Studies on Neurokinin Antagonists. 1. The Design of Novel Tripeptides Possessing the Glutaminyl-D-tryptophylphenylalanine Sequence as Substance P Antagonists†

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To discover a novel and low molecular weight substance P (SP) antagonist we postulated that the essential binding domain of peptide ligands was only a small portion in the whole structure. On the basis of this assumption, we selected the known octapeptide SP antagonist D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-D-Trp-Phe-NH_2 (1) as a lead and synthesized its fragment tripeptides which were evaluated for their activity to block ^{3}H-SP binding on guinea pig lung membranes. The protected tripeptide 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 (**4a**)], corresponding to the Gln-D-Trp-Phe part of **1**, exhibited 7-fold potent inhibitory activity in comparison with **1**. Studies on structure–activity relationships revealed that the D-tryptophan, L-phenylalanine, and benzyl ester were quite important to maintain the high binding affinity. It was also indicated that **4a** antagonized the SP-induced contraction of isolated guinea pig trachea strips (IC_{50} = 4.7 × 10^{-6} M).

Introduction

Substance P (SP) is a peptide comprising eleven amino acids. In 1931 von Euler and Gaddum[1] suggested its existence in the extract of mammalian and avian guts. The confirmed structure of SP was finally sequenced by Chang et al.[2] in 1971 as Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2. SP is a member of the tachykinin family of peptides which have similar amino acid sequences at the carboxy terminal. Three particular peptides within the family, namely SP, neurokinin A (NKA),[3] and neurokinin B (NKB),[4] are termed neurokinins. The receptors of the neurokinins are now classified into the following three subtypes, NK_1, NK_2, and NK_3, which have high affinity to SP, NKA, and NKB, respectively.[5] Numerous physiological activities of SP such as vasodilation, salivation, contraction of smooth muscle, and so forth have been reported as reviewed.[6] Conventional research on the etiology of asthma has focused on the allergic reaction as mediated by histamine, leukotrienes, prostaglandins, PAF, and so on. Recently, however, Barnes and Lundberg have proposed another mechanism in which SP participates.[7] SP is distributed in sensory nerves C-fibers in the human airway. The stimuli to the epithelium of the airway results in the release of SP from its nerve endings by the so called axon reflex mechanism. The physiological actions of released SP lead to the pathological features of asthma, e.g. bronchoconstriction, mucus hypersecretion, and microvascular leakage. This mechanism is referred as "neurogenic inflammation" by Barnes et al.

Standing on the foundation of these roles of SP in asthma, we assumed that an antagonist of SP would be able to modulate the SP-induced functions in the airway and consequently could be developed as a new type of antiasthmatic drug totally different from the currently available antihistamines, mediator release inhibitors, and bronchodilators.

Recently the first nonpeptide SP antagonist, or in other words a NK_1-selective antagonist, (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (CP-96,345) has been reported.[8] Before this important discovery, enormous numbers of peptide SP antagonists had been reported,[9] but most of them are analogues of SP in which the component amino acids are conventionally replaced with one or more amino acids, as exemplified by Spantide [(D-Arg^1,D-Trp^{7,9},Leu^{11})-SP].[10] In addition another type of peptide SP antagonist, in which some peptide bonds are replaced by nonpeptide bonds, has been reported.[11] Nevertheless,

† 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: WSCD, 1-ethyl-3-[3-(*N*,*N*-dimethylamino)-propyl]carbodiimide; HOBT, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; DMF, dimethylformamide; IPE, diisopropyl ether.

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Table I. Fragment Tripeptides and Their Activity in SP Binding Assay

compd	structure	inhibition of ^{3}H-SP binding (IC_{50}, μM)[a]
1	D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-D-Trp-Phe-NH_2	0.6 (n = 3)
2a	H-D-Pro-Gln-Gln-OH	>100
3a	<Glu-Gln-D-Trp-OH[b]	>100
5a	<Glu-D-Trp-Phe-OH[b]	>100
6a	H-D-Trp-Phe-D-Trp-OH	100
7a	H-Phe-D-Trp-D-Trp-OH	58
8a	HCl·H-D-Trp-D-Trp-Phe-NH_2	10 (n = 2)

[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. [b] <Glu: pyroglutamic acid.

Table II. Protected Fragment Peptides and Their Activity in SP Binding Assay

compd	structure	inhibition of ^{3}H-SP binding (IC_{50}, μM)[a]
2b	Boc-D-Pro-Gln-Gln-OBzl	100
2c	Boc-D-Pro-Gln-Gln-NH_2	>100
2d	Z-D-Pro-Gln-Gln-OBzl	10
3b	Z-Gln-Gln-D-Trp-OH	>100
3c	Z-Gln-Gln-D-Trp-NH_2	100
4a	Boc-Gln-D-Trp(CHO)-Phe-OBzl	0.09 (n = 3)
4b	Boc-Gln-D-Trp(CHO)-Phe-OH	>100
4c	HCl·H-Gln-D-Trp(CHO)-Phe-OBzl	0.21
5b	Boc-Gln-D-Trp-Phe-OH	>100
5c	Boc-Gln-D-Trp-Phe-NH_2	38.7
6b	Z-D-Trp-Phe-D-Trp-OH	23.8 (n = 2)
6c	Z-D-Trp-Phe-D-Trp-NH_2	>100
6d	Z-D-Trp-Phe-D-Trp-OBzl	10
7b	Z-Phe-D-Trp-D-Trp-OH	>100
7c	Z-Phe-D-Trp-D-Trp-NH_2	2.5 (n = 2)
7d	Z-Phe-D-Trp-D-Trp-OBzl	100
8b	Boc-D-Trp-D-Trp-Phe-NH_2	17.6 (n = 2)
8c	Boc-D-Trp-D-Trp-Phe-OBzl	>100

[a] See footnote *a* in Table I.

these peptide or peptide-like compounds seem to have some disadvantages as drugs: (1) susceptibility against enzyme degradation, (2) partial agonistic activity,[9,12] and (3) histamine-releasing activity.[13] Thus we desired to discover a new type of low molecular weight SP antagonist which would overcome these disadvantages. Here in this paper we report the discovery of a novel tripeptide SP antagonist.

Strategy for Research

Among the various compounds reported as SP antagonists we selected the octapeptide D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-D-Trp-Phe-NH_2 (1), a truncated analogue of SP, as a lead compound for our study. The reason for the selection was based on the report that the octapeptide was the most potent and specific antagonist against SP in guinea pig trachea.[12] Aiming to find a novel antagonist with low molecular weight, we hypothesized that if 1 exerts its antagonistic activity by binding to the SP receptor, the whole molecule would not be necessarily required; instead,

Scheme I. Synthetic Routes of Fragment Tripeptides and Their Protected Compounds

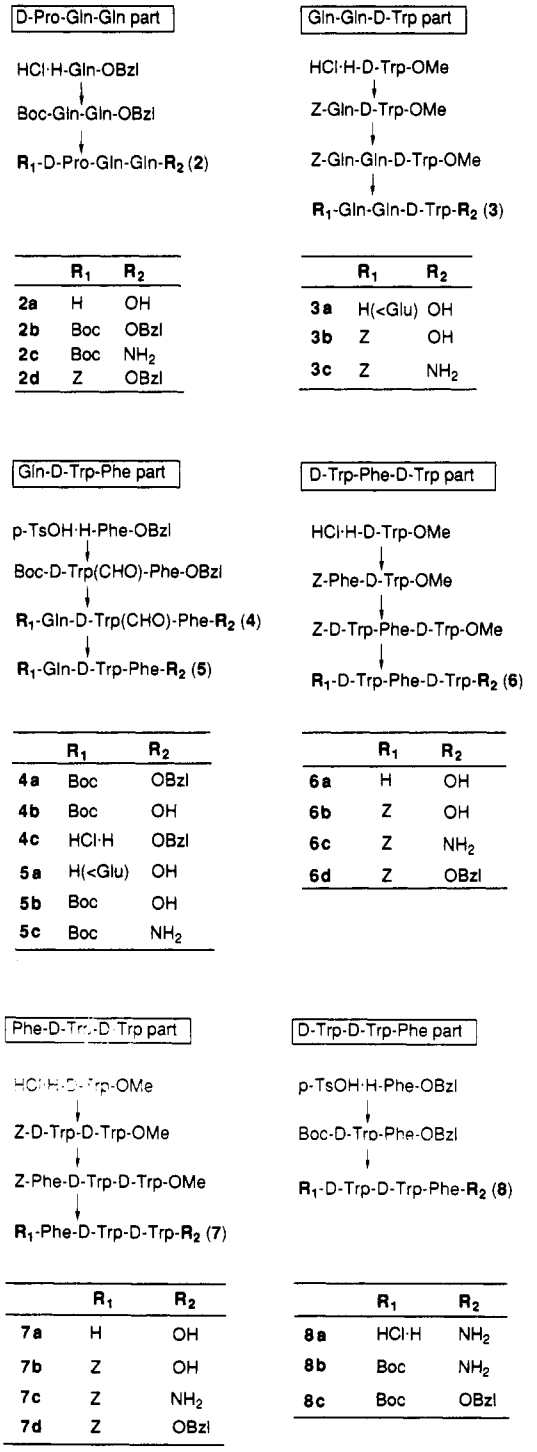

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Table III. Modification of Glutamine Part of **4a**

Boc-R_3-D-Trp(CHO)-Phe-OBzl

compd	R_3	inhibition of ^{3}H-SP binding (IC_{50}, μM)[a]
9a	D-Gln	0.14
9b	Thr	0.11 (n = 2)
9c	Ser	0.11 (n = 2)
9d	Tyr	0.26
9e	Gly	0.42 (n = 2)

[a] See footnote *a* in Table I.

we hypothesized that the essential binding domain in **1** might be as small as a few amino acids.[14] On the basis of this hypothesis we attempted to synthesize a variety of fragment peptides of **1** which have three amino acid units in order to define the structural requirements for the receptor binding of **1**. To assess the activity of the fragment peptides synthesized, we employed a receptor binding assay using guinea pig lung membranes and tritium-labeled SP as a radioactive ligand.

Chemistry

The objective fragment tripeptides and their related compounds, listed respectively in Tables I and II, were synthesized by the conventional solution method according to Scheme I. The WSCD–HOBT method[15] was exclusively used as a coupling reagent on account of its efficiency and the ease of product isolation. In the synthesis of series **2**, **4**, and **8**, Boc (*tert*-butyloxycarbonyl) was used as a temporary amino protecting group. In series **4**, the indole of D-tryptophan was protected by a formyl (CHO) group,[16] and the Boc group was removed with 4 N HCl in dioxane. On the contrary, an unprotected D-tryptophan was used for the synthesis of series **8**, and in this case the removal of the Boc group was carried out with TFA in the presence of 1,2-ethanedithiol and dimethyl sulfide[17] to avoid alkylation at the indole nucleus. In the synthesis of series **3**, **6**, and **7**, Z (benzyloxycarbonyl) was used as an amino protecting group, which was removed by catalytic hydrogenation.

Tripeptide esters **2b**, **2d**, **4a**, and **8c** were prepared stepwise from the benzyl esters of the corresponding carboxy terminal amino acids. Deprotection of the Boc group in **4a** produced **4c**. Tripeptide amides **2c**, **3c**, **5c**, **6c**, **7c**, and **8b** were obtained by ammonolysis of the corresponding benzyl or methyl esters. During the preparation of **5c** by this procedure, the formyl group of the indole was removed simultaneously. Tripeptide acids **3b**, **5b**, **6b**, and **7b** were also prepared by alkaline hydrolysis of the corresponding methyl or benzyl ester. Tripeptide acid **4b** was obtained from **4a** by catalytic hydrogenation. Benzyl esters **6d** and **7d** were produced by esterification of **6b** and **7b** with benzyl bromide, respectively.

Among the objective fragment tripeptides, **2a**, **6a**, and **7a** were derived respectively from the corresponding Z-protected tripeptides **2d**, **6b**, and **7b** by catalytic hydrogenation, whereas **8a** was obtained by deprotection of the Boc in **8b** with TFA in the presence of 1,2-ethanedithiol and dimethyl sulfide. The tripeptides **3a** and **5a** having a pyroglutamic acid moiety at the amino terminal were synthesized by initial deprotection of **3b** and **5b**, followed by heating in acidic conditions.[18]

The analogues of **4a** listed in Tables III–VI were prepared similarly as described for **4a** except **10b** which was synthesized by the TFA–1,2-ethanedithiol–dimethyl sulfide method for Boc deprotection. The starting esters of phenylalanine, e.g. *p*-chlorobenzyl, and cyclohexylmethyl esters, were prepared by heating a mixture of the corresponding alcohol, phenylalanine, and *p*-toluenesulfonic acid. *O*-Benzyl-(2*S*,4*R*)-hydroxyproline benzyl ester was prepared from commercially available Boc-*O*-benzylhydroxyproline via two-step reactions, namely esterifica-

Table IV. Modification of D-Tryptophan Part of **4a**

Boc-Gln-R_4-Phe-OBzl

compd	R_4	inhibition of ^{3}H-SP binding (IC_{50}, μM)[a]
10a	Trp(CHO)	0.36
10b	D-Trp	0.55
10c	Phe	1.0
10d	D-Phe	0.38
10e	D-Met	0.79
10f	D-Leu	1.0

[a] See footnote *a* in Table I.

