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Further Synthesis of Enkephalinol Analogs containing the Dipeptide Unit Tyr–Arg (Kyotorphin)[1]

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In order to obtain enkephalin derivatives with high analgesic activity, four enkephalinol analogs having the Tyr–D-Arg unit in the *N*-terminal position were synthesized. Of these, H–Tyr–D-Arg–Gly–MePhe–Met(O)-ol exhibited analgesic activity comparable to that of morphine after intravenous administration to mice.

Keywords——enkephalinol; kyotorphin; methanesulfonic acid deprotection; sodium borohydride reduction; analgesic effect

Previously, we synthesized eight Met- and Leu-enkephalin analogs containing the dipeptide unit, Tyr–Arg (kyotorphin).[2] Of these, H–Tyr–D-Arg–Gly–Phe–Met–OH was found to possess an analgesic effect 2.4 times higher than that of morphine on a molar basis, when injected intracisternally. This compound was also found to produce analgesia when administered intravenously. Recently, it has been reported that some *C*-terminal amino alcohol derivatives, such as H–Tyr–D-Ala–Gly–MePhe–Met(O)-ol[3] and H–Tyr–D-Met(O)–Gly–MePhe–ol,[4] were found to be several times more analgesic than morphine in experimental animals after s.c. administration.

We have therefore synthesized four peptides: H–Tyr–D-Arg–Gly–MePhe–Met(O)–ol, Me–Tyr–D-Arg–Gly–MePhe–Met(O)–ol, H–Tyr–D-Arg–Gly–MePhe–Leu–ol and H–Tyr–D-Arg–Gly–MePhe–Ile–ol, using readily available α-amino alcohols.[5,6]

The first compound, H–Tyr–D-Arg–Gly–MePhe–Met(O)–ol, was synthesized starting with Z(OMe)–Met–ol[6] prepared by the reduction of Z(OMe)–Met–OPCP with $NaBH_4$. This, after removal of the Z(OMe) group with TFA,[7] was condensed successively with Z(OMe)–MePhe–OH and Boc–Gly–OH by the mixed anhydride procedure as shown in Fig. 1.

Attempts to crystallize Boc–Gly–MePhe–Met–ol have been unsuccessful. This peptide was oxidized to the corresponding sulfoxide, Boc–Gly–MePhe–Met(O)–ol, by sodium metaperi-

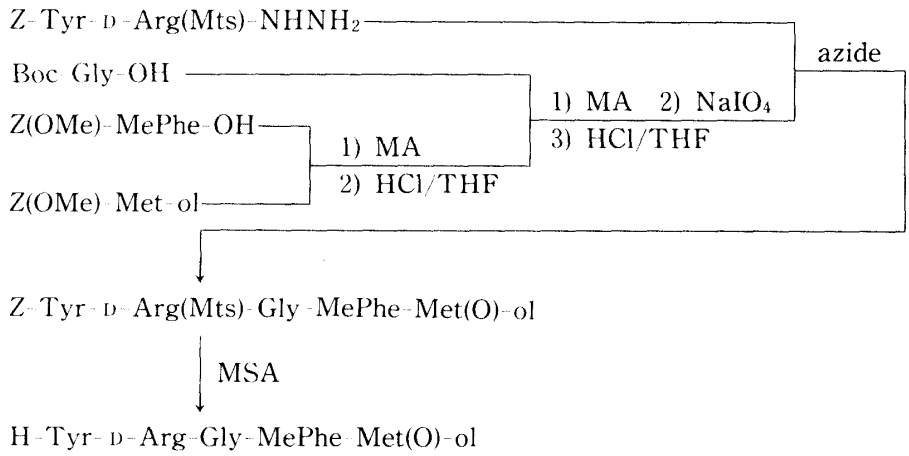

Fig. 1. Synthetic Scheme for [D-Arg[2], MePhe[4], Met(O)[5]]enkephalinol

odate.[8] The Boc group was removed from the resulting powder by TFA treatment as usual and the resulting peptide was condensed with Z–Tyr–D-Arg(Mts)–$NHNH_2$[2] by Rudinger's azide procedure.[9]

