

[Chem. Pharm. Bull.]
33(1) 377-382 (1985)

Synthesis and Analgesic Activity of Neo-kyotorphin Analogs¹⁾

KOUKI KITAGAWA,*^a NOBUYUKI KAWAI,^a SHINYA KIYAMA,^a
TADASHI AKITA,^a YOSHIAKI KISO,^b HIROSHI UEDA,^c
GE MING,^c and HIROSHI TAKAGI^c

*Faculty of Pharmaceutical Sciences, University of Tokushima,^a Shomachi,
Tokushima 770, Japan, Kyoto College of Pharmacy,^b Yamashina-ku,
Kyoto 607, Japan, and Faculty of Pharmaceutical Sciences,
Kyoto University,^c Sakyo-ku, Kyoto 606, Japan*

(Received May 25, 1984)

Seven analogs which contain D-amino acids or the sterically hindered amino acid Pro, and two short-chain analogs of neo-kyotorphin (**1**), an analgesic pentapeptide isolated from bovine brain, were synthesized by the conventional method and screened for analgesic activity. All the analogs exhibited significantly enhanced analgesic effect upon intracisternal administration in mice. In particular, [D-Ser²]- and [Pro²]-neo-kyotorphin exhibited almost 10 times more potent activity than neo-kyotorphin.

Keywords—peptide synthesis; neo-kyotorphin; analgesic peptide; D-amino acid replacement; analgesic activity; naloxone antagonism

Neo-kyotorphin, an analgesic pentapeptide, was isolated from bovine brain and its structure was determined to be H-Thr-Ser-Lys-Tyr-Arg-OH (**1**) by Takagi *et al.*^{2,3)} We have already reported the conventional synthesis of this pentapeptide, confirming its structure.^{4,5)} Neo-kyotorphin has an analgesic activity upon intracisternal administration in mice, but the mechanism of this effect is not clear.⁶⁾ Kyotorphin,^{7,8)} the C-terminal dipeptide of neo-kyotorphin, has an analgesic activity which is fully antagonized by naloxone pre-treatment.⁹⁾ In contrast, neo-kyotorphin was reported to be partially antagonized by naloxone.⁶⁾ These preliminary observations suggest that different analgesic mechanisms may be involved in the actions of these two structurally related peptides.

In the hope of obtaining more potent analogs of neo-kyotorphin, we designed various analogs which were expected to resist the action of degrading enzymes such as aminopeptidases and chymotrypsin-like peptidases. Namely, D-amino acids and/or the sterically hindered amino acid proline were introduced into the peptide chain of neo-kyotorphin. In this paper, we report the syntheses and analgesic activities of various neo-kyotorphin analogs listed in Table I.

Syntheses of Analogs

The analogs, **2**–**6**, were synthesized in almost the same manner as previously reported for the synthesis of neo-kyotorphin.⁴⁾ Namely, the protecting groups removable at the final deprotection by the use of the 1 M TFMSA-thioanisole-TFA system¹⁰⁾ were employed for Lys and Arg. Azide coupling at position 2 (Ser residue) was used to obtain the fully protected pentapeptides.

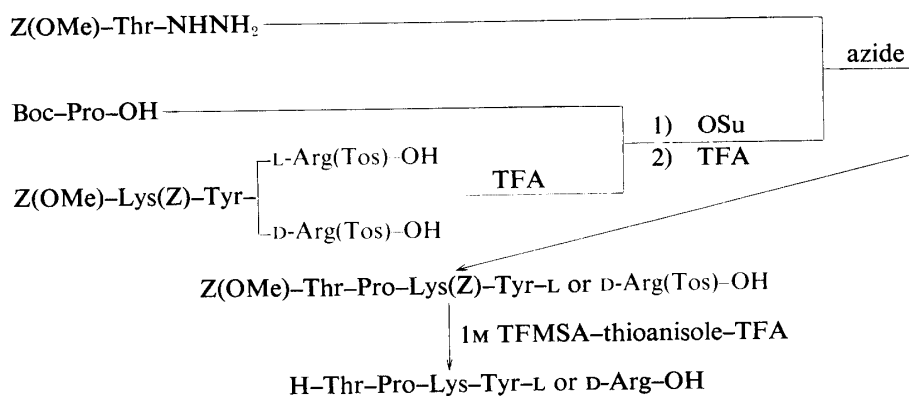
For the two Pro²-containing analogs, **7** and **8**, Boc-Pro-OH and Z(OMe)-Thr-NHNH₂ were introduced in a stepwise manner by the HOSu active ester and azide methods as shown in Fig. 1.

