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New Protecting Groups for the Indole Ring of Tryptophan in Peptide Synthesis: 2,4,6-Trimethoxybenzenesulfonyl and 4-Methoxy-2,3,6-trimethylbenzenesulfonyl Groups[1]

Tsunehiko Fukuda, Mitsuhiro Wakimasu, Shigeru Kobayashi, and Masahiko Fujino*

Chemistry Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka, 532, Japan

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Five substituted benzenesulfonyl groups, *p*-toluenesulfonyl, *p*-methoxybenzenesulfonyl, 2,4-dimethoxybenzenesulfonyl, 2,4,6-trimethoxybenzenesulfonyl, and 4-methoxy-2,3,6-trimethylbenzenesulfonyl, were introduced at N^{in} of tryptophan and their protecting group properties were investigated. Among them, 2,4,6-trimethoxybenzenesulfonyl and 4-methoxy-2,3,6-trimethylbenzenesulfonyl are stable to trifluoroacetic acid, but can be readily removed by hydrogen fluoride or methanesulfonic acid, and suppress decomposition and modification of the tryptophan residue during peptide synthesis. These protecting groups were successfully used in syntheses of bombesin, a potent analog of luteinizing hormone-releasing hormone by the solution method and dynorphin by the solid-phase method.

Keywords——peptide synthesis; tryptophan; protecting group; 2,4,6-trimethoxybenzenesulfonyl group; 4-methoxy-2,3,6-trimethylbenzenesulfonyl group

Oxidation and alkylation of the indole ring of the trytophan (Trp) residue are the two main side reactions observed frequently during the acidolytic removal of protecting groups in peptide synthesis. The former can be effectively suppressed by addition of antioxidants such as 1,2-ethanedithiol[2] to the reaction medium, while the latter, especially *t*-butylation[3] or *p*-methoxybenzylation,[4] has been recognized as a serious problem on the basis of recent investigations in several laboratories.[5,6] According to these reports, only 60% of Trp was recovered from *tert*-butoxycarbonyl-tryptophan (Boc–Trp–OH)[6] and 30% from *p*-methoxybenzyloxycarbonyl-tryptophan[4] when they were treated with trifluoroacetic acid. Masui *et al.*[6] considered that N^{in}-*tert*-butylation occurred first, thus making the carbon atoms of the resulting N^{in}-modified indole ring more susceptible to further attack by Bu^t cations. The addition of sulfur compounds such as 1,2-ethanedithiol or dimethyl sulfide to trifluoroacetic acid (TFA) solution was proposed[4,6,7] to avoid these alkylations. The effectiveness of these scavengers, however, remains to be fully characterized and, in addition, their unpleasant odor makes them inconvenient to use. The formyl,[8] benzyloxycarbonyl,[9] and 2,4-dichlorobenzyloxycarbonyl[9] groups, proposed as protecting groups of the indole ring of Trp, are not stable enough to serve as a persistent protecting group under the experimental conditions of peptide synthesis.[10] Recently, Shimonishi *et al.*[11] reported the dimerization of Trp residues in TFA or hydrogen fluoride (HF) as another type of by-product. As a consequence of all these facts, the development of an N^{in}-protecting group of Trp in peptide synthesis is urgently needed. Benzenesulfonyl groups might be used for this purpose because of the stability of the sulfonamide bond. In addition, the electron-withdrawing property of these groups should inactivate the indol ring against the attack of alkyl cation, thus furnishing ideal protection for the indole of Trp.

In this paper we describe the evaluation of *p*-toluenesulfonyl (Tos), *p*-methoxybenzenesulfonyl (Mbs),[12] 2,4-dimethoxybenzenesulfonyl (Dmb), 2,4,6-trimethoxybenzenesulfonyl (Mtb),[13] and 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr)[13] as protecting groups (Fig. 1). The syntheses of bombesin, a highly active luteinizing hormone-releasing hormone (LH–RH)

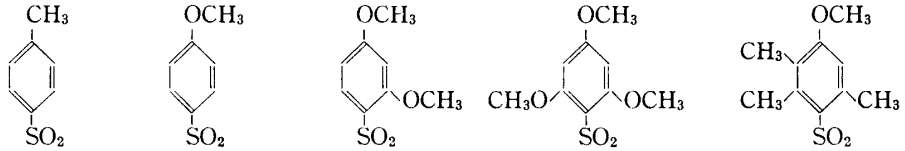

Fig. 1. N^{in}-Protecting Groups for Tryptophan

analog, by the solution method and dynorphine by the solid-phase method are also reported using Mtb and Mtr as protecting groups of the indole of Trp.

Synthesis and Properties of N^{in}-Protected Trp Derivatives

The introduction of the substituted-benzenesulfonyl group at N^{in} of Trp and the preparation of Boc derivatives were carried out by two methods (Fig. 2). In method A, Tos-chloride