Z and Mts[10] were removed from the resulting protected peptide by MSA treatment.[11] In order to suppress a possible side reaction at the Tyr residue, *i.e.*, *O*-mesitylene sulfonylation,[8] a mixture of scavengers, thioanisole–*o*-cresol, was employed. The deblocked peptide was converted to the corresponding acetate by treatment with Dowex 1 (acetate form) and purified by gel-filtration on Sephadex G-15, followed by column chromatography on carboxymethyl (CM)-cellulose. In the latter step, gradient elution was employed to elute the desired compound with ammonium acetate buffers. After lyophilization, homogeneity of the product was ascertained by thin layar chromatography (TLC) acid hydrolysis and elemental analysis.

The second compound, Me–Tyr–D-Arg–Gly–MePhe–Met(O)–ol, was synthesized starting with H–Gly–MePhe–Met(O)–ol (Fig. 2). This tripeptide was condensed successively with Z(OMe)–D-Arg(Mts)–OH and Boc–MeTyr(Bzl)–OH[12] by the DCC–HOSu[13] and the TCP[14] ester procedures, respectively. Deprotection and purification of the resulting protected peptide, Boc–MeTyr–D-Arg(Mts)–Gly–MePhe–Met(O)–ol, were performed in essentially the same manner as mentioned above.

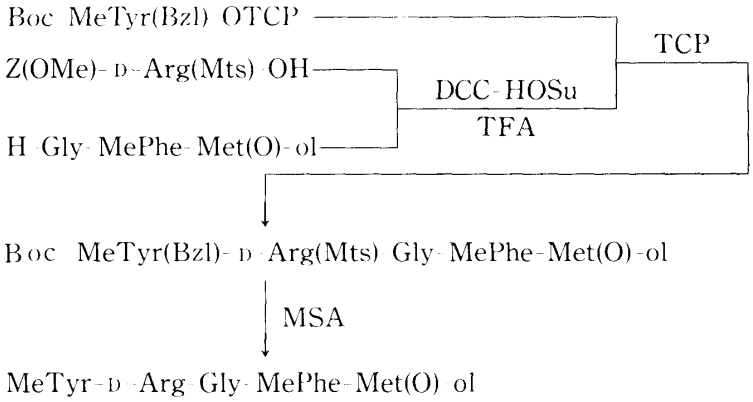

Fig. 2. Synthetic Scheme for Me–Tyr–D-Arg–Gly–MePhe–Met(O)-ol

The third compound H–Tyr–D-Arg–Gly–MePhe–Leu–ol was synthesized starting with Z(OMe)–Leu–ol prepared by the reduction of the corresponding active ester of Z(OMe)–Leu–OH with $NaBH_4$ (Fig. 3). Z(OMe)–Leu–ol, after removal of the Z(OMe) group with TFA, was condensed with Z(OMe)–MePhe–OH by the mixed anhydride procedure. The resulting peptide, Z(OMe)–MePhe–Leu–ol, was obtained as an oily compound, from which the Z(OMe) group was cleaved with HCl–dioxane to afford the hydrochloride as a crystalline compound. The *N*-

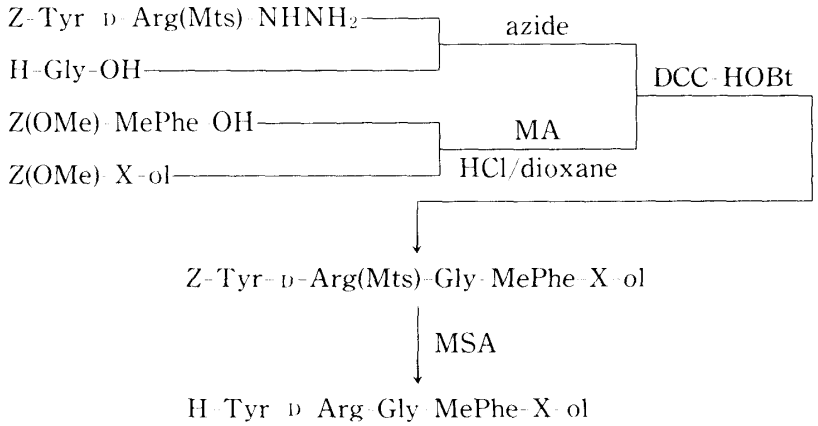

Fig. 3. Synthetic Scheme for H–Tyr–D-Arg–Gly–MePhe–X-ol (X=Leu or Ile)

