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Studies on Peptides. CXXXI.[1,2] Synthesis of Adrenorphin and Enkephalin Analogs

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Adrenorphin, H–Tyr–Gly–Gly–Phe–Met–Arg–Arg–Val–NH_2, and its D-Met(O)^2-analog were synthesized. In addition, analogs of [Arg^6, Phe^7]enkephalin substituted at the 2nd position by D-Ala and D-Met(O) were also synthesized, together with four other enkephalin analogs. Among these compounds, [D-Met(O)^2]adrenorphin exhibited high analgesic activity (29.1 times that of morphine) antagonized by naloxone, when administered intracisternally to mice.

Keywords——adrenorphin; [Arg^6, Phe^7]enkephalin analog; enkephalin tryptamine analog; N^G-mesitylenesulfonylarginine; thioanisole-mediated deprotection; analgesic activity

Since Hughes *et al.*[3] characterized two pentapeptides, Met–enkephalin and Leu–enkephalin, as endogenous opioid peptides, several analogous peptides of proenkephalin origin[4] have been isolated and subsequently various highly active enkephalin analogs have been synthesized. Analogs are generally designed so as to resist the actions of various proteolytic enzymes. Thus, synthetic studies demonstrated that, for instance, replacement of the Gly residue (position 2) of enkephalin by D-Ala[5] or D-Met[6] brought about a significant increase of analgesic activity. In the present studies, we examined the analgesic activities of analogs of two naturally occurring opioid peptides, adrenorphin[7] and [Arg^6, Phe^7]-enkephalin.[8] In addition, four enkephalin analogs, *i.e.*, [L- and D-Pro^2]Leu–enkephalin and [L- and D-Met(O)^2]tryptamine derivatives, were synthesized and assayed.

Adrenorphin (also named metrophamide)[9] is the C-amidated octapeptide, H–Tyr–Gly–

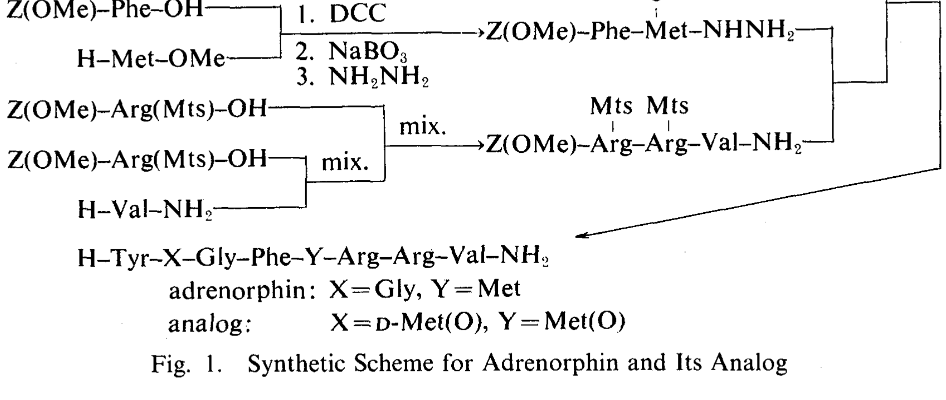

Fig. 1. Synthetic Scheme for Adrenorphin and Its Analog

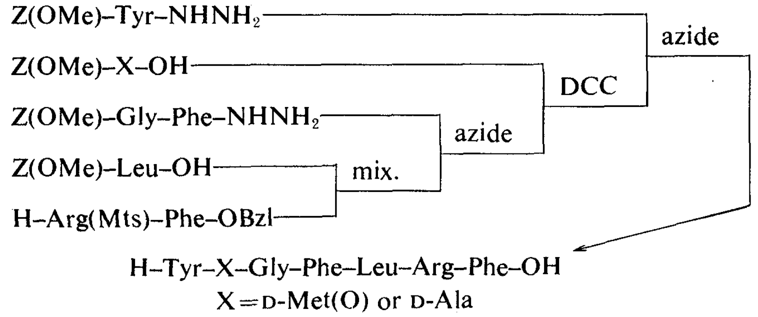

Fig. 2. Synthetic Scheme for [Arg^{6}, Phe^{7}]Enkephalin Analogs

Table I. Analgesic Activity of Synthetic Peptides

Synthetic peptides	ED$_{50}$ nmol	Relative potency
H–Tyr–Gly–Gly–Phe–Met–Arg–Arg–Val–NH$_2$	0.899	0.8
H–Tyr–D-Met(O)–Gly–Phe–Met(O)–Arg–Arg–Val–NH$_2$	0.024	29.1
H–Tyr–D-Met(O)–Gly–Phe–Leu–Arg–Phe–OH	0.093	7.5
H–Tyr–D-Ala–Gly–Phe–Leu–Arg–Phe–OH	$>$11.5	
H–Tyr–D-Met(O)–Gly–tryptamine	0.214	3.3
H–Tyr–Met(O)–Gly–tryptamine	$>$19.0	
H–Tyr–Pro–Gly–Phe–Leu–OH	$>$16.8	
H–Tyr–D-Pro–Gly–Phe–Leu–OH	$>$16.8	
Morphine (standard)	0.698	1.0

