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Use of the 4-Methoxy-2,6-dimethylbenzenesulfonyl (Mds) Group to Synthesize Dynorphin [1-13] and Related Peptides¹⁾

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The syntheses of a tridecapeptide corresponding to the amino acid sequence of dynorphin [1-13], H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH, and related truncated peptides, [4-13], [8-11], and [8-13], using the 4-methoxy-2,6-dimethylbenzenesulfonyl (Mds) group for the protection of the guanidino function of arginine, are described.

To synthesize dynorphin[1-13], three fragments, 1-3, 4-10, and 11-13, were prepared and used as building blocks for the final construction of the amino acid sequence of this peptide. Final deprotection of the fully protected peptide was achieved by treatment with trifluoroacetic acid-thioanisole at 50°C for 2 h and the purification of this synthetic peptide was effected by column chromatography on CM-cellulose. Other truncated peptides, [4-13], [8-11], and [8-13], were synthesized in the same manner as described for dynorphin [1-13]. The applicability of the Mds protecting group for the synthesis of arginine-containing peptides was confirmed.

Keywords—dynorphin[1-13]; N^ε-4-methoxy-2,6-dimethylbenzenesulfonylarginine; trifluoroacetic acid-thioanisole deprotection; HONB-DCC method; HOBT-DCC method; truncated peptide

Dynorphin is a leucine-enkephalin-containing peptide isolated from porcine pituitary extracts. The structure of the N-terminal tridecapeptide dynorphin [1-13] was elucidated as H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH by Goldstein *et al.*²⁾ This tridecapeptide contains three arginine residues in its sequence.

Very recently, we introduced the 4-methoxy-2,6-dimethylbenzenesulfonyl (Mds) group as a new protecting group for the guanidino function of arginine.³⁾ This group could be removed cleanly by treatment with trifluoroacetic acid-thioanisole⁴⁾ at 50°C for 1-2 h. In order to demonstrate the usefulness of this group in peptide chemistry, we have already reported the syntheses of substance P and two LH-RH analogs.³⁾

This paper deals with further application of the Mds group for the syntheses of dynorphin [1-13] and related fragment peptides [4-13], [8-11], and [8-13]. Our synthetic scheme for dynorphin [1-13] is outlined in Fig. 1.

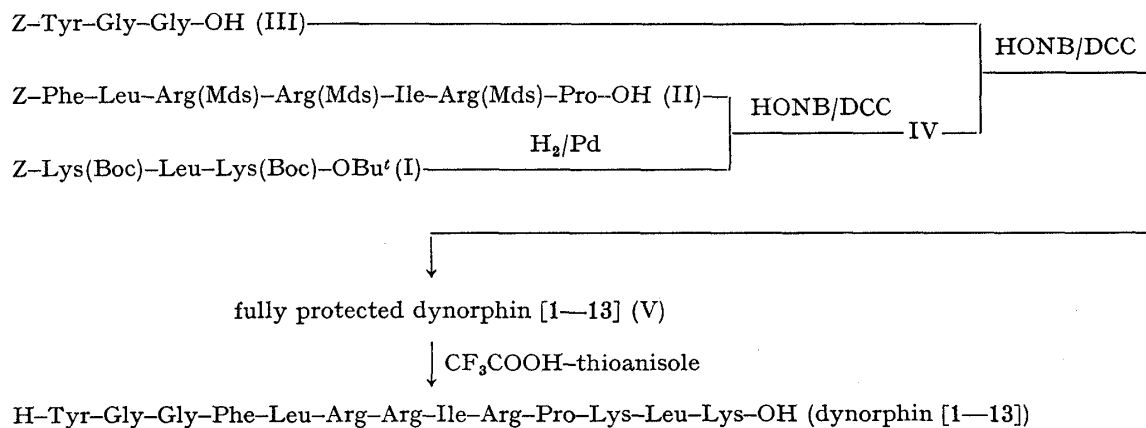


Fig 1 Synthetic Route to Dynorphin [1-13]

The α -amino function of the intermediates was protected by the Z group, in addition to the Mds protection of the guanidino function of arginine, and the side chain of Lys was protected by the Boc group.

