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Studies on Analgesic Oligopeptides. IV.[1,2] Synthesis and Analgesic Activity of N-Terminal Shorter Analogs of [D-Arg^2]Dermorphin and Des-Tyr^1-Dermorphin Analogs

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Des-Tyr^1-dermorphin, des-Tyr^1-[D-Arg^2]dermorphin, and the N-terminal di-, tri-, tetra- and hexapeptide amides of [D-Arg^2]dermorphin were synthesized by a conventional solution method and their analgesic activities were assayed by means of the tail pressure test after subcutaneous administration (*s.c.*) to mice. The des-Tyr^1 analogs of both dermorphin and [D-Arg^2]dermorphin did not show analgesic activity even at a dose of up to 50 mg/kg, *s.c.* The N-terminal tetrapeptide amide, H–Tyr–D-Arg–Phe–Gly–NH_2, showed extremely potent activity, being 31 times more active than morphine on a molar basis, whereas the N-terminal tri- and dipeptide amides showed no activity even at a dose of up to 40 mg/kg, *s.c.*

Keywords——des-Tyr^1-dermorphin; des-Tyr^1-[D-Arg^2]dermorphin; [D-Arg^2]dermorphin; N-terminal shorter analog; peptide synthesis; analgesic activity; subcutaneous administration; tail pressure test

Dermorphin (H–Tyr–D-Ala–Phe–Gly–Tyr–Pro–Ser–NH_2), isolated from the skin of South American frogs, has been shown to have a potent analgesic activity.[3] Although a number of dermorphin-related peptides have been synthesized, only a few analogs substituted at position 2 with high potency have been reported.[4] Recently, we found that D-Arg^2 derivatives in the N-terminal tetrapeptide segment[1,5] and [D-Arg^2]dermorphin[1] show highly potent analgesic activity. In this paper, we describe the synthesis and analgesic activity after subcutaneous (*s.c.*) administration of des-Tyr^1-dermorphin, des-Tyr^1-[D-Arg^2]dermorphin

TABLE I. Analgesic Activity of Des-Tyr^1-Dermorphin and N-Terminal Shorter Analogs of [D-Arg^2]Dermorphin after Subcutaneous Administration in Mice

Analog No.	Peptide	ED_{50}[a] (mg/kg, *s.c.*)	Relative potency[b]
I	H–D-Ala–Phe–Gly–Tyr–Pro–Ser–NH_2	50<	—
II	H–D-Arg–Phe–Gly–Tyr–Pro–Ser–NH_2	50<	—
III	H–Tyr–D-Arg–Phe–Gly–Tyr–Pro–NH_2	2.7 (1.9—3.9)	6.5
IV	H–Tyr–D-Arg–Phe–Gly–Tyr–NH_2	7.2 (3.3—15.8)	2.1
V	H–Tyr–D-Arg–Phe–Gly–NH_2	0.4 (0.3—0.7)	31.0
VI	H–Tyr–D-Arg–Phe–NH_2	40<	—
VII	H–Tyr–D-Arg–NH_2	40<	—
	Morphine[c]	6.2 (4.1—9.4)	1.0
	Dermorphin[c]	4.6 (3.4—6.3)	3.3
	[D-Arg^2]Dermorphin[c]	3.5 (2.8—4.4)	5.1

a) 95% confidence limits are given in parentheses. *b*) ED_{50} value of each peptide compared with that of morphine on a molar basis. *c*) Data cited from ref. 1.

and five shorter analogs of [D-Arg^2]dermorphin truncated at the C-terminal (Table I).