Table V. Modification of Phenylalanine Part of **4a**

Boc-Gln-D-Trp(CHO)-R_5-OBzl

compd	R_5	inhibition of ^{3}H-SP binding (IC_{50}, μM)[a]
11a	Gly	*b*
11b	Leu	10
11c	Pro	*b*
11d	Val	*b*
11e	Hyp(Bzl)	0.45
11f	D-Phe	0.77
11g	Tyr	0.39
11h	MePhe	0.24
11i	Tic	0.14

[a] See footnote *a* in Table I. [b] No inhibition at 10^{-4} M.

Table VI. Modification of Benzyl Ester Part of **4a**

Boc-Gln-D-Trp(CHO)-Phe-R_6

compd	R_6	inhibition of ^{3}H-SP binding (IC_{50}, μM)[a]
12a	OMe	10
12b	OiPr	1.7
12c	OCH_2CH_2Ph	0.5
12d	OCH_2Ph(*p*-Cl)	1.0
12e	$OCH_2C_6H_{11}$	0.4

[a] See footnote *a* in Table I.

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tion with benzyl bromide followed by Boc deprotection.

Results

Activity of the Fragment Tripeptides. To elucidate the essential binding domain in the structure of 1 for receptor recognition, we first examined the binding affinity of the six fragment tripeptides **2a**, **3a**, **5a**, **6a**, **7a**, and **8a**, by which the whole amino acid sequence of 1 is covered. The carboxy terminal of each tripeptide was a free carboxylic acid except **8a**, which was amidated. The amino terminal glutamine of **3a** or **5a** was converted into a pyroglutamic acid in consideration of naturally existing neuropeptides. As can be seen in Table I, none of these fragment tripeptides exhibited significant binding affinity, although **8a** showed only weak activity ($IC_{50} = 1.0 \times 10^{-5}$ M).

Activity of the Protected Fragment Tripeptides. From the above results we presumed that the ionic functional group(s) such as a free carboxylic acid or an amine in the fragment tripeptides could disturb their interaction with the SP receptor. This speculation led us to evaluate the activity of the fragment tripeptides bearing protecting groups at either or both the carboxy and the amino termini. The corresponding amine and carboxylic acid were respectively protected with a Boc or a Z group and with amide or a benzyl ester. Among the evaluated 18 compounds summarized in Table II, 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 (**4a**)] was indicated to be the most potent compound. The IC_{50} value of **4a** was calculated as 9×10^{-8} M, being about 7 times more potent than 1. The compound lacking the Boc group (**4c**) still retained about half the binding affinity of **4a**, but the removal of the benzyl ester (**4b**) resulted in a complete loss of the activity. Among the other protected tripeptides representing the different amino acid sequences in 1, Z-D-Pro-Gln-Gln-OBzl (**2d**), Boc-Gln-D-Trp-Phe-NH_2 (**5c**), Z-D-Trp-Phe-D-Trp-OH (**6b**), Z-D-Trp-Phe-D-Trp-OBzl (**6d**), Z-Phe-D-Trp-D-Trp-NH_2 (**7c**), and Boc-D-Trp-D-Trp-Phe-NH_2 (**8b**) exhibited significantly potent binding affinities but were all weaker than **4a**.

It may be concluded from the above results that the Gln-D-Trp-Phe part is the most important in the whole structure of 1 for its receptor binding, albeit the carboxy terminal has to be protected with a benzyl ester. This structural feature was of interest to us in our study on discovering a novel and low molecular weight SP antagonist.

Structure–Activity Relationships. In order to obtain a more potent antagonist we attempted to partly modify the chemical structure of **4a**. Firstly, the Boc-Gln part was converted into another Boc-protected amino acid. From the results summarized in Table III, the substitution with D-glutamine (**9a**), threonine (**9b**), and serine (**9c**) all retained the potent activities, whereas the activities of tyrosine (**9d**) and glycine (**9e**) were to some extent attenuated but still potent.

Secondly, the results on the modification at the D-Trp-(CHO) moiety are listed in Table IV. The compound (**10a**) which has a L-configurational tryptophan and the compound (**10b**) which excluded the formyl group exhibited 4 times and 6 times less activity in comparison with **4a**, respectively. Substitution with other D-amino acids such as D-Phe (**10d**), D-Met (**10e**), and D-Leu (**10f**) turned out to significantly attenuate the activity. Thus D-tryptophan with a formyl group at the indole nitrogen in the structure of **4a** was shown to be essential to the binding affinity.

Subsequently, the phenylalanine part was modified into a variety of amino acids and the results were summarized in Table V. Substitution with D-phenylalanine (**11f**) resulted in an 8-fold loss of activity. The compounds substituted with an aromatic amino acid such as tyrosine (**11g**), *N*-methylphenylalanine (MePhe) (**11h**), or 1,2,3,4-tetrahydroisoquinoline-3(*S*)-carboxylic acid (Tic) (**11i**) still maintained significant binding affinity, while the compounds having an aliphatic or a neutral amino acid **11a** (Gly), **11b** (Leu), **11c** (Pro), **11d** (Val), and **11e** [Hyp(Bzl)] turned out to be only weakly active. It was consequently indicated that the phenylalanine with L-configuration seemed to be required for the binding affinity.

Finally, the benzyl ester part was modified and the results are shown in Table VI. The importance of the benzene ring on this part was proved by the great loss of binding affinity in the methyl ester (**12a**) and the isopropyl ester (**12b**) derivatives. The compound with a cyclohexylmethyl ester (**12e**) exhibited significant but reduced binding affinity compared to **4a**. An introduction of a chloro atom at the para position (**12d**) brought a remarkable loss of the binding affinity and an elongation of the ester chain with one methylene unit (**12c**) resulted in about 5-fold loss of the activity in comparison with **4a**.

It can be recognized from the described results that the D-tryptophan formylated at its indole nitrogen and both benzene rings in the phenylalanine and the benzyl ester appear to be required in the structure of **4a** for exhibiting the potent binding affinity.

Biological Activity of 4a. The following in vitro experiment revealed that **4a** was pharmacologically profiled as an actual SP antagonist. Namely this compound inhibited the SP-induced contraction of the isolated guinea pig tracheal strips ($IC_{50} = 4.7 \times 10^{-6}$ M) without exhibiting any agonistic activity at the maximum concentration (3.2×10^{-5} M). In addition this compound had no effect on the histamine-induced contraction in the same preparation. In in vivo experiments, however, **4a** did not inhibit the SP-induced bronchoconstriction in guinea pigs when administered intraveneously. This result may be explained on the basis of an insufficient antagonistic activity or rapid degradation of the molecule within the body. The details of this issue will be discussed in the following paper.

Discussion

Since the involvement of SP in asthma was implied, research on discovering a novel SP antagonist makes sense in terms of contributing not only to the elucidation of the pathological function of SP but also to the production of a new remedy for asthma. At the very beginning of our study we postulated the following working hypothesis. If a peptide ligand exerts its biological activity through binding to the corresponding receptor, essential parts for the receptor binding must be in the peptide sequence and could be picked up by an appropriate method. Once the essential binding parts were disclosed, we were much closer to reaching a novel antagonist with a molecular weight which is dramatically reduced in comparison with the mother peptide. On the basis of this hypothesis, we synthesized and evaluated the fragment tripeptides of the lead octapeptide 1 which had been indicated to be the most potent SP antagonist on guinea pig trachea strips.

Contrary to our expectation, none of the fragment tripeptides of 1 in which the carboxy and amino termini are unprotected exhibited any potent inhibitory activity for SP binding to its receptor. However, the protected fragment peptide [Boc-Gln-D-Trp(CHO)-Phe-OBzl (**4a**)] which represents the Gln-D-Trp-Phe part in 1 showed the most potent activity for inhibition of SP binding, whereas the compound (**4b**) lacking the benzyl ester was inactive. It may be concluded from these results that the Gln-D-Trp-

Phe part in the structure of 1 seemed to play an important role for the receptor binding, although the assistance of the benzyl ester in the carboxy terminal is required. These findings suggest that the SP binding site in its receptor consists of a hydrophobic environment and also that the role of the benzyl ester in **4a** appears to provide a hydrophobic nature around its carboxy terminal or an additional binding affinity. This assumption could be supported by the following two facts. Firstly the lead 1 and other peptide type SP antagonists have hydrophobic amino acids, especially D-tryptophan at the 7-, 9-, and 10-positions[12] and SP itself consist of all neutral or hydrophobic amino acids except for several amino acids at the amino terminal. Secondly it is known that the modification of the amidated carboxy terminal of the SP analogue into carboxylic acid results in a significant loss of the activity.[19]

The structure–activity relationships on **4a** and its related analogues showed that the D-Trp(CHO)-Phe-OBzl part along with their absolute configurations was essential for the receptor binding on the basis of significant attenuation of the activity by any modifications at this part. On the other hand the Boc-Gln part seemed to accept a variety of chemical modifications, in other words to be the variable parts in **4a**. It has not been elucidated yet, however, which part in the structure of **4a**, particularly the essential part such as D-Trp, Phe, or the benzyl group, corresponds to the part in SP that mainly contributes to the receptor binding.

It is of interest that the tripeptide compound **4a** discovered in this paper exerts antagonistic activity against SP in the in vitro test. We are now going on a study to search for more potent SP antagonists. The results will be presented in the following papers.[20]

Experimental Section

Instruments and Materials. Melting points were measured on Mel-Temp apparatus (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 (Bruker); chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. 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 precoated silica gel plates 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 (9:1); C, $CHCl_3$–MeOH (10:1); D, $CHCl_3$–MeOH (20:1); E, $CHCl_3$–MeOH (30:1); F, $CHCl_3$–MeOH–AcOH (8:1:1); G, *n*-BuOAc–*n*-BuOH–AcOH–H_2O (80:15:40:24); H, $CHCl_3$–MeOH (4:1), I, *n*-BuOH–AcOH–H_2O (6:3:2). 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 protected amino acid derivatives were purchased from Peptide Institute (Minoh, Japan): Boc-Gln-OH, Boc-D-Gln-OH, Z-Gln-OH, Boc-Gly-OH, Boc-D-Trp-OH, Boc-D-Trp(CHO)-OH, Boc-Trp(CHO)-OH, Boc-Phe-OH, Boc-D-Phe-OH, Boc-D-Met-OH, Boc-D-Leu-OH, Boc-Hyp(Bzl)-OH, *p*-TsOH·H-Phe-OBzl, *p*-TsOH·H-Gly-OBzl, *p*-TsOH·H-Leu-OBzl, and HCl·H-Pro-OBzl. And the following ones were from Kokusan Chemical Works, Ltd. (Tokyo, Japan): Z-Phe-OH, Boc-D-Pro-OH, Z-D-Pro-OH, Z-D-Trp-OH, Boc-Thr-OH, Boc-Ser-OH, Boc-Tyr-OH, HCl·H-Phe-OMe, *p*-TsOH·H-Val-OBzl, *p*-TsOH·H-Tyr-OBzl, and *p*-TsOH·H-D-Phe-OBzl. The ester HCl·H-D-Trp-OMe was purchased from Bachem Feinchemikalien AG (Switzerland). These materials were used without further purification. The following amino acid esters were synthesized according to the methods described in literatures: HCl·H-Gln-OBzl,[21] *p*-TsOH·H-MePhe-OBzl,[22] HCl·H-Tic-OBzl,[23] HCl·H-Phe-OiPr,[24] HCl·H-Phe-OCH_2CH_2Ph.[25] The reagents WSCD and HOBT were purchased from Eibeiss Co. Ltd. (Yokohama, Japan). TFA was purchased from Nacalai Tesque (Kyoto, Japan). The octapeptide 1 was purchased from Bachem Feinchemikalien AG (Switzerland).

Receptor Binding Assay. The preparation of guinea pig lung membranes[26] and the receptor binding assay[27] were carried out according to the method described in the literature with certain modifications. Male Hartley strain guinea pigs were sacrificed by decapitation. The trachea and lungs were removed and homogenized in buffer (0.25 M sucrose, 50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA) by using Polytoron (Kinematica). The homogenate was centrifuged (1000*g*, 10 min) to remove tissue clumps and the supernatant was centrifuged again (14000*g*, 20 min) to yield pellets. The pellets were resuspended in buffer (5 mM Tris-HCl, pH 7.5), homogenized with a Teflon homogenizer, and centrifuged (14000*g*, 20 min) to yield pellets which were referred as crude membrane fractions. The obtained pellets were stored at −70 °C until use. Frozen crude membrane fractions were thawed and resuspended in a medium (50 mM Tris-HCl pH 7.5, 5 mM $MnCl_2$, 0.02% BSA, 2 μg/mL of chymotrypsin, 4 μg/mL of leupeptin, 40 μg/mL of bacitracin).

Binding assays were performed by incubating aliquots (100 μL) of the membrane fractions with ^{3}H-SP (final concentration, 1 nM) with or without test compounds in the same buffer solution at 4 °C for 0.5 h in a final volume of 500 μL. At the end of the incubation period, reaction mixture was quickly filtered through a Whatman GF/B glass filter (pretreated with 1% poly(ethelenimine) for 3 h prior to use) with the aid of water aspirator. The filters were then washed four times with 5 mL of the buffer (50 mM Tris-HCl, pH 7.5). The radioactivity was counted in 5 mL of Aquasol-2 in Packerd scintillation counter (Packerd TRI-CARB 4530). All data presented are specific binding defined as that displacable by 5 μM unlabeled SP. The inhibitory concentration of an antagonist that gave 50% displacement of the specific binding of ^{3}H-SP (IC_{50}) was estimated from the displacement curve. The assays were performed in duplicate.