As shown in Fig. 1, three peptide fragments, Z-Lys(Boc)-Leu-Lys(Boc)-OBu^t (I), Z-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-OH (II), and Z-Tyr-Gly-Gly-OH (III) were chosen for construction of the total sequence. All peptides were prepared by stepwise chain elongation starting from the carboxy-end amino acid using HONB-activated ester⁵⁾ for fragments I and III and the HOBT-DCC method⁶⁾ for fragment II. The synthetic routes to these fragments are shown in Figs. 2–4.

In synthesizing fragment I, we chose Z-Lys(Boc)-OBu^t as a starting material and used the POCl₃ method.⁷⁾ However, some degree of racemization can occur in this method. For this reason, Z-Leu-Lys-OH obtained by treatment of Z-Leu-Lys(Boc)-OBu^t with TFA was compared with Z-D-Leu-Lys-OH by high performance liquid chromatography (HPLC)⁸⁾ and we found that Z-Leu-Lys(Boc)-OBu^t contained a small amount of the D-Lys isomer (*ca.* 5%). However, Z-Leu-Lys(Boc)-OBu^t was successfully crystallized and the D-isomer could be removed by recrystallization (checked by HPLC).⁸⁾

In synthesizing fragment II, all the protected amino acids were introduced by the HOBT-DCC procedure. Since Z-Pro-OBu^t was chosen as a starting material, it was necessary to