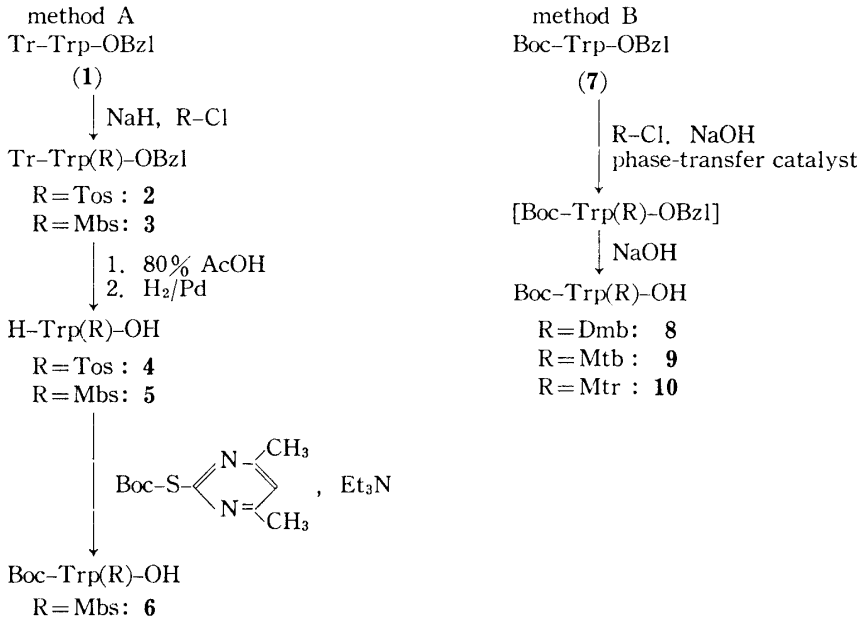

Fig. 2. Synthesis of N^{in}-Protected Tryptophan Derivatiyes

and Mbs-chloride were allowed to react with Tr–Trp–OBzl (**1**) in the presence of NaH to give arylsulfonyl derivatives (**2** and **3**) Compounds **2** and **3** were treated with 80% AcOH to remove the trityl group and then subjected to hydrogenolysis to give H–Trp(R)–OH (R=Tos: **4**; Mbs: **5**). Compound **5** was converted to Boc–Trp(Mbs)–OH (**6**).

In method B, Illi's procedure[14] for the acylation of indole using the phase transfer catalyst was applied to the arylsulfonylation of Boc–Trp–OBzl (**7**). Dmb-,[15] Mtb-,[16] and Mtr-chloride[13] were allowed to react with **7** in the presence of pulverized NaOH and a catalytic amount of cetyltrimethylammonium chloride in CH_2Cl_2 to give Boc–Trp(R)–OBzl, which was hydrolyzed to (Boc–Trp(R)–OH (R=Dmb: **8**; Mtb: **9**; Mtr: **10**). Method B was briefer than method A.

The N^{in}-protected Trp derivatives (**4**—**6** and **8**—**10**) were treated with HF[17] in the presence of 1,2-ethanedithiol and anisole at 0°C for 1 h, and with methanesulfonic acid (MSA)[18] in the presence of 1,2-ethanedithiol and thioanisole at room temperature for 1 h to assess the removability of the protecting groups (Table I). Under HF treatment, Tos and Dmb of **4** and **8** were unsatisfactory protecting groups. However, more than 80% of Trp was recovered from the Mbs (**5** and **6**), Mtb (**9**), and Mtr (**10**) derivatives. The TLC analysis of the products from

Table I. Recovery of Tryptophan after the Treatment of N^{in}-Protected Tryptophan Derivatives with HF and MSA[a]

	H–Trp(R)–OH		Boc–Trp(R)–OH			
R:	Tos (**4**)	Mbs (**5**)	Mbs (**6**)[b]	Dmb (**8**)	Mtb (**9**)	Mtr (**10**)
HF	19.0%	86.5%	84.1%	60.7%	83.7%	82.6%
MSA	—	—	—	—	34.8%	109.6%

a) Measured by amino acid analysis. For details see "Experimental."
b) Previously treated with TFA.

5, **6**, **9**, and **10** showed a single spot of Trp. When compound **6** was treated with TFA at room temperature for 1 h to remove the Boc group, prior to the HF treatment, the recovery of Trp was in good agreement with that obtained from the treatment of **5** with HF. This result indicated that the Trp residue was not modified during TFA treatment of **6**. The effectiveness of the N^{in}-protecting groups against alkylation was confirmed by H^1-NMR spectroscopy. Boc–Trp–OH and **9** were treated with neat TFA for 1 h at room temperature and TFA was removed by evaporation The NMR spectrum of the whole residue derived from Boc–Trp–OH clearly showed the Bu^t group attached to the indole ring. It was estimated from the integral curve that about 50% of the Bu^t cation generated from the Boc group attacked the indole ring. No such signal was observed in the spectrum of the product derived from compound **9** treated under the same conditions. In the case of MSA treatment, the Mtr group was readily cleaved, but compound **9** generated unknown by-product together with Trp. All of these protecting groups were found to be stable against TFA, alkaline conditions, and hydrogenolysis. The only exception was the formation of a very small amount of an unknown compound when **10** was exposed to TFA at room temperature for 15 h.

In order to study the properties of these protecting groups in the peptide chain, the tripeptides, Z–Gly–Trp(Mbs)–Gly–OBzl (**13**) and Z–Lys(Z)–Trp(Mtb)–Gly–OBzl (**14**), were prepared as model peptides by the conventional solution method, using **6** and **9** as starting materials (Fig. 3). The dipeptides, Boc–Trp(R)–Gly–OBzl (R=Mbs: **11** and Mtb: **12**), were

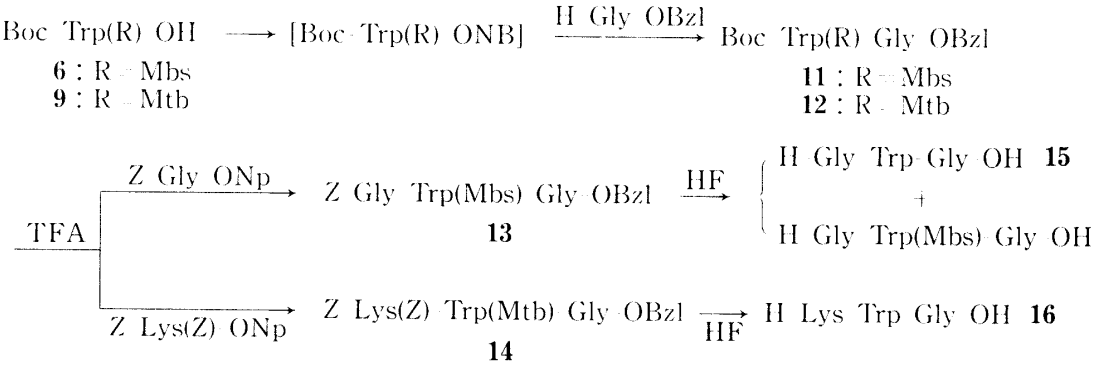

Fig. 3. Synthesis of Tripeptide

prepared by the HONB[19] active ester method, and treated with neat TFA. The resulting TFA salts were each coupled with Z–Gly–OH or Z–Lys(Z)–OH to give **13** and **14**, respectively. Compound **13** was then treated with HF under the conditions described above. The deblocked peptide was converted to acetate by passage through a column of Amberlite IRA-410 (acetate form), and purified by partition chromatography on Sephadex G-25, giving H–Gly–Trp–Gly–OH (**15**) in 60% yield. But in this case, the existence of a moderate amount of by-product, H–Gly–Trp(Mbs)–Gly–OH,[20] was observed in the crude product by TLC analysis. In a