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Contractile Response of Isolated Guinea Pig Trachea. Male albino guinea pigs weighing 300–500 g were stunned and bled, and the trachea was rapidly removed. A zig–zag strip preparation of trachea was made carefully and placed in an organ bath (15 mL) filled with warmed (37 °C) and oxygenated (95% O_2 and 5% CO_2) standard Tyrode solution under a resting tension of 0.5 g. After a 60-min equilibration period, the response to agonist (SP, 1.0×10^{-8} M, or histamine, 3.2×10^{-7} M) was recorded two or three times to obtain a stable contraction response, which was used as control. After the tension of the preparation returned to basal levels by washing, the test compound was added and 10 min later contraction was induced with SP. The contractile response obtained in the presence of compound was compared with the control response.

Synthesis of New Amino Acid Ester Derivatives: *O*-Benzyl-(2*S*,4*R*)-hydroxyproline Benzyl Ester Hydrochloride (13). To a solution of Boc-Hyp(Bzl)-OH (5.0 g, 15.56 mmol) in DMF (50 mL) were added diisopropylethylamine (2.33 g, 18.0 mmol) and benzyl bromide (3.08 g, 18.0 mmol) under ice-cooling. The solution was stirred at the temperature for 3 h and then at room temperature overnight. After concentration, the product was extracted with EtOAc. The organic layer was successively washed with water, diluted sodium hydrogen carbonate solution, water, 0.5 N hydrochloric acid, and brine, dried, and evaporated under reduced pressure. The obtained syrup (5.68 g) was treated with a mixture of anisole (6 mL) and TFA (40 mL) under ice-cooling for 10 min and at room temperature for 15 min. After concentration of the solvent, 4 N hydrochloric acid (6.9 mL) in dioxane was added to the residue, and the mixture was concentrated again to give **13** as a crystalline solid (4.45 g, 92.7%): mp 142–143 °C (Et_2O); $[\alpha]^{25}_D = -14.43°$ (c = 1.02, MeOH); IR (Nujol) 2700–2400, 1740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.10–2.24 (1 H, m), 2.45–2.56 (1 H, m), 3.30–3.57 (2 H, m), 4.43 (1 H, m), 4.52 (2 H, s), 4.5–4.6 (1 H, m), 5.21 (1 H, d, J = 12 Hz), 5.28 (1 H, d, J = 12 Hz), 7.28–7.50 (10 H, m), 10.04 (2 H, br s); R_f = 0.43 (system B). Anal. ($C_{19}H_{22}ClNO_3$) C, H, N.

Phenylalanine *p*-Chlorobenzyl Ester *p*-Toluenesulfonate (14). A mixture of phenylalanine (1.65 g, 10 mmol), *p*-chlorobenzyl alcohol (7.12 g, 50 mmol), and *p*-toluenesulfonic acid hydrate (2.09 g, 11 mmol) in toluene (50 mL) was heated under reflux with a Dean–Stark apparatus for 5 h. After cooling, Et_2O was added to the mixture. The crystalline precipitates obtained were collected by filtration and washed with Et_2O to give **14** (4.59 g, 99.5%): mp 187–189 °C; $[\alpha]^{25}_D = -3.75°$ (c = 1.03, MeOH); IR (Nujol) 3250, 1750, 1600, 1520 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.29 (3 H, s), 3.04 (1 H, dd, J = 8, 14 Hz), 3.22 (1 H, dd, J = 6, 14 Hz), 4.37 (1 H, m), 5.13 (2 H, s), 7.1–7.7 (13 H, m), 8.51 (3 H, br s); R_f = 0.62 (system A). Anal. ($C_{23}H_{24}ClNO_5S$) C, H, N, S.

Phenylalanine Cyclohexylmethyl Ester *p*-Toluenesulfonate (15). Similarly prepared as **14**: 88.1% yield; mp 157–159 °C; $[\alpha]^{25}_D = +8.93°$ (c = 0.80, MeOH); IR (Nujol) 1735, 1515, 1240, 1180 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.5–1.7 (11 H, m), 2.30 (3 H, s), 3.0 (1 H, dd, J = 8, 14 Hz), 3.2 (1 H, dd, J = 6, 14 Hz), 3.86 (2 H, d, J = 6 Hz), 4.33 (1 H, dd, J = 6, 8 Hz), 7.15 (2 H, d, J = 8 Hz), 7.2–7.5 (5 H, m), 7.55 (2 H, d, J = 8 Hz), 8.48 (3 H, br s); R_f = 0.76 (system A). Anal. ($C_{23}H_{31}NO_5S$) H, N, S; C: calcd, 63.72; found, 64.33.

Synthesis of Protected Dipeptides: Boc-D-Trp(CHO)-Phe-OBzl (16). To a solution of Boc-D-Trp(CHO)-OH (2.99 g, 9.0 mmol), *p*-TsOH·H-Phe-OBzl (3.85 g, 9.0 mmol), and HOBT (1.29 g, 9.0 mmol) in a mixed solvent of CH_2Cl_2 (60 mL) and DMF (15 mL) was added WSCD (1.53 g, 9.9 mmol) under ice-cooling. The solution was stirred at the temperature for 3 h. The reaction mixture was 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, dried, and evaporated under reduced pressure. The residue obtained was crystallized from a mixed solvent of EtOAc and IPE (1:1) to give **16** (4.95 g, 96.5%): mp 146–147 °C; $[\alpha]^{25}_D = -9.19°$ (c = 1.18, DMF); IR (Nujol) 3340, 1732 (sh), 1710, 1686, 1650, 1545, 1528 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 (9 H, s), 2.65–2.85 (2 H, m), 2.90 (1 H, dd, J = 6, 14 Hz), 3.15 (1 H, dd, J = 9, 14 Hz), 4.2–4.5 (1 H, m), 4.5–4.85 (1 H, m), 5.15 (2 H, s), 6.83 (1 H, d, J = 8 Hz), 7.25 (5 H, s), 7.40 (5 H, s), 7.2–7.85 (4 H, m), 8.20 (1 H, br s), 8.62 (1 H, d, J = 8 Hz), 9.3–9.8 (1 H, br s); R_f = 0.65 (system E). Anal. ($C_{33}H_{35}N_3O_6$) C, H, N.

The following protected dipeptides (**17–40**) were prepared similarly as **16** from the starting materials which are commercially available or known in the literature.

Boc-Gln-Gln-OBzl (17): 94.1% yield; mp 159–160 °C; $[\alpha]^{25}_D = -13.30°$ (c = 1.1, DMF): IR (Nujol) 3380, 3200, 2660, 1755, 1660, 1620 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.37 (9 H, s), 1.6–2.2 (8 H, m), 3.93 (1 H, m), 4.26 (1 H, m), 5.11 (2 H, s), 6.78 (2 H, s), 6.86 (1 H, d, J = 8 Hz), 7.25 (2 H, s), 7.30 (5 H, s), 8.31 (1 H, d, J = 7 Hz); R_f = 0.33 (system A). Anal. ($C_{22}H_{32}N_4O_7$) C, H, N.

Z-Gln-D-Trp-OMe (18): 83.6% yield: mp 209–210 °C (EtOH); $[\alpha]^{25}_D = -6.98°$ (c = 1.02, DMF); IR (Nujol) 3420, 3300, 3220 (sh), 1756, 1690, 1660, 1630, 1555, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.6–2.2 (4 H, m), 3.12 (2 H, d, J = 8 Hz), 3.55 (3 H, s), 3.9–4.15 (1 H, m), 4.4–4.75 (1 H, m), 5.0 (2 H, s), 6.70 (2 H, br s), 6.9–7.55 (6 H, m), 7.35 (5 H, s), 8.19 (1 H, d, J = 8 Hz), 10.83 (1 H, s); R_f = 0.38 (system C). Anal. ($C_{25}H_{28}N_4O_6$) C, H, N.

Z-Phe-D-Trp-OMe (19): 91.9% yield: mp 185–186 °C (EtOH); $[\alpha]^{25}_D = -1.10°$ (c = 1.05, DMF); IR (Nujol) 3420, 3330, 3300, 1738, 1690, 1655, 1550 (sh), 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.6–2.85 (2 H, m), 3.0–3.2 (2 H, m), 3.60 (3 H, s), 4.2–4.7 (2 H, m), 4.95 (2 H, s), 6.9–7.5 (11 H, m), 7.30 (5 H, s), 8.45 (1 H, d, J = 8 Hz), 10.85 (1 H, s); R_f = 0.8 (system C). Anal. ($C_{29}H_{29}N_3O_5$) C, H, N.

Z-D-Trp-D-Trp-OMe (20): 74.5% yield; mp 188–189 °C (EtOH); $[\alpha]^{25}_D = +13.13°$ (c = 0.94, DMF); IR (Nujol) 3400, 3330, 1746, 1698, 1650, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.9–3.3 (4 H, m), 3.56 (3 H, s), 4.25–4.7 (2 H, m), 4.95 (2 H, s), 6.9–7.7 (16 H, m), 8.35 (1 H, d, J = 8 Hz), 10.76 (1 H, s), 10.83 (1 H, s); R_f = 0.60 (system C). Anal. ($C_{31}H_{30}N_4O_5$) C, H, N.

Boc-D-Trp-Phe-OBzl (21): 80.3% yield; mp 145–146 °C (EtOH–H_2O); $[\alpha]^{25}_D = +0.92°$ (c = 1.08, DMF); IR (Nujol) 3400 (sh), 3360, 1730, 1690, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 (9 H, s), 2.5–3.3 (4 H, m), 4.00–4.35 (1 H, m), 4.35–4.75 (1 H, m), 5.08 (2 H, s), 6.55 (1 H, d, J = 8.5 Hz), 6.8–7.7 (15 H, m), 8.36 (1 H, d, J = 8.5 Hz); R_f = 0.50 (system E). Anal. ($C_{32}H_{35}N_3O_5$) C, H, N.

Boc-D-Trp(CHO)-Gly-OBzl (22): 91.7% yield; mp 85–87 °C (EtOAc–IPE); $[\alpha]^{25}_D = +4.33°$ (c = 1.11, DMF); IR (Nujol) 3360, 1732, 1720, 1685, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (9 H, s), 3.20 (2 H, d, J = 10 Hz), 4.02 (2 H, d, J = 7 Hz), 4.58 (1 H, m), 5.15 (2 H, s), 5.30 (1 H, d, J = 10 Hz), 6.78 (1 H, t, J = 7 Hz), 7.35 (8 H, s), 7.6–7.7 (2 H, m), 8.35 (1 H, m), 9.10 (1 H, br s); R_f = 0.85 (system A). Anal. ($C_{26}H_{29}N_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Leu-OBzl (23): 92% yield; mp 107–109 °C (Et_2O); $[\alpha]^{25}_D = -3.88°$ (c = 0.85, DMF); IR (Nujol) 3320, 1740, 1715, 1690, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (6 H, d, J = 6 Hz), 1.1–1.6 (3 H, m), 1.38 (9 H, s), 3.21 (2 H, d, J = 6 Hz), 4.4–4.7 (2 H, m), 5.13 (2 H, s), 5.1–5.2 (1 H, m), 6.50 (1 H, d, J = 9 Hz), 7.35 (5 H, s), 7.2–7.75 (5 H, m), 8.3 (1 H, m), 9.1 (1 H, br s); R_f = 0.80 (system A). Anal. ($C_{30}H_{37}N_3O_6$) H, N; C: calcd, 67.27; found, 66.70.

Boc-D-Trp(CHO)-Pro-OBzl (24): amorphous solid; used for the next reaction without further purification.

Boc-D-Trp(CHO)-Val-OBzl (25): 83.5% yield; mp 120–122 °C (EtOH–H_2O); $[\alpha]^{25}_D = +6.87°$ (c = 0.873, DMF); IR (Nujol) 3350, 1730, 1705, 1690, 1650, 1554, 1528 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.80 (6 H, d, J = 8 Hz), 1.23 (9 H, s), 2.0 (1 H, sept, J = 8 Hz), 2.85–3.08 (2 H, m), 4.1–4.6 (2 H, m), 5.17 (2 H, s), 6.9 (1 H, d, J = 8 Hz), 7.40 (5 H, s), 7.4–7.5 (2 H, m), 7.6–7.85 (2 H, m), 8.0–8.4 (2 H, m), 9.4 (1 H, br s); R_f = 0.65 (system D). Anal. ($C_{29}H_{35}N_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Hyp(Bzl)-OBzl (26). Prepared from **13**: amorphous solid; used for the next reaction without further purification.

Boc-D-Trp(CHO)-D-Phe-OBzl (27): 88.1% yield; mp 146 °C dec (EtOH); $[\alpha]^{25}_D = +12.97°$ (c = 1.03, DMF); IR (Nujol) 3350, 1725, 1680, 1660, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.26 (9 H, s), 2.7–3.2 (4 H, m), 4.1–4.8 (2 H, m), 5.10 (2 H, s), 6.8–7.1 (1 H, m), 7.2–7.5 (2 H, m), 7.25 (5 H, s), 7.35 (5 H, s), 7.5–7.8 (2 H, m), 8.2 (1 H, m), 8.50 (1 H, d, J = 9 Hz), 9.4 (1 H, br s); R_f = 0.92 (system F). Anal. ($C_{33}H_{35}N_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Tyr-OBzl (28): 89.7% yield; mp 126–127 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = –9.80° (*c* = 1.01, DMF); IR (Nujol) 3480, 3320, 1720, 1690, 1655, 1545, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.29 (9 H, s), 2.6–3.2 (4 H, m), 4.2–4.7 (2 H, m), 5.12 (2 H, s), 6.64 (2 H, d, *J* = 8 Hz), 6.7–7.0 (1 H, m), 7.03 (2 H, d, *J* = 8 Hz), 7.3–7.9 (9 H, m), 8.0–8.3 (1 H, m), 8.4–8.6 (1 H, m), 9.22 (1 H, s), 9.4 (1 H, br s); R_f = 0.83 (system F). Anal. ($C_{33}H_{35}N_3O_7 \cdot 2H_2O$) C, H, N.