Gly–Phe–Met–Arg–Arg–Val–NH$_2$, independently characterized by Matsuo *et al.*7) and Weber *et al.*9) in 1983; solid-phase syntheses were also reported by these authors. We synthesized adrenorphin and [D-Met(O)^{2}, Met(O)^{5}]adrenorphin by the solution-phase method according to the scheme illustrated in Fig. 1. First, for the synthesis of adrenorphin, three fragments, Z(OMe)–Arg(Mts)–Arg(Mts)–Val–NH$_2$, Z(OMe)–Phe–Met(O)–NHNH$_2$ and Z(OMe)–Tyr–Gly–Gly–NHNH$_2$, were synthesized by the known amide-forming reactions and they were assembled successively by the azide procedure10) according to the scheme. The protected octapeptide was treated with 1 M TFMSA–thioanisole/TFA11) to remove all protecting groups. *m*-Cresol was employed as an additional scavenger to suppress O-sulfonation at the Tyr residue.12) The deprotected peptide, after conversion to the corresponding acetate by Amberlite CG-4B and incubation with dithiothreitol, was purified by ion-exchange chromatography on CM-cellulose. Its purity was ascertained by amino acid analysis, after acid hydrolysis and enzymatic digestion.

Next, for the synthesis of [D-Met(O)^{2}, Met(O)^{5}]adrenorphin, the necessary N-terminal tripeptide, Z(OMe)–Tyr–D-Met(O)–Gly–NHNH$_2$, was prepared in a stepwise manner and condensed with the TFA-treated sample of Z(OMe)–Phe–Met(O)–Arg(Mts)–Arg(Mts)–Val–NH$_2$ used in the former adrenorphin synthesis. For deprotection, MSA,13) instead of 1 M TFMSA–thioanisole/TFA, was employed, since the latter is known to have an ability partially to reduce Met(O).14) The deprotected peptide, after conversion to the corresponding acetate, was purified in essentially the same manner as described above.

Next, as analogs of a naturally occurring opioid peptide, [Arg^{6}, Phe^{7}]enkephalin, we synthesized two heptapeptides, H–Tyr–D-Met(O)–Gly–Phe–Leu–Arg–Phe–OH and H–Tyr–D-Ala–Gly–Phe–Leu–Arg–Phe–OH, using the thioanisole mediated deprotecting procedure mentioned above, as shown in Fig. 2.

In addition, using the available derivative, Z(OMe)–Tyr–D-Met(O)–Gly–NHNH$_2$, we prepared H–Tyr–D-Met(O)–Gly–tryptamine and its L-isomer, since H–Tyr–D-Met(O)–Gly–*N*-methylphenethylamide[15] is known to have relatively high analgesic activity. Casomorphin[16] possesses the Pro residue at the 2nd position and this Pro residue in peptides is known to resist the action of leucine aminopeptidase (LAP). We also synthesized L- and D-Pro analogs of Leu–enkephalin.

Analgesic effects of the synthetic peptides were evaluated by means of the tail-pinch test.[17] Each sample was intracisternally administered to mice. The ED_{50} values and relative potency with respect to morphine are listed in Table I. The peptides possessing D-Met(O) exhibited higher activity than the D-Ala-analogs. It seems noteworthy that the D-$Met(O)^2$-analog of adrenorphin exhibited markedly high activity, and that the effects of analogs possessing D-Met(O) were all antagonized by naloxone.

Experimental

General experimental methods employed in this paper are essentially the same as described in Part 88[18] of this series. *Rf* values in thin layer chromatography (TLC) performed on silica gel (Kieselgel G, Merck No. 7731) refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O = 8 : 3 : 1, Rf_2 $CHCl_3$–MeOH–AcOH = 9 : 1 : 0.5, Rf_3 $CHCl_3$–MeOH = 10 : 0.5, Rf_4 *n*-BuOH–AcOH–pyridine–H_2O = 4 : 1 : 1 : 2, Rf_5 *n*-BuOH–AcOH–AcOEt–H_2O = 1 : 1 : 1 : 1.

Derivatives of Amino Acids, Dipeptides and Tripeptides——Several starting materials prepared easily by known methods were characterized as shown in Tables II, III and IV.

