 H, br s); R_f = 0.50 (system C); MS (FAB) m/z 698.4 (M + H)$^+$.

Boc-His-D-Trp(CHO)-Phe-NMeBzl (4f). A solution of Boc-His(Tos)-OH (0.82 g, 2.0 mmol) in DMF (10 mL) was cooled to −15 °C. *N*-Methylmorpholine (202 mg, 2.0 mmol) and isobutyl chloroformate (273 mg, 2.0 mmol) were added thereto, and the mixture was stirred at this temperature for 10 min. To this solution was added a solution of HCl·H-D-Trp(CHO)-Phe-NMeBzl (1.04 g, 2.0 mmol), which was prepared from 9 according to the method described in 4a, and *N*-methylmorpholine (202 mg, 2.0 mmol) in DMF (10 mL). The mixture was stirred at the temperature for 1 h, and then the solvent was evaporated under reduced pressure. The residue obtained was diluted in water and extracted with EtOAc. The organic layer was washed successively with 2% hydrochloric acid, water, 2% sodium hydrogen carbonate solution, and brine, and evaporated under reduced pressure leading to Boc-His(Tos)-D-Trp(CHO)-Phe-NMeBzl as an amorphous solid (1.64 g, 93.7%). This crude product (1.64 g, 1.9 mmol) was dissolved in DMF (20 mL), and pyridine hydrochloride (2.68 g, 23.2 mmol) was added. The mixture was stirred at room temperature for 2 h, and then the solvent was evaporated under reduced pressure. The residue obtained was diluted in water and extracted with EtOAc. The organic layer was washed successively with 2% hydrochloric acid, water, 2% sodium hydrogen carbonate solution, and brine and evaporated under reduced pressure. The residue (1.28 g) was chromatographed on a silica gel column (60 g) eluting with $CHCl_3$–MeOH (20:1). The product obtained was triturated with Et_2O to give 4f as an amorphous solid (1.04 g, 77.0%): $[\alpha]^{25}_D$ = +13.88° (c = 1.04, DMF); IR (Nujol) 3300, 1710, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.31 (9 H, s), 2.5–3.1 (6 H, m), 2.76 and 2.84 (3 H, 2 s), 3.9–5.1 (5 H, m), 6.5–6.9 (1 H, m), 6.56 (1 H, s), 6.9–7.7 (14 H, m), 7.45 (1 H, s), 7.7–8.3 (2 H, m), 8.6–8.8 (1 H, m), 9.2 (1 H, br s), 11.6 (1 H, br s); R_f = 0.41 (system A). Anal. ($C_{40}H_{45}N_7O_6 \cdot 0.5Et_2O$) C, H, N.

Synthesis of Tripeptides Having an Acyl at Amino Terminal. HCl·H-Thr-D-Trp(CHO)-Phe-NMeBzl (5l). To a solution of 4a (17.6 g, 25.7 mmol) in CH_2Cl_2 (120 mL) was added 4 N hydrochloric acid (120 mL) in dioxane under ice-cooling. The solution was stirred at this temperature for 10 min and at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was triturated with IPE. The resulting precipitates were filtered and dried to give 5l (16.0 g, quantitative yield) as an amorphous solid: $[\alpha]^{25}_D$ = −4.41° (c = 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 0.77 (3 H, t, J = 6 Hz), 2.80 and 2.88 (3 H, 2 s), 2.6–3.0 (4 H, m), 3.5–3.8 (2 H, m), 4.15–5.1 (5 H, m), 6.95–7.4 (13 H, m), 7.4–7.8 (2 H, m), 8.10 (3 H, br s), 8.6–9.0 (2 H, m), 9.1–9.7 (1 H, br s); R_f = 0.20 (system A).