Analog **9** and **10**, short-chain peptides, were prepared from Z(OMe)-Lys(Z)-Tyr-Arg(Tos)-OH and Z(OMe)-Ser(Bzl)-Lys(Z)-Tyr-Arg(Tos)-OH, respectively.

TABLE I. Analgesic Effects Produced by Neo-kyotorphin Analogs

Compound			ED ₅₀ (nmol/mouse)	Antagonized by naloxone ^{a)}
1	Neo-kyotorphin (NK)	Thr-Ser-Lys-Tyr-Arg	32.3	—
2	[D-Arg ⁵]-NK	D	8.8	+
3	[D-Lys ³]-NK	D	15.5	+
4	[D-Ser ²]-NK	D	2.7	+
5	[D-Lys ³ , D-Arg ⁵]-NK	D D	7.2	+
6	[D-Ser ² , D-Arg ⁵]-NK	D D	11.6	+
7	[Pro ²]-NK	Pro	3.8	+
8	[Pro ² , D-Arg ⁵]-NK	Pro D	5.1	—
9	NK ₃₋₅	Lys-Tyr-Arg	9.6	+
10	NK ₂₋₅	Ser-Lys-Tyr-Arg	19.3	+
	Kyotorphin	Tyr-Arg	42.4	+

a) 0.5 mg/kg s.c.

Fig. 1. Synthetic Scheme for [Pro²]-, [Pro², D-Arg⁵]-neo-kyotorphin

All the protected peptides were deprotected with the 1 M TFMSA-thioanisole in TFA system in the presence of *o*-cresol to suppress the possible O-sulfonylation.¹¹⁾ The deprotected peptides were converted to the acetates and purified on Sephadex G-15 followed by partition chromatography on Sephadex G-25.¹²⁾ In the partition chromatography, not all the deprotected peptides were eluted by the upper phase of the solvent system, so the lower phase of the same solvent system was used after 500 ml of the upper phase had been passed. Each fraction of lower phase was checked by thin layer chromatography (TLC) and fractions giving a single ninhydrin-positive spot were pooled. After removal of solvents by evaporation and repeated lyophilization, a homogeneous peptide was obtained as a powder. The homogeneity of each peptide was assessed by TLC, amino acid analysis of an acid hydrolysate and elemental analysis. The physico-chemical data for deprotected peptides are listed in Tables III and IV.

Analgesic Activity of Synthetic Analogs

The analgesic effects of synthetic analogs were examined by the tail-pinch analgesic test according to Ueda *et al.*¹³⁾ The peptides were dissolved in distilled water (10 μ l) and administered with a J-shaped needle into the cisterna magna of unanesthetized mice. Each assay was done with 7–14 mice. The ED₅₀ values of the analogs are listed in Table I. All the investigated neo-kyotorphin analogs, including short-chain peptides, produced dose-dependent analgesia and exhibited significantly enhanced activity compared to neo-

kyotorphin.

As regards the mono-substituted analogs, **2** and **3**, having D-Arg at position 5 and D-Lys at position 3, respectively, exhibited only 2—4 times more potent activity than neo-kyotorphin. However, analogs **4** and **7**, having D-Ser or Pro at position 2, showed almost 10 times more potent analgesic activity. Compared to the L-Arg⁵-containing analogs, an enhanced effect of the corresponding D-Arg⁵-containing analogs was observed only in **2** and **5**. These results suggest that substitution at position 2 was fairly effective in stabilizing the molecule towards degrading enzymes, especially aminopeptidases.