Boc-D-Trp(CHO)-MePhe-OBzl (29): 76.6% yield; mp 105–107 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = –40.70° (*c* = 1.01, DMF); IR (Nujol) 3400, 1725, 1715, 1690, 1640, 1520 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 (9 H, s), 2.5–3.4 (4 H, m), 2.85 (3 H, s), 4.4–4.8 (1 H, m), 4.9–5.4 (1 H, m), 5.17 (2 H, s), 7.0–7.7 (5 H, m), 7.27 (5 H, s), 7.38 (5 H, s), 7.9–8.4 (1 H, m), 9.4 (1 H, br s); R_f = 0.72 (system E). Anal. ($C_{34}H_{37}N_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Tic-OBzl (30): amorphous solid, used for the next reaction without purification.

Boc-Trp(CHO)-Phe-OBzl (31): 81.1% yield; mp 144–146 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = –11.42° (*c* = 1.24, DMF); IR (Nujol) 3350, 1740, 1715, 1690, 1660, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 (9 H, s), 2.8–3.2 (4 H, m), 4.2–4.4 (1 H, m), 4.5–4.8 (1 H, m), 5.12 (2 H, s), 6.95 (1 H, d, *J* = 8 Hz), 7.29 (5 H, s), 7.36 (5 H, s), 7.2–7.8 (4 H, m), 8.2 (1 H, m), 8.50 (1 H, d, *J* = 8 Hz), 9.5 (1 H, br s); R_f = 0.58 (system E). Anal. ($C_{33}H_{35}N_3O_6$) C, H, N.

Boc-Phe-Phe-OBzl (32): 95.2% yield; mp 121–123 °C; $[\alpha]^{25}_D$ = –10.19° (*c* = 1.01, DMF); IR (Nujol) 3340, 1740, 1690, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (9 H, s), 2.95–3.2 (4 H, m), 4.3 (1 H, m), 4.7–5.1 (2 H, m), 5.13 (2 H, s), 6.40 (1 H, d, *J* = 8 Hz), 6.9–7.4 (5 H, m); 7.20 (5 H, s), 7.30 (5 H, s); R_f = 0.78 (system E). Anal. ($C_{30}H_{34}N_2O_5$) H, N; C: calcd, 71.69; found, 72.23.

Boc-D-Phe-Phe-OBzl (33): 92.4% yield; mp 125–126 °C (EtOAc–IPE); $[\alpha]^{25}_D$ = –8.12° (*c* = 1.05, DMF); IR (Nujol) 3340, 1740, 1690, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (9 H, s), 2.95–3.2 (4 H, m), 4.4 (1 H, m), 4.7–5.2 (2 H, m), 5.10 (2 H, s), 6.43 (1 H, d, *J* = 9 Hz), 6.8–7.2 (5 H, m), 7.24 (5 H, s), 7.35 (5 H, s); R_f = 0.78 (system E). Anal. ($C_{30}H_{34}N_2O_5$) H, N; C: calcd, 71.69; found, 72.22.

Boc-D-Met-Phe-OBzl (34): 92.5% yield; mp 101 °C (EtOAc); $[\alpha]^{25}_D$ = –7.61° (*c* = 1, DMF); IR (Nujol) 3340, 1740, 1690, 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (9 H, s), 1.8–2.1 (2 H, m), 2.07 (3 H, s), 2.3–2.65 (2 H, m), 3.13 (2 H, d, *J* = 6 Hz), 4.1–4.5 (1 H, m), 4.8–5.3 (2 H, m), 5.20 (2 H, s), 6.73 (1 H, d, *J* = 9 Hz), 7.0–7.5 (5 H, m), 7.33 (5 H, s); R_f = 0.9 (system A). Anal. ($C_{26}H_{34}N_2O_5S$) C, H, N, S.

Boc-D-Leu-Phe-OBzl (35): 93.9% yield; mp 97 °C (EtOAc–IPE); $[\alpha]^{25}_D$ = –0.54° (*c* = 1.10, DMF); IR (Nujol) 3350, 1740 (sh), 1730, 1690, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (6 H, d, *J* = 7 Hz), 1.40 (9 H, s), 1.5–1.8 (3 H, m), 3.10 (2 H, d, *J* = 7 Hz), 4.2 (1 H, m), 4.8–5.0 (2 H, m), 5.10 (2 H, s), 6.67 (1 H, d, *J* = 8 Hz), 7.0–7.4 (5 H, m), 7.35 (5 H, s); R_f = 0.67 (system D). Anal. ($C_{27}H_{36}N_2O_5$) C, H, N.

Boc-D-Trp(CHO)-Phe-OMe (36): 86.2% yield; mp 114–116 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = –10.20° (*c* = 1.10, DMF); IR (Nujol) 3320, 1740, 1710, 1700, 1680, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.28 (9 H, s), 2.6–3.3 (4 H, m), 3.65 (3 H, s), 4.1–4.8 (2 H, m), 6.83 (1 H, d, *J* = 9 Hz), 7.2–7.6 (3 H, m), 7.24 (5 H, s), 7.6–7.9 (1 H, m), 8.0–8.4 (1 H, m), 8.54 (1 H, d, *J* = 9 Hz), 9.4 (1 H, br s); R_f = 0.85 (system F). Anal. ($C_{27}H_{31}N_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Phe-OiPr (37): 89.6% yield; mp 100–103 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = –4.13° (*c* = 1.05, DMF); IR (Nujol) 3340, 1725, 1710, 1690, 1650, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.12 (6 H, d, *J* = 6 Hz), 1.27 (9 H, s), 2.6–3.2 (4 H, m), 4.1–4.7 (2 H, m), 4.91 (1 H, sept, *J* = 6 Hz), 6.87 (1 H, d, *J* = 9 Hz), 7.2–7.6 (3 H, m), 7.25 (5 H, s), 7.6–7.9 (1 H, m), 8.0–8.3 (1 H, m), 8.53 (1 H, d, *J* = 9 Hz), 9.4 (1 H, br s); R_f = 0.87 (system F). Anal. ($C_{29}H_{35}N_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Phe-OCH$_2$CH$_2$Ph (38): 90.5% yield; mp 141–142 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = –6.16° (*c* = 0.97, DMF); IR (Nujol) 3400, 1740, 1720, 1680, 1670, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.26 (9 H, s), 2.6–3.1 (4 H, m), 2.88 (2 H, t, *J* = 6 Hz), 4.2–4.8 (2 H, m), 4.28 (2 H, t, *J* = 6 Hz), 6.83 (1 H, d, *J* = 8 Hz), 7.1–7.6 (3 H, m), 7.20 (5 H, s), 7.28 (5 H, s), 7.6–7.9 (1 H, m), 7.9–8.3 (1 H, m), 8.53 (1 H, d, *J* = 8 Hz), 9.4 (1 H, br s); R_f = 0.50 (system E). Anal. ($C_{34}H_{37}N_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Phe-OCH$_2$Ph(*p*-Cl) (39). Prepared from **14**: 91.9% yield; mp 157–158 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +1.51° (*c* = 1.03, DMF); IR (Nujol) 3350, 1740, 1720, 1680, 1660, 1545 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.29 (9 H, s), 2.6–3.3 (4 H, m), 4.1–4.8 (2 H, m), 5.14 (2 H, s), 6.93 (1 H, d, *J* = 9 Hz), 7.2–7.9 (4 H, m), 7.26 (5 H, s), 7.43 (4 H, s), 8.2 (1 H, s), 8.58 (1 H, d, *J* = 8 Hz), 9.4 (1 H, br s); R_f = 0.85 (system F). Anal. ($C_{33}H_{34}ClN_3O_6$) C, H, N.

Boc-D-Trp(CHO)-Phe-OCH$_2$C$_6$H$_{11}$ (40). Prepared from **15**: 93.5% yield; mp 78–80 °C (*n*-hexane); $[\alpha]^{25}_D$ = –7.33° (*c* = 1.07, DMF); IR (Nujol) 3350, 1710, 1690, 1650, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.7–1.8 (10 H, m), 1.28 (9 H, s), 2.6–3.2 (5 H, m), 3.87 (2 H, d, *J* = 6 Hz), 4.0–4.8 (2 H, m), 6.6–6.9 (1 H, m), 7.1–7.8 (4 H, m), 7.26 (5 H, s), 7.9–8.3 (1 H, m), 8.53 (1 H, d, *J* = 9 Hz), 9.4 (1 H, br s); R_f = 0.95 (system E). Anal. ($C_{33}H_{41}N_3O_6$) C, H, N.

Synthesis of Protected Tripeptides from Boc-Protected Dipeptides: Boc-Gln-D-Trp(CHO)-Phe-OBzl (4a). To a solution of **16** (4.86 g, 8.53 mmol) in CH_2Cl_2 (50 mL) was added 4 N hydrochloric acid (50 mL) in dioxane under ice-cooling. The solution was stirred at the 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-OBzl were collected by filtration. This hydrochloride (4.70 g, 8.53 mmol), Boc-Gln-OH (2.1 g, 8.53 mmol), and HOBT (1.15 g, 8.53 mmol) were dissolved in a mixed solvent of CH_2Cl_2 (60 mL) and DMF (10 mL). To the solution was added WSCD (1.41 g, 8.66 mmol) under ice-cooling. The reaction mixture was stirred at the temperature for 1.5 h and then at room temperature for 1.5 h. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue obtained was diluted with water to form precipitates which were collected, washed successively with water, diluted sodium hydrogen carbonate solution, and water, and dried. This crude product was recrystallized from EtOAc to give **4a** (5.7 g, 95.8%) as crystals: mp 202–203.5 °C (EtOAc); $[\alpha]^{25}_D$ = +2.88° (*c* = 1.11, DMF); IR (Nujol) 3440, 3300, 1720, 1660 (sh), 1645 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.5–1.8 (2 H, m), 1.85–1.95 (2 H, m), 2.7–3.1 (4 H, m), 3.90 (1 H, br s), 4.45–4.8 (2 H, m), 5.10 (2 H, s), 6.70 (2 H, br s), 7.1–7.7 (4 H, m), 7.20 (5 H, s), 7.35 (5 H, s), 7.55 (1 H, m), 7.95–8.25 (2 H, m), 8.65 (1 H, d, *J* = 6 Hz), 9.3 (1 H, br s); R_f = 0.64 (system A). Anal. ($C_{38}H_{43}N_5O_8$) C, H, N.

The following protected tripeptides, **2b**, **2d**, **9a–e**, **10a**, **10c–f**, **11a–i**, and **12a–e**, were prepared similarly as **4a**.

Boc-D-Pro-Gln-Gln-OBzl (2b). Prepared from **17** and Boc-D-Pro-OH: 51.3% yield; mp 202–203 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +5.43° (*c* = 1.01, DMF); IR (Nujol) 3430, 3300, 3210, 1735, 1710, 1660, 1640 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.30 (9 H, s), 1.60–2.3 (12 H, m), 3.3 (2 H, m), 4.0–4.5 (3 H, m), 5.15 (2 H, s), 6.75 (2 H, s), 7.20 (2 H, s), 7.40 (5 H, s), 7.8–8.6 (2 H, m); R_f = 0.43 (system F). Anal. ($C_{27}H_{39}N_5O_8$) C, H, N.

Z-D-Pro-Gln-Gln-OBzl (2d). Prepared from **17** and Z-D-Pro-OH: 73.4% yield (amorphous solid); mp 187–188 °C; $[\alpha]^{25}_D$ = +3.34° (*c* = 1.05, DMF); IR (Nujol) 3430, 3200, 1730, 1710, 1635, 1550 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.6–2.2 (12 H, m), 3.4–3.5 (2 H, m), 4.25 (3 H, m), 5.0 (2 H, s), 5.10 (2 H, s), 6.78 (2 H, s), 7.27 (2 H, m), 7.35 (10 H, m), 8.0–8.4 (2 H, m); R_f = 0.36 (system A). Anal. ($C_{30}H_{37}N_5O_8$) C, H, N.

Boc-D-Gln-D-Trp(CHO)-Phe-OBzl (9a). Prepared from **16** and Boc-D-Gln-OH: 72.6% yield; mp 170–172 °C (EtOH); $[\alpha]^{25}_D$ = +5.87° (*c* = 0.99, DMF); IR (Nujol) 3300, 1720, 1660, 1640, 1550, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 (9 H, s), 1.5–2.2 (4 H, m), 2.6–3.2 (4 H, m), 3.6–4.1 (1 H, m), 4.4–4.9 (2 H, m), 5.12 (2 H, s), 6.6–7.0 (2 H, m), 7.0–7.7 (5 H, m), 7.25 (5 H, s), 7.36 (5 H, s), 7.90 (1 H, d, *J* = 9 Hz), 8.0–8.3 (1 H, m), 8.76 (1 H, d, *J* = 8 Hz), 9.2 (1 H, br s); R_f = 0.77 (system F). Anal. ($C_{38}H_{43}N_5O_8$) C, H, N.