Synthesis of Adrenorphin and Its Analog——Z(OMe)–Phe–Met(O)–Arg(Mts)–Arg(Mts)–Val–NH_2: A TFA-treated sample of Z(OMe)–Arg(Mts)–Arg(Mts)–Val–NH_2 (2.80 g, 2.92 mmol) was dissolved in DMF (30 ml) containing Et_3N (0.40 ml, 2.92 mmol). The azide [prepared from 1.72 g (3.50 mmol) of Z(OMe)–Phe–Met(O)–$NHNH_2$] in DMF (30 ml) and Et_3N (0.48 ml, 3.50 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C overnight. Additional azide (prepared from 0.2 eq of the hydrazide) in DMF (6 ml) and Et_3N (0.2 eq) were added, and the mixture, after being stirred overnight, was concentrated. The residue was purified by recrystallization from MeOH and AcOEt; yield 2.06 g (56%), mp 133—136 °C, $[\alpha]_D^{18}$ $-1.1°$ ($c = 0.9$, DMF), Rf_1 0.64. Amino acid ratios in 6 N HCl hydrolysate: Phe 0.98, Met 0.77, Arg 2.28, Val 1.00 (recovery of Val 83%). *Anal.* Calcd for $C_{58}H_{82}N_{12}O_{13}S \cdot H_2O$: C, 54.87; H, 6.67; N, 13.24. Found: C, 54.73; H, 6.66; N, 13.26.

Z(OMe)–Tyr–Gly–Gly–Phe–Met(O)–Arg(Mts)–Arg(Mts)–Val–NH_2: The azide [prepared from 0.62 g (1.30 mmol) of Z(OMe)–Tyr–Gly–Gly–$NHNH_2$] in DMF (15 ml) and Et_3N (0.14 ml, 1.08 mmol) were added to an ice-chilled solution of the pentapeptide [derived from 1.35 g (1.08 mmol) of the above Z(OMe)-derivative] in DMF (20 ml). The mixture, after being stirred at 4 °C overnight, was concentrated and the product was purified by recrystallization from MeOH; yield 0.85 g (51%), mp 160—164 °C, $[\alpha]_D^{20}$ $-10.7°$ ($c = 0.7$, DMF), Rf_1 0.64. Amino acid ratios in 6 N HCl hydrolysate: Tyr 1.07, Gly 2.28, Phe 1.04, Met 0.96, Arg 2.24, Val 1.00 (recovery of Val 87%). *Anal.* Calcd for $C_{71}H_{97}N_{15}O_{17}S_3 \cdot H_2O$: C, 55.13; H, 6.45; N, 13.58. Found: C, 55.32; H, 6.43; N, 13.33.

H–Tyr–Gly–Gly–Phe–Met–Arg–Arg–Val–NH_2: The above protected adrenorphin (200 mg, 0.13 mmol) was

Table II. Characterization of Amino Acid Derivatives

	mp (°C)	$[\alpha]_D$ (MeOH)	Formula	Analysis (%) Calcd (Found)		
				C	H	N
Z(OMe)–D-Ala–OH	81—82	+14.0	$C_{12}H_{15}NO_5$	56.91	5.97	5.53
				(57.13	5.85	5.61)
Z(OMe)–D-Met–OH	70—72	+25.0	$C_{14}H_{19}NO_5S$	53.66	6.11	4.47
				(53.53	6.18	4.48)
Z(OMe)–D-$\overset{\mathrm{O}}{\overset{\vert}{\mathrm{M}}}$et–OH	123—125	+32.0	$C_{14}H_{19}NO_6S$	51.05	5.81	4.25
				(50.82	5.90	4.41)
Z(OMe)–D-Pro–OH DCHA	135—137	+28.0	$C_{26}H_{40}N_2O_5$	67.79	8.75	6.08
				(67.99	8.84	6.09)
Z(OMe)–D-Pro–ONp	Oil	+19.1	$C_{20}H_{20}N_2O_7$	59.99	5.04	7.00
				(60.26	5.40	6.69)

TABLE III. Characterization of Dipeptide Derivatives

	Proc.	Yield (%)	mp (°C)	$[\alpha]_D$	Formula	Analysis (%) Calcd (Found) C	H	N
Z(OMe)–$\overset{\text{Mts}}{\text{Arg}}$–Val–$NH_2$	Mixed	66	139—142	+8.5 MeOH	$C_{29}H_{42}N_6O_7S\cdot$ $1/2\,H_2O$	55.48 (55.34	6.90 6.99	13.39 13.15)
Z(OMe)–Phe–Met–OMe	DCC	63	111—113	−16.0 DMF	$C_{24}H_{30}N_2O_6S$	60.74 (60.69	6.37 6.52	5.90 6.11)
Z(OMe)–Phe–$\overset{\text{O}}{\text{Met}}$–OMe	$NaBO_3$	35	129—131	−10.6 MeOH	$C_{24}H_{30}N_2O_7S$	58.76 (58.91	6.16 5.90	5.71 5.71)
Z(OMe)–Phe–$\overset{\text{O}}{\text{Met}}$–$NHNH_2$	NH_2NH_2	88	197—200	−17.2 DMF	$C_{23}H_{30}N_4O_6S$	56.31 (56.04	6.16 5.99	11.42 11.33)
Z(OMe)–D-$\overset{\text{O}}{\text{Met}}$–Gly–OMe	DCC	81	100—103	+27.0 MeOH	$C_{16}H_{24}N_2O_7S$	50.99 (50.81	6.04 6.05	7.00 7.23)
Z(OMe)–Gly–Phe–OBzl	Mixed	97	83—84	−2.0 MeOH	$C_{27}H_{28}N_2O_6$	68.05 (68.23	5.92 5.93	5.88 5.99)
Z(OMe)–Gly–Phe–$NHNH_2$	NH_2NH_2	85	134—136	−9.0 DMF	$C_{20}H_{24}N_4O_5$	59.99 (59.96	6.27 6.04	14.13 13.99)
Z(OMe)–$\overset{\text{Mts}}{\text{Arg}}$–Phe–OBzl	Mixed	97	133—134	−9.0 MeOH	$C_{40}H_{47}N_5O_8S$	63.39 (63.48	6.25 6.14	9.24 9.18)
Z(OMe)–Phe–Leu–OBzl	DCC	81	96—98	−28.0 MeOH	$C_{31}H_{36}N_2O_6$	69.91 (69.74	6.81 6.72	5.26 5.33)