As regards the short-chain peptides, analog **9**, lacking the N-terminal Thr-Ser sequence, exhibited potent activity compared to neo-kyotorphin and kyotorphin.

Although the analgesic effect induced by neo-kyotorphin was not antagonized by naloxone treatment (0.5 mg/kg mice), the analgesic effects produced by the investigated analogs were completely antagonized by naloxone except for that of analog **8**. The short-chain peptides, whose action was antagonized by naloxone, might possess a kyotorphin-like analgesic mechanism.

Moreover, preliminary experiments indicated that the analogs did not bind to the opiate receptors. The nature of the analgesic mechanism of these analogs is not clear, but the present results suggest that different mechanisms may be involved.

TABLE II. Physical Data for Protected Peptides

	Yield (%)	mp (°C)	[α] _D ²⁰ (°)	Formula	Analysis (%)		
					Calcd	Found	
					C	H	N
Dipeptide							
Z(OMe)-D-Lys(Z)-Tyr-OEt	56	126—129	+1.0	C ₃₄ H ₄₁ N ₃ O ₉	64.24	6.50	6.61
					(64.34)	6.83	6.81)
Z(OMe)-D-Lys(Z)-Tyr-NHNH ₂	85	214—216	+3.0 ^{a)}	C ₃₂ H ₃₉ N ₅ O ₈	61.82	6.32	11.27
					(61.62)	6.31	10.97)
Z(OMe)-Thr-D-Ser-OMe	56	129—130	-3.0	C ₁₇ H ₂₄ N ₂ O ₈	53.12	6.29	7.29
					(53.03)	6.39	7.19)
Z(OMe)-Thr-D-Ser-NHNH ₂	82	204—206	-1.0 ^{a)}	C ₁₆ H ₂₄ N ₄ O ₇	49.99	6.29	14.58
					(49.42)	6.56	15.01)
Tripeptide: Z(OMe)-X-Tyr-Y-OH							
X=L-Lys(Z), Y=D-Arg(Tos)	70	133—135	-28.0	C ₄₅ H ₅₅ N ₇ O ₁₂ S·H ₂ O	57.18	6.19	10.37
					(57.03)	5.80	10.03)
X=D-Lys(Z), Y=L-Arg(Tos)	89	104—106	+1.0	C ₄₅ H ₅₅ N ₇ O ₁₂ S	58.87	6.04	10.68
					(58.80)	6.30	10.24)
X=D-Lys(Z), Y=D-Arg(Tos)	80	135—137	+4.0	C ₄₅ H ₅₅ N ₇ O ₁₂ S	58.87	6.04	10.68
					(58.40)	6.11	10.10)
Pentapeptide: Z(OMe)-Thr-X-Y-Tyr-Z-OH							
X=L-Ser, Y=L-Lys(Z), Z=D-Arg(Tos)	65	115—117	-14.0	C ₅₂ H ₆₇ N ₉ O ₁₆ S·H ₂ O	55.55	6.19	11.21
					(55.54)	6.29	11.07)
X=L-Ser, Y=D-Lys(Z), Z=L-Arg(Tos)	69	104—106	-13.0	C ₅₂ H ₆₇ N ₉ O ₁₆ S·H ₂ O	55.55	6.19	11.21
					(55.46)	6.02	10.90)
X=D-Ser, Y=L-Lys(Z), Z=L-Arg(Tos)	49	133—135	-3.0	C ₅₂ H ₆₇ N ₉ O ₁₆ S·3/2H ₂ O	55.11	6.22	11.12
					(55.03)	6.19	10.95)
X=L-Ser, Y=D-Lys(Z), Z=D-Arg(Tos)	49	105—107	-8.0	C ₅₂ H ₆₇ N ₉ O ₁₆ S·H ₂ O	55.55	6.19	11.21
					(55.84)	6.46	10.63)
X=D-Ser, Y=L-Lys(Z), Z=D-Arg(Tos)	72	117—119	-9.0	C ₄₂ H ₆₇ N ₉ O ₁₆ ·H ₂ O	55.55	6.19	11.21
					(55.50)	6.09	10.78)

a) (c=1, DMF); others are (c=1, MeOH).