Ac-Thr-D-Trp(CHO)-Phe-NMeBzl (5a). To a cooled (−15 °C) solution of 5l (16.0 g, 25.8 mmol) and triethylamine (5.22 g, 51.6 mmol) in CH_2Cl_2 (200 mL) was added acetic anhydride (2.63 g, 25.8 mmol) dropwise, and the reaction mixture was stirred at the temperature for 20 min. The mixture was washed successively with water, sodium hydrogen carbonate solution, water, 0.5 N hydrochloric acid, and brine, dried, and evaporated under reduced pressure. The residue obtained was recrystallized with 65% aqueous ethanol (300 mL) to give 5a (13.3 g, 82.4%): mp 181–183 °C; $[\alpha]^{25}_D$ = +20.16° (c = 1.0, DMF); IR (Nujol) 3450, 3260, 1720 (sh), 1698, 1660 (sh), 1640, 1550 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.75 (3 H, br s), 1.85 (3 H, s), 2.79 and 2.88 (3 H, 2 s), 2.7–3.1 (4 H, m), 3.75 (1 H, br s), 4.10 (1 H, br s), 4.3–4.8 (4 H, m), 4.96 (1 H, m), 7.0–7.4 (13 H, m), 7.6–7.8 (2 H, m), 7.9–8.3 (2 H, m), 8.68 and 8.77 (1 H, 2 d, J = 8 Hz), 9.15 and 9.61 (1 H, 2 s); R_f = 0.58 (system A). Anal. ($C_{35}H_{39}N_5O_6 \cdot H_2O$) C, H, N.

The following compounds, 5b–d, were prepared from the corresponding Boc analogs by deprotection of the Boc and subsequent acetylation similarly as 5a.

Ac-Ser-D-Trp(CHO)-Phe-NMeBzl (5b). Prepared from 4b: 80.0% yield; mp 125 °C (EtOH–H_2O) dec; $[\alpha]^{25}_D$ = +16.86° (c = 1.10, DMF); IR (Nujol) 3300, 1710, 1640, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.82 (3 H, s), 2.5–3.1 (4 H, m), 2.77 and 2.85 (3 H, 2 s), 3.40 (2 H, m), 4.0–5.1 (6 H, m), 6.9–7.7 (14 H, m), 7.80 (1 H, d, J = 8 Hz), 7.9–8.2 (2 H, m), 8.62 (1 H, m), 9.2 (1 H, br s); R_f = 0.57 (system B). Anal. ($C_{34}H_{37}N_5O_6 \cdot 0.33EtOH$) C, H, N.

Ac-Gln(Me$_2$)-D-Trp(CHO)-Phe-NMeBzl (5c). Prepared from 4c: 76.2% yield (amorphous solid); $[\alpha]^{25}_D$ = +19.06° (c = 1.0, DMF); IR (Nujol) 3300, 1710, 1645 (sh), 1640, 1545 (sh), 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.3–2.1 (4 H, m), 1.79 (3 H, s), 2.5–3.2 (4 H, m), 2.63 (3 H, s), 2.73 (3 H, s), 2.82 and 2.90 (3 H, 2 s), 4.0–5.2 (5 H, m), 6.9–7.6 (13 H, m), 7.6–8.3 (4 H, m), 8.5–8.9 (1 H, m), 9.3 (1 H, br s); R_f = 0.53 (system B). Anal. ($C_{38}H_{44}N_6O_6 \cdot 0.5H_2O$) C, H, N.

Ac-His-D-Trp(CHO)-Phe-NMeBzl·HCl (5d). Prepared from 4f and converted into hydrochloride by treatment with hydrochloric acid in dioxane after silica gel chromatography ($CHCl_3$–MeOH, 10:1): 44.3% yield (amorphous solid); IR (Nujol) 3270, 1710, 1640, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.77 (3 H, s), 2.5–3.1 (6 H, m), 2.78 and 2.85 (3 H, 2 s), 4.2–5.1 (5 H, m), 6.9–7.4 (13 H, m), 7.4–7.8 (2 H, m), 7.8–8.3 (3 H, m), 8.5–8.9 (1 H, m), 8.89 (1 H, s), 9.3 (1 H, br s), 14.4 (2 H, br s); R_f = 0.35 (system A). Anal. ($C_{37}H_{40}ClN_7O_5 \cdot 1.5H_2O \cdot 0.1Et_2O$) C, H, N.