TABLE IV. Characterization of Tripeptide Derivatives

	Proc.	Yield (%)	mp (°C)	$[\alpha]_D$	Formula	Analysis (%) Calcd (Found) C	H	N
Z(OMe)–$\overset{\text{Mts}}{\text{Arg}}$–$\overset{\text{Mts}}{\text{Arg}}$–Val–$NH_2$	Mixed 1+2	84	119—123	−5.7 MeOH	$C_{44}H_{64}N_{10}O_{10}S_2\cdot$ $1/2\,H_2O$	54.69 (54.69	6.78 6.79	14.50 14.27)
Z(OMe)–Tyr–D-$\overset{\text{O}}{\text{Met}}$–Gly–OMe	Azide 1+2	64	172—174	+2.0 DMF	$C_{26}H_{33}N_3O_9S\cdot H_2O$	53.73 (53.64	6.07 5.99	7.23 7.20)
Z(OMe)–Tyr–D-$\overset{\text{O}}{\text{Met}}$–Gly–$NHNH_2$	NH_2NH_2	99	164—166	≑0 DMF	$C_{25}H_{33}N_5O_8S\cdot$ $1/2\,H_2O$	52.44 (52.44	5.99 6.01	12.23 12.17)
Z(OMe)–Leu–$\overset{\text{Mts}}{\text{Arg}}$–Phe–OBzl	Mixed 1+2	92	143—144	−19.1 DMF	$C_{46}H_{58}N_6O_9S$	63.43 (63.45	6.71 6.53	9.65 9.41)
Z(OMe)–Tyr–$\overset{\text{O}}{\text{Met}}$–Gly–$NHNH_2$	NH_2NH_2	82	183—185	−13.0 DMF	$C_{25}H_{33}N_5O_8S\cdot H_2O$	51.63 (51.49	6.07 5.87	12.04 11.93)
Z(OMe)–Gly–Phe–Leu–OBzl	DCC 1+2	76	53—56	−19.0 MeOH	$C_{33}H_{39}N_3O_7$	67.22 (67.29	6.67 6.48	7.13 7.00)

treated with 1 M TFMSA–thioanisole in TFA (5.2 ml) in the presence of *m*-cresol (0.21 ml, 15 eq) in an ice-bath for 90 min, then dry ether was added. The resulting powder was retreated with the above acid under identical conditions. The deprotected peptide was dissolved in H_2O (30 ml) and the solution was treated with Amberlite CG-4B (acetate form, approximately 1.0 g) for 30 min. The resin was removed by filtration. Dithiothreitol (0.40 g, 20 eq) was added and the solution was incubated at 37 °C for 2 d. The solution was then applied to a column of Sephadex G-15 (3.2 × 104 cm), which was eluted with 0.5 N AcOH. The fractions (7.5 ml each) corresponding to the front peak (tube Nos. 49—63, determined by ultraviolet (UV) absorption measurement at 275 nm) were combined and the solvent was removed by lyophilization. The resulting powder was then applied to a column of CM-cellulose (2.4 × 8.0 cm), which was eluted first with H_2O (240 ml), then with a gradient formed from 0.1 M $AcONH_4$ (pH 7.0, 1000 ml) through a mixing flask containing 0.01 M $AcONH_4$ (pH 6.0, 200 ml). Individual fractions (6 ml each) were examined by UV absorption measurement at 275 nm and the desired fractions in the gradient eluates (tube Nos. 329—384) were combined. The solvent and the salt were removed by repeated lyophilization to give a fluffy white powder; yield

68.5 mg (53%), $[\alpha]_D^{20}$ -17.0° ($c=0.5$, H_2O), Rf_4 0.61, Rf_5 0.85. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.95, Gly 2.00, Phe 0.96, Met 0.95, Arg 1.92, Val 0.86 (recovery of Gly 86%). Amino acid ratios in LAP digest: Tyr 1.00, Gly 2.00, Phe 1.06, Met 0.96, Arg 2.02, Val 0.93 (recovery of Gly 75%). *Anal.* Calcd for $C_{44}H_{69}N_{15}O_9S \cdot 3CH_3COOH \cdot 2.5H_2O$: C, 49.65; H, 7.17; N, 17.37. Found: C, 49.41; H, 7.12; N, 17.51.