TABLE III. Physical Data for Deprotected Peptides

Compound	Yield (%)	$[\alpha]_D^{20}$ (°)	Formula	Analysis (%)		
				Calcd (Found)		
				C	H	N
2	56	-21.0 (<i>c</i> = 1.1, H ₂ O)	C ₂₈ H ₄₇ N ₉ O ₉ · 2AcOH · 4 ¹ / ₂ H ₂ O	44.95 (44.90)	7.55 7.62	14.75 14.75)
3	43	-6.5 (<i>c</i> = 1.2, H ₂ O)	C ₂₈ H ₄₇ N ₉ O ₉ · 2AcOH · 3H ₂ O	46.42 (46.25)	7.43 7.25	15.23 14.95)
4	91	-0.6 (<i>c</i> = 1.7, H ₂ O)	C ₂₈ H ₄₇ N ₉ O ₉ · 2AcOH · 4H ₂ O	45.43 (45.44)	7.51 7.23	14.90 14.90)
5	64	+9.4 (<i>c</i> = 1.9, H ₂ O)	C ₂₈ H ₄₇ N ₉ O ₉ · 2AcOH · H ₂ O	48.53 (48.67)	7.26 7.43	15.92 15.88)
6	84	-7.7 (<i>c</i> = 1.9, H ₂ O)	C ₂₈ H ₄₇ N ₉ O ₉ · 2AcOH · 5H ₂ O	44.99 (44.23)	7.58 7.55	14.59 14.77)
7	66	-58.7 (<i>c</i> = 1.2, H ₂ O)	C ₃₀ H ₄₉ N ₉ O ₈ · 2AcOH · 3H ₂ O	48.73 (48.73)	7.57 7.24	15.04 14.91)
8	59	-67.4 (<i>c</i> = 1.0, H ₂ O)	C ₃₀ H ₄₉ N ₉ O ₈ · 2AcOH · H ₂ O	50.92 (50.72)	7.42 7.46	15.72 15.68)
9	50	-21.8 (<i>c</i> = 0.6, H ₂ O)	C ₂₁ H ₃₅ N ₇ O ₅ · 2AcOH · 2H ₂ O	48.30 (48.42)	7.62 7.81	15.77 16.23)
10	42	+13.3 (<i>c</i> = 0.8, H ₂ O)	C ₂₄ H ₄₀ N ₈ O ₇ · 2AcOH · 4H ₂ O	45.15 (45.26)	7.58 6.98	15.03 15.06)

Experimental

The melting points are uncorrected. Rotations were determined with a Union PM-201 polarimeter. Acid hydrolyses were carried out in 6N HCl and amino acid analyses were performed on a Hitachi 835 amino acid analyzer. TLC was carried out on silica gel (pre-coated Silica gel 60 F₂₅₄, Merck). Solvent systems used were $Rf_1 = \text{CHCl}_3 : \text{MeOH} : \text{H}_2\text{O}$ (8 : 3 : 1), $Rf_2 = n\text{-BuOH} : \text{AcOH} : \text{pyridine} : \text{H}_2\text{O}$ (4 : 1 : 1 : 2).

Syntheses of Analogs, 2—6—The synthesis of analog 2 is described in this section as an example. The protected di-, tri-, and pentapeptides used for the syntheses of 3—6 were prepared in essentially the same manner. The physical data are listed in Table II.

Z(OMe)-Lys(Z)-Tyr-D-Arg(Tos)-OH: The azide (prepared from 3.10 g of Z(OMe)-Lys(Z)-Tyr-NHNH₂ in a usual manner) was added to a solution of H-D-Arg(Tos)-OH (2.46 g) in *N,N*-dimethylformamide (DMF) (20 ml) containing Et₃N (1.05 ml). After being stirred for 48 h at 4 °C, the solution was concentrated and the residue was dissolved in AcOEt and 10% citric acid. The organic phase was washed with 10% citric acid, saturated NaCl and H₂O, then dried over Na₂SO₄, and AcOEt was evaporated off. The residue was purified by silica gel column chromatography and recrystallized twice from MeOH-ether.