(Hydroxyacetyl)-Thr-D-Trp(CHO)-Phe-NMeBzl (5e). To a solution of 5l (1.0 g, 1.61 mmol), hydroxyacetic acid (147 mg, 1.93 mmol), and HOBT (216 mg, 1.6 mmol) in DMF (20 mL) was added WSCD (299 mg, 1.93 mmol) under ice-cooling. The solution was stirred at this temperature for 1 h and at room temperature for 6 h, and then the solvent was evaporated under reduced pressure. The residue obtained was diluted with water and extracted with EtOAc. The organic layer was washed successively with sodium hydrogen carbonate solution, water, 0.5 N hydrochloric acid, and brine and evaporated under reduced pressure to give 5e as an amorphous solid (1.27 g, 93.8%): $[\alpha]^{25}_D$ = +16.96° (c = 1.1, DMF); IR (Nujol) 3300, 1710, 1640, 1535 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.71 (3 H, br), 2.80 and 2.89 (3 H, 2 s), 2.6–3.1 (4 H, m), 3.18 (1 H, m), 3.86 (2 H, s), 4.1–4.4 (2 H, m), 4.5–4.8 (3 H, m), 4.82–5.05 (2 H, m), 5.7 (1 H, m), 7.0–7.4 (13 H, m), 7.4–7.6 (1 H, m), 7.7 (1 H, m), 7.9–8.3 (2 H, m), 8.70 and 8.80 (1 H, 2 d, J = 8 Hz), 9.15 and 9.60 (1 H, 2 s); R_f = 0.38 (system A). Anal. ($C_{35}H_{39}N_5O_7 \cdot 1.5H_2O$) C, H, N.

H-Gly-Thr-D-Trp(CHO)-Phe-NMeBzl (5f). The Z-protected intermediate was prepared from 5l and Z-Gly-OH similarly as 5e, in 55.7% yield (amorphous solid): $[\alpha]^{25}_D$ = +6.40° (c = 1.0, DMF); IR (Nujol) 3300, 1710, 1640 (sh), 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.80 (3 H, d, J = 6 Hz), 2.6–3.1 (4 H, m), 2.77 and

2.84 (3 H, 2 s), 3.70 (2 H, d, J = 6 Hz), 3.8 (1 H, m), 4.1 (1 H, m), 4.3–5.0 (5 H, m), 4.92 (2 H, s), 6.9–7.7 (16 H, m), 7.27 (5 H, s), 8.0 (2 H, m), 8.6 (1 H, t, J = 6 Hz), 9.2 (1 H, br s); R_f = 0.41 (system A). A solution of the Z-protected intermediate (0.56 g, 0.72 mmol) in a mixed solvent of EtOH (30 mL) and AcOH (10 mL) was hydrogenated over 5% Pd (0.35 g) on charcoal under atmospheric pressure. The solvent was evaporated, and the residue was dissolved in water. The solution was then lyophilized to give **5f** as an amorphous solid (0.41 g, 88%): $[\alpha]^{20}_D$ = +11.9° (c = 1.0, DMF); IR (Nujol) 3300, 1720 (sh), 1690, 1660 (sh), 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.80 (3 H, d, 6 Hz), 2.6–3.0 (4 H, m), 2.80 and 2.97 (3 H, 2 s), 3.27 (2 H, br s), 3.8–5.0 (7 H, m), 6.9–7.3 (15 H, m), 7.4–7.7 (2 H, m), 7.8–8.2 (3 H, br s), 8.6 (1 H, m), 9.2 (1 H, br s); R_f = 0.58 (system F); MS (FAB) m/z 641.3 (M + H)$^+$. Anal. ($C_{35}H_{40}N_6O_6 \cdot 2H_2O \cdot 0.3AcOH$) C, H, N.