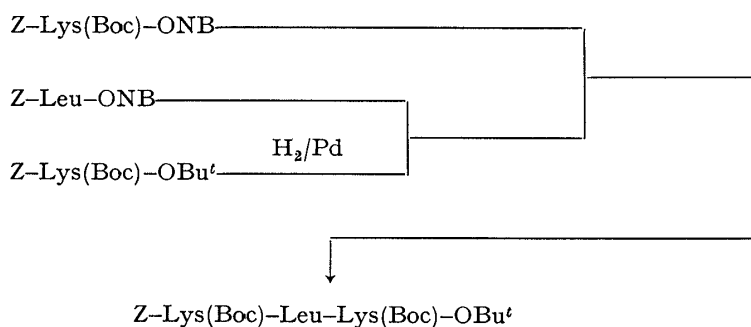


Fig. 2. Preparation of the Protected Tripeptide I

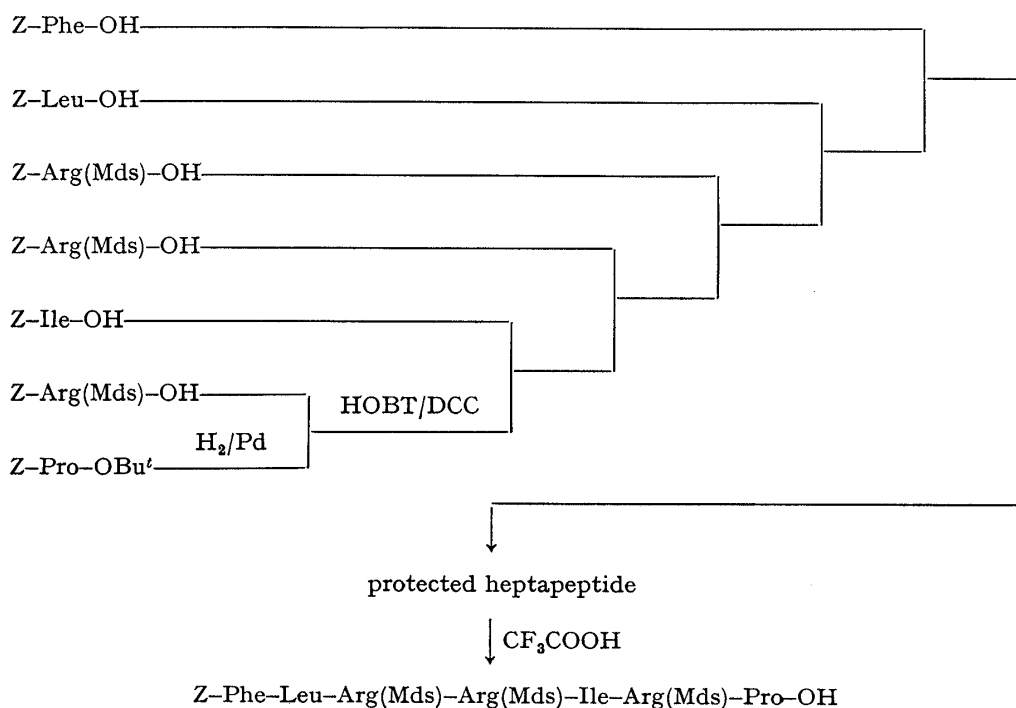


Fig. 3. Preparation of the Protected Heptapeptide II

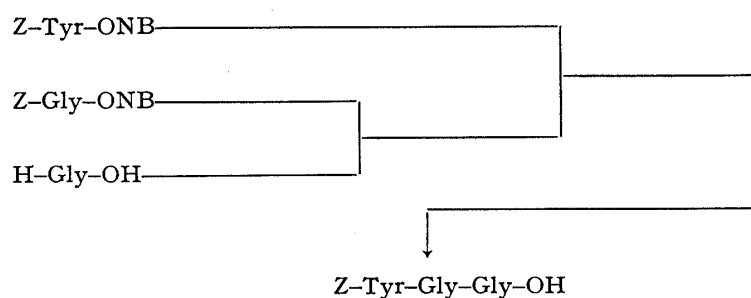


Fig. 4. Preparation of the Protected Tripeptide III

treat Z-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-OBu' with TFA to obtain the free acid. During treatment with TFA at room temperature for 30 min, some cleavage of the Mds group was observed (checked by thin-layer chromatography (TLC)). However, the free acid II was soluble in organic solvents (ethyl acetate and chloroform) and could be purified by column chromatography on silica gel. This high solubility of fragment II may be due to the Mds group.

In synthesizing the entire amino acid sequence of the tridecapeptide, the protected tripeptide I was hydrogenated over Pd-black as a catalyst in MeOH, and the resulting free base of I was condensed with fragment II by the HONB-DCC procedure in DMF, giving Z-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-Lys(Boc)-Leu-Lys(Boc)-OBu' (IV). IV was obtained in 93.3% yield. The Z group of the decapeptide IV was removed by hydrogenation, and the free base was coupled to fragment III by the HONB-DCC method to afford the protected tridecapeptide, Z-Tyr-Gly-Gly-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-Lys(Boc)-Leu-Lys(Boc)-OBu' (V). The protected tridecapeptide was obtained in 86.8% yield. To remove all the protecting groups, V was treated with TFA-thioanisole (9:1) at 50°C for 2 h, and the resulting product was immediately converted into the corresponding acetate with Amberlite IRA 410 (acetate form), then purified on a carboxymethyl-cellulose column by gradient elution using pH 6.8 ammonium acetate buffer (0.005–0.6 M). Dynorphin [1–13] was obtained as a white powder in 56.3% yield. Other truncated peptides of dynorphin were prepared from the corresponding materials in the same manner as described for dynorphin [1–13]. The final product thus obtained exhibited a single spot on TLC and paper electrophoresis. Amino acid analyses of the acid hydrolysate and aminopeptidase-M hydrolysate gave results which agreed well with the theoretical values. The opiate activity of the synthetic dynorphin [1–13] was measured by the method of Pert and Snyder⁹⁾ with some modifications (the use of a membrane fraction from rat brain homogenate); the results are summarized in Table I.

TABLE I. Receptor Assay of Synthetic Dynorphin [1–13]

Compound	Inhibition of ³ H-naloxone stereospecific binding (IC ₅₀)	
	– NaCl	+ 100 mM NaCl
	(nM)	(nM)
Morphine	11	710
Met-enkephalin	19	500
β _h -Endorphin	6.5	580
Dynorphin [1–13]	6.2	380

In the receptor assay, dynorphin [1–13] exhibited almost the same activity as β_h-endorphin. Immunological studies of dynorphin using these synthetic peptides are under way and the results will be reported in another paper.¹⁰⁾ The present results clearly indicate that Mds is a useful protecting group in peptide synthesis.