Z(OMe)-Thr-Ser-Lys(Z)-Tyr-D-Arg(Tos)-OH: The above prepared tripeptide (1.38 g) was treated with TFA (3.0 ml)-anisole (0.7 ml). The dried TFA salt was dissolved in DMF (15 ml) containing Et₃N (0.21 ml). To this ice-chilled solution, the azide (prepared from 1.15 g of Z(OMe)-Thr-Ser-NHNH₂ in a usual manner) was added. The reaction mixture was stirred for 24 h at 4 °C, then DMF was evaporated off. The residue was dissolved in AcOEt and 10% citric acid and the organic phase was washed with 10% citric acid and H₂O, then dried over Na₂SO₄, and AcOEt was evaporated off. Trituration with ether gave the solid product, which was recrystallized twice from MeOH-ether.

H-Thr-Ser-Lys-Tyr-D-Arg-OH, 2: The above prepared pentapeptide (550 mg) was treated with TFA (12 ml) in the presence of thioanisole (3.1 ml, 50 eq) and *o*-cresol (2.7 ml, 50 eq) in an ice-bath for 30 min, then TFMSA (1.2 ml) was added. The reaction mixture was further stirred for 1.5 h with ice-cooling, then TFA was evaporated off at 35 °C. After being washed with *n*-hexane, the oily residue was dried over KOH pellets, then dissolved in H₂O (50 ml) and treated with Amberlite IR-400 (5 g, acetate form) for 30 min. The resin was removed by filtration and washed with a small amount of 10% AcOH. This solution was washed with ether and the pH was adjusted to 10 with 5% NH₄OH. After 30 min, the pH was adjusted to 7 with 3% AcOH and the solution was lyophilized. The lyophilized material was dissolved in 3% AcOH (3 ml) and applied to a column of Sephadex G-15 (3.0 × 105 cm), which was eluted with 3% AcOH. The ultraviolet (UV) absorption at 275 nm was determined in each fraction (10 ml), and the crude product (295 mg) was obtained from first main fraction after lyophilization. This material was dissolved in the upper phase of *n*-BuOH : AcOH : H₂O (4 : 1 : 5, 5 ml) and applied to a column of Sephadex G-25 (2.4 × 90 cm), which was equilibrated with the same solvent system. After 500 ml of the solvent had been passed, the lower phase of the same

TABLE IV. Amino Acid Analyses of Deprotected Peptides

Compound	Thr	Ser	Lys	Tyr	Arg	Pro
2	0.97	0.95	1.02	1.00	0.85	
3	1.01	0.98	0.96	1.00	0.90	
4	1.04	0.99	1.00	1.00	1.03	
5	1.03	0.92	1.16	1.00	0.99	
6	1.00	0.95	1.14	1.00	0.96	
7	0.91		0.94	1.00	0.99	0.95
8	0.91		1.00	1.00	1.02	0.95
9			0.83	1.00	0.85	
10		0.95	1.09	1.00	0.95	

solvent system was employed. Each fraction (8 ml) was checked by TLC using ninhydrin as a staining reagent. From the fractions showing a single ninhydrin positive spot, a homogeneous material was obtained after repeated lyophilization. Physical data are listed in Tables III and IV.

Syntheses of Analogs, 7 and 8—Boc-Pro-Lys(Z)-Tyr-Arg(Tos)-OH: Z(OMe)-Lys(Z)-Tyr-Arg(Tos)-OH (2.75 g) was treated with TFA (6 ml)-anisole (1.5 ml) in a usual manner. The dried TFA salt was dissolved in DMF (30 ml) containing Et₃N (1.26 ml). Boc-Pro-OSu (0.94 g) was added to the above solution and the reaction mixture was stirred for 6 h. DMF was evaporated off, and the residue was dissolved in AcOEt and washed with 10% citric acid, saturated NaCl and H₂O, then dried over Na₂SO₄. AcOEt was evaporated off and the residue was purified by silica gel column chromatography and recrystallized from MeOH-ether. Yield 2.1 g (74%), mp 150–153 °C, $[\alpha]_D^{20}$ –27.0° (*c*=1, MeOH), *R*_f 0.18. *Anal.* Calcd for C₄₆H₆₂N₈O₁₂S·3/2H₂O: C, 56.48; H, 6.70; N, 11.45. Found: C, 56.69; H, 6.63; N, 11.29.