Syntheses of the analogs were performed by the conventional solution method. For the syntheses of two des-Tyr^1-hexapeptides (I and II), Boc–D-Ala–Phe–Gly–OH (**1**) and Boc–D-Arg(Tos)–Phe–Gly–OH (**3**), both of which were obtained from their ethyl ester derivatives,[1] were coupled to H–Tyr–Pro–Ser–NH_2[6] by the use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide[7] in the presence of 1-hydroxybenztriazole[8] (WSCI–HOBt method) to give Boc–D-Ala–Phe–Gly–Tyr–Pro–Ser–NH_2 (**2**) and Boc–D-Arg(Tos)–Phe–Gly–Tyr–Pro–Ser–NH_2 (**4**), respectively. Treatment of **2** with 4 N hydrogen chloride in dioxane gave I and deprotection of **4** by treatment with a mixture of trifluoromethanesulfonic acid and thioanisole[9] gave II. For the syntheses of III and IV, Boc–Tyr–D-Arg(Tos)–Phe–Gly–OH[1] was coupled to H–Tyr–Pro–NH_2 and H–Tyr–NH_2 by means of the WSCI–HOBt method to give the corresponding hexa- (**5**) and penta- (**6**) peptide intermediates, followed by treatment with the deblocking reagent described above. Boc–Tyr–D-Arg(Tos)–Phe–Gly–OEt[1] was converted to its amide (**7**) by treatment with saturated ammonia in methanol and then deprotected as described above to give V. For the syntheses of VI, Boc–Tyr–D-Arg(Tos)–Phe–OMe (**9**) was built up in a stepwise manner, and converted to the corresponding amide (**10**), then this was deprotected as described above. In order to synthesize VII, Boc–D-Arg(Tos)–NH_2 (**11**) was prepared from Boc–D-Arg(Tos)–OH and ammonia by means of the mixed anhydride method.[10] Compound **11**, after removal of the Boc group by using 4 N HCl in dioxane, was coupled with Boc–Tyr–OH to give Boc–Tyr–D-Arg (Tos)–NH_2 (**12**). Deprotection of **12** with the deblocking reagent described above gave VII. All the analogs were purified by column chromatography on carboxymethyl cellulose and Toyopearl HW-40 or Sephadex G-10. These analogs were shown to be homogeneous on thin-layer chromatography and gave the expected amino acid values on amino acid analysis.

Analgesic activity of synthetic analogs following *s.c.* administration was assessed by means of the tail pressure test[11] in mice and compared with that of morphine (Table I). Both des-Tyr^1-dermorphin and des-Tyr^1-[D-Arg^2]dermorphin were essentially inactive at a dose of up to 50 mg/kg, suggesting that the Tyr^1 residue in dermorphin or [D-Arg^2]dermorphin is required for the activity. Removal of Ser^7 gave III, which exhibited approximately the same activity as [D-Arg^2]dermorphin. Further removal of Pro,^{6} caused a significant decrease of potency. The N-terminal tetrapeptide amide (V), however, exhibited extremely potent activity: its potency is 31 times that of morphine and 6 times that of the tetrapeptide free acid[1,5] on a molar basis. In contrast, the N-terminal tetrapeptide amide in the dermorphin sequence has been shown to lack any significant activity following *s.c.* administration.[12] The tri- (VI) and dipeptide amides (VII) were inactive. However, the tripeptide amide has been shown to possess opioid activities in *in vitro* assays[13] and following intracerebroventricular administration it showed analgesic activity amounting to one-tenth that of the parent peptide.[14] These results suggest that the activity patterns of [D-Arg^2]dermorphin analogs having various peptide chain lengths differ from those of dermorphin analogs.[15] Analog V seems to be the most active compound in the series of tetrapeptide analogs of [D-Arg^2]dermorphin.[1] Its high potency may be caused in part by increased stability to proteolytic enzymes, because the tetrapeptide free acid was easily hydrolyzed, liberating Gly, by a purified carboxypeptidase.[16] Studies on the stability of [D-Arg^2]dermorphin analogs to enzymes are in progress.