Experimental

All melting points were taken by the capillary method and are uncorrected. Rotations were determined with a Perkin-Elmer model 141 polarimeter. Acid hydrolyses were carried out in 6 N HCl 110°C for 24 h and aminopeptidase-M hydrolysis was carried out in 0.1 M Tris-HCl buffer (pH 7.5) at 37°C for 24 h. Amino acid analyses were performed on a Hitachi 835 amino acid analyzer. Solutions were concentrated in a rotary evaporator under reduced pressure at a temperature of 30–40°C. Catalytic hydrogenations were performed at room temperature with palladium (Pd) black as a catalyst in MeOH. The purity of the products was tested by thin-layer chromatography on silica gel (precoated silica gel plate 60F₂₅₄, Merck) or cellulose (Avicel, Funakoshi Yakuhin Co. Ltd.) plates. Solvent systems used were CHCl₃-MeOH-AcOH (9:1:0.5, *R*_f¹), *n*-BuOH-pyridine-AcOH-H₂O (30:20:6:24, *R*_f²), AcOEt-*n*-BuOH-AcOH-H₂O (1:1:1:1, *R*_f³). *R*_f values are given for silica gel unless otherwise mentioned.

4-Methoxy-2,6-dimethylbenzenesulfonyl Chloride (Mds-Cl)—This compound was synthesized according to the method of Buchanan *et al.*,¹¹ but the oily product showed 2 spots on TLC (CHCl₃-*n*-hexane (1:1), *R*_f 0.51, 0.34) and the ¹H nuclear magnetic resonance (NMR) spectrum in CDCl₃ showed resonances at δ 6.67, 3.85, and 2.70 together with 6.73, 3.96, 2.62, and 2.35. From these results, we concluded that the product contained some 2-methoxy-4,6-dimethylbenzenesulfonyl chloride (27% by ¹H NMR). For this reason, the oily material was purified by column chromatography on silica gel (CHCl₃-*n*-hexane (1:9)) and the crystalline product was obtained in 56.5% yield. mp 27–28°C, *R*_f (CHCl₃-*n*-hexane (1:1)) 0.51. *Anal.* Calcd for C₉H₁₁ClO₃S: C, 46.05; H, 4.72; Cl, 15.11; S, 13.66. Found: C, 46.12; H, 4.68; Cl, 15.01; S, 13.37.

Z-Arg(Mds)-OH·CHA—Z-Arg-OH (3.08 g) was dissolved in a mixture of 4 N aqueous NaOH-acetone (10 ml–40 ml) and cooled to 5°C with ice. To this was added Mds-Cl (4.20 g) dissolved in acetone (20 ml) and the mixture was stirred at room temperature for 3 h. After the usual work-up, cyclohexylamine (1.12 ml) was added and the crystals formed from AcOEt were filtered and recrystallized from acetonitrile: yield 3.70 g (61.1%), mp 140–141°C, [α]_D²⁵ +5.7° (*c*=0.5 in MeOH), *R*_f¹ 0.25. *Anal.* Calcd for C₂₃H₃₀N₄O₇S·C₆H₁₃N: C, 57.50; H, 7.15; N, 11.56; S, 5.29. Found: C, 57.23; H, 6.96; N, 11.66; S, 5.32.

Z-Lys(Boc)-OBut (Ia)—Z-Lys(Boc)-OH (12.1 g) was dissolved in a mixture of *tert*-BuOH (80 ml) and pyridine (32 ml) and the solution was cooled to –5°C. To this was added POCl₃ (3.2 ml) and the solution was stirred at 0°C for 1 h then at room temperature for an additional 15 h. After acidification with 10% citric acid, the product was extracted with AcOEt. The organic layer was washed with aqueous NaHCO₃ and dried over anhydr. Na₂SO₄. After removal of the solvent by evaporation, the product was obtained as an oil: yield 13.2 g (96.0%).

Z-Leu-Lys(Boc)-OBut (Ib)—Compound Ia (12.8 g) was hydrogenated and the resulting free base was coupled with Z-Leu-ONB prepared from Z-Leu-OH (7.5 g), HONB (5.6 g), and DCC (6.4 g). The solution was stirred for 15 h then worked up as usual. The product was crystallized from petroleum ether and recrystallized from AcOEt-petroleum ether: yield 11.4 g (70.1%), mp 84–86°C, [α]_D²⁵ –22.7° (*c*=1.2 in MeOH), *R*_f¹ 0.79. *Anal.* Calcd for C₂₉H₄₇N₅O₇: C, 63.36; H, 8.62; N, 7.64. Found: C, 63.58; H, 8.77; N, 7.63.