Boc-Thr-D-Trp(CHO)-Phe-OBzl (9b). Prepared from **16** and Boc-Thr-OH: 73.9% yield; mp 158–160 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +6.51° (*c* = 0.78, DMF); IR (Nujol) 3340, 3290 (sh), 1720, 1685, 1640, 1540 (sh), 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.83 (3 H, d, *J* = 6 Hz), 1.33 (9 H, s), 2.7–3.2 (4 H, m), 3.7–4.1 (2 H, m), 4.4–5.0 (3 H, m), 5.10 (2 H, s), 6.2–6.5 (1 H, m), 7.2–7.8 (4 H, m), 7.21 (5 H, s), 7.33 (5 H, s), 7.9–8.4 (2 H, m), 8.62 (1 H, d, *J* = 9 Hz), 9.3 (1 H, br s); R_f = 0.81 (system C). Anal. ($C_{37}H_{42}N_4O_8$) C, H, N.

Boc-Ser-D-Trp(CHO)-Phe-OBzl (9c). Prepared from **16** and Boc-Ser-OH: 86.3% yield; mp 164–166 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +3.23° (*c* = 0.90, DMF); IR (Nujol) 3200, 1700 (br), 1640, 1550, 1525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 2.7–3.2 (4 H, m), 3.35–3.65 (2 H, m), 3.8–4.2 (1 H, m), 4.4–4.9 (3 H, m), 5.12 (2 H, s), 6.60 (1 H, br s), 7.2–7.7 (4 H, m), 7.23 (5 H, s), 7.36 (5 H, s), 7.9–8.3 (2 H, m), 8.67 (1 H, d, *J* = 8 Hz), 9.3 (1 H, br s); R_f = 0.75 (system C). Anal. ($C_{36}H_{40}N_4O_8 \cdot H_2O$) C, H, N.

Boc-Tyr-D-Trp(CHO)-Phe-OBzl (9d). Prepared from **16** and Boc-Tyr-OH: 78.7% yield; mp 213–215 °C (DMF–Et_2O); $[\alpha]^{25}_D$ = +9.11° (*c* = 0.94, DMF); IR (Nujol) 3450, 3290, 1755, 1715, 1640, 1560 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.25 (9 H, s), 2.3–2.6 (2 H, m), 2.6–3.2 (4 H, m), 3.9–4.3 (1 H, m), 4.4–5.0 (2 H, m), 5.12 (2 H, s), 6.4–6.7 (1 H, m), 6.53 (2 H, d, *J* = 9 Hz), 6.86 (2 H, d, *J* = 9 Hz), 7.2–7.8 (4 H, m), 7.26 (5 H, s), 7.35 (5 H, s), 8.0–8.4 (2 H, m), 8.6–8.9 (1 H, m), 9.08 (1 H, s), 9.3 (1 H, br s); R_f = 0.75 (system C). Anal. ($C_{42}H_{44}N_4O_8$) C, H, N.

Boc-Gly-D-Trp(CHO)-Phe-OBzl (9e). Prepared from **16** and Boc-Gly-OH: 74.0% yield; mp 78–80 °C (EtOAc–*n*-hexane); $[\alpha]^{25}_D$ = −1.44° (*c* = 0.83, DMF); IR (Nujol) 3290, 1750, 1710, 1650, 1555 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 2.6–3.3 (4 H, m), 3.49 (2 H, d, *J* = 6 Hz), 4.4–4.9 (2 H, m), 5.13 (2 H, s), 6.9 (1 H, br s), 7.2–7.8 (4 H, m), 7.24 (5 H, s), 7.37 (5 H, s), 7.97 (1 H, d, *J* = 9 Hz), 8.2 (1 H, m), 8.76 (1 H, d, *J* = 9 Hz), 9.3 (1 H, br s); R_f = 0.30 (system C). Anal. ($C_{35}H_{38}N_4O_7$) C, H, N.

Boc-Gln-Trp(CHO)-Phe-OBzl (10a). Prepared from **31** and Boc-Gln-OH: 91.8% yield; mp 206–208 °C (DMF–EtOAc); $[\alpha]^{25}_D$ = −16.9° (*c* = 1.08, DMF); IR (Nujol) 3450, 3300, 1745 (sh), 1724, 1706, 1690, 1658 (sh), 1640, 1544, 1520 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.5–2.15 (4 H, m), 2.8–3.1 (4 H, m), 3.7–4.0 (1 H, m), 4.4–4.8 (2 H, m), 5.10 (2 H, s), 6.7–6.9 (2 H, m), 7.1–7.4 (3 H, m), 7.5–7.7 (2 H, m), 7.22 (5 H, s), 7.31 (5 H, s), 7.8–8.3 (2 H, m), 8.65 (1 H, d, *J* = 8 Hz), 9.3 (1 H, br s); R_f = 0.50 (system C). Anal. ($C_{38}H_{43}N_5O_8$) H, N; C: calcd, 65.41; found, 66.16.

Boc-Gln-Phe-Phe-OBzl (10c). Prepared from **32** and Boc-Gln-OH: 92.2% yield; mp 199–200 °C (EtOAc); $[\alpha]^{25}_D$ = −17.52° (*c* = 1.01, DMF); IR (Nujol) 3450, 3330, 1744, 1690, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.35 (9 H, s), 1.5–2.2 (4 H, m), 2.7–3.1 (4 H, m), 3.7–4.05 (1 H, m), 4.45–4.75 (2 H, m), 5.08 (2 H, s), 6.75 (2 H, s), 6.8–7.0 (1 H, m), 7.18 (5 H, s), 7.24 (5 H, s), 7.30 (5 H, s), 7.80 (1 H, d, *J* = 8 Hz), 8.65 (1 H, d, *J* = 8 Hz); R_f = 0.48 (system B). Anal. ($C_{35}H_{42}N_4O_7$) C, H, N.

Boc-Gln-D-Phe-Phe-OBzl (10d). Prepared from **33** and Boc-Gln-OH: 78.1% yield; mp 173–174 °C (EtOAc); $[\alpha]^{25}_D$ = −3.58° (*c* = 1.03, DMF); IR (Nujol) 3350, 1730, 1680, 1642 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.4–2.1 (4 H, m), 2.5–3.2 (4 H, m), 3.75–4.1 (1 H, m), 4.4–4.8 (2 H, m), 5.12 (2 H, s), 6.70 (2 H, s), 6.6–6.85 (1 H, m), 6.95–7.2 (5 H, m), 7.25 (5 H, s), 7.32 (5 H, s), 7.89 (1 H, d, *J* = 9 Hz), 8.55 (1 H, d, *J* = 9 Hz); R_f = 0.72 (system A). Anal. ($C_{35}H_{42}N_4O_7$) C, H, N.

Boc-Gln-D-Met-Phe-OBzl (10e). Prepared from **34** and Boc-Gln-OH: 87.9% yield; mp 168–170 °C (EtOAc); $[\alpha]^{25}_D$ = −10.59° (*c* = 1.02, DMF); IR (Nujol) 3450, 3340, 3220, 1725, 1685, 1660, 1640, 1540 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.7–2.3 (8 H, m), 1.95 (3 H, s), 3.0 (2 H, m), 3.9 (1 H, m), 4.2–4.7 (2 H, m), 5.13 (2 H, s), 6.75 (2 H, br s), 6.9 (1 H, d, *J* = 6 Hz), 7.23 (5 H, s), 7.38 (5 H, s), 7.86 (1 H, d, *J* = 8 Hz), 8.35 (1 H, d, *J* = 8 Hz); R_f = 0.69 (system F). Anal. ($C_{31}H_{42}N_4O_7S$) C, H, N.

Boc-Gln-D-Leu-Phe-OBzl (10f). Prepared from **35** and Boc-Gln-OH: 82.6% yield; mp 176–177 °C (EtOAc); $[\alpha]^{25}_D$ = −7.99° (*c* = 0.95, DMF); IR (Nujol) 3320, 1720, 1685, 1635 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.71 (6 H, d, *J* = 6 Hz), 1.1–1.45 (3 H, m), 1.36 (9 H, s), 1.6–2.2 (4 H, m), 2.82 (1 H, dd, *J* = 2, 15 Hz), 3.06 (1 H, dd, *J* = 5, 15 Hz), 3.75–4.05 (1 H, m), 4.2–4.7 (2 H, m), 5.03 (2 H, s), 6.75 (2 H, s), 6.7–6.9 (1 H, m), 7.20 (5 H, s), 7.32 (5 H, s), 7.76 (1 H, d, *J* = 9 Hz), 8.42 (1 H, d, *J* = 9 Hz); R_f = 0.74 (system A). Anal. ($C_{32}H_{44}N_4O_7$) C, H, N.

Boc-Gln-D-Trp(CHO)-Gly-OBzl (11a). Prepared from **22** and Boc-Gln-OH: 90.0% yield; mp 192–193 °C dec (EtOH); $[\alpha]^{25}_D$ = +10.8° (*c* = 0.97, DMF); IR (Nujol) 3300, 1750 (sh), 1710, 1685, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.35 (9 H, s), 1.5–2.1 (4 H, m), 2.8–3.3 (2 H, m), 3.97 (2 H, d, *J* = 6 Hz), 3.9 (1 H, m), 4.72 (1 H, m), 5.2 (2 H, s), 6.6–7.8 (12 H, m), 8.1–8.3 (2 H, m), 8.6 (1 H, m), 9.35 (1 H, br s); R_f = 0.65 (system F). Anal. ($C_{31}H_{37}N_5O_8$) C, H, N.

Boc-Gln-D-Trp(CHO)-Leu-OBzl (11b). Prepared from **23** and Boc-Gln-OH: 91.1% yield; mp 204 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = −1.42° (*c* = 0.98, DMF); IR (Nujol) 3450 (sh), 3370 (sh), 3290, 1740 (sh), 1720, 1660 (sh), 1645, 1550, 1535 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.7–0.8 (6 H, m), 1.35 (9 H, s), 1.5–2.2 (7 H, m), 2.9–3.2 (2 H, m), 3.9–5.0 (3 H, m), 5.1 (2 H, s), 6.6–7.0 (2 H, m), 7.1–7.9 (10 H, m), 8.0–8.5 (3 H, m), 9.4 (1 H, br s); R_f = 0.60 (system E). Anal. ($C_{35}H_{45}N_5O_8$) C, H, N.

Boc-Gln-D-Trp(CHO)-Pro-OBzl (11c). Prepared from **24** and Boc-Gln-OH: 71.4% yield (amorphous solid); $[\alpha]^{25}_D$ = −31.33° (*c* = 1.02, DMF); IR (Nujol) 1745, 1705, 1690–1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 and 1.40 (9 H, s), 1.7–2.4 (8 H, m), 3.15 (2 H, d, *J* = 7 Hz), 2.9–3.2 (1 H, m), 3.4–3.8 (1 H, m), 4.0–4.4 (2 H, m), 5.10 (2 H, s), 5.0–5.2 (1 H, m), 5.6–6.1 (2 H, m), 6.63 (1 H, m), 7.2–7.7 (10 H, m), 8.0–8.5 (1 H, m), 9.2 (1 H, br s); R_f = 0.6 (system A). Anal. ($C_{34}H_{41}N_5O_8$) C, H; N: calcd, 10.81; found, 10.03.

Boc-Gln-D-Trp(CHO)-Val-OBzl (11d). Prepared from **25** and Boc-Gln-OH: 74.2% yield; mp 200–201 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +2.87° (*c* = 1.01, DMF); IR (Nujol) 3350, 3200, 1730, 1710, 1690, 1664, 1550, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.72 (6 H, d, *J* = 7 Hz), 1.33 (9 H, s), 1.5–2.1 (5 H, m), 2.8–3.1 (2 H, m), 3.7–4.3 (2 H, m), 4.7–5.9 (1 H, m), 5.12 (2 H, s), 6.65–6.9 (2 H, m), 7.1–7.85 (5 H, m), 7.36 (5 H, s), 8.0–8.5 (3 H, m), 9.35 (1 H, br s); R_f = 0.76 (system C). Anal. ($C_{34}H_{43}N_5O_8 \cdot 0.5H_2O$) H, N; C: calcd, 62.85; found, 62.03.

Boc-Gln-D-Trp(CHO)-Hyp(Bzl)-OBzl (11e). Prepared from **26** and Boc-Gln-OH: 63.3% yield (amorphous solid); $[\alpha]^{25}_D$ = −13.42° (*c* = 1.11, DMF); IR (Nujol) 3250, 1730 (sh), 1710, 1660, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (9 H, s), 1.8–2.6 (6 H, m), 3.1–3.6 (3 H, m), 3.6–4.3 (4 H, m), 4.40 (1 H, d, *J* = 9 Hz), 4.55 (1 H, d, *J* = 9 Hz), 5.0–5.3 (1 H, m), 5.18 (2 H, s), 5.7–6.6 (3 H, m), 7.1–7.8 (15 H, m), 8.4 (1 H, m), 9.1 (1 H, s); R_f = 0.4 (system B). Anal. ($C_{41}H_{47}N_5O_9$) H, N; C: calcd, 65.32; found, 64.60.