Experimental

Melting points were determined with a Yanaco model MP-S3 micro melting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-140 polarimeter. Amino acid analyses were performed on a

Hitachi model 835 amino acid analyzer. TLC was performed on silica gel plates (Kieselgel GF_{254}, Merck) with the following solvent systems: Rf^1, 1-BuOH–AcOH–H_2O (4:1:5, upper phase); Rf^2, 1-BuOH–pyridine–AcOH–H_2O (15:10:3:12). The Boc group of all intermediates was removed by 4 N HCl–DOX treatment before TLC.

Boc–D-Ala–Phe–Gly–OH (1)——A 2 N NaOH solution (0.58 ml) was added to a solution of Boc–D-Ala–Phe–Gly–OEt[1] (422 mg) in MeOH (5 ml), and the mixture was stirred at room temperature for 50 min, then diluted with H_2O (50 ml) and washed twice with AcOEt (50 ml). The aqueous phase was chilled, acidified with solid citric acid and extracted twice with AcOEt (50 ml). The extract was washed with H_2O, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The resulting residue was reprecipitated from AcOEt–pet. ether; yield 306 mg (78%), mp 108—110 °C, $[\alpha]_D^{23}$ +4.1° (c=1, MeOH), Rf^1 0.30. *Anal.* Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 57.71; H, 6.81; N, 10.33.

Boc–D-Ala–Phe–Gly–Tyr–Pro–Ser–NH_2 (2)——WSCI (105 mg) was added to a solution of **1** (197 mg), $CF_3COOH \cdot$H–Tyr–Pro–Ser–NH_2[6] (240 mg) and HOBt (68 mg) in DMF (3 ml) containing TEA (0.08 ml) at 0 °C. The mixture was stirred at 5 °C for 40 h, then diluted with H_2O (20 ml) and extracted twice with 1-BuOH (20 ml). The extract was washed with 1-BuOH-saturated 1 N AcOH (×3), 1-BuOH-saturated 1 N NH_4OH (×3) and 1-BuOH-saturated H_2O (×3), then evaporated to dryness *in vacuo*. The resulting residue was reprecipitated from AcOEt–pet. ether; yield 310 mg (84%), mp 139—143 °C, $[\alpha]_D^{23}$ −15.5° (c=1, MeOH), Rf^1 0.32. *Anal.* Calcd for $C_{36}H_{49}N_7O_{10}$: C, 58.45; H, 6.68; N, 13.25. Found: C, 58.68; H, 6.71; N, 13.13.

H–D-Ala–Phe–Gly–Tyr–Pro–Ser–NH_2 (I)——Compound **2** (200 mg) was treated with 4 N HCl–DOX (5 ml) at room temperature for 30 min, then the solution was evaporated to dryness *in vacuo*. The resulting residue was dissolved in H_2O (5 ml) and treated with Dowex 1×2 (AcOH form) resin (8 g by wet weight) for 30 min. After removal of the resin by filtration, the filtrate was lyophilized. The product was dissolved in H_2O (0.5 ml) and the solution was applied to a column (2×11 cm) of CM-cellulose. The column was eluted first with H_2O (100 ml) and then with a linear gradient formed from 0.1 M pyridinium acetate buffer (pH 5.15, 300 ml) through a mixing chamber containing 0.05 M pyridinium acetate buffer (pH 5.15, 300 ml). Fractions of 5.2 ml each were collected and tube Nos. 60—80 (numbering from the starting point of gradient elution) were pooled, and evaporated to dryness *in vacuo*. The residue was lyophilized from H_2O; yield 120 mg (54%), $[\alpha]_D^{23}$ −44.4° (c=0.5, H_2O), Rf^1 0.28, Rf^2 0.67. *Anal.* Calcd for $C_{31}H_{41}N_7O_8 \cdot 2CH_3COOH \cdot 7/2H_2O$: C, 51.09; H, 6.86; N, 11.92. Found: C, 50.92; H, 6.63; N, 12.07. Amino acid analysis (6 N HCl): Ser 0.91; Gly 0.98; Ala 1.00; Tyr 0.97; Phe 0.98; Pro 1.02; NH_3 1.03 (recovery 78%).