Z-Lys(Boc)-Leu-Lys(Boc)-OBut (I)—Compound Ib (10.0 g) was hydrogenated and the free base obtained was coupled with Z-Lys(Boc)-ONB prepared from Z-Lys(Boc)-OH (6.93 g), HONB (3.60 g), and DCC (4.20 g). After the usual work-up, the material was crystallized from ether: yield 13.35 g (94.3%), mp 53–54°C, [α]_D²⁵ –29.8° (*c*=1.0 in MeOH), *R*_f¹ 0.72. *Anal.* Calcd for C₄₀H₆₇N₅O₁₀: C, 61.75; H, 8.68; N, 9.13. Found: C, 61.36; H, 8.73; N, 8.75.

Z-Arg(Mds)-Pro-OBut (IIa)—Z-Pro-OBut (13.8 g) was hydrogenated and the resulting product was coupled with Z-Arg(Mds)-OH prepared from Z-Arg(Mds)-OH·CHA (25.0 g) in the presence of HOBT (6.19 g) and DCC (9.3 g). After the usual work-up, the oily product was purified by column chromatography on silica gel (7.5×17 cm, 1% MeOH/CHCl₃): yield 17.6 g (65.1%), *R*_f¹ 0.61.

Z-Ile-Arg(Mds)-Pro-OBut (IIb)—Compound IIa (15.0 g) was hydrogenated and the free amine obtained was coupled to Z-Ile-OH (6.1 g) by the HOBT-DCC method. After the usual work-up, the material was precipitated from ether-petroleum ether: yield 16.0 g (90.4%), mp 79–80°C, [α]_D²⁵ –46.8° (*c*=0.9 in MeOH), *R*_f¹ 0.72. *Anal.* Calcd for C₃₈H₅₆N₆O₉S: C, 59.04; H, 7.30; N, 10.87; S, 4.15. Found: C, 59.17; H, 7.61; N, 10.73; S, 3.97.

Z-Arg(Mds)-Ile-Arg(Mds)-Pro-OBut (IIc)—Compound IIb (7.0 g) was hydrogenated and the resulting free base was coupled with Z-Arg(Mds)-OH prepared from Z-Arg(Mds)-OH·CHA (5.5 g) by the HOBT-DCC method. After the usual work-up, the product was purified by column chromatography on silica gel (6×13 cm, 3% MeOH/CHCl₃). The desired fractions were pooled and concentrated. The resulting residue was triturated with ether to give a precipitate: yield 9.1 g (89.1%), mp 103–107°C, [α]_D²⁵ –39.5° (*c*=1.0 in MeOH), *R*_f¹ 0.71. *Anal.* Calcd for C₅₃H₇₈N₁₀O₁₃S₂: C, 56.46; H, 6.97; N, 12.43; S, 5.69. Found: C, 56.19; H, 7.07; N, 12.08; S, 5.45.

Z-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-OBut (IId)—Compound IIc (7.0 g) was hydrogenated and the free base was condensed with Z-Arg(Mds)-OH prepared from Z-Arg(Mds)-OH·CHA (3.76 g) by the HOBT-DCC method. After the usual work-up, the material was purified by column chromatography on silica gel (6×10 cm, 5% MeOH/CHCl₃). The purified product was triturated with ether to give a precipitate: yield 5.0 g (54.3%), mp 127–130°C, [α]_D²⁵ –33.3° (*c*=1.0 in MeOH), *R*_f¹ 0.69. *Anal.* Calcd for C₆₉H₁₀₀N₁₄-

O₁₇S₃: C, 55.11; H, 6.80; N, 13.23; S, 6.49. Found: C, 54.90; H, 6.87; N, 12.90; S, 6.27.

Z-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-OBu^t (IId)—Compound IId (4.50 g) was hydrogenated and the free amine was coupled with Z-Leu-OH (0.85 g) by the HOBT-DCC procedure. After the usual work-up, the material was triturated with ether to give a precipitate: yield 4.50 g (92.8%), mp 125–128°C, $[\alpha]_D^{25} -37.7^\circ$ ($c=0.9$ in MeOH), R_f^1 0.55. Anal. Calcd for C₇₄H₁₁₁N₁₅O₁₈S₃: C, 55.72; H, 7.02; N, 13.17; S, 6.03. Found: C, 55.56; H, 6.92; N, 12.99; S, 5.94.