terminal tripeptide, Z-Tyr-D-Arg(Mts)-Gly-OH, was prepared by the 2+1 coupling method, *i.e.*, the azide condensation of Z-Tyr-D-Arg(Mts)-$NHNH_2$ with the triethylammonium salt of H-Gly-OH, and then condensed with H-MePhe-Leu-ol obtained above by the DCC-HOBt[15] procedure to afford Z-Tyr-D-Arg(Mts)-Gly-MePhe-Leu-ol. The corresponding Ile-ol compound, Z-Tyr-D-Arg(Mts)-Gly-MePhe-Ile-ol, was similarly prepared. The deprotection and purification of these two enkephalinol analogs were performed in essentially the same manner as described above.

The analgesic effects of these four synthetic enkephalinol analogs are listed in Table I. In terms of the tail-flick test, H-Tyr-D-Arg-Gly-MePhe-Met(O)-ol exhibited analgesic activity comparable to that of morphine when administered intravenously. However, the corresponding *N*-methylated compound MeTyr-D-Arg-Gly-MePhe-Met(O)-ol, was less active. The corresponding Leu-ol, and Ile-ol compounds were also less active. H-Tyr-D-Arg-Gly-MePhe-Leu-ol seems to be toxic. After intravenous administration, all of the mice tested (5) died of respiratory collapse within a few minutes.

Table I. Analgesic Effects of Enkephalinol Analogs intravenously administered to Mice

Compound	ED_{50} (mg/kg) (95% confidence limits)
H-Tyr-D-Arg-Gly-MePhe-Met(O)-ol	0.62(0.31—1.22)
Me-Tyr-D-Arg-Gly-MePhe-Met(O)-ol	2.8 (1.4 —5.8)
H-Tyr-D-Arg-Gly-Mephe-Leu-ol	≒10
H-Tyr-D-Arg-Gly-MePhe-Ile-ol	>20
Morphine	0.85(0.56—1.28)

(tail-flick method)

The analgesic effect of H-Tyr-D-Arg-Gly-MePhe-Met(O)-ol was also evaluated by the tail-pinch method. The ED_{50} value of this peptide (5.2 mg/kg) was comparable to that of morphine (4.0 mg/kg) in the mouse after intravenous administration.

Experimental

General experimental procedures were essentially the same as those described in the previous paper.[2] Thin layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$-MeOH-H_2O (8: 3: 1), Rf_2 $CHCl_3$-MeOH-AcOH (95: 5: 3), Rf_3 *n*-BuOH-AcOH-pyridine-H_2O 4: 1: 1: 2), Rf_4 *n*-BuOH-AcOH-AcOEt-H_2O (1: 1: 1: 1).

Z(OMe)-MePhe-OH·DCHA——CH_3I (5 ml) and NaH (1.32 g, 55 mmol) were added to a solution of Z(OMe)-Phe-OH (3.29 g, 10 mmol) in THF (50 ml) under ice cooling. The mixture was stirred at room temperature for 4 h, then the solvent was removed by evaporation and the residue was dissolved in H_2O. This solution was washed with AcOEt, and acidified with citric acid. The resulting precipitate was extracted with AcOEt. The extract was washed with 5% $Na_2S_2O_3$ and H_2O-NaCl, dried over Na_2SO_4 and concentrated. The residue was dissolved in ether (20 ml) and dicyclohexylamine (2 ml, 10 mmol) was added. The solvent was evaporated off and the residue was triturated with petroleum ether. The resulting solid was recrystallized from AcOEt and petroleum ether; yield 4.37 g, (90%), mp 100—102°C, $[\alpha]_D^{24}$ −18.0° (c=0.3, MeOH), Rf_2 0.34. *Anal.* Calcd for $C_{31}H_{44}N_2O_5$: C, 70.96; H, 8.45; N, 5.34. Found: C, 70.94; H, 8.42; N, 5.30.