similar manner, H–Lys–Trp–Gly–OH (**16**) was obtained from **14** in 89% yield. The UV absorption spectrum of **16** was virtually identical with that of H–Lys–D-Trp–Gly–OH (**17**) prepared by the conventional method, thus showing excellent recovery of the Trp residue. HPLC analysis[21] of **16** using **17** as the diastereoisomeric standard showed that no racemization occurred during the synthesis of compound **9** and its coupling procedure.

The results of these experiments demonstrate that the Mtb group can be removed easily even when incorporated in the peptide chain. The N^{in}-Mbs group in the peptide chain, however can not be removed cleanly by HF under the conditions described above. This indicates that the Mbs group is unsuitable for practical use. On the other hand, the Mtr group is more susceptible to acid than the Mtb group.[13] From these several results, Mtb and Mtr are considered to be suitable as protecting groups for Trp.

Applications of N^{in}-Mtb and -Mtr Groups to the Synthesis of Biologically Active Peptides

Bombesin[22] (**31**) was synthesized for the purpose of applying the N^{in}-Mtb protection-HF deblocking method to the conventionation solution method (Fig. 4). The fragment (6—11 (**23**), prepared by stepwise elongation followed by hydrogenolytic cleavage of the benzyl ester,

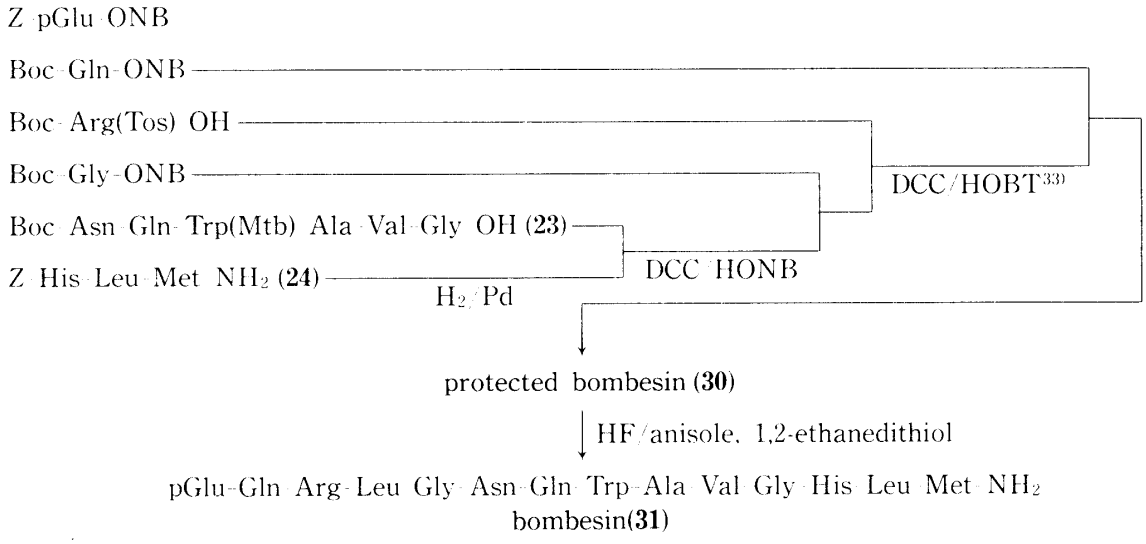

Fig. 4. Synthesis of Bombesin

was coupled with the C-terminal tripeptide amide[23] (**24**) by the DCC/HONB method[19] to give fragment (6—14) (**25**). The fragment (6—14) was then elongated to the protected bombesin (**30**) by stepwise coupling of Boc-amino acids. Although the intermediates containing a Trp residue were repeatedly treated with neat TFA, no side reactions were observed. The protected peptide (**30**) thus obtained was treated with HF in the presence of 1,2-ethanedithiol and anisole at 0°C for 1 h. The deblocked peptide was converted to acetate, which was purified by partition chromatography on Sephadex G-25 and preparative HPLC[24] to give pure bombesin (**31**) in a yield 47% from **30**. The purity of the product was confirmed by TLC, HPLC,[25] and amino acid analysis, and its contractile activity on rat uterus was as potent as that of the standard material.[26]

The Mtr group was then used in the synthesis of des-Gly[10]-[D-Leu[6]]–LH–RH-ethylamide[27] (**35**), a potent LH–RH analog (Fig. 5). Z–Ser–Tyr–D-Leu–OH was coulped with H–Leu–Arg-(Mbs)–Pro–NH–C_2H_5 by the DCC/HONB method to give hexapeptide ethylamide (**32**). The Mbs group masking the N^g of Arg had been previously reported[12] to be susceptible to MSA. After the hydrogenolytic cleavage of the Z group, Boc–Trp(Mtr)–OH (**10**) was introduced to give the protected heptapeptide ethylamide (**33**). Compound **33** was treated with TFA followed