Boc-Gln-D-Trp(CHO)-D-Phe-OBzl (11f). Prepared from **27** and Boc-Gln-OH: 77.7% yield; mp 208–209 °C (DMF-EtOAc); $[\alpha]^{25}_D$ = +15.8° (*c* = 0.94, DMF); IR (Nujol) 3430 (sh), 3310, 3220 (sh), 1724, 1690, 1660, 1640, 1525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.35 (9 H, s), 1.5–2.1 (4 H, m), 2.8–3.1 (4 H, m), 3.7–4.0 (1 H, m), 4.4–4.8 (2 H, m), 5.10 (2 H, s), 6.6–6.9 (2 H, m), 7.1–7.45 (13 H, m), 7.5–7.8 (2 H, m), 8.0–8.3 (2 H, m), 8.60 (1 H, d, *J* = 8 Hz), 9.3 (1 H, br s); R_f = 0.62 (system A). Anal. ($C_{38}H_{43}N_5O_8$) C, H, N.

Boc-Gln-D-Trp(CHO)-Tyr-OBzl (11g). Prepared from **28** and Boc-Gln-OH: 64.9% yield; mp 203 °C dec (DMF–EtOAc); $[\alpha]^{25}_D$ = −0.25° (*c* = 1.18, DMF); IR (Nujol) 3320, 1740 (sh), 1700, 1680, 1646, 1515 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.5–2.1 (4 H, m), 2.7–3.0 (4 H, m), 4.1–4.4 (1 H, m), 4.4–4.9 (2 H, m), 5.10 (2 H, s), 6.61 (2 H, d, *J* = 8 Hz), 7.01 (2 H, d, *J* = 8 Hz), 6.7 (2 H, m), 7.30 (5 H, s), 7.0–7.8 (5 H, m), 7.9–8.3 (2 H, m), 8.5–8.7 (1 H, m), 9.2 (1 H, s), 9.3 (1 H, br s); R_f = 0.41 (system C). Anal. ($C_{38}H_{43}N_5O_9 \cdot 0.75H_2O$) C, H, N.

Boc-Gln-D-Trp(CHO)-MePhe-OBzl (11h). Prepared from **29** and Boc-Gln-OH: 30.9% yield (amorphous solid); $[\alpha]^{25}_D$ = −27.57° (*c* = 1.07, DMF); IR (Nujol) 3420, 3350, 3200, 1740, 1710, 1660, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.34 (9 H, s), 1.5–2.1 (4 H, m), 2.5–3.4 (4 H, m), 2.81 (3 H, s), 3.8–4.2 (1 H, m), 4.8–5.4 (2 H, m), 5.16 (2 H, s), 6.75 (2 H, s), 7.0–7.7 (5 H, m), 7.24 (5 H, s), 7.38 (5 H, s), 8.0–8.4 (2 H, m), 9.3 (1 H, br s); R_f = 0.13 (system E). Anal. ($C_{39}H_{45}N_5O_8$) C, H, N.

Boc-Gln-D-Trp(CHO)-Tic-OBzl (11i). Prepared from **30** and Boc-Gln-OH: 36.8% yield (amorphous solid); $[\alpha]^{25}_D$ = +1.42° (*c* = 0.98, DMF): IR (Nujol) 3300, 1710, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35 and 1.43 (9 H, s), 1.9–2.4 (4 H, m), 3.0–3.3 (4 H, m), 4.3 (3 H, m), 4.86 and 5.02 (2 H, s), 5.2–5.5 (2 H, m), 5.8 (1 H, m), 6.3 (1 H, m), 6.9–7.4 (13 H, m), 7.6 (2 H, m), 8.1 (1 H, m), 8.92 (1 H, s); R_f = 0.50 (system A). Anal. ($C_{39}H_{43}N_5O_8 \cdot 2H_2O$) C, H, N.

Boc-Gln-D-Trp(CHO)-Phe-OMe (12a). Prepared from **36** and Boc-Gln-OH: 60.2% yield; mp 165–167 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +1.70° (*c* = 1.10, DMF); IR (Nujol) 3310, 1710, 1690, 1650 (sh), 1540, 1525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.4–2.1 (4 H, m), 2.6–3.1 (4 H, m), 3.63 (3 H, m), 3.7–4.1 (1 H, m), 4.3–4.8 (2 H, m), 6.6–6.9 (2 H, m), 7.0–7.5 (4 H, m), 7.25 (5 H, s), 7.5–7.7 (1 H, m), 7.9–8.3 (2 H, m), 8.64 (1 H, d, *J* = 8 Hz), 9.3 (1 H, br s); R_f = 0.66 (system C). Anal. ($C_{32}H_{39}N_5O_8 \cdot 0.67H_2O$) C, H, N.

Boc-Gln-D-Trp(CHO)-Phe-OiPr (12b). Prepared from **37** and Boc-Gln-OH: 86.4% yield; 213–216 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +6.08° (*c* = 0.97, DMF); IR (Nujol) 3450, 3350, 1715, 1690, 1660, 1645, 1545 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.07 (3 H, d, *J* = 7 Hz), 1.17 (3 H, d, *J* = 7 Hz), 1.32 (9 H, s), 1.5–2.2 (4 H, m), 2.6–3.2 (4 H, m), 3.8–4.1 (1 H, m), 4.3–4.9 (2 H, m), 4.88 (1 H, sept, *J* = 7 Hz), 6.6–7.0 (2 H, m), 7.0–7.6 (4 H, m), 7.23 (5 H, s), 7.6–7.8 (1 H, m), 7.9–8.3 (2 H, m), 8.70 (1 H, d, *J* = 8 Hz), 9.8 (1 H, br s); R_f = 0.74 (system F). Anal. ($C_{34}H_{43}N_5O_8$) C, H, N.

Boc-Gln-D-Trp(CHO)-Phe-OCH_2CH_2Ph (12c). Prepared from **38** and Boc-Gln-OH: 89.9% yield; mp 157–159 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +3.63° (*c* = 1.0, DMF); IR (Nujol) 3330, 1725, 1710, 1690, 1645, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.5–2.2 (4 H, m), 2.6–3.1 (6 H, m), 3.7–4.2 (1 H, m), 4.27 (2 H, t, *J* = 6 Hz), 4.4–4.9 (2 H, m), 6.6–6.9 (2 H, m), 7.0–7.8 (5 H, m), 7.22 (5 H, s), 7.28 (5 H, s), 7.9–8.3 (2 H, m), 8.61 (1 H, d, *J* = 8 Hz), 9.3 (1 H, br s); R_f = 0.69 (system F). Anal. ($C_{39}H_{45}N_5O_8$) C, H, N.

Boc-Gln-D-Trp(CHO)-Phe-OCH_2Ph(*p*-Cl) (12d). Prepared from **39** and Boc-Gln-OH: 86.1% yield; mp 214–216 °C (DMF–EtOAc); $[\alpha]^{25}_D$ = −9.35° (*c* = 1.04, DMF); IR (Nujol) 3310, 1725, 1710, 1685, 1640, 1545 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 (9 H, s), 1.4–2.2 (4 H, m), 2.6–3.2 (4 H, m), 3.8–4.1 (1 H, m), 4.4–4.9 (2 H, m), 5.11 (2 H, s), 6.6–6.9 (2 H, m), 7.0–7.7 (9 H, m), 7.23 (5 H, s), 7.9–8.4 (2 H, m), 8.73 (1 H, d, *J* = 9 Hz), 9.3 (1 H, br s); R_f = 0.69 (system F). Anal. ($C_{38}H_{42}ClN_5O_8$) C, H, N.

Boc-Gln-D-Trp(CHO)-Phe-$OCH_2C_6H_{11}$ (12e). Prepared from **40** and Boc-Gln-OH: 78.1% yield; mp 198–201 °C (DMF–EtOAc); $[\alpha]^{25}_D$ = +2.79° (*c* = 1.11, DMF); IR (Nujol) 3330, 1725, 1710, 1690, 1645, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.6–2.1 (14 H, m), 1.33 (9 H, s), 2.7–3.3 (5 H, m), 3.7–4.1 (1 H, m), 3.84 (2 H, d, *J* = 6 Hz), 4.3–4.9 (2 H, m), 6.6–6.9 (2 H, m), 7.0–7.8 (5 H, m), 7.25 (5 H, s), 7.9–8.4 (2 H, m), 8.5–8.8 (1 H, m), 9.3 (1 H, br s); R_f = 0.69 (system F). Anal. ($C_{38}H_{49}N_5O_8 \cdot 0.5H_2O$) C, H, N.

Boc-D-Trp-D-Trp-Phe-OBzl (8c). A mixture of **21** (1.76 g, 3.26 mmol), TFA (9 mL), 1,2-ethanedithiol (3 mL), and dimethyl sulfide (4 mL) was stirred under ice-cooling for 1 h and at room temperature for an additional 2 h. The mixture was concentrated to half volume under reduced pressure, and 4 N hydrochloric acid (1.7 mL) in dioxane was added to the residue. The mixture was evaporated again. The residue obtained was triturated with Et_2O and filtered to give HCl·H-D-Trp-Phe-OBzl (1.68 g). This hydrochloride was coupled with Boc-D-Trp-OH (0.99 g, 3.26 mmol) by using WSCD (0.55 g, 3.26 mmol) and HOBT (0.44 g, 3.26 mmol) similarly as the synthesis of **4a** to give **8c** (2.14 g, 90.3%): mp 142–144 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +16.63° (*c* = 1.01, DMF); IR (Nujol) 3430, 3350, 1750, 1690, 1640 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.23 (9 H, s), 2.6–3.1 (6 H, m), 3.9–4.25 (1 H, m), 4.25–4.75 (2 H, m), 5.03 (2 H, m), 6.6–7.6 (11 H, m), 7.14 (5 H, s), 7.23 (5 H, s), 7.73 (1 H, d, *J* = 8 Hz), 8.51 (1 H, d, *J* = 8 Hz), 10.64 (2 H, s); R_f = 0.41 (system E). Anal. ($C_{43}H_{45}N_5O_6$) C, H, N.

Boc-Gln-D-Trp-Phe-OBzl (10b). Prepared similarly as **8c** except using Boc-Gln-OH: 67.2% yield; mp 195–197 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = −1.28° (*c* = 1.01, DMF); IR (Nujol) 3420, 3340, 3300, 3230, 1736, 1688, 1665, 1640, 1620 cm^{-1}; ^1H NMR (DSMO-d_6) δ 1.33 (9 H, s), 1.5–2.1 (4 H, m), 2.7–3.05 (4 H, m), 3.8–4.0 (1 H, m), 4.4–4.7 (2 H, m), 5.07 (2 H, s), 6.6–6.9 (3 H, m), 6.9–7.6 (5 H, m), 7.20 (5 H, s), 7.31 (5 H, s), 7.85 (1 H, d, *J* = 8 Hz), 8.36 (1 H, d, *J* = 8 Hz); R_f = 0.86 (system F). Anal. ($C_{37}H_{43}N_5O_7$) C, H, N.

Synthesis of Protected Tripeptides from Z-Protected Dipeptides: Z-Gln-Gln-D-Trp-OMe (41). To a solution of **18** (0.50 g, 1 mmol) in a mixed solvent of MeOH (27 mL) and water (3 mL) was added 1 N hydrochloric acid (1.1 mL). The resulting solution was hydrogenated at atmospheric pressure with 10% Pd (0.1 g) on charcoal. After removal of the catalyst, the solvent was evaporated and the residue obtained was triturated with Et_2O to form precipitates. The precipitates which were identified as HCl·Gln-D-Trp-OMe were filtered and dried. This hydrochloride (0.38 g), Z-Gln-OH (280 mg, 1 mmol), and HOBT (0.135 g, 1 mmol) were dissolved in DMF (10 mL). WSCD (0.17 g, 1.1 mmol) was added to the solution under ice-cooling. The solution was stirred at this temperature for 2 h and at room temperature for 6 h. The reaction mixture was concentrated, diluted in water, and extracted with EtOAc. The organic layer was washed successively with water, diluted sodium hydrogen carbonate solution, 0.5 N hydrochloric acid, and brine and dried. Concentration of the solvent gave **41** (0.52 g, 85.4%) as an amorphous solid: mp 232 °C dec; $[\alpha]^{25}_D$ = −9.32° (*c* = 1.02, DMF); IR (Nujol) 3420, 3300, 1732, 1690 (sh), 1650, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.6–2.2 (8 H, m), 3.05–3.2 (2 H, m), 3.60 (3 H, s), 3.85–4.15 (1 H, m), 4.2–4.65 (2 H, m), 5.1 (2 H, s), 6.75 (2 H, s), 6.95–7.6 (8 H, m), 7.35 (5 H, s), 8.0 (1 H, d, *J* = 8 Hz), 8.34 (1 H, d, *J* = 8 Hz), 10.85 (1 H, s); R_f = 0.43 (system H). Anal. ($C_{30}H_{36}N_6O_8 \cdot 0.5H_2O$) C, H, N.

The following protected tripeptides, **42** and **43**, were prepared similarly as **41**.

Z-D-Trp-Phe-D-Trp-OMe (42). Prepared from **19** and Z-D-Trp-OH: 92.1% yield; mp 207–209 °C (EtOH); $[\alpha]^{25}_D$ = +19.20° (*c* = 0.95, DMF); IR (Nujol) 3410, 3300, 3260, 3080, 1730, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.6–2.9 (4 H, m), 2.9–3.2 (2 H, m), 3.60 (3 H, s), 4.1–4.4 (1 H, m), 4.5–4.8 (2 H, m), 4.90 (2 H, s), 6.9–7.4 (19 H, m), 7.4–7.7 (2 H, m), 8.20 (1 H, d, *J* = 8 Hz), 8.45 (1 H, d, *J* = 8 Hz), 10.7 (1 H, s), 10.83 (1 H, s); R_f = 0.57 (system C). Anal. ($C_{40}H_{39}N_5O_6$) C, H, N.