H-β-Ala-Thr-D-Trp(CHO)-Phe-NMeBzl·HCl (5g). The Z-protected intermediate was prepared from **5l** and Z-β-Ala-OH, in 82.3% yield (amorphous solid); $[\alpha]^{25}_D$ = +15.65° (c = 1.02, DMF); IR (Nujol) 3300, 1710, 1690, 1640, 1535 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.75 (3 H, d, J = 6 Hz), 2.36 (2 H, t, J = 7 Hz), 2.5–3.3 (6 H, m), 2.70 and 2.84 (3 H, 2 s), 3.5–3.9 (1 H, m), 3.9–4.2 (1 H, m), 4.2–5.0 (5 H, m), 4.96 (2 H, s), 6.8–7.5 (19 H, m), 7.5–7.8 (2 H, m), 7.8–8.2 (2 H, m), 8.61 (1 H, m), 9.2 (1 H, br s); R_f = 0.58 (system B). A solution of the Z-protected intermediate (0.59 g, 0.75 mmol) in AcOH (18 mL) was hydrogenated over 10% Pd (0.2 g) on charcoal under atmospheric pressure. The mixture was filtered and evaporated under reduced pressure. The residue was treated with 4 N hydrochloric acid (0.8 mL) in dioxane and evaporated to dryness. The residue was triturated with ether, filtered, and dried to give **5g** (0.48 g, 92.9%) as an amorphous solid: $[\alpha]^{20}_D$ = +10.8° (c = 1.0, DMF); IR (Nujol) 3300, 1710 (sh), 1640, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.82 (3 H, d, J = 6 Hz), 2.5–3.1 (8 H, m), 2.78 and 2.85 (3 H, 2 s), 3.1–5.1 (10 H, m), 6.8–7.3 (11 H, m), 7.3–7.7 (2 H, m), 7.7–8.2 (4 H, m), 8.3–8.6 (1 H, m), 9.2 (1 H, br s); R_f = 0.39 (system E); MS (FAB) m/z 655.3 (M + H)$^+$. Anal. ($C_{36}H_{43}ClN_6O_6 \cdot 2.5H_2O$) C, H, N, Cl.

(*N*,*N*-Diethylamino)-β-Ala-Thr-D-Trp(CHO)-Phe-NMeBzl·HCl (5h). To a solution of **5l** (0.62 g, 1.0 mmol), *N*,*N*-diethylamino-β-alanine hydrochloride (182 mg, 1.0 mmol), and HOBT (135 mg, 1.0 mmol) in DMF (10 mL) was added WSCD (155 mg, 1.0 mmol) under ice-cooling. The solution was stirred at this temperature for 2 h and at room temperature for 6 h. The solvent was evaporated under reduced pressure. The residue obtained was diluted with sodium hydrogen carbonate solution and extracted with EtOAc. The organic layer was washed with brine and dried. After filtration, 4 N hydrochloric acid (0.3 mL) in dioxane was added to the filtrate. The resulting solution was extracted with water (50 mL) and the aqueous layer was seperated and was washed once with *n*-BuOAc. The aqueous layer was then lyophilized to give **5h** as an amorphous solid (480 mg, 64.3%): $[\alpha]^{20}_D$ = +14.08 (c = 0.93, DMF); IR (Nujol) 3300, 1710, 1660 (sh), 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.80 (3 H, d, J = 6 Hz), 1.17 (6 H, t, J = 7 Hz), 2.77 and 2.83 (3 H, 2 s), 2.6–3.3 (12 H, m), 3.77 (1 H, m), 4.0–4.4 (3 H, m), 4.5–4.8 (2 H, m), 4.95 (1 H, m), 7.0–7.4 (13 H, m), 7.5–7.8 (2 H, m), 8.0–8.3 (2 H, m), 8.65 (1 H, m), 9.3 (1 H, br s), 10.45 (1 H, br s); R_f = 0.1 (system D). Anal. ($C_{40}H_{51}ClN_6O_6 \cdot 2.5H_2O$) H, N; C: calcd 62.04; found 61.44.