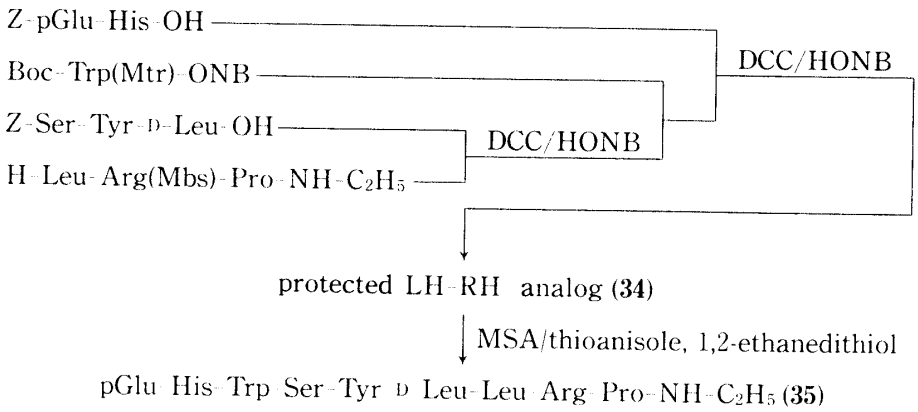

Fig. 5. Synthesis of an LH-RH Analog

by the addition of HCl. The protected nonapeptide ethylamide (**34**) was obtained by the coupling of Z–pGlu–His–OH with the HCl salt of heptapeptide ethylamide by means of the DCC/HONB method. Compound **34** was exposed to MSA in the presence of 1,2-ethanedithiol and thioanisole at room temperature for 1 h, and then passed through a column of Amberlite IRA-410 (acetate form). The resulting acetate was purified by column chromatography on CM-cellulose, Amberlite XAD-2, and finally Sephadex LH-20, giving a pure LH–RH analog (**35**). The analytical data for the product (**35**) were in good accord with those of the authentic compound synthesized previously.[27]

Finally, dynorphin[29] (**36**) was synthesized using the N^{in}-Mtb group in order to demonstrate the applicability of these Trp protecting groups to the solid-phase procedure[28] (Fig. 6). The

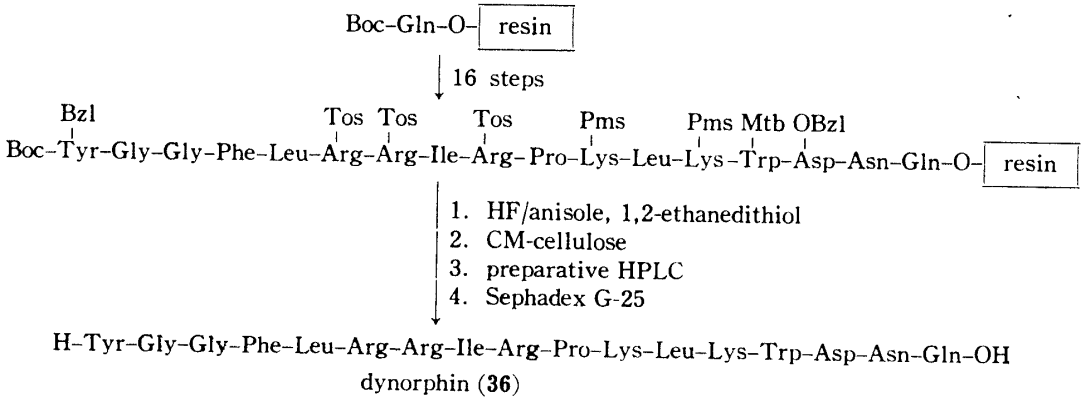

Fig. 6. Solid-Phase Synthesis of Dynorphin

protected peptide was synthesized on Merrifield's solid support by means of a symmetrical Boc-amino acid anhydride according to the method of Li *et al.*[30] The following amino acid derivatives with side chain functional groups were used: Boc–Arg(Tos)–OH, Boc–Lys(Pms)–OH, Boc–Trp(Mtb)–OH (**9**), and Boc–Tyr(Bzl)–OH. Among them, the Pms (*p*-tolylmethylsulfonyl) group had been proposed by us[31] as an amino protecting group that was easily removed by HF. The operations of the synthetic procedure were carried out manually. After the completion of the synthesis, the peptide resin was treated with HF. The deblocked crude peptide was removed from the resin and purified by ion-exchange chromatography on CM-cellulose, preparative HPLC, and gel-filtration on Sephadex G-25 successively to give pure dynorphin (**36**). The product showed identical physicochemical properties with the reference material[32] prepared by the solution method.

From these results, we conclude that substituted benzenesulfonyl groups such as Mtb

and Mtr can be used to protect the indole ring of Trp. Such groups should make it possible to synthesize peptides containing Trp without the need for special precautions.

Experimental

Melting points were taken in open capillaries and are uncorrected. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. H^1-NMR spectra were obtained on a Varian EM-360 spectrometer, and UV spectra on a Hitachi EPS-3T spectrophotometer. Acid hydrolysis was carried out in 4% thioglycolic acid-containing 6 N HCl at 110°C for 24 h. Amino acid analysis was performed on a Hitachi Model 835-50 amino acid analyzer. All chemicals and solvents were of reagent grade and were used without further purification. Evaporation was carried out in a rotary evaporator under reduced pressure at temperatures below 45°C. The solvent systems used for TLC on silica gel (precoated silica gel plate $60F_{254}$, Merck) or cellulose (Avicel, Funakoshi Yakuhin Co. Ltd.) were *n*-BuOH–AcOEt–AcOH–H_2O (1: 1: 1: 1, Rf^1); *n*-BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24, Rf^2); *n*-BuOH–pyridine–AcOH–H_2O (4: 1: 1: 2, Rf^3); *n*-BuOH–AcOH–H_2O (4: 1: 1, Rf^4).