Z(OMe)–Tyr–D-Met(O)–Gly–Phe–Met(O)–Arg(Mts)–Arg(Mts)–Val–NH_2: A TFA treated sample of Z(OMe)–Phe–Met(O)–Arg(Mts)–Arg(Mts)–Val–NH_2 (0.40 g, 0.32 mmol) was dissolved in DMF (10 ml) containing Et_3N (44 μl, 0.32 mmol). The azide [prepared from 0.21 g (0.38 mmol) of Z(OMe)–Tyr–D-Met(O)–Gly–$NHNH_2$] in DMF (10 ml) and Et_3N (44 μl, 0.32 mmol) were added to the above ice-chilled solution and the mixture, after being stirred at 4 °C overnight, was concentrated. The product was purified by recrystallization from MeOH and EtOH; yield 0.19 g (36%), mp 166—169 °C, $[\alpha]_D^{20}$ $+6.6$° ($c=0.5$, DMF), Rf_1 0.49. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.96, Met 1.23, Met(O) 0.31, Gly 1.05, Phe 1.05, Arg 2.04, Val 1.00 (recovery of Val 89%). *Anal.* Calcd for $C_{74}H_{103}N_{15}O_{18}S_4 \cdot 1.5H_2O$: C, 54.00; H, 6.49; N, 12.77. Found: C, 53.92; H, 6.38; N, 12.56.

H–Tyr–D-Met(O)–Gly–Phe–Met(O)–Arg–Arg–Val–NH_2: The above protected octapeptide amide (100 mg, 62 μmol) was treated with MSA (1.3 ml) in the presence of *m*-cresol (0.13 ml, 20 eq) in an ice-bath for 30 min, then at room temperature for 60 min. *n*-Hexane was added to precipitate the product, which was treated with ether. The resulting powder was dissolved in H_2O (30 ml) and the solution, after being treated with Amberlite CG-4B (acetate form, approximately 1.0 g) for 30 min, was filtered and the filtrate was lyophilized. The residue was purified in essentially the same manner as described in the above adrenorphin synthesis by gel-filtration on Sephadex G-10, followed by ion-exchange chromatography on CM-cellulose; yield 42.1 mg (62%), $[\alpha]_D^{30}$ $+4.7$° ($c=0.4$, H_2O), Rf_4 0.81, Rf_5 0.90. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.97, Met 1.67, Gly 1.00, Phe 1.02, Arg 1.88, Val 0.86 (recovery of Gly 73%). *Anal.* Calcd for $C_{47}H_{75}N_{15}O_{11}S_2 \cdot 3CH_3COOH \cdot 2H_2O$: C, 48.72; H, 7.02; N, 16.08. Found: C, 48.55; H, 7.03; N, 16.01.

[Arg^6, Phe^7]Enkephalin Analogs——Z(OMe)–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl: A TFA-treated sample of Z(OMe)–Leu–Arg(Mts)–Phe–OBzl (8.71 g, 10.0 mmol) was dissolved in DMF (50 ml) containing Et_3N (1.4 ml, 10.0 mmol). The azide [prepared from 4.00 g (10.0 mmol) of Z(OMe)–Gly–Phe–$NHNH_2$] in DMF (70 ml) and Et_3N (1.4 ml, 10.0 mmol) were added to the above ice-chilled solution and the mixture was stirred at 4 °C overnight. The product was isolated as usual and precipitated from DMF with AcOEt; yield 7.96 g (88%), mp 169—170 °C, $[\alpha]_D^{28}$ -11.0° ($c=1.0$, DMF), Rf_2 0.82. *Anal.* Calcd for $C_{57}H_{70}N_8O_{11}S \cdot H_2O$: C, 62.62; H, 6.64; N, 10.25. Found: C, 62.83; H, 6.48; N, 10.28.

Z(OMe)–D-Met(O)–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl: Z(OMe)–D-Met(O)–OH (0.99 g, 3.0 mmol) was condensed with H–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl [derived from 2.73 g (3.0 mmol) of the Z(OMe)-derivative] by using DCC (0.68 g, 3.3 mmol), and the product was purified by precipitation from DMF with AcOEt; yield 2.93 g (80%), mp 201—202 °C, $[\alpha]_D^{26}$ $+2.0$° ($c=1.0$, DMF), Rf_2 0.77. *Anal.* Calcd for $C_{62}H_{79}N_9O_{13}S_2$: C, 60.92; H, 6.51; N, 10.31. Found: C, 60.62; H, 6.57; N, 10.21.