Z-Phe-D-Trp-D-Trp-OMe (43). Prepared from **20** and Z-Phe-OH: 81.4% yield; mp 201–202 °C (EtOH); $[\alpha]^{25}_D$ = +11.33° (*c* = 1.02, DMF); IR (Nujol) 3420, 3300, 1740, 1680, 1642, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5–2.8 (2 H, m), 2.9–3.3 (4 H, m), 3.60 (3 H, s), 4.3 (1 H, m), 4.5–4.8 (2 H, m), 4.97 (2 H, s), 7.0–7.8 (21 H, m), 8.3 (1 H, d, *J* = 8 Hz), 8.5 (1 H, d, *J* = 8 Hz), 10.80 (2 H, s); R_f = 0.70 (system C). Anal. ($C_{40}H_{39}N_5O_6$) C, H, N.

Synthesis of Tripeptide Acids: Z-Gln-Gln-D-Trp-OH (3b). To a solution of **41** (1.8 g, 3.0 mmol) in a mixed solvent of THF (60 mL) and water (20 mL) was added 1 N sodium hydroxide (3.3 mL). The solution was stirred at room temperature for 2 h. After removal of THF by evaporation, the residual solution was acidified with 10% aqueous citric acid. The precipitates formed were collected to give **3b** (1.54 g, 87.6%): mp 239 °C dec (EtOH–H_2O); $[\alpha]^{25}_D$ = −10.30° (*c* = 1.00, DMF); IR (Nujol) 3400 (sh), 3300, 1690 (sh), 1650, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.6–2.3 (8 H, m), 2.9–3.5 (2 H, m), 3.9–4.6 (3 H, m), 5.0 (2 H, s), 6.7 (2 H, br s), 6.9–7.6 (13 H, m), 7.92 (1 H, d, *J* = 8 Hz), 8.10 (1 H, d, *J* = 8 Hz), 10.8 (1 H, s), 12.6 (1 H, s); R_f = 0.05 (system H). Anal. ($C_{29}H_{34}N_6O_8 \cdot 0.6H_2O$) C, H, N.

The following tripeptide acids, **5b**, **6b**, and **7b**, were prepared similarly as **3b**.

Boc-Gln-D-Trp-Phe-OH (5b). Prepared from **4a**: 80.2% yield; mp 168–170 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +4.35° (*c* = 0.64, DMF); IR (Nujol) 3320, 1715, 1690, 1645, 1545, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.4–2.2 (4 H, m), 2.6–3.5 (4 H, m), 3.7–4.1 (1 H, m), 4.3–4.8 (2 H, m), 6.6–7.6 (13 H, m), 7.86 (1 H, d, *J* = 8 Hz), 8.36 (1 H, d, *J* = 8 Hz), 10.70 (1 H, s), 12.7 (1 H, br s); R_f = 0.67 (system G). Anal. ($C_{30}H_{37}N_5O_7 \cdot 0.5H_2O$) C, H, N.

Z-D-Trp-Phe-D-Trp-OH (6b). Prepared from **42**: 86.3% yield; mp 146 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = +25.11° (*c* = 0.62, DMF); IR (Nujol) 3430, 3320, 1720, 1680, 1640, 1550 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.6–3.5 (6 H, m), 4.2–4.8 (3 H, m), 4.95 (2 H, s), 7.0–7.45 (20 H, m), 7.55–7.7 (2 H, m), 8.21 (1 H, d, *J* = 8 Hz), 8.40 (1 H, d, *J* = 8 Hz), 10.75 (1 H, s), 10.86 (1 H, s); R_f = 0.69 (system C). Anal. ($C_{39}H_{37}N_5O_6 \cdot H_2O$) C, H, N.

Z-Phe-D-Trp-D-Trp-OH (7b). Prepared from **43**: 89.1% yield; mp 186 °C dec (EtOH–H_2O); $[\alpha]^{25}_D$ = +10.96° (*c* = 0.52, DMF); IR (Nujol) 3420, 3320, 1730, 1710, 1680, 1644, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5–3.4 (6 H, m), 4.15–4.8 (3 H, m), 4.95 (2 H, s), 6.95–7.4 (20 H, m), 7.5–7.75 (2 H, m), 8.2–8.4 (2 H, m), 10.78 (2 H, s); R_f = 0.55 (system H). Anal. ($C_{39}H_{37}N_5O_6 \cdot 0.5H_2O$) C, H, N.

Esterification of Tripeptide Acids: Z-D-Trp-Phe-D-Trp-OBzl (6d). To a solution of **6b** (195 mg, 0.29 mmol) in DMF (5 mL) were added benzyl bromide (86 mg, 0.50 mmol) and diisopropylethylamine (59 mg, 0.46 mmol). The mixture was stirred at room temperature for 8 h and then concentrated, diluted in water, and extracted with EtOAc. The organic layer was washed successively with diluted sodium hydrogen carbonate solution, 0.5 N HCl, and brine and dried. The crude product was crystallized from EtOAc–IPE to give **6d** (197 mg, 89.1%): mp 191–192 °C; $[\alpha]^{25}_D$ = +13.07° (*c* = 0.83, DMF); IR (Nujol) 3450, 3330, 1732, 1685 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5–2.9 (4 H, m), 3.0–3.3 (2 H, m), 4.3 (1 H, m), 4.5–4.8 (2 H, m), 4.85 (1 H, d, *J* = 13 Hz), 4.92 (1 H, d, *J* = 13 Hz), 5.04 (1 H, d, *J* = 13 Hz), 5.12 (1 H, d, *J* = 13 Hz), 6.9–7.2 (24 H, m), 7.53 (1 H, d, *J* = 7.4 Hz), 7.61 (1 H, d, *J* = 7.4 Hz), 8.31 (1 H, d, *J* = 8.6 Hz), 8.59 (1 H, d, *J* = 7.6

Hz), 10.75 (1 H, s), 10.89 (1 H, s); R_f = 0.88 (system A). Anal. ($C_{46}H_{43}N_5O_6$) C, H, N.

Z-Phe-D-Trp-D-Trp-OBzl (7d). Prepared similarly as **6d** from **7b**: 94.7% yield; mp 171–172 °C (EtOAc); $[\alpha]^{25}_D$ = +9.24° (*c* = 0.92, DMF); IR (Nujol) 3430, 3300, 1735, 1680 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.4–2.7 (2 H, m), 2.8–3.3 (4 H, m), 4.27 (1 H, m), 4.55–4.75 (2 H, m), 4.89 (1 H, d, *J* = 12.9 Hz), 4.96 (1 H, d, *J* = 12.9 Hz), 4.98 (1 H, d, *J* = 12.6 Hz), 5.05 (1 H, d, *J* = 12.6 Hz), 6.95–7.4 (24 H, m), 7.51 (1 H, d, *J* = 7.6 Hz), 7.65 (1 H, d, *J* = 7.5 Hz), 8.35 (1 H, d, *J* = 8.4 Hz), 8.64 (1 H, d, *J* = 7.0 Hz), 10.80 (1 H, s), 10.87 (1 H, s); R_f = 0.90 (system A). Anal. ($C_{46}H_{43}N_5O_6$) C, H, N.

Synthesis of Tripeptide Amides: Boc-D-Pro-Gln-Gln-NH$_2$ (2c). The benzyl ester **2b** (2.5 g, 4.45 mmol) was dissolved in a solution of ammonia in MeOH (24%, 50 mL). The solution was left standing overnight at room temperature in a sealed tube. After evaporation, the crude product was purified on a column of Toyopearl HW-40 (Tosoh, Japan) eluting with water. Lyophilization of the fractions which contained the desired product gave **2c** (1.68 g, 80.2%) as an amorphous solid: mp 230–231 °C dec; $[\alpha]^{25}_D$ = +22.6° (*c* = 1.03, DMF); IR (Nujol) 3300, 1690 (sh), 1660 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.6–2.3 (12 H, m), 3.3–3.4 (2 H, m), 4.0–4.3 (3 H, m), 6.7 (2 H, s), 7.05 (2 H, s), 7.25 (2 H, s), 7.6–8.4 (2 H, m); R_f = 0.35 (system G). Anal. ($C_{20}H_{34}N_6O_7 \cdot H_2O$) C, H, N.

The following tripeptide amides, **3c**, **5c**, **6c**, **7c**, and **8b**, were prepared similarly as **2c** from the corresponding methyl or benzyl esters:

Z-Gln-Gln-D-Trp-NH$_2$ (3c). Prepared from **41**: 67.2% yield; mp 267 °C dec; $[\alpha]^{25}_D$ = −4.70° (*c* = 1.03, DMF); IR (Nujol) 3440, 3310, 1670, 1630, 1550 (sh), 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.6–2.3 (8 H, m), 2.8–3.3 (2 H, m), 3.8–4.7 (3 H, m), 5.08 (2 H, s), 6.75 (2 H, br s), 7.0–7.8 (15 H, m), 8.0–8.2 (2 H, m), 10.72 (1 H, s); R_f = 0.55 (system G). Anal. ($C_{29}H_{35}N_7O_7 \cdot 0.9H_2O$) C, H, N.

Boc-Gln-D-Trp-Phe-NH$_2$ (5c). Prepared from **4a**: 83.4% yield; mp 210–212 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = −12.24° (*c* = 0.92, DMF); IR (Nujol) 3420, 3300, 3220, 1690, 1640, 1540, 1525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.33 (9 H, s), 1.4–2.1 (4 H, m), 2.6–3.2 (4 H, m), 3.7–4.1 (1 H, m), 4.3–4.7 (2 H, m), 6.6–7.6 (10 H, m), 7.22 (5 H, s), 7.7–8.0 (1 H, m), 8.1–8.4 (1 H, m), 10.73 (1 H, s); R_f = 0.58 (system H). Anal. ($C_{30}H_{38}N_6O_6$) C, H, N.

Z-D-Trp-Phe-D-Trp-NH$_2$ (6c). Prepared from **42**: 80.2% yield; mp 256–258 °C dec (amorphous solid); $[\alpha]^{25}_D$ = +35.85° (*c* = 0.99, DMF); IR (Nujol) 3450, 3310, 1690, 1645, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.6–3.3 (6 H, m), 4.1–4.8 (3 H, m), 4.90 (2 H, s), 6.9–7.8 (23 H, m), 8.1–8.4 (2 H, m), 10.75 (2 H, br s); R_f = 0.44 (system C). Anal. ($C_{39}H_{38}N_6O_5$) C, H, N.

Z-Phe-D-Trp-D-Trp-NH$_2$ (7c). Prepared from **43**: 83.1% yield; mp 240 °C dec (EtOH–H_2O); $[\alpha]^{25}_D$ = +38.3° (*c* = 1.01, DMF); IR (Nujol) 3420, 3300, 1708 (sh), 1680, 1642, 1540 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.5–3.3 (6 H, m), 4.1–4.75 (3 H, m), 4.92 (2 H, m), 6.85–7.5 (21 H, m), 7.5–7.7 (2 H, m), 8.0 (1 H, d, *J* = 8 Hz), 8.36 (1 H, d, *J* = 8 Hz), 10.70 (2 H, s); R_f = 0.55 (system C). Anal. ($C_{39}H_{38}N_6O_5$) C, H, N.

Boc-D-Trp-D-Trp-Phe-NH$_2$ (8b). Prepared from **8c**: 78.1% yield; mp 135–143 °C dec (EtOH–H_2O); IR (Nujol) 3330, 1680, 1665, 1625, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.26 (9 H, s), 2.6–3.1 (6 H, m), 3.9–4.25 (1 H, m), 4.25–4.7 (2 H, m), 6.5–6.7 (1 H, m), 6.7–7.6 (12 H, m), 7.14 (5 H, s), 7.84 (1 H, d, *J* = 8 Hz), 8.20 (1 H, d, *J* = 8 Hz), 10.63 (2 H, s); R_f = 0.20 (system D). Anal. ($C_{36}H_{40}N_6O_5 \cdot 2H_2O$) C, H, N.

Synthesis of Unprotected Tripeptides: H-D-Pro-Gln-Gln-OH (2a). A solution of **2d** (0.20 g, 0.34 mmol) in a mixed solvent of DMF (20 mL) and AcOH (2 mL) was hydrogenated at atmospheric pressure with 10% Pd(OH)$_2$ (20 mg) on charcoal. After removal of the catalyst, the solvent was evaporated under reduced pressure. The residue was triturated with Et_2O. The powder obtained was washed succesively with EtOH and Et_2O to give **2a** (87 mg, 69.6%) as an amorphous solid: mp 225–227 °C dec; $[\alpha]^{25}_D$ = −6.66° (*c* = 0.54, 10% AcOH); IR (Nujol) 3300, 1660 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.6–2.2 (12 H, m), 2.9–3.1 (2 H, m), 3.8 (1 H, m), 4.0 (1 H, m), 4.2 (1 H, m), 6.74 (2 H, s), 7.33 (2 H, s), 8.00 (1 H, d, *J* = 7.2 Hz), 8.42 (1 H, d, *J* = 8 Hz); R_f = 0.21 (system I). Anal. ($C_{15}H_{25}N_5O_6 \cdot 0.5H_2O \cdot 0.33EtOH$) C, H, N.