Succinyl-Thr-D-Trp(CHO)-Phe-NMeBzl (5i). To a solution of **5l** (1.0 g, 1.6 mmol) and triethylamine (324 mg, 3.2 mmol) in CH_2Cl_2 (25 mL) was added succinic anhydride (0.16 g, 1.6 mmol) at −15 °C. The solution was stirred at this temperature for 2 h. The solvent was evaporated under reduced pressure. The residue obtained was diluted with 0.5 N hydrochloric acid and extracted with EtOAc. The organic layer was washed with brine and evaporated under reduced pressure. This crude product was purified by silica gel chromatography eluting with $CHCl_3$–MeOH (10:1) to give **5i** as an amorphous solid (0.77 g, 70.0%): $[\alpha]^{25}_D$ = +16.81° (c = 1.0, DMF); IR (Nujol) 3300, 1710, 1640, 1540 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.84 (3 H, d J = 6 Hz), 2.35 (4 H, s), 2.6–3.1 (7 H, m), 3.7–5.1 (8 H, m), 6.9–7.4 (12 H, m), 7.4–7.9 (3 H, m), 7.9–8.3 (2 H, m), 8.6–8.9 (1 H, m), 9.2 (1 H, br s); R_f = 0.76 (system D). Anal. ($C_{37}H_{41}N_5O_8 \cdot H_2O$) C, H, N.

Glutaryl-Thr-D-Trp(CHO)-Phe-NMeBzl (5j). Similarly prepared as **5i** except using glutaric anhydride: 81.4% yield (amorphous solid); $[\alpha]^{25}_D$ = +28.5° (c = 1.0, DMF); IR (Nujol) 3300, 1710, 1635, 1540 (sh) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.82 (3 H, d, J = 6 Hz), 1.5–1.9 (2 H, m), 1.9–2.4 (4 H, m), 2.75 and 2.82 (3 H, 2 s), 3.7–4.0 (1 H, m), 4.0–5.2 (7 H, m), 6.9–7.4 (12 H, m), 7.4–7.8 (3 H, m), 7.9–8.3 (2 H, m), 8.4–8.8 (1 H, m), 9.3 (1 H, br s); R_f = 0.47 (system D); MS (FAB) m/z 736.2 (M + K)$^+$.

(Morpholin-4-ylcarbonyl)-Thr-D-Trp(CHO)-Phe-NMeBzl (5k). To a solution of **5l** (1.0 g, 1.61 mmol) and triethylamine (0.40 g, 3.93 mmol) in CH_2Cl_2 (25 mL) was added morpholine-4-carbonyl chloride (282 mg, 1.89 mmol). The solution was stirred at room temperature overnight. The solvent was evaporated under reduced pressure. The residue obtained was diluted with water and extracted with EtOAc. The organic layer was washed successively with sodium hydrogen carbonate solution, water, 0.5 N hydrochloric acid, and brine and evaporated under reduced pressure. The residue (0.97 g) was chromatographed on a silica gel column (35 g) eluting with $CHCl_3$–MeOH (25:1) to give **5k** as an amorphous solid (0.67 g, 60.9%): $[\alpha]^{20}_D$ = +33.60° (c = 1.1, DMF); IR (Nujol) 3400, 3280, 1710, 1660 (sh), 1640, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.80 (3 H, t, J = 6 Hz), 2.77 (2 H, m), 2.83 (3 H, s), 2.8 (2 H, m), 3.28 (4 H, m), 3.50 (4 H, br s), 3.65–4.1 (2 H, m), 4.2–5.1 (5 H, m), 6.12 (1 H, d, J = 7 Hz), 6.95–7.4 (13 H, m), 7.4–7.6 (2 H, m), 8.1 (6 H, m), 8.6 (1 H, m), 9.25 (1 H, br s); R_f = 0.45 (system A). Anal. ($C_{38}H_{44}N_6O_7 \cdot H_2O$) C, H, N.