Z(OMe)-MePhe-Met-ol——Z(OMe)-Met-ol (2.28 g, 7.63 mmol) was treated with TFA-anisole (6 ml-4 ml) in an ice-bath for 60 min, then petroleum ether was added. The resulting oily precipitate was washed with petroleum ether, dried over KOH pellets *in vacuo* and then dissolved in THF (5 ml) containing Et_3N (1.06 ml, 7.63 mmol). The mixed anhydride [prepared from 4.00 g, (7.63 mmol) of Z(OMe)-MePhe-OH·DCHA as usual] in THF (10 ml) was added to the above ice-chilled solution and the mixture was stirred in an ice-bath for 2 h and then for an additional 3 h at room temperature. The solvent was evaporated off and the residue was dissolved in AcOEt. This solution was washed with 0.1 N HCl, 5% $NaHCO_3$ and H_2O-NaCl, dried over Na_2SO_4 and then concentrated to give an oily product. Rf_1 0.77. Attempts to solidify this compound have been unsuccessful. yield 3.34 g (95%).

HCl·H-MePhe-Met-ol——Z(OMe)-MePhe-Met-ol (4.61 g, 10 mmol) dissolved in THF (5 ml) was treated with 6.2 N HCl-THF (20 ml) in the presence of dimethylsulfide (3 ml) for 1 h. The product was precipitated

by addition of dry ether, and then recrystallized from EtOH and ether; yield 2.67 g (80%), mp 152—154°C, $[\alpha]_D^{24}$ +14.4° (c=0.2 MeOH) (lit.[3] +12.6 in MeOH). Rf_1 0.61. *Anal.* Calcd for $C_{15}H_{25}N_2O_2SCl \cdot 1/4H_2O$: C, 53.40; H, 7.62; N, 8.30. Found: C, 53.67; H, 7.44; N, 8.04.

Boc-Gly-MePhe-Met-ol——In the usual manner, a mixed anhydride [prepared from 1.04 g, (8 mmol) of Boc-Gly-OH] in THF (10 ml) was added to a solution of HCl·H-MePhe-Met-ol (2.66 g, 8 mmol) and Et_3N (1.12 ml, 8 mmol) in DMF (15 ml). After being stirred at 0°C for 2 h and then at room temperature for 3 h, the solution was concentrated, and the residue was dissolved in AcOEt. This solution was washed with 0.1 N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then concentrated to give an oily residue. Rf_1 0.63, yield 3.60 g (99%).

Boc-Gly-MePhe-Met(O)-ol——To a solution of Boc-Gly-MePhe-Met-ol (3.60 g, 7.9 mmol) in MeOH (40 ml), $NaIO_4$ (1.71 g, 8 mmol) in H_2O (40 ml) was added. After being stirred at room temperature for 3 h, the solution was filtered. The filtrate was concentrated and the residue was dissolved in AcOEt. This solution was washed with 0.1 N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then evaporated to dryness. The residue was triturated with n-hexane; yield 3.63 g (98%), mp 74—78°C, $[\alpha]_D^{24}$ −54.2° (c=0.2, MeOH), Rf_1 0.39. *Anal.* Calcd for $C_{22}H_{35}N_3O_6S$: C, 56.27; H, 7.51; N, 8.95. Found: C, 56.29; H, 7.40; N, 8.29.

HCl·H-Gly-MePhe-Met(O)-ol——A solution of Boc-Gly-MePhe-Met(O)-ol (2.58 g, 5.4 mmol) in THF (10 ml) was treated with 6.2 N HCl–THF (20 ml), and the mixture was stirred at room temperature for 1 h. After addition of ether, the resulting powder was collected by filtration and recrystallized from EtOH and ether; yield 1.87 g (84%), mp 95—99°C, $[\alpha]_D^{24}$ −46.5° (c=0.4, MeOH) Rf_1 0.29. *Anal.* Calcd for $C_{17}H_{27}N_3O_4S \cdot HCl \cdot 1/2H_2O$: C, 49.20; H, 7.04; N, 10.13. Found: C, 48.94; H, 7.21; N, 9.71.