<Glu-Gln-D-Trp-OH (3a). A solution of **3b** (0.87 g, 1.5 mmol) in a mixed solvent of DMF (36 mL) and AcOH (12 mL) was hydrogenated at atmospheric pressure with 10% Pd (0.17 g) on charcoal. After removal of the catalyst, the solvent was evaporated under reduced pressure. The residue was dissolved in 10% aqueous AcOH (25 mL), and the solution was heated at 60 °C for 6 h. After cooling of the reaction mixture, the precipitates formed were filtered to give **3a** (0.22 g, 33.9%): mp 260–261 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = −2.95° (*c* = 0.57, DMF); IR (Nujol) 3480, 3280, 1720, 1660, 1640, 1562 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.6–2.3 (6 H, m), 3.0–3.4 (4 H, m), 3.9–4.6 (3 H, m), 6.7 (1 H, br), 6.9–7.6 (6 H, m), 7.83 (1 H, s), 7.9–8.2 (2 H, m), 10.8 (1 H, s), 12.6 (1 H, br s); R_f = 0.25 (system G). Anal. ($C_{21}H_{25}N_5O_6$) C, H, N.

<Glu-D-Trp-Phe-OH (5a). A mixture of **5b** (0.20 g, 0.35 mmol), TFA (3.5 mL), 1,2-ethanedithiol (0.35 mL), and dimethyl sulfide (3.5 mL) was stirred at room temperature for 3 h. After concentration, 4 N hydrochloric acid (0.4 mL) in dioxane was added to the residue and the mixture was concentrated again. The residue was triturated with Et_2O to form a powder, which was filtered and dried. This product was dissolved in a mixed solvent of EtOH (5 mL) and water (5 mL), and the solution was heated under reflux for 2 h. The reaction mixture was evaporated and diluted with water. The precipitates formed were collected by filtration and dried to give **5a** (75 mg, 64.1%): mp 168–170 °C (EtOH–H_2O); $[\alpha]^{25}_D$ = −0.37° (*c* = 0.53, DMF); IR (Nujol) 3320, 1735, 1650, 1550 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.35–1.5 (1 H, m), 1.9–2.1 (3 H, m), 2.6–2.9 (3 H, m), 3.0–3.2 (1 H, m), 3.95 (1 H, m), 4.5 (1 H, m), 4.65 (1 H, m), 6.9–7.1 (3 H, m), 7.2–7.4 (6 H, m), 7.54 (1 H, d, *J* = 7 Hz), 7.71 (1 H, s), 8.03 (1 H, d, *J* = 8 Hz), 8.57 (1 H, d, *J* = 8 Hz), 10.7 (1 H, s), 12.9 (1 H, br s); R_f = 0.67 (system G). Anal. ($C_{25}H_{26}N_4O_5 \cdot 0.5H_2O$) C, H, N.

H-D-Trp-Phe-D-Trp-OH (6a). A solution of **6b** (0.70 g, 1.0 mmol) in a mixed solvent of MeOH–water–AcOH (16:1:3, 60 mL) was hydrogenated at atmospheric pressure with 10% Pd (0.14 g) on charcoal. After removal of the catalyst, the solvent was evaporated under reduced pressure. The residue obtained was crystallized from water to give **6a** (0.32 g, 57.1%): mp 222 °C dec (EtOH–H_2O); $[\alpha]^{25}_D$ = −13.57° (*c* = 0.52, AcOH); IR (Nujol) 3430, 3270, 1690, 1640, 1570 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.7–3.2 (6 H, m), 3.5–3.7 (1 H, m), 4.3–4.9 (2 H, m), 5.2 (3 H, br s), 6.9–7.7 (15 H, m), 8.3–8.5 (2 H, m), 10.8 (2 H, s); R_f = 0.5 (system G). Anal. ($C_{31}H_{31}N_5O_4 \cdot 0.5H_2O$) C, H, N.

H-Phe-D-Trp-D-Trp-OH (7a). Prepared similarly as **6a** from **7b**: 53.6% yield; mp 188 °C dec (EtOH–H_2O); $[\alpha]^{25}_D$ = +6.89° (*c* = 0.53, AcOH); IR (Nujol) 3430, 3250, 1690 (sh), 1645, 1560, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.6–3.3 (6 H, m), 3.4–3.6 (1 H, m), 4.3–4.7 (2 H, m), 4.9 (3 H, br s), 6.9–7.7 (15 H, m), 8.12 (1 H, d, *J* = 8 Hz), 8.30 (1 H, d, *J* = 8 Hz), 10.75 (2 H, s); R_f = 0.49 (system G). Anal. ($C_{31}H_{31}N_5O_4 \cdot 1.5H_2O$) C, H, N.

HCl·H-D-Trp-D-Trp-Phe-NH$_2$ (8a). A mixture of **8b** (1.0 g, 1.57 mmol), TFA (16 mL), 1,2-ethanedithiol (1.6 mL), and dimethyl sulfide (16 mL) was stirred at room temperature for 3 h. After concentration, 4 N hydrochloric acid (1.6 mL) in dioxane was added to the residue, and the mixture was concentrated again. The residue was triturated with Et_2O to form a crude powder, which was dissolved in EtOH. To the solution was added a mixed solvent of EtOAc and Et_2O to form precipitates again. These precipitates were collected and recrystallized from a mixed solvent of $CHCl_3$ and MeOH to give **8a** (0.50 g, 75.6%): mp 250–253 °C dec; $[\alpha]^{25}_D$ = +0.79° (*c* = 0.75, DMF); IR (Nujol) 3450, 3300, 1675, 1645, 1545 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.6–3.5 (6 H, m), 3.8–4.1 (1 H, m), 4.4–4.8 (2 H, m), 6.8–7.9 (12 H, m), 7.23 (5 H, s), 8.10 (3 H, br s), 8.50 (1 H, d, *J* = 9 Hz), 8.89 (1 H, d, *J* = 8 Hz), 10.81 (1 H, s), 10.97 (1 H, s); R_f = 0.75 (system G). Anal. ($C_{31}H_{33}ClN_6O_3 \cdot 3H_2O$) C, H, N; Cl: calcd, 6.19, found, 5.76.

Boc-Gln-D-Trp(CHO)-Phe-OH (4b). A solution of **4a** (0.70 g, 1.0 mmol) in a mixed solvent of DMF (18 mL) and AcOH (2 mL) was hydrogenated at atmospheric pressure with 10% Pd (0.14 g) on charcoal. After filtration of the catalyst, the solvent was evaporated under reduced pressure. The residue obtained was diluted with water to form precipitates, which were collected by filtration to give **4b** (0.40 g, 65.6%): mp 187 °C dec (EtOH–H_2O); $[\alpha]^{25}_D$ = +6.45° (*c* = 1.05, DMF); IR (Nujol) 3440 (sh), 3320, 1710, 1690, 1660, 1640, 1545 (sh), 1525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30 (9 H, s), 1.6–2.1 (4 H, m), 2.6–3.2 (4 H, m), 3.7–4.0 (1 H, m), 4.35–4.8 (2 H, m), 6.7 (2 H, br s), 7.22 (5 H, s), 7.0–7.7 (5 H, m), 7.8–8.25 (2 H, m), 8.47 (1 H, d, *J* = 8 Hz), 9.2 (1 H, br s); R_f =

0.31 (system F). Anal. ($C_{31}H_{37}N_5O_8$) C, H, N.

HCl·H-Gln-D-Trp(CHO)-Phe-OBzl (4c). A solution of **4a** (0.5 g) in 4 N hydrochloric acid (10 mL) in dioxane was stirred under ice-cooling for 0.5 h and then at room temperature for 2 h. The reaction mixture was concentrated, and the residue was triturated with Et_2O. The resulting precipitates were filtered and dried to give **4c** (0.40 g, 99.0%) as an amorphous solid: $[\alpha]^{25}_D$ = +14.40° (c = 0.92, DMF); IR (Nujol) 3200 (br), 1735 (sh), 1710 (sh), 1690, 1675 (sh), 1660, 1605, 1545 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.5–2.2 (4 H, m), 2.6–3.3 (4 H, m), 3.6–4.0 (1 H, m), 4.4–5.0 (2 H, m), 5.14 (2 H, s), 6.90 (1 H, br s), 7.27 (5 H, s), 7.38 (5 H, s), 7.0–7.8 (5 H, m), 8.33 (4 H, br s), 8.7–9.2 (2 H, m), 9.3 (1 H, br s); R_f = 0.13 (system F). Anal. ($C_{33}H_{36}ClN_5O_6 \cdot 0.25HCl \cdot 1.3H_2O$) C, H, N, Cl.

Benz[*f*]isoquinoline Analogues as High-Affinity σ Ligands

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This paper describes the synthesis of some conformationally restricted 4-phenylpiperidine analogues and their affinities for the guinea pig cerebellum σ recognition site ([^{3}H]-DTG) and the rat striatum dopamine D_2 receptor ([^{3}H]-(−)-sulpiride) in order to develop potent selective σ ligands as tools in the investigation of this site in psychosis. It was found that both hexa- and octahydrobenz[*f*]isoquinolines with lipophilic N-substituents had high affinities for the σ site. Notably, *trans*-3-cyclohexyl-1,2,3,4,4a,5,6,10b-octahydrobenz[*f*]isoquinoline (**26**) had an affinity of 0.25 nM making it the highest affinity σ ligand reported to date. Moreover, it is at least 10 000-fold selective over the D_2 receptor and could prove to be a valuable tool in the study of σ sites. Other analogues such as 1*H*-indeno[2,1-*c*]pyridines and 1*H*-benzo[3,4]cyclohepta[1,2-*c*]pyridines also displayed high σ site affinity.

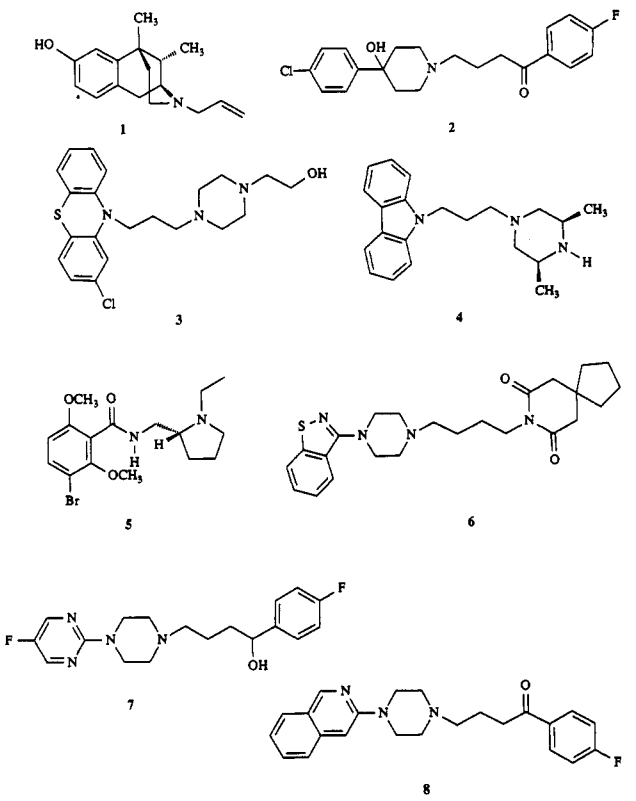
Introduction

The existence of σ receptors was first postulated by Martin et al.[1] as a result of a study of the actions of *N*-allylnormetazocine (SKF-10047, 1) (Chart I), which had been shown to elicit psychotomimetic effects in humans.[2] It was found that 1 caused autonomic stimulation and "canine delirium" in the chronic spinal dog leading to the suggestion that these effects involved a unique subtype of opiate receptor, which was termed σ. However, unlike conventional opiate receptors, behavioral effects appeared to be confined to the dextrorotatory (+) isomer and were not antagonized by opiate antagonists such as naloxone or naltrexone.[3-9] The origin of these effects is, however, unclear since 1 also interacts strongly with the NMDA ion-channel complex.[10,11]

Nevertheless, there is evidence that implicates the involvement of σ sites in psychosis. Firstly, some traditional and clinically useful neuroleptics such as haloperidol (**2**) and perphenazine (**3**), which act as dopamine D_2 receptor antagonists, also have a high affinity for the σ site.[12,13] Secondly, several new atypical antipsychotic agents, such as rimcazole (BW 234U, **4**),[14-18] remoxipride (**5**),[19-24] tiaspirone (BMY 13859, **6**),[25] BMY 14802 (**7**),[26] and cinuperone (HR 375, **8**),[27] may not act primarily at D_2 receptors but have a reasonable affinity for the σ site,[28-31] and furthermore appear to be devoid of the extrapyramidal side effects associated with D_2 antagonists. Thirdly, post mortem brain tissue studies have shown that schizophrenics contained a significantly lower number of σ sites than normal patients,[32] which seems to be due to haloperidol treatment,[33] and may be relevant to its antipsychotic action.

Although σ sites have been implicated in regulation of neurotransmitter release,[34] smooth muscle contraction,[34-36] intestinal alkaline secretion,[37-39] control of motor behavior,[40-42] and modulation of phosphoinositide turnover[43] (though the latter is probably due to muscarinic receptor blockade[44,45]), the poor receptor selectivity of the known σ ligands makes convincing functional correlations difficult to obtain.[46] Our aim, therefore, was to develop potent, selective σ ligands as tools in the investigation of the role of this recognition site in psychosis.

Chart I



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