Tr-Trp(Tos)-OBzl (2)——To a solution of Tr–Trp–OBzl (1) (2.15 g, 4 mmol) in DMF (10 ml) was added NaH (50% in mineral oil; 240 mg, 5 mmol) under N_2, and the mixture was stirred at room temperature for 2 h. To this was added *p*-toluenesulfonyl chloride (950 mg, 5 mmol) and the mixture was stirred for 15 h, then AcOEt (50 ml) was added and stirring was continued for 1 h. Water (30 ml) was added carefully. The organic layer was taken, washed with 5% $NaHCO_3$ and water, and dried over Na_2SO_4. The solvent was evaporated and the resulting residue was purified by column chromatography on silica gel using toluene as the solvent: 1.70 g (61.5%) mp 101—103°C, $[\alpha]_D^{24}$ +47.6° (c=0.5, DMF). *Anal.* Calcd for $C_{44}H_{38}N_2O_4S$: C, 76.49; H, 5.54; N, 4.06; S, 4.64. Found: C, 76.51; H, 5.55; N, 3.51; S, 4.67.

Tr-Trp(Mbs)-OBzl (3)——This compound was prepared from compound **1** and *p*-methoxybenzenesulfonyl chloride in the same manner as compound **2**: 100% mp 96°C, $[\alpha]_D^{21}$ +43.1° (c=0.5, DMF). *Anal.* Calcd for $C_{44}H_{38}N_2O_5S$: C, 74.79; H, 5.42; N, 3.96; S, 4.54. Found: C, 75.01; H, 5.35; N, 3.93; S, 4.50.

H-Trp(Tos)-OH (4)——In 80% AcOH (20 ml) was suspended compound **2** (1.3 g, 1.88 mmol) and the mixture was stirred at room temperature. After a while **2** was dissolved and triphenylmethanol precipitated out. After 1 h the solution was filtered, and the filtrate was subjected to catalytic hydrogenolysis at room temperature for 4 h in the presence of palladium black. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was recrystallized from water: 585 mg (86.9%) mp 227°C (dec.), $[\alpha]_D^{21}$ −29.2° (c=0.5, AcOH). *Anal.* Calcd for $C_{18}H_{18}N_2O_4S \cdot 1/2\ H_2O$: C, 58.83; H, 5.21; N, 7.63; S, 8.73. Found: C, 58.88; H, 5.23; N, 7.23; S, 8.72.

H-Trp(Mbs)-OH (5)——This compound was prepared from **3** in the same manner as compound **4**: 88.7% mp 218—220°C, $[\alpha]_D^{24}$ −34.8° (c=0.5, DMF). *Anal.* Calcd for $C_{18}H_{18}N_2O_5S \cdot 1/2H_2O$: C, 56.38; H, 5.00; N, 7.31. Found: C, 56.43; H, 4.92; N, 7.07.

Boc-Trp(Mbs)-OH (6)——To a solution of compound **5** (375 mg, 1 mmol) and Et_3N (0.12 ml) in water (2 ml)–dioxane (2 ml) was added 2-*t*-butoxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine (290 mg, 1.2 mmol), and the mixture was stirred vigorously at room temperature for 15 h. The solution was diluted with water (10 ml) and extracted with Et_2O (5 ml). The organic layer was extracted with a small amount of 5% $NaHCO_3$. The aqueous layers were combined and acidified with 10% aqueous citric acfd. This was extracted with AcOEt (20 ml). The organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated and the residue was triturated with petroleum ether to give crystals: 425 mg (95.4%) mp 83—84°C, $[\alpha]_D^{24}$ −28.1° (c=0.5, DMF). *Anal.* Calcd for $C_{23}H_{26}N_2O_7S$: C, 58.21; H, 5.50; N, 5.90; S, 6.76. Found: C, 58.17; H, 5.85; N, 5.46; S, 6.81.

Boc-Trp(Dmb)-OH (8)——To a solution of Boc–Trp–OBzl (7) (789 mg, 2 mmol) and cetyltrimethylammonium chloride (6.4 mg, 0.02 mmol) in CH_2Cl_2 (10 ml) was added dropwise 2,4-dimethoxybenzenesulfonyl chloride (710 mg, 3 mmol) dissolved in CH_2Cl_2 (3 ml) in the presence of pulverized NaOH (200 mg, 5 mmol) and the mixture was stirred vigorously at room temperature for 30 min. To this was added 1 N HCl (10 ml) under cooling. The organic layer was taken, washed with water and then dried over Na_2SO_4. The solvent was removed by evaporation, giving Boc–Trp(Dmb)–OBzl. Without further purification, the product was dissolved in EtOH (10 ml). To this was added 1 N NaOH (2.2 ml) dropwise under cooling. After stirring for 1 h at room temperature, EtOH was evaporated off. The resulting aqueous solution was diluted with H_2O (20 ml) and extracted with Et_2O (20 ml). The aqueous layer was cooled and brought to pH 3 with 1 N HCl. The solution was extracted with AcOEt and the organic layer was washed with H_2O, then dried over Na_2SO_4. The solvent was evaporated and the residue was triturated with petroleum ether to give a solid: 737 mg (73%) mp 100°C (dec.) $[\alpha]_D^{22}$ −9.43° (c=0.5, DMF). *Anal.* Calcd for $C_{24}H_{28}N_2O_8S$: C, 57.13; H, 5.59; N, 5.55; S, 6.36. Found: C, 57.56; H, 5.86; N, 5.30; S, 6.36.

2,4,6-Trimethoxybenzenesulfonyl Chloride (Mtb-Cl)——To a solution of 1,3,5-trimethoxybenzene (18.7 g, 0.11 mmol) in CCl_4 (30 ml) was added dropwise $ClSO_3H$ (8.64 ml, 0.13 mol). The mixture was left at room temperature for 30 min, and then cooled. In the course of the addition, 2,4,6-trimethoxybenzenesulfonic

acid precipitated out. The crystals, which were collected by filtration, were added to ice-water (100 ml) containing K_2CO_3 (80 g). The precipitate was collected by filtration and dried at 100°C over P_2O_5. The resulting potassium salt was allowed to react with $POCl_3$ (28 ml, 0.3 mol) at 100°C for 2 h and the mixture was poured into ice-water. The solution was extracted with $CHCl_3$ (3×100 ml). The organic extracts were combined, washed with water, and dried over Na_2SO_4. After evaporation of the solvent, the residue was triturated with Et_2O to give crystals, which were recrystallized from $CHCl_3$–Et_2O: 10.4 g (35.5%) mp 133—134°C. (lit.[16] mp 134—136°C).