Z(OMe)–Tyr–D-Met(O)–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl: The azide [prepared from 0.72 g (2.0 mmol) of Z(OMe)–Tyr–$NHNH_2$] in DMF (20 ml) and Et_3N (0.3 ml, 2.0 mmol) were added to an ice-chilled solution of H–D-Met(O)–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl [prepared from 2.44 g (2.0 mmol) of the Z(OMe)-derivative] and the mixture was stirred at 4 °C overnight. The product was isolated as usual and purified by precipitation from DMF with AcOEt; yield 2.07 g (75%), mp 150—151 °C, $[\alpha]_D^{26}$ -8.9° ($c=1.0$, DMF), Rf_1 0.81. *Anal.* Calcd for $C_{71}H_{88}N_{10}O_{15}S_2$: C, 61.54; H, 6.40; N, 10.11. Found: C, 61.49; H, 6.41; N, 10.10.

H–Tyr–D-Met(O)–Gly–Phe–Leu–Arg–Phe–OH: The above protected heptapeptide (200 mg, 144 μmol) was treated with MSA (3.0 ml) in the presence of *m*-cresol (0.3 ml, 20 eq) in an ice-bath for 60 min, then at room temperature for 60 min. *n*-Hexane was added and the precipitate was treated with ether. A solution of the resulting powder in 2 N AcOH (15 ml) was treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min. The resin was removed by filtration and the filtrate was lyophilized. The product was next purified by partition chromatography on Sephadex G-10 (3.0 × 58 cm) using the upper phase of *n*-BuOH–AcOH–H_2O as an eluant. The desired fractions (8 ml each, tube Nos. 25—38 determined by UV absorption at 280 nm) were combined, the solvent was removed by evaporation and the residue was lyophilized to give a fluffy powder; yield 114 mg (78%), $[\alpha]_D^{18}$ $+7.3$° ($c=0.4$, 2 N AcOH), Rf_5 0.56. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.94, Met 0.92, Gly 1.03, Phe 2.00, Leu 1.00, Arg 1.01 (recovery of Phe 80%). *Anal.* Calcd for $C_{46}H_{64}N_{10}O_{10}S \cdot CH_3COOH \cdot 2.5H_2O$: C, 54.68; H, 6.98; N, 13.29. Found: C, 54.64; H, 6.90; N, 13.23.

Z(OMe)–D-Ala–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl: Z(OMe)–D-Ala–OH (0.76 g, 3.0 mmol) was condensed with H–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl [prepared from 2.73 g (3.0 mmol) of the Z(OMe)-derivative] by using DCC (0.68 g, 3.3 mmol) and the product was purified by precipitation from DMF with AcOEt; yield 2.72 g (79%), mp 192—193 °C, $[\alpha]_D^{26}$ -10.8° ($c=1.0$, DMF), Rf_2 0.69. *Anal.* Calcd for $C_{60}H_{75}N_9O_{12}S$: C, 62.87; H, 6.59; N, 11.00. Found: C, 62.72; H, 6.56; N, 10.92.

Z(OMe)–Tyr–D-Ala–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl: The azide [prepared from 0.72 g (2.0 mmol) of Z(OMe)–Tyr–$NHNH_2$] was allowed to react with H–D-Ala–Gly–Phe–Leu–Arg(Mts)–Phe–OBzl [derived from 2.29 g (2.0 mmol) of the Z(OMe)-derivative]. The product was isolated as stated above and purified by precipitation from DMF with AcOEt; yield 2.10 g (80%), mp 173—175 °C, $[\alpha]_D^{26}$ -21.0° ($c=1.0$, DMF), Rf_1 0.79. *Anal.* Calcd for

$C_{69}H_{84}N_{10}O_{14}S$: C, 63.29; H, 6.47; N, 10.70. Found: C, 63.10; H, 6.58; N, 10.40.

H–Tyr–D-Ala–Gly–Phe–Leu–Arg–Phe–OH: The above protected heptapeptide (200 mg, 153 μmol) was treated with 1 M TFMSA–thioanisole in TFA (3.1 ml) in the presence of *m*-cresol (0.3 ml, 20 eq) in an ice-bath for 90 min, then dry ether was added. The deprotected peptide was converted to the corresponding acetate and purified by partition chromatography on Sephadex G-10 (3.0 × 58 cm) as described in the above purification of the D-Met(O)-analog; yield 103 mg (67%), $[\alpha]_D^{18}$ +10.1° (c=0.5, 2 N AcOH), Rf_5 0.54. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.97, Ala 1.12, Gly 1.03, Phe 2.00, Leu 1.02, Arg 1.02 (recovery of Phe 93%). *Anal.* Calcd for $C_{44}H_{60}N_{10}O_9 \cdot 2CH_3COOH \cdot 3H_2O$: C, 55.05; H, 7.12; N, 13.38. Found: C, 54.77; H, 6.66; N, 13.57.