Z-Tyr-D-Arg(Mts)-Gly-MePhe-Met(O)-ol——HCl·H-Gly-MePhe-Met(O)-ol (0.81 g, 2 mmol) was dissolved in DMF (5 ml) containing Et_3N (0.28 ml, 2 mmol). To this ice-chilled solution, the azide [prepared from 1.34 g, (2 mmol) of Z-Tyr-D-Arg (Mts)-$NHNH_2$] in DMF (5 ml) and Et_3N (0.28 ml, 2 mmol) were added. After being stirred at 4°C for 48 h, the solution was concentrated and the residue was treated with ether. The resulting powder was washed with 0.2 N AcOH and H_2O and precipitated from DMF with ether, yield 1.54 g (77%), mp 145—150°C, $[\alpha]_D^{24}$ −22.1° (c=0.4, MeOH), Rf_1 0.44. *Anal.* Calcd for $C_{49}H_{64}N_8O_{11}S_2 \cdot H_2O$: C, 57.51; H, 6.50; N, 10.95. Found: C, 57.72; H, 6.20; N, 10.65.

H-Tyr-D-Arg-Gly-MePhe-Met(O)-ol——The above protected peptide (502 mg, 0.5 mmol) was treated with MSA–TFA (3 ml–1 ml) in the presence of thioanisole–o-cresol (1: 1, v/v, 1 ml) in an ice-bath for 15 min and at room temperature for 60 min, then dry ether was added. The resulting oily precipitate was washed with ether, dissolved in H_2O (10 ml) and treated with Dowex 1 (acetate form, approximately 3 g) for 30 min. The resin was removed by filtration, and the filtrate was lyophilized. The residue was subjected to gel-filtration on Sephadex G-15 (3×110 cm), which was eluted wth 0.2 N AcOH. Individual fractions (5 ml each) were collected and the ultraviolet (UV) absorption at 275 nm was determined. The desired fractions (tube Nos. 65—104) were combined and the solvent was removed by lyophilization. The resulting powder was dissolved in H_2O (30 ml) and applied to a column of CM-cellulose (1.8×50 cm), which was first eluted with H_2O (300 ml) and then with a gradient formed from 0.5 M NH_4OAc (pH 6.9, 1000 ml) through a mixing flask containing H_2O (1000 ml). Individual fractions (10 ml each) were collected and the UV absorption at 275 nm was determined. The desired fractions (tube Nos. 52—74) were combined and the solvent was removed by lyophilization. For desalting, the product was dissolved in 0.2 N AcOH and the solution was applied to a column of Sephadex G-15 (3×110 cm), which was eluted with the same solvent. Individual fractions (5 ml each) were collected and the desired fractions (tube Nos. 73—86) were collected in essentially the same manner as described above. Lyophilization gave a white fluffy powder; yield 150 mg (37%), $[\alpha]_D^{24}$ +12.9° (c=0.6, 0.2 N AcOH), Rf_3 0.58, Rf_4 0.60. Amino acid ratios in 3 N Tos-OH hydrolysate; Tyr 0.95, Arg 0.98, Gly 1.00 (average recovery 89%). *Anal.* Calcd for $C_{32}H_{48}N_8O_7S \cdot 2CH_3COOH \cdot 2.5H_2O$: C, 50.63; H, 7.20; N, 13.12. Found: C, 50.67; H, 6.62; N, 12.60.