Boc–D-Arg(Tos)–Phe–Gly–OH (3)——The title compound was obtained from Boc–D-Arg(Tos)–Phe–Gly–OEt[1] (331 mg) in the same manner as described for the preparation of **1**; yield 250 mg (79%), mp 64—67 °C, $[\alpha]_D^{23}$ −15.6° (c=1, MeOH), Rf^1 0.29. *Anal.* Calcd for $C_{29}H_{40}N_6O_8S$: C, 55.05; H, 6.37; N, 13.29. Found: C, 54.71; H, 6.10; N, 13.08.

Boc–D-Arg(Tos)–Phe–Gly–Tyr–Pro–Ser–NH_2 (4)——The title compound was obtained from **3** (190 mg) and $CF_3COOH \cdot$H–Tyr–Pro–Ser–NH_2 (144 mg) by means of the WSCI–HOBt method as described for the preparation of **2**; yield 270 mg (92%), mp 140—144 °C, $[\alpha]_D^{23}$ −23.5° (c=1, MeOH), Rf^1 0.33. *Anal.* Calcd for $C_{46}H_{62}N_{10}O_{12}S$: C, 56.43; H, 6.38; N, 14.31. Found: C, 56.51; H, 6.74; N, 14.02.

H–D-Arg–Phe–Gly–Tyr–Pro–Ser–NH_2 (II)——CF_3SO_3H (0.23 ml) was added to a solution of **4** (300 mg), *o*-cresol (100 mg) and thioanisole (0.36 ml) in CF_3COOH (5 ml). The mixture was stirred at room temperature for 90 min, then concentrated to a small volume *in vacuo*. To this solution, abs. ether (30 ml) was added. The resulting oil was washed well with abs. ether and dried *in vacuo*. The crude peptide was purified by column chromatography on CM-cellulose in a manner similar to that described above. The CM-cellulose column was eluted first with H_2O (100 ml) and then with a linear gradient formed from 0.35 M pyridinium acetate buffer (pH 5.15, 300 ml) through a mixing chamber containing 0.1 M pyridinium acetate buffer (pH 5.15, 300 ml). Fractions of 5.2 ml each were collected and tube Nos. 72—90 were pooled, and lyophilized. The partially purified product was applied to a column (2.5×45 cm) of Toyopearl HW-40, which was eluted with 2% AcOH. Fractions of 6 ml each were collected and tube Nos. 33—40 were pooled, and evaporated to dryness *in vacuo*. The residue was lyophilized from H_2O; yield 140 mg (51%), $[\alpha]_D^{23}$ −45.1° (c=0.5, H_2O), Rf^1 0.28, Rf^2 0.60. *Anal.* Calcd for $C_{34}H_{48}N_{10}O_8 \cdot 5/2CH_3COOH \cdot 3/2H_2O$: C, 51.93; H, 6.82; N, 15.53. Found: C, 51.92; H, 6.74; N, 15.49. Amino acid analysis (6 N HCl): Ser 0.92; Gly 1.00; Tyr 0.96; Phe 0.98; Arg 1.00; Pro 1.01; NH_3 1.03 (recovery 80%).

Boc–Tyr–D-Arg(Tos)–Phe–Gly–Tyr–Pro–NH_2 (5)——The title compound was obtained from Boc–Tyr–D-Arg(Tos)–Phe–Gly–OH[1] (796 mg) and HBr·H–Tyr–Pro–NH_2 [derived from Z–Tyr–Pro–NH_2[17] (412 mg) by treatment with 25% HBr–AcOH] in the same manner as described for the preparation of **2**; yield 900 mg (85%), mp 138—144 °C, $[\alpha]_D^{23}$ −11.6° (c=1, MeOH), Rf^1 0.53. *Anal.* Calcd for $C_{52}H_{66}N_{10}O_{12}$ S: C, 59.19; H, 6.30; N, 13.27. Found: C, 58.82; H, 6.24; N, 12.85.