Z-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-OBu^t (IIe)—Compound IIe (4.00 g) was hydrogenated and the resulting residue was coupled with Z-Phe-OH (0.88 g) by the HOBT-DCC method. After the usual work-up, the resulting residue was triturated with ether to give a precipitate: yield 4.20 g (85.5%), mp 134–137°C, $[\alpha]_D^{25} -37.1^\circ$ ($c=0.9$ in MeOH), R_f^1 0.55. Anal. Calcd for C₈₃H₁₂₀N₁₆O₁₉S₃·H₂O: C, 56.63; H, 6.87; N, 12.73; S, 5.47. Found: C, 56.35; H, 6.66; N, 12.71; S, 5.47.

Z-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-OH (II)—Compound II (4.0 g) was treated with TFA at room temperature for 30 min. After concentration, the residue was triturated with ether to give a precipitate, which was further purified by column chromatography on silica gel (5 × 8 cm, CHCl₃-MeOH-AcOH=30:2:1). The material was triturated with ether to give a precipitate: yield 3.0 g (77.5%), mp 131–134°C, $[\alpha]_D^{25} -31.4^\circ$ ($c=0.9$ in MeOH), R_f^1 0.39. Anal. Calcd for C₇₉H₁₁₂N₁₆O₁₉S₃·H₂O: C, 55.68; H, 6.63; N, 13.15; S, 5.65. Found: C, 55.57; H, 6.50; N, 13.14; S, 5.60.

Z-Gly-Gly-OH (IIIa)—Glycine (15.0 g) and NaHCO₃ (16.8 g) were dissolved in water (100 ml) and to this was added Z-Gly-ONB prepared from Z-Gly-OH (40.0 g) in THF (400 ml). The mixture was stirred for 40 h. THF was evaporated off, and the remaining solution was acidified with 6N HCl to give crystals, which were collected by filtration and washed well with water: yield 44.4 g (87.3%), mp 169–172°C, R_f^1 0.12. Anal. Calcd for C₁₂H₁₄N₂O₅: C, 54.13; H, 5.30; N, 10.52. Found: C, 53.78; H, 5.10; N, 10.25.

Z-Tyr-Gly-Gly-OH (III)—Compound IIIa (20.0 g) was hydrogenated in a mixture of AcOH (400 ml) and water (100 ml). The solution was filtered and evaporated to dryness. The crystals formed from MeOH were collected by filtration (9.3 g). This product was dissolved in water (75 ml) together with NaHCO₃ (5.92 g). To this was added Z-Tyr-ONB (36.2 g) dissolved in THF (300 ml) and the mixture was stirred for 24 h. After removal of THF by evaporation, the remaining solution was acidified with 6N HCl and the product was extracted with *n*-BuOH. The extract was concentrated to provide a residue, which was triturated with AcOEt to give crystals. These were collected and recrystallized from aqueous acetonitrile: yield 22.0 g (76.7%), mp 203–206°C, $[\alpha]_D^{25} -19.8^\circ$ ($c=1.0$ in DMF), R_f^1 0.22. Anal. Calcd for C₂₁H₂₃N₃O₇: C, 58.73; H, 5.40; N, 9.79. Found: C, 58.19; H, 5.47; N, 9.63.

Z-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-Lys(Boc)-Leu-Lys(Boc)-OBu^t (IV)—Compound I (0.78 g) was hydrogenated and the resulting free base was coupled with compound II (1.53 g) in the presence of HONB (0.36 g) and DCC (0.41 g). The solution was stirred for 15 h then worked up as usual. The residue was triturated with ether to give a precipitate: yield 1.95 g (93.3%), mp 125–127°C, $[\alpha]_D^{25} -39.1^\circ$ ($c=0.9$ in MeOH), R_f^1 0.51. Anal. Calcd for C₁₁₁H₁₇₁N₂₀O₂₆S₃·2H₂O: C, 57.12; H, 7.56; N, 12.00; S, 4.12. Found: C, 57.08; H, 7.48; N, 12.44; S, 4.18.