Tryptamine-Containing Analogs——Z(OMe)–Tyr–Met(O)-Gly–Tryptamine: The azide [prepared from 1.69 g (3.0 mmol) of Z(OMe)–Tyr–Met(O)–Gly–$NHNH_2$] in DMF (30 ml) was allowed to react with tryptamine [prepared from 0.98 g (5.0 mmol) of the HCl salt] in DMF (20 ml) as usual and the product was purified by recrystallization from MeOH and AcOEt; yield 1.37 g (66%), mp 115—117 °C, $[\alpha]_D^{28}$ −15.9° (c=1.0, DMF), Rf_2 0.21. *Anal.* Calcd for $C_{35}H_{41}N_5O_8S \cdot H_2O$: C, 59.22; H, 6.11; N, 9.87. Found: C, 59.27; H, 5.96; N, 9.96.

H–Tyr–Met(O)–Gly–Tryptamine: Z(OMe)–Tyr–Met(O)–Gly–tryptamine (200 mg, 0.29 mmol) was treated with TFA (1 ml) in the presence of anisole (0.31 ml, 10 eq) and EDT (0.55 ml, 20 eq) in an ice-bath for 2 h, then dry ether was added. The resulting powder, after conversion to the corresponding acetate as usual, was purified by partition chromatography on Sephadex G-10 (3.0 × 58 cm) as described in the synthesis of [Arg^6, Phe^7]–enkephalin analogs; yield 72 mg (42%), $[\alpha]_D^{18}$ −2.6° (c=0.4, 0.1 N AcOH), Rf_5 0.48. Amino acid ratios in LAP digest: Try 0.97, Met (0) 0.98, Gly 1.00 (recovery of Gly 98%). *Anal.* Calcd for $C_{28}H_{33}N_5O_5S \cdot CH_3COOH \cdot 1/2H_2O$: C, 56.35; H, 6.42; N, 11.74. Found: C, 56.14; H, 6.43; N, 11.57.

Z(OMe)–Tyr–D-Met(O)–Gly–Tryptamine: The azide [prepared from 676 mg (1.20 mmol) of Z(OMe)–Tyr–D-Met(O)–Gly–$NHNH_2$] in DMF–DMSO (1 : 1, 20 ml) was allowed to react with tryptamine [prepared from 393 mg (2.0 mmol) of the HCl salt] in DMF (10 ml) and the product was purified by precipitation from DMF with AcOEt; yield 638 mg (77%), mp 180—181 °C, $[\alpha]_D^{28}$ +3.0° (c=1.0, DMF), Rf_1 0.69. *Anal.* Calcd for $C_{35}H_{41}N_5O_8S \cdot H_2O$: C, 59.22; H, 6.11; N, 9.87. Found: C, 59.34; H, 5.85; N, 9.78.

H–Tyr–D-Met(O)–Gly–Tryptamine: The above Z(OMe)-derivative (200 mg, 0.29 mmol) was treated with TFA (1 ml) in the presence of anisole (0.31 ml, 10 eq) and EDT (0.55 ml, 20 eq) in an ice-bath for 2 h and the product was purified as described above by partition chromatography on Sephadex G-10; yield 80 mg (47%), $[\alpha]_D^{18}$ +77.0° (c=0.4, 0.1 N AcOH), Rf_5 0.53. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.96, Met 0.99, Gly 1.00 (recovery of Gly 92%). *Anal.* Calcd for $C_{28}H_{37}N_5O_5S \cdot CH_3COOH \cdot H_2O$: C, 55.51; H, 6.49; N, 11.56. Found: C, 55.89; H, 6.32; N, 11.70.

Casomorphin Analogs——Z(OMe)–Pro–Gly–Phe–Leu–OBzl: The title compound was prepared by condensation of Z(OMe)–Pro–ONp (1.32 g, 3.3 mmol) and H–Gly–Phe–Leu–OBzl [derived from 1.79 g (3.0 mmol) of the Z(OMe)-derivative] and purified by recrystallization from MeOH and diisopropyl ether; yield 1.73 g (84%), mp 159—161 °C, $[\alpha]_D^{29}$ −23.0° (c=1.0, MeOH), Rf_3 0.53. *Anal.* Calcd for $C_{38}H_{46}N_4O_8$: C, 66.45; H, 6.75; N, 8.16. Found: C, 66.39; H, 6.70; N, 8.42.

Z(OMe)–Tyr–Pro–Gly–Phe–Leu–OBzl: Z(OMe)–Tyr–$NHNH_2$ (431 mg, 1.2 mmol) in DMF (10 ml) was condensed, *via* the azide, with H–Pro–Gly–Phe–Leu–OBzl [derived from 824 mg (1.2 mmol) of the above Z(OMe)-derivative] in DMF (10 ml) and the product was purified by precipitation from MeOH with ether; yield 475 mg (46%), mp 78—81 °C, $[\alpha]_D^{18}$ −33.2° (c=0.9, MeOH), Rf_2 0.73. *Anal.* Calcd for $C_{47}H_{55}N_5O_{10} \cdot 1/2H_2O$: C, 65.72; H, 6.57; N, 8.15. Found: C, 65.80; H, 6.76; N, 8.38.