H–Tyr–D-Arg–Phe–Gly–Tyr–Pro–NH_2 (III)——Compound **5** (250 mg) was treated with the CF_3SO_3H–thioanisole system and purified by column chromatography on CM-cellulose in the same manner as described for the preparation of II. The partially purified product was applied to a column (2.5×90 cm) of Sephadex G-10, which was eluted with 2% AcOH. Fractions of 5 ml each were collected and tube Nos. 56—62 were pooled, and evaporated to dryness *in vacuo*. The residue was lyophilized from H_2O; yield 115 mg (45%), $[\alpha]_D^{23}$ +6.2° (c=1, H_2O),

Rf^1 0.32, Rf^2 0.73. *Anal.* Calcd for $C_{40}H_{52}N_{10}O_8 \cdot 3CH_3COOH \cdot 5H_2O$: C, 51.58; H, 6.96; N, 13.08. Found: C, 51.41; H, 6.64; N, 13.30. Amino acid analysis (6 N HCl): Gly 1.02; Tyr 1.86; Phe 0.98; Arg 1.00; Pro 1.03; NH_3 1.02 (recovery 75%).

Boc–Tyr–D-Arg(Tos)–Phe–Gly–Tyr–NH_2 (6)——The title compound was obtained from Boc–Tyr–D-Arg(Tos)–Phe–Gly–OH (796 mg) and HCl·H–Tyr–NH_2 (217 mg) in the same manner as described for the preparation of **2**; yield 810 mg (85%), mp 145—149 °C, $[\alpha]_D^{23} -8.3°$ ($c=1$, MeOH), Rf^1 0.61. *Anal.* Calcd for $C_{47}H_{59}N_9O_{11}S$: C, 58.92; H, 6.21; N, 13.16. Found: C, 58.71; H, 6.00; N, 12.83.

H–Tyr–D-Arg–Phe–Gly–Tyr–NH_2 (IV)——Compound **6** (200 mg) was treated with the CF_3SO_3H–thioanisole system and purified by using columns of CM-cellulose and Toyopearl HW-40 in the same manner as described for the preparation of II; yield 85 mg (45%), $[\alpha]_D^{23} +33.7°$ ($c=1$, H_2O), Rf^1 0.36, Rf^2 0.62. *Anal.* Calcd for $C_{35}H_{45}N_9O_7 \cdot 5/2CH_3COOH \cdot 5/2H_2O$: C, 53.44; H, 6.73; N, 14.02. Found: C, 53.13; H, 6.44; N, 14.26. Amino acid analysis (6 N HCl): Gly 1.03; Tyr 1.87; Phe 0.98; Arg 1.00; NH_3 1.04 (recovery 78%).

Boc–Tyr–D-Arg(Tos)–Phe–Gly–NH_2 (7)——NH_3 gas was bubbled through a solution of Boc–Tyr–D-Arg(Tos)–Phe–Gly–OEt[1)] (824 mg) in MeOH (20 ml) under ice-cooling for 20 min. The resulting solution was allowed to stand at room temperature for 2 d and then evaporated to dryness *in vacuo*. The resulting residue was reprecipitated from MeOH–abs. ether; yield 735 mg (92%), mp 141—144 °C, $[\alpha]_D^{23} -4.7°$ ($c=1$, MeOH), Rf^1 0.51. *Anal.* Calcd for $C_{38}H_{50}N_8O_9S$: C, 57.41; H, 6.34; N, 14.10. Found: C, 57.03; H, 6.27; N, 13.74.