Boc-MeTyr (Bzl)-OTCP——CH_3I (17 ml, 0.27 mol) and NaH (4.35 g, 0.18 mol) were added to a solution of Boc-Tyr(Bzl)-OH (12.36 g, 33.3 mmol) in THF (90 ml) under ice cooling. The mixture was stirred at room temperature for 18 h, then the solvent was evaporated off and the residue was dissolved in H_2O. The aqueous phase was washed with AcOEt and then acidified with citric acid. The resulting precipitate was extracted with AcOEt. The extract was washed with 5% $Na_2S_2O_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The residue was dissolved in THF (50 ml) and then HOTCP (6.58 g, 33 mmol) and DCC (6.87 g, 33.3 mmol) were added. After being stirred at room temperature for 18 h, the solution was filtered and the filtrate was concentrated. The residue was triturated with n-hexane and recrystallized from n-hexane; yield 16.58 g (88%), mp 71—73°C, $[\alpha]_D^{24}$ −58.7° (c=0.3, MeOH) Rf_1 0.92. *Anal.* Calcd or $C_{28}H_{28}NO_5Cl_3$: C, 59.53; H, 4.99; N, 2.48. Found: C, 59.92; H, 5.08; N, 2.54.

Z(OMe)-D-Arg(Mts)-Gly-MePhe-Met(O)-ol——HOSu (130 mg, 1.13 mmol), HCl·H-Gly-MePhe-Met(O)-ol (459 mg, 1.13 mmol), and Et_3N (0.16 ml, 1.13 mmol) were added to a solution of Z(OMe)-D-Arg(Mts)-OH [prepared from 700 mg, (1.13 mmol) of the DCHA salt as usual] in THF (3 ml). To this mixture, DCC (233 mg, 1.13 mmol) was added and the whole was stirred at room temperature for 18 h. The solution was filtered, and the filtrate was concentrated. The residue was dissolved in AcOEt. This solution was washed with 0.1 N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then concentrated. The residue was

triturated with ether and precipitated with THF and ether; yield 650 mg (66%), mp 108—112°C, $[\alpha]_D^{24}$ −23.8° (c=0.3, MeOH). Rf_1 0.54. *Anal.* Calcd for $C_{41}H_{57}N_7O_{10}S_2 \cdot 1.5H_2O$: C, 54.77; H, 6.73; N, 10.91. Found: C, 54.65; H, 6.33; N, 10.75.

Boc-MeTyr(Bzl)-D-Arg(Mts)-Gly-MePhe-Met(O)-ol——In the usual manner, Z(OMe)-D-Arg(Mts)–Gly–MePhe–Met(O)–ol (523 mg, 0.6 mmol) was treated with TFA–anisole (0.8 ml–0.3 ml) at 0°C for 1 h, then dry ether was added. The resulting powder was dissolved in DMF (3 ml), together with Boc–MeTyr(Bzl)–OTCP (339 mg, 0.6 mmol), HOBt (81 ml, 0.6 mmol) and Et_3N (0.17 ml, 1.2 mmol). After being stirred at room temperature for 24 h, the solution was concentrated and the residue was triturated with ether. The resulting powder was recrystallized from EtOH and ether. Yield 477 mg (74%), mp 119—123°C, $[\alpha]_D^{24}$ −36.9° (c=0.3, MeOH). Rf_1 0.61. *Anal.* Calcd for $C_{54}H_{74}N_8O_{11}S_2 \cdot H_2O$: C, 59.32; H, 7.01; N, 10.25. Found: C, 59.51; H, 6.68; H, 10.76.

Me-Tyr-D-Arg-Gly-MePhe-Met(O)-ol——The above protected peptide (323 mg, 0.3 mmol) was treated with MSA–TFA (2 ml–0.5 ml) in the presence of thioanisole (0.3 ml) and *o*-cresol (0.3 ml) in an ice-bath for 15 min and at room temperature for 1 h. The deprotected peptide was converted to the corresponding acetate, and purified by column chromatography on Sephadex G-15 followed by CM-cellulose as described for the purification of H–Tyr-D-Arg–Gly–MePhe–Met(O)–ol; yield 83 mg (52%), $[\alpha]_D^{24}$ +33.7° (c=0.1, 0.2 N AcOH) Rf_4 0.67. *Anal.* Calcd for $C_{33}H_{50}N_8O_7S \cdot CH_3COOH \cdot 1.5H_2O$: C, 53.28; H, 7.28; N, 14.20. Found: C, 53.59; H, 6.84; N, 14.51.