Z-Tyr-Gly-Gly-Phe-Leu-Arg(Mds)-Arg(Mds)-Ile-Arg(Mds)-Pro-Lys(Boc)-Leu-Lys(Boc)-OBu^t (V)—Compound IV (230 mg) was hydrogenated and the free base obtained was coupled to compound III (43 mg) by the HONB-DCC method. The solution was stirred for 15 h then worked up as usual. The residue was triturated with ether to give a precipitate: yield 225 mg (86.8%), mp 153–155°C, $[\alpha]_D^{25} -25.0^\circ$ ($c=1.0$ in MeOH), R_f^1 0.51. Anal. Calcd for C₁₂₄H₁₈₆N₂₃O₃₀S₃·H₂O: C, 57.43; H, 7.31; N, 12.42; S, 3.71. Found: C, 57.16; H, 7.36; N, 12.71; S, 3.62.

H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH (Dynorphin [1–13])—Compound V (150 mg) was treated with TFA-thioanisole (9:1, 3 ml) at 50°C for 2 h. The solution was evaporated to dryness, and the residue was triturated with ether to give a precipitate, which was dissolved in water and passed through a column (1 × 10 cm) of Amberlite IRA 410 (acetate form). The eluates were then applied to a column (2.2 × 4 cm) of CM-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient: 0.005/0.6 M = 300 ml/300 ml). The fractions (205–315 ml) containing the desired product were combined and lyophilized: yield 60 mg (56.3%), $[\alpha]_D^{25} -62.9^\circ$ ($c=0.5$ in 1% AcOH), R_f^2 (cellulose) 0.64, R_f^3 (cellulose) 0.59. Paper electrophoresis (pH 1.9, HCOOH-AcOH buffer, 500 V, 50 min) 1.00 × Arg. Amino acid ratios in acid hydrolysate: Lys 2.08(2); Arg 3.16(3); Pro 1.10(1); Gly 1.94(2); Ile 0.86(1); Leu 2.05(2); Tyr 0.82(1); Phe 1.00(1) (average recovery 71%). Amino acid ratios in aminopeptidase-M hydrolysate: Lys 2.00(2); Arg 3.17(3); Pro 1.07(1); Gly 1.90(2); Ile 1.00(1); Leu 1.93(2); Tyr 0.95(1); Phe 1.13(1) (average recovery 73%).

H-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH (Dynorphin [4–13])—Compound IV (150 mg) was treated with TFA-thioanisole (9:1, 1.5 ml) at 50°C for 2 h. TFA was evaporated off, and the residue was triturated with ether to give a precipitate, which was dissolved in water and converted to the acetate by passage through a column of Amberlite IRA 410 (acetate form). The acetate was further applied to a column (2.2 × 5.5 cm) of CM-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient: 0.005/0.6 M = 300 ml/300 ml). The fractions (220–310 ml) containing the desired product were combined and lyophilized: yield 45 mg (43.0%), $[\alpha]_D^{25} -78.6^\circ$ ($c=0.5$ in 1% AcOH), R_f^2 (cellulose) 0.55. Amino acid ratios in acid hy-

drollysate: Lys 1.97(2); Arg 2.73(3); Pro 1.08(1); Ile 0.86(1); Leu 1.93(2); Phe 1.00(1) (average recovery 79%).

Z-Ile-Arg(Mds)-Pro-OH (VIa)—Compound IIB (1.5 g) was treated with TFA at room temperature for 30 min. The solution was evaporated to dryness and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with 1 N HCl, then dried over anhydr. Na_2SO_4 , and concentrated. The residue was triturated with ether to give a precipitate: yield 1.06 g (76.2%), mp 101–103°C, $[\alpha]_D^{25} -38.1^\circ$ ($c=1.0$ in MeOH), R_f^1 0.52. *Anal.* Calcd for $\text{C}_{34}\text{H}_{48}\text{N}_6\text{O}_9\text{S}$: C, 56.94; H, 6.75; N, 11.73; S, 4.47. Found: C, 56.76; H, 7.09; N, 11.50; S, 4.40.