H–Tyr–D-Arg–Phe–Gly–NH_2 (V)——Compound **7** (200 mg) was treated with the CF_3SO_3H–thioanisole system and purified on a CM-cellulose column in the same manner as described for the preparation of II. The partially purified product was applied to a column (2.5 × 90 cm) of Sephadex G-10, which was eluted with 2% AcOH. Fractions of 6.2 ml each were collected and tube Nos. 30—40 were pooled, and evaporated to dryness *in vacuo*. The residue was lyophilized from H_2O; yield 129 mg (68%), $[\alpha]_D^{23} +43.1°$ ($c=1$, H_2O), Rf^1 0.35, Rf^2 0.69. *Anal.* Calcd for $C_{26}H_{36}N_8O_5 \cdot 7/2CH_3COOH$: C, 52.79; H, 6.71; N, 14.92. Found: C, 53.02; H, 6.73; N, 14.70. Amino acid analysis (6 N HCl): Gly 1.05; Tyr 0.98; Phe 1.04; Arg 1.00; NH_3 0.97 (recovery 72%).

Boc–D-Arg(Tos)–Phe–OMe (8)——DCC (454 mg) was added to a solution of Boc–D-Arg(Tos)–OH (857 mg), HOBt (270 mg) and HCl·H–Phe–OMe (430 mg) in DMF (5 ml) containing TEA (0.31 ml) at 0 °C, and the mixture was stirred at 5 °C overnight. A few drops of AcOH were added and the whole was stirred for a further 30 min at room temperature. DC-urea formed was filtered off. The filtrate was diluted with H_2O (50 ml) and extracted twice with AcOEt (50 ml). The extract was washed well with 1 N citric acid (× 3), 1 N $NaHCO_3$ (× 3) and H_2O (× 3), then evaporated to dryness *in vacuo*. The resulting residue was reprecipitated from AcOEt–pet. ether; yield 1.12 g (95%), mp 84—88 °C, $[\alpha]_D^{23} -6.6°$ ($c=1$, MeOH), Rf^1 0.55. *Anal.* Calcd for $C_{28}H_{39}N_5O_7S$: C, 57.03; H, 6.66; N, 11.88. Found: C, 57.26; H, 6.97; N, 11.66.

Boc–Tyr–D-Arg(Tos)–Phe–OMe (9)——Compound **8** (590 mg) was treated with 4 N HCl–DOX (5 ml) at room temperature for 30 min, then the solution was evaporated to dryness *in vacuo*. The excess HCl was removed by repeated evaporation with fresh DOX *in vacuo*. The resulting residue was dissolved in DMF (5 ml) containing TEA (0.16 ml), then Boc–Tyr–OH (282 mg) and HOBt (135 mg) were added, followed by DCC (227 mg) at 0 °C. After being stirred at 5 °C overnight, the mixture was worked up in the same manner as described for the preparation of **8**; yield 720 mg (96%), mp 110—114 °C, $[\alpha]_D^{23} +3.4°$ ($c=0.5$, MeOH), Rf^1 0.68. *Anal.* Calcd for $C_{37}H_{48}N_6O_9S$: C, 59.02; H, 6.43; N, 11.16. Found: C, 59.14; H, 6.60; N, 10.85.

Boc–Tyr–D-Arg(Tos)–Phe–NH_2 (10)——The title compound was obtained from **9** (500 mg) in the same manner as described for the preparation of **7**; yield 450 mg (92%), mp 128—132 °C, $[\alpha]_D^{23} +4.4°$ ($c=1$, MeOH), Rf^1 0.60. *Anal.* Calcd for $C_{36}H_{47}N_7O_8S$: C, 58.60; H, 6.42; N, 13.29. Found: C, 58.40; H, 6.57; N, 13.09.

H–Tyr–D-Arg–Phe–NH_2 (VI)——Compound **10** (200 mg) was treated with the CF_3SO_3H–thioanisole system and purified on columns of CM-cellulose and Toyopearl HW-40 in the same manner as described for the preparation of II; yield 95 mg (57%), $[\alpha]_D^{23} +47.9°$ ($c=1$, H_2O), Rf^1 0.31, Rf^2 0.65. *Anal.* Calcd for $C_{24}H_{33}N_7O_4 \cdot 2CH_3COOH \cdot 3/2H_2O$: C, 53.32; H, 7.03; N, 15.54. Found: C, 53.43; H, 6.98; N, 15.19. Amino acid analysis (6 N HCl): Tyr 0.96; Phe 1.02; Arg 1.00; NH_3 1.01 (recovery 76%).