Z-Tyr-D-Arg(Mts)-Gly-OH——To a solution of H–Gly–OH (338 mg, 4.5 mmol) in H_2O (3 ml) containing Et_3N (0.63 ml, 4.5 mmol), the azide [prepared from 2.00 g, (3 mmol) of Z–Tyr-D-Arg(Mts)–$NHNH_2$] in DMF (4 ml) and Et_3N (0.42 ml, 3 mmol) were added. After being stirred at 4°C for 48 h, the solution was concentrated and the residue was treated with 0.2 N AcOH and ether. The resulting powder was recrystallized from MeOH and ether; yield 1.87 g (88%), mp 125—130°C, $[\alpha]_D^{24}$ +16.9° (c=0.4, MeOH) Rf_1 0.23. *Anal.* Calcd for $C_{34}H_{42}N_6O_9S \cdot H_2O$: C, 56.03; H, 6.09; N, 11.53. Found: C, 56.33; H, 6.46; N, 11.22.

HCl·H-MePhe-Leu-ol——Z(OMe)–Leu–ol (0.84 g, 3 mmol) was treated with TFA–anisole (2.3 ml–1.6 ml) as usual and *n*-hexane was added. The precipitate was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 h and dissolved in THF (4 ml) containing Et_3N (0.35 ml). To this ice-chilled solution, the mixed anhydride [prepared from 1.31 g, (2.5 mmol) of the DCHA salt of Z(OMe)–MePhe–OH] in THF (4 ml) was added and the mixture was stirred in an ice-bath for 1 h then at room temperature for 3 h. The solvent was evaporated off and the residue was dissolved in AcOEt. This solution was washed with 0.1 N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The oily residue was treated with 2.5 N HCl–dioxane in the presence of anisole (1.5 ml) at room temperature for 1 h. Ether was added and the resulting powder was recrystallized from EtOH and ether; yield 0.40 g (51%), mp 207—210°C, $[\alpha]_D^{24}$ +10.0° (c=0.2, MeOH), Rf_1 0.54. *Anal.* Calcd for $C_{16}H_{27}N_2O_2Cl$: C, 61.03; H, 8.64; N, 8.90. Found: C, 60.88; H, 8.47; N, 8.79.

Z-Tyr-D-Arg(Mts)-Gly-MePhe-Leu-ol——DCC (165 mg, 0.8 mmol) was added to a mixture of Z–Tyr–D-Arg(Mts)–Gly–OH (569 mg, 0.8 mmol), HOBt (108 mg, 0.8 mmol), HCl·H–MePhe–Leu–ol (255 mg, 0.8 mmol) and Et_3N (0.11 ml, 0.8 mmol) in DMF (2 ml) and the mixture, after being stirred at room temperature for 24 h, was filtered. The filtrate was concentrated and the residue was dissolved in AcOEt. This solution was washed with 1 N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then concentrated. Trituration of the residue with ether afforded a powder; yield 560 mg (72%), mp 132—136°C, $[\alpha]_D^{24}$ +3.0° (c=0.3, MeOH), Rf_1 0.61. *Anal.* Calcd for $C_{50}H_{66}N_8O_{10}S \cdot 1.5H_2O$: C, 60.16; H, 6.97; N, 11.23. Found: C, 60.30; H, 6.90; N, 11.50.

H-Tyr-D-Arg-Gly-MePhe-Leu-ol——The above protected peptide (286 mg, 0.29 mmol) was treated with MSA–TFA (3 ml–1 ml) in the presence of thioanisole (1 ml) in an ice-bath for 15 min and at room temperature for 1 h. The deprotected peptide was converted to the corresponding acetate and purified by gel filtration on Sephadex G-15, followed by column chromatography on CM-cellulose as described above; yield 95 mg (50%), $[\alpha]_D^{24}$ +25.2° (c=0.3, 0.2 N AcOH). Rf_4 0.63. Amino acid ratios in 3 N Tos-OH hydrolysate; Tyr 0.97, Arg 1.04, Gly 1.00 (average recovery 87%). *Anal.* Calcd for $C_{33}H_{50}N_8O_6 \cdot 2CH_3COOH \cdot 4H_2O$: C, 52.46; H, 7.85; N, 13.23. Found: C, 52.08; H, 7.91; N, 12.96.