Z-Ile-Arg(Mds)-Pro-Lys(Boc)-OH (VI)—Z-Lys(Boc)-OH (0.54 g) was hydrogenated in MeOH. After removal of the solvent by evaporation, the residue was dissolved in aqueous DMF together with NaHCO_3 (65 mg). To this was added Z-Ile-Arg(Mds)-Pro-ONB prepared from compound VIa (500 mg) and the solution was stirred for 15 h. After the usual work-up, the material was purified by column chromatography on silica gel (3×4 cm, CHCl_3 -MeOH-AcOH=50:2:1). The purified product was triturated with ether to give a precipitate: yield 0.33 g (50.0%) mp 80–82°C, $[\alpha]_D^{25} -27.6^\circ$ ($c=1.0$ in MeOH), R_f^1 0.52. *Anal.* Calcd for $\text{C}_{45}\text{H}_{68}\text{N}_8\text{O}_{12}\text{S}$: C, 57.18; H, 7.25; N, 11.86; S, 3.39. Found: C, 56.91; H, 7.47; N, 11.41; S, 3.25.

H-Ile-Arg-Pro-Lys-OH (Dynorphin [8–11])—Compound VI (200 mg) was treated with TFA-thioanisole (9:1, 2 ml) at 50°C for 2 h. After the usual work-up, the product was applied to a column (3×8 cm) of CM-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient: 0.005/0.2 M=300 ml/300 ml). The desired fractions (150–220 ml) were combined and lyophilized: yield 110 mg (82.2%) $[\alpha]_D^{25} -43.3^\circ$ ($c=0.4$ in 1% AcOH), R_f^2 (cellulose) 0.39. Amino acid ratios in acid hydrolysate: Lys 1.00(1); Arg 0.89(1); Pro 1.10(1); Ile 0.95(1) (average recovery 68%).

Z-Ile-Arg(Mds)-Pro-Lys(Boc)-Leu-Lys(Boc)-OBut (VII)—Compound I (434 mg) was hydrogenated and the free base obtained was coupled to compound VIa (400 mg) by the HONB-DCC method. After the usual work-up, the material was triturated with ether to give a precipitate: yield 725 mg (97.8%) mp 79–80°C, $[\alpha]_D^{25} -44.3^\circ$ ($c=1.0$ in MeOH), R_f^1 0.69. *Anal.* Calcd for $\text{C}_{66}\text{H}_{107}\text{N}_{10}\text{O}_{16}\text{S}$: C, 59.66; H, 8.12; N, 10.54; S, 2.41. Found: C, 59.73; H, 8.43; N, 10.80; S, 2.46.

H-Ile-Arg-Pro-Lys-Leu-Lys-OH (Dynorphin [8–13])—Compound VII (500 mg) was treated with TFA-thioanisole (9:1, 5 ml) at 50°C for 2 h. After the usual work-up, the material was applied to a column (3×9.5 cm) of CM-cellulose, which was eluted with pH 6.8 ammonium acetate buffer (gradient: 0.005/0.2 M=300 ml/300 ml). The fractions (360–500 ml) containing the desired product were pooled and lyophilized: yield 170 mg (48.4%) $[\alpha]_D^{25} -75.0^\circ$ ($c=0.6$ in 1% AcOH), R_f^2 (cellulose) 0.46. Amino acid ratios in acid hydrolysate: Lys 2.24(2); Arg 1.00(1); Pro 1.22(1); Ile 0.96(1); Leu 1.22(1) (average recovery 66%).

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References and Notes

- 1) Amino acids, peptides and their derivatives in this paper are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Mds=4-methoxy-2,6-dimethyl-benzenesulfonyl, OBut=*tert*-butyl ester, HONB=N-hydroxy-5-norbornene-2,3-dicarboximide, HOBT=N-hydroxybenzotriazole, DCC=N,N'-dicyclohexylcarbodiimide, THF=tetrahydrofuran, DMF=dimethylformamide, TFA=trifluoroacetic acid, CHA=cyclohexylamine.
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- 8) Column: Toyo Soda LS-410 (0.4×20 cm); solvent, CH_3CN -0.1 M ammonium acetate buffer (pH 6.8)=1:4; flow rate, 0.9 ml/min; elution time, Z-Leu-Lys-OH=23.6 min, Z-D-Leu-Lys-OH=25.2 min.
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