Z(OMe)-Thr-Pro-Lys(Z)-Tyr-Arg(Tos)-OH: Boc-Pro-Lys(Z)-Tyr-Arg(Tos)-OH (1.43 g) was treated with TFA (3 ml)-anisole (0.8 ml) and the dried TFA salt was dissolved in DMF (15 ml) containing Et₃N (0.21 ml). The azide (prepared from 0.89 g of Z(OMe)-Thr-NHNH₂ in a usual manner) was then added. After 24 h at 4 °C, DMF was evaporated off and the residue was dissolved in AcOEt and washed with 10% citric acid, saturated NaCl and H₂O, then dried over Na₂SO₄. AcOEt was evaporated off and the residue was further purified by silica gel column chromatography and recrystallized from MeOH-ether. Yield 0.53 g (32%), mp 141–144 °C, $[\alpha]_D^{20}$ –36.0° (*c*=1, MeOH), *R*_f 0.19. *Anal.* Calcd for C₅₄H₆₉N₉O₁₅S·H₂O: C, 57.18; H, 6.30; N, 11.13. Found: C, 56.97; H, 6.26; N, 10.92.

H-Thr-Pro-Lys-Tyr-Arg-OH, **7**: The fully protected pentapeptide (390 mg) was treated with TFA (5 ml) in the presence of thioanisole (4.3 ml, 100 eq) and *o*-cresol (3.8 ml, 100 eq) with ice-cooling for 30 min, then TFMSA (1 ml) was added. The reaction mixture was treated in the same manner as described for analog **2**, then purified on Sephadex G-15 followed by partition chromatography on Sephadex G-25.

Boc-Pro-Lys(Z)-Tyr-D-Arg(Tos)-OH: The title compound was prepared in the same manner as described for Boc-Pro-Lys(Z)-Tyr-Arg(Tos)-OH. Yield 79%, mp 119–122 °C, $[\alpha]_D^{20}$ –33.0° (*c*=1, MeOH), *R*_f 0.20. *Anal.* Calcd for C₄₆H₆₂N₈O₁₂S·H₂O: C, 57.01; H, 6.65; N, 11.56. Found: C, 57.04; H, 6.63; N, 11.31.

Z(OMe)-Thr-Pro-Lys(Z)-Tyr-D-Arg(Tos)-OH: The title compound was prepared in the same manner as described for Z(OMe)-Thr-Pro-Lys(Z)-Tyr-Arg(Tos)-OH. Yield 59%, mp 114–117 °C, $[\alpha]_D^{20}$ –42.0° (*c*=1, MeOH), *R*_f 0.21. *Anal.* Calcd for C₅₄H₆₉N₉O₁₅S·3/2H₂O: C, 56.73; H, 6.34; N, 11.03. Found: C, 56.98; H, 6.39; N, 10.99.

H-Thr-Pro-Lys-Tyr-D-Arg-OH, **8**: The fully protected pentapeptide (560 mg) was treated with 1 M TFMSA-thioanisole-*o*-cresol-TFA system in the same manner as described previously and purified on Sephadex G-15 followed by partition chromatography on Sephadex G-25. Physical data for analogs **7** and **8** are listed in Tables III and IV.

Syntheses of Short-Chain Peptides, 9 and 10—H-Lys-Tyr-Arg-OH, **9**: Z(OMe)-Lys(Z)-Tyr-Arg(Tos)-OH (460 mg) was treated with 1 M TFMSA-thioanisole-*o*-cresol-TFA in the same manner as described above and purified on Sephadex G-15 followed by partition chromatography on Sephadex G-25.