Boc–D-Arg(Tos)–NH_2 (11)——Isobutylchloroformate (0.15 ml) was added to a solution of Boc–D-Arg(Tos)–OH (429 mg) and NMM (0.12 ml) in tetrahydrofuran (THF, 10 ml) at −20 °C. After 10 min, cooled 30% NH_4OH (0.5 ml) was added and the mixture was stirred in an ice-bath for 3 h. The resulting solution was evaporated to dryness and the residue was dissolved in AcOEt. The solution was worked up in the same manner as described for the preparation of **8**; yield 360 mg (84%), mp 72—75 °C, $[\alpha]_D^{23} -6.6°$ ($c=1$, MeOH), Rf^1 0.36. *Anal.* Calcd for $C_{18}H_{29}N_5O_5S$: C, 50.57; H, 6.84; N, 16.38. Found: C, 50.65; H, 6.84; N, 15.92.

Boc–Tyr–D-Arg(Tos)–NH_2 (12)——The title compound was obtained from Boc–Tyr–OH (282 mg) and HCl·H–D-Arg(Tos)–NH_2 [derived from compound **11** (428 mg)] in the same manner as described for the preparation of **8**; yield 505 mg (85%), mp 112—115 °C, $[\alpha]_D^{23} +19.7°$ ($c=0.5$, MeOH), Rf^1 0.50. *Anal.* Calcd for $C_{27}H_{38}N_6O_7S$: C, 54.90; H, 6.48; N, 14.23. Found: C, 54.72; H, 6.21; N, 13.95.

H–Tyr–D-Arg–NH_2 (VII)——Compound **12** (200 mg) was treated with the CF_3SO_3H–thioanisole system and purified on a CM-cellulose column in the same manner as described for the preparation of II; yield 74 mg (46%), $[\alpha]_D^{23} +65.1°$ ($c=1$, H_2O), Rf^1 0.14, Rf^2 0.37. *Anal.* Calcd for $C_{15}H_{24}N_6O_3 \cdot 2CH_3COOH \cdot H_2O$: C, 48.09; H, 7.22; N,

17.71. Found: C, 48.05; H, 6.92; N, 17.54. Amino acid analysis (6 N HCl): Tyr 0.96; Arg 1.00; NH_3 1.05 (recovery 71%).

Analgesic Assay——Male Std-ddy strain mice (20—25 g) were used. Mice were injected subcutaneously with a test compound dissolved in Ringer's solution. The analgesic effect was assessed by means of the tail pressure test as described previously.[5,11] Changes in responsive tail pressure were expressed as a percentage of maximun possible effect (% MPE) as follows: % MPE $=(P_t - P_o/100 - P_o)$ where P_o is pre-drug responsive pressure (mmHg) and P_t is responsive pressure (mmHg) after drug administration. The ED_{50} values and 95% confidence limits were determined by the method of Litchfield and Wilcoxon.[18]

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References and Notes

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2) Amino acids, peptides and their derivatives in this study are of L-configuration unless otherwise stated. Abbreviations used are: Boc = *tert*-butoxycarbonyl, Z = carbobenzoxy, Me = methyl, Et = ethyl, Tos = tosyl, DCC = dicyclohexylcarbodiimide, WSCI = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, HOBt = 1-hydroxybenztriazole, TEA = triethylamine, NMM = *N*-methylmorpholine, DMF = dimethylformamide, AcOEt = ethyl acetate, DOX = dioxane, CM- = carboxymethyl-, TLC = thin-layer chromatography.
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