Boc-Trp(Mtb)-OH (9)——This compound was prepared from compound **7** and Mtb-Cl in the same manner as compound **8**: 91.2% mp 82—84°C, $[\alpha]_D^{24}$ −15.4° (c=0.5, DMF). *Anal.* Calcd for $C_{25}H_{30}N_2O_9S$: C, 56.17; H, 5.66; N, 5.24; S, 6.00. Found: C, 56.37; H, 5.85; N, 4.91; S, 6.07.

Boc-Trp(Mtr)-OH (10)——This compound was prepared from compound **7** and Mtr-Cl in the same manner as compound **8**: 79.4% mp 88—90°C, $[\alpha]_D^{22}$ −24.8° (c=0.5, DMF). *Anal.* Calcd for $C_{26}H_{32}N_2O_7S$: C, 60.44; H, 6.24; N, 5.42; S, 6.21. Found: C, 61.07; H, 6.25; N, 5.17; S, 5.83. When this compound was treated with TFA at room temperature for 15 h, the formation of an unknown product (Rf^1=0.61, silica gel) was observed in addition to H–Trp(Mtr)–OH (Rf^1=0.70).

Measurement of the Recovery of Trp in the Treatment of Compounds 4—6 and 8—10 with HF or MSA——The protected Trp derivatives (**4**—**6** and **8**—**10**) (0.1 mmol each) were treated with HF (1 ml) in the presence of 1,2-ethanedithiol (0.5 ml) and anisole (0.1 ml) at 0°C for 1 h. After evaporation of HF, the residue was dissolved in AcOH (15 ml), which was evaporated off again. The residue was dissolved in water (20 ml), and the insolubles were filtered off. The filtrate was extracted with Et_2O. The aqueous layer was diluted to 50 ml and subjected to amino acid analysis to determine the recovery of Trp. Compound **8** was previously treated with TFA (0.5 ml) in the reaction vessel for HF. After evaporation of TFA, the residue was treated with HF as described above.

Compounds **9** and **10** (0.1 mmol each) were treated with MSA (0.5 ml) in the presence of 1,2-ethanedithiol (0.025 ml) and thioanisole (0.06 ml) at room temperature for 1 h. The reaction mixture was diluted with water to 50 ml, and subjected to amino acid analysis. The results are listed in Table I.

Evaluation of the Effectiveness of N^{in}-Protecting Group against Alkylation by H^1-NMR Analysis——Compound **9** (60 mg) and Boc–Trp–OH (60 mg) were treated with TFA (0.5 ml) at room temperature for 1 h. After evaporation of TFA, the residue was flushed twice with TFA. The residue was dissolved in TFA and subjected to H^1-NMR analysis. The product derived from Boc–Trp–OH showed signals (multiplet) corresponding to Bu^t groups in the region of 0.91—1.20 ppm (external reference: tetramethylsilane), while that derived from **9** showed no signal in this region.

Boc-Trp(Mbs)-Gly-OBzl (11)——To a solution of compound **6** (717 mg, 1.5 mmol) and HONB (297 mg, 1.65 mmol) in CH_3CN (10 ml) was added DCC (340 mg, 1.65 mmol) at 0°C. The mixture was stirred at 0°C for 1 h and at room temperature for 15 h. The precipitate was filtered off, and H–Gly–OBzl–Tos–OH (557 mg, 1.65 mmol) and Et_3N (0.23 ml) were added to the filtrate. The mixture was stirred at room temperature for 5 h. The solution was concentrated and the residue was dissolved in AcOEt (20 ml). This solution was washed successively with 5% $NaHCO_3$, 1 N HCl and water, then dried over Na_2SO_4. The solution was evaporated to dryness and the residue was solidified with petroleum ether: 850 mg (91.1%) mp 72—74°C, $[\alpha]_D^{24}$ −15.0° (c=0.5, DMF). *Anal.* Calcd for $C_{32}H_{35}N_3O_8S$: C, 61.82; H, 5.67; N, 6.76; S, 5.16. Found: C, 62.32; H, 5.74; N, 6.81; S, 4.60.

Z-Gly-Trp(Mbs)-Gly-OBzl (13)——Compound **11** (627 mg, 1 mmol) was treated with TFA (6 ml) at room temperature for 20 min. The mixture was evaporated to dryness and the residue was triturated with Et_2O. The resulting powder was collected by filtration. This TFA salt was dissolved in CH_3CN (20 ml) together with Z–Gly–ONp (330 mg, 1 mmol) and *N*-ethylmorpholine (0.3 ml), and the mixture was stirred at room temperature for 15 h. The solvent was evaporated and the residue was crystallized from Et_2O. This was recrystallized from AcOEt–Et_2O: 580 mg (85.4%) mp 130—131°C, $[\alpha]_D^{24}$ −7.10° (c=0.5, DMF). *Anal.* Calcd for $C_{37}H_{36}N_4O_9S$: C, 62.34; H, 5.09; N, 7.86; S, 4.50. Found: C, 62.24; H, 5.02; N, 7.85; S, 4.78.