H–Tyr–Pro–Gly–Phe–Leu–OH: The above protected pentapeptide (180 mg, 0.21 mmol) was treated with TFA–anisole (0.21 ml–70 μl) in an ice-bath for 90 min, then dry ether was added. The resulting powder was dissolved in 80% MeOH (10 ml) and hydrogenated over a Pd catalyst for 5 h. The product was purified by partition chromatography on Sephadex G-10 as described above; yield 74 mg (59%), $[\alpha]_D^{18}$ −39.6° (c=0.5, 1 N AcOH), Rf_5 0.57. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.87, Pro 1.01, Gly 1.05, Phe 1.00, Leu 1.07 (recovery of Phe 85%). *Anal.* Calcd for $C_{31}H_{41}N_5O_7 \cdot CH_3COOH$: C, 60.44; H, 6.91; N, 10.68. Found: C, 60.28; H, 6.94; N, 10.94.

Z(OMe)–D-Pro–Gly–Phe–Leu–OBzl: Z(OMe)–D-Pro–ONp (1.32 g, 3.3 mmol) was allowed to react with H–Gly–Phe–Leu–OBzl [derived from 1.79 g (3.0 mmol) of the Z(OMe)-derivative] in DMF (20 ml) as usual and the product was purified by precipitation from MeOH with ether; yield 0.79 g (38%), mp 155—157 °C, $[\alpha]_D^{29}$ +13.1° (c=1.0, MeOH), Rf_3 0.59. *Anal.* Calcd for $C_{38}H_{46}N_4O_8$: C, 66.46; H, 6.75; N, 8.16. Found: C, 66.34; H, 6.90; N, 8.44.

Z(OMe)–Tyr–D-Pro–Gly–Phe–Leu–OBzl: Z(OMe)–Tyr–$NHNH_2$ (270 mg, 0.75 mmol) in DMF (8 ml) was condensed, *via* the azide, with H–D-Pro–Gly–Phe–Leu–OBzl [derived from 515 mg (0.75 mmol) of the Z(OMe)-derivative] in DMF (8 ml) as usual and the product was purified by precipitation from AcOEt with ether; yield 419 mg (63%), mp 84—87 °C, $[\alpha]_D^{29}$ −8.0° (c=1.0, MeOH), Rf_2 0.67. *Anal.* Calcd for $C_{47}H_{55}N_5O_{10}$: C, 66.41; H, 6.52; N, 8.24. Found: C, 66.41; H, 6.52; N, 8.02.

H–Tyr–D-Pro–Gly–Phe–Leu–OH: The above protected pentapeptide (152 mg, 0.18 mmol) was treated with TFA–anisole (0.17 ml–58 μl) in an ice-bath for 60 min, then hydrogenated over a Pd catalyst for 5 h, and the product was purified by partition chromatography on Sephadex G-10 as described above; yield 17.8 mg (23%), $[\alpha]_D^{20}$ +102.4° (c=0.4, 1 N AcOH), Rf_4 0.70. Amino acid ratios in 6 N HCl hydrolysate: Tyr 0.90, Pro 1.02, Gly 0.99, Phe 0.99, Leu 1.00 (recovery of Leu 98%). *Anal.* Calcd for $C_{31}H_{41}N_5O_7 \cdot 1.5H_2O$: C, 59.79; H, 7.12; N, 11.24. Found: C, 59.73; H, 6.82; N, 10.96.

Bioassay——Male ddk mice (18—22 g) were used and synthetic peptides were intracisternally administered. The analgesic effect was examined by means of the tail-pinch test, in which the base of the mouse tail including the anal mucosa was pinched by an artery clip (2 mm) with a constant pressure of 500 g. Mices which bit the clip within 1 s after its application were selected and used. Analgesic effect was regarded as positive when the animal did not bite the clip within 6 s, after peptide administration. The analgesic effects of all the peptides used had maximally developed within 5 to 15 min after intracisternal injection. The results are shown in Table I.

References and Notes

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2) Unless otherwise stated, amino acids, peptides and their derivatives mentioned in this paper are of the L-configuration. Diastereomeric Met(O) [*Chem. Pharm. Bull.*, **26**, 650 (1978)] was employed. The following abbreviations were used: Z = benzyloxycarbonyl, Z(OMe) = *p*-methoxybenzyl oxycarbonyl, Bzl = benzyl, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, MSA = methanesulfonic acid, DCC = dicyclohexylcarbodiimide, HOBT = *N*-hydroxybenzotriazole, DMF = *N,N*-dimethylformamide, Np = *p*-nitrophenyl, EDT = ethanedithiol, DCHA = dicyclohexylamine.
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