Z(OMe)-Ser(Bzl)-Lys(Z)-Tyr-Arg(Tos)-OH: Z(OMe)-Lys(Z)-Tyr-Arg(Tos)-OH (0.73 g) was treated with TFA (1.5 ml)-anisole (0.4 ml) and the dried TFA salt was dissolved in DMF (20 ml) containing Et₃N (0.34 ml). To this solution, Z(OMe)-Ser(Bzl)-ONb (0.41 g) was added and the reaction mixture was stirred for 5 h. DMF was evaporated off, and the residue was dissolved in AcOEt, washed with 10% citric acid, saturated NaCl and H₂O, and then dried over Na₂SO₄. AcOEt was evaporated off and the residue was triturated with ether. The formed precipitate was collected and recrystallized from AcOEt-ether. Yield 0.51 g (58%), mp 95–97 °C, $[\alpha]_D^{20}$ –9.0° (*c*=1, MeOH), *R*_f 0.20. *Anal.* Calcd for C₅₅H₆₆N₈O₁₄S·H₂O: C, 59.34; H, 6.16; N, 10.07. Found: C, 58.89; H, 5.93; N, 9.98.

H-Ser-Lys-Tyr-Arg-OH, **10**: The above protected tetrapeptide (380 mg) was treated with 1 M TFMSA-

thioanisole-*o*-cresol-TFA in the same manner as described above and purified on Sephadex G-15 followed by partition chromatography on Sephadex G-25. Physical data for analogs **9** and **10** are listed in Tables III and IV.

Acknowledgement We wish to express our gratitude to Professor Haruaki Yajima of Kyoto University for his encouragement during the course of this investigation.

References and Notes

- 1) Amino acids and their derivatives with no prefix were of L-configuration. The abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *J. Biol. Chem.*, **247**, 977 (1972). Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Tos = *p*-toluenesulfonyl, Bzl = benzyl, OSu = *N*-hydroxysuccinimidyl, ONb = *N*-hydroxy-5-norbornene-2,3-dicarboximidyl, DCC = *N,N'*-dicyclohexylcarbodiimide, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, Et₃N = triethylamine, DMF = dimethylformamide.
- 2) H. Takagi, H. Shiomi, K. Fukui, K. Hayashi, Y. Kiso, and K. Kitagawa, *Life Sci.*, **31**, 1733 (1982).
- 3) K. Fukui, H. Shiomi, H. Takagi, K. Hayashi, Y. Kiso, and K. Kitagawa, *Neuropharmacol.*, **22**, 191 (1983).
- 4) K. Kitagawa, N. Kawai, Y. Kiso, T. Akita, K. Fukui, H. Amano, and H. Takagi, *Chem. Pharm. Bull.*, **31**, 2349 (1983).
- 5) Y. Kiso, K. Kitagawa, N. Kawai, T. Akita, H. Takagi, H. Amano, and K. Fukui, *FEBS Lett.*, **155**, 281 (1983).
- 6) K. Fukui, H. Amano, Y. Kiso, K. Kitagawa, and H. Takagi, *Pharmaceutical Research*, **1984**, 39.
- 7) H. Takagi, H. Shiomi, H. Ueda, and H. Amano, *Nature* (London), **282**, 410 (1979).
- 8) H. Shiomi, H. Ueda, and H. Takagi, *Neuropharmacol.*, **20**, 633 (1981).
- 9) H. Takagi, H. Shiomi, H. Ueda, and H. Amano, *Eur. J. Pharmacol.*, **55**, 109 (1979).
- 10) H. Yajima and N. Fujii, *J. Am. Chem. Soc.*, **103**, 5867 (1981).
- 11) H. Yajima, M. Takeyama, J. Kanaki, O. Nishimura, and M. Fujino, *Chem. Pharm. Bull.*, **26**, 3752 (1978).
- 12) D. Yamashiro, *Nature* (London), **201**, 76 (1964).
- 13) H. Ueda, H. Amano, H. Shiomi, and H. Takagi, *Eur. J. Pharmacol.*, **56**, 265 (1979).