H-Gly-Trp-Gly-OH (15)——Compound **13** (340 mg, 0.5 mmol) was treated with HF (5 ml) in the presence of 1,2-ethanedithiol (1.5 ml) and anisole (1.5 ml) at 0°C for 1 h. After evaporation of HF, water (10 ml) and Et_2O (10 ml) were added and the insolubles were filtered off. The aqueous layer was taken and washed with Et_2O. The aqueous solution was passed through a column of Amberlite IRA-410 (acetate form, 1×10 cm) and the effluent and washings were combined and concentrated. The residue was purified by partition chromatography on a column of Sephadex G-25 (4×45 cm) using *n*-BuOH–AcOH–H_2O (4: 1: 5) as an eluent. The fractions containing the desired product were collected and the solvent was evaporated. The residue was crystallized from EtOH–Et_2O: 98 mg (61.6%) mp 245—246°C (dec.), $[\alpha]_D^{21}$ +3.76° (c=0.5, AcOH). Rf^4=0.29 (silica gel). *Anal.* Calcd for $C_{15}H_{18}N_4O_4 \cdot 1/2AcOH \cdot 1/2H_2O$: C, 53.77; H, 5.92; N, 15.68. Found: C, 53.48, H, 5.88; N, 15.15.

Z-Lys(Z)-Trp(Mtb)-Gly-OBzl (14)——To a solution of compound **9** (504 mg, 1 mmol), H–Gly–OBzl·Tos–OH (371 mg, 1.1 mmol), HONB (228 mg, 1.2 mmol) and Et_3N (0.16 ml) in CH_3CN (10 ml) was added DCC (227 mg, 1.1 mmol) at 0°C. The mixture was stirred at 0°C for 2 h and then at room temperature for 15 h. After the usual work-up, Boc–Trp(Mtb)–Gly–OBzl (**12**) was obtained as an oil: 453 mg (67%). Compound

12 was treated with TFA (15 ml) as described above. The resulting TFA salt was dissolved in CH_3CN (10 ml) followed by addition of Z-Lys(Z)-ONp (391 mg, 0.73 mmol) and *N*-ethylmorpholine (0.1 ml). The mixture was stirred at room temperature for 48 h. After the usual work-up, the product was crystallized from AcOEt–Et_2O: 303 mg (48.4%) mp 98—100°C, $[\alpha]_D^{26}$ −12.9° (c=0.5, DMF). *Anal.* Calcd for $C_{51}H_{55}N_5O_{13}S$: C, 62.62; H, 5.67; N, 7.16; S, 3.19. Found: C, 62.67; H, 5.60; N, 7.24; S, 3.19.

H-Lys-Trp-Gly-OH (16)——Compound **14** (190 mg, 0.2 mmol) was treated with HF (5 ml) in the presence of 1,2-ethanedithiol (0.8 ml) and anisole (0.8 ml) at 0°C for 1 h. The reaction mixture was worked up and purified by column chromatography on Sephadex G-25 in the same manner as for compound **15**. The fractions containing the desired compound were collected and the solvent was evaporated. The residue was lyophilized from water: 80 mg (89.9%) $[\alpha]_D^{22}$ +31.4° (c=0.5, 5% AcOH), Rf^2=0.58 (cellulose), Rf^4=0.15 (cellulose). Amino acid analysis: Lys 1.00(1); Trp 0.84(1); Gly 1.03(1). *Anal.* Calcd for $C_{19}H_{27}N_5O_4 \cdot AcOH \cdot 4H_2O$: C, 48.36; H, 7.54; N, 13.34. Found: C, 47.98; H, 7.23; N, 13.26.

Z-D-Trp-Gly-OBzl——To a solution of Z-D-Trp-ONB (449.6 mg, 1.1 mmol) and H-Gly-OBzl·Tos-OH (371.1 mg, 1.1 mmol) in CH_3CN (10 ml) was added Et_3N (0.16 ml) and the mixture was stirred for 5 h at room temperature. After the usual work-up, the residue was triturated with petroleum ether to give crystals: 310 mg (63.9%) mp 75°C, $[\alpha]_D^{20}$ +28.8° (c=0.5, DMF). *Anal.* Calcd for $C_{28}H_{27}N_3O_5$: C, 69.26; H, 5.61; N, 8.65. Found: C, 69.16; H, 5.77; N, 8.64.

Z-Lys(Z)-D-Trp-Gly-OH——Z-D-Trp-Gly-OBzl (290 mg, 0.6 mmol) was hydrogenated in a mixture of MeOH (10 ml)–H_2O (5 ml)–AcOH (5 ml) in the presence of palladium black at room temperature for 5 h. The solution was filtered and the filtrate was concentrated to dryness. The residue was triturated with Et_2O to give a solid, which was collected by filtration. The resulting dipeptide was dissolved in DMF (10 ml) together with Et_3N (0.14 ml). To this was added Z-Lys(Z)-ONp (384 mg, 0.72 mmol) and the mixture was stirred at room temperature for 3 h. The solution was concentrated and the residue was purified by column chromatography on silica gel (10 g) with $CHCl_3$–MeOH–AcOH (18: 2: 1) as an eluent: 200 mg (50%) mp 154—156°C, $[\alpha]_D^{20}$ +11.4° (c=0.5, DMF). *Anal.* Calcd for $C_{35}H_{39}N_5O_8$: C, 63.91; H, 5.98; N, 10.65. Found: C, 63.55; H, 6.04; N, 10.21.

H-Lys-D-Trp-Gly-OH (17)——Z-Lys(Z)-D-Trp-Gly-OH (180 mg, 0.27 mmol) was hydrogenated in MeOH (15 ml)–H_2O (2 ml)–AcOH (1 ml) using palladium black as a catalyst at room temperature for 2 h. The mixture was filtered and the filtrate was concentrated to dryness. The residue was lyophilized from waster to give a powder: 110 mg (87.3%), $[\alpha]_D^{20}$ +40.0° (c=0.5, 5% AcOH), Rf^2=0.53 (cellulose), Rf^4=0.10 (cellulose). *Anal.* Calcd for $C_{19}H_{27}N_5O_4 \cdot AcOH \cdot H_2O$: C, 53.94; H, 7.12; N, 14.98. Found: C, 54.19; H, 7.58; N, 14.56.