HCl·H-MePhe-Ile-ol——Z(OMe)–Ile–ol (0.70 g, 2.5 mmol) was treated with TFA–anisole (2.3 ml–1.6 ml) as usual and *n*-hexane was added. The precipitate was washed with *n*-hexane, dried *in vacuo* and dissolved in THF (4 ml) containing Et_3N (0.35 ml, 2.5 mmol). To this ice-chilled solution, the mixed anhydride [prepared from 1.31 g, (2.5 mmol) of the DCHA salt of Z(OMe)–MePhe–OH as usual] in THF (4 ml) was added and the mixture was stirred in an ice-bath for 2 h then at room temperature for 18 h. The solvent was evaporated off and the residue was dissolved in AcOEt. This solution was washed with 0.1 N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and concentrated. The oily residue was treated with 2.5 N HCl–dioxane in the presence of anisole (1.5 ml) as stated above. Ether was added and the resulting powder was recrystallized from EtOH and ether; yield 0.36 g (46%), mp 206—209°C, $[\alpha]_D^{24}$ +4.2° (c=0.5, MeOH), Rf_1 0.54. *Anal.* Calcd for $C_{16}H_{27}N_2O_2Cl \cdot 1/4H_2O$: C, 60.17; H, 8.68; N, 8.77. Found: C, 60.36; H, 8.76; N, 8.53.

Z-Tyr-D-Arg(Mts)-Gly-MePhe-Ile-ol——DCC (165 mg, 0.8 mmol) was added to a mixture of Z–Tyr-D-Arg(Mts)–Gly–OH (569 mg, 0.8 mmol), HOBt (108 mg, 0.8 mmol), HCl·H–MePhe–Ile–ol (252 mg, 0.8 mmol)

and Et_3N (0.11 ml, 0.8 mmol) in DMF (4 ml) and the mixture, after being stirred at room temperature for 24 h, was filtered. The filtrate was concentrated and the residue was dissolved in AcOEt. This solution was washed with 1 N HCl, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then concentrated. Trituration of the residue with ether afforded a powder; yield 544 mg (70%), mp 130—135°C, $[\alpha]_D^{24}$ +4.8° (c=0.3, MeOH), Rf_1 0.61. *Anal.* Calcd for $C_{50}H_{66}N_8O_{10}S \cdot H_2O$: C, 60.72; H, 6.92; N, 11.33. Found: C, 60.55; H, 6.65; N, 11.29.

H-Tyr-D-Arg-Gly-MePhe-Ile-ol——The above protected peptide (191 mg, 0.2 mmol) was treated with MSA–TFA (2.5 ml–1 ml) in the presence of thioanisole–*o*-cresol (1: 1, v/v, 1 ml) in an ice-bath for 15 min and at room temperature for 1 h. The deprotected peptide was converted to the corresponding acetate and purified by gel filtration on Sephadex G-15, followed by column chromatography on CM-cellulose as described above; yield 64 mg (49%) $[\alpha]_D^{24}$ +34.2° (c=0.1, 0.2 N AcOH), Rf_4 0.63. Amino acid ratios in 3 N Tos-OH hydrolysate; Tyr 0.93, Arg 0.97, Gly 1.00 (average recovery 85%). *Anal.* Calcd for $C_{33}H_{50}N_8O_6 \cdot 2CH_3COOH \cdot 2H_2O$: C, 54.80; H, 7.71; N, 13.82. Found: C, 55.22; H, 7.30; N, 13.58.

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References and Notes

1) The following abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, HOBt=1-hydroxybenzotriazole HOSu: *N*-hydroxysuccinimide, MSA=methanesulfonic acid, DMF=dimethylformamide THF=tetrahydrofuran, TFA=trifluoroacetic acid, Mts=mesitylene-2-sulfonyl, OPCP=pentachlorophenyl ester, OTCP=2,4,5-trichlorophenyl ester, DCHA=dicyclohexylamine, Met(O)-ol=methioninol sulfoxide, Leu-ol=leucinol, Ile-ol=isoleucinol.
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