Boc-Val-Gly-OBzl (18)——To a solution of Boc-Val-ONB (4.91 g, 13 mmol) and H-Gly-OBzl·Tos-OH (4.82 g, 14.3 mmol) in CH_3CN (20 ml), was added Et_3N (2.0 ml) and the mixture was stirred at room temperature for 15 h. After the usual work-up, the product was purified by recrystallization from AcOEt–petroleum ether: 4.06 g (85.7%) mp 77—78°C, $[\alpha]_D^{22}$ −9.50° (c=0.5, DMF). *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.64; H, 7.86; N, 7.74.

Boc-Ala-Val-Gly-OBzl (19)——Compound **18** (3.8 g, 10.4 mmol) was treated with TFA (20 ml) as described above. The resulting TFA salt was allowed to react in the presence of *N*-ethylmorpholine (1.92 ml) with Boc-Ala-ONB [prepared from Boc-Ala-OH (2.08 g, 11 mmol) and HONB (2.15 g, 12 mmol) using DCC (2.42 g, 12 mmol) in CH_3CN (20 ml)]. The mixture was stirred at room temperature for 24 h. After the usual work-up, the crude product was recrystallized from AcOEt–petroleum ether: 3.95 g (87.2%) mp 146°C, $[\alpha]_D^{22}$ −20.6° (c=0.5, DMF). *Anal.* Calcd for $C_{22}H_{33}N_3O_6$: C, 60.67; H, 7.76; N, 9.65. Found: C, 60.98; H, 7.78; N, 9.76.

Boc-Trp(Mtb)-Ala-Val-Gly-OBzl (20)——Compound **19** (2.18 g, 5 mmol) was treated with TFA (15 ml) as described above. The resulting TFA salt was dissolved in CH_3CN (20 ml) together with Et_3N (0.7 ml). This was mixed with a solution of Boc-Trp(Mtb)-ONB [prepared from compound **9** (2.94 g, 5.5 mmol) and HONB (1.08 g, 6 mmol) using DCC (1.24 g, 6 mmol) in CH_3CN (10 ml)]. The mixture was stirred at room temperature for 15 h. After the usual work-up, the crude product was recrystallized from AcOEt–Et_2O: 3.50 g (82.2%) mp 115—117°C, $[\alpha]_D^{24}$ −6.74° (c=0.5, DMF). *Anal.* Calcd for $C_{42}H_{53}N_5O_2S$: C, 59.21; H, 6.27; N, 8.22; S, 3.76. Found: C, 59.36; H, 6.45; N, 8.14; S, 3.70.

Boc-Gln-Trp(Mtb)-Ala-Val-Gly-OBzl (21)——Compound **20** (3.40 g, 3.99 mmol) was treated with TFA (15 ml) at room temperature for 20 min. The mixture was concentrated and the residue was triturated with ether to give a solid, which was collected by filtration. The resulting TFA salt was dissolved in DMF (10 ml) together with Et_3N (0.66 ml) and Boc-Gln-ONB (1.79 g, 4.4 mmol), and the mixture was stirred at room temperature for 15 h. After the usual work-up, the material was recrystallized twice from MeOH–Et_2O: 2.60 g (66.4%) mp 210—211°C, $[\alpha]_D^{24}$ −7.73° (c=0.5, DMF). *Anal.* Calcd for $C_{47}H_{61}N_7O_{14}S \cdot 1/2H_2O$: C, 57.07; H, 6.32; N, 9.91; S, 3.24. Found: C, 57.08; H, 6.23; N, 9.98; S, 3.10.

Boc-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-OBzl (22)——Compound **21** (2.54 g, 2.59 mmol) was treated with TFA (15 ml) as described above. The resulting TFA salt was dissolved in DMF (10 ml) together with Boc-Asn-ONp (1.10 g, 3.21 mmol) and Et_3N (0.44 ml), and the mixture was stirred at room temperature for 48 h. To this was added Et_2O (50 ml), and the resulting precipitate was collected by filtration. The product was

washed with hot CH_3CN; 2.70 g (95.4%) mp 236°C (dec.), $[\alpha]_D^{24}$ −25.4° (c=0.5, DMF). *Anal.* Calcd for $C_{51}H_{67}N_9O_{16}S$: C, 55.98; H, 6.17; N, 11.52; S, 2.93. Found: C, 55.86; H, 6.18; N, 11.55; S, 2.95.

Boc-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-OH (23)——Compound **22** (2.62 g, 2.39 mmol) was hydrogenated over palladium black as a catalyst. The mixture was filtered and the filtrate was concentrated to dryness. The residue was triturated with AcOEt to give crystals: 2.39 g (99.6%) mp 220°C (dec.), $[\alpha]_D^{24}$ −24.7° (c=0.5, DMF). *Anal.* Calcd for $C_{44}H_{61}N_9O_{16}S\cdot 1/2H_2O$: C, 52.16; H, 6.17; N, 12.44; S, 3.17. Found: C, 52.24; H, 6.22; N, 12.54; S, 3.55.

Boc-His-Leu-Met-NH_2 (24)——This compound was prepared in the manner described by Bernard *et al.*[23] mp 159—161°C, $[\alpha]_D^{25}$ −27.3° (c=0.5, DMF). (lit.[23] mp 163—164°C, $[\alpha]_D^{24}$ −29.9° (c=0.5, DMF)).

Boc-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-His-Leu-Met-NH_2 (25)——To a solution of compound **23** (2.01 g, 2 mmol) and HONB (432 mg, 2.4 mmol) was added DCC (618 mg, 3 mmol) at 0°C. The mixture was stirred at 0°C for 2 h and at room temperature for 15 h, then filtered to give a solution of active ester. Compound **24** (1.10 g, 2.2 mmol) was treated with TFA (10 ml) as usual. The resulting TFA salt and Et_3N (0.56 ml) were added to the solution of active ester, and the mixture was stirred at room temperature for 48 h, then Et_2O (50 ml) was added. The resulting precipitate was filtered and reprecipitated from 85% CH_3CN: 2.37 g (85.4%) mp 250°C (dec.), $[\alpha]_D^{24}$ −18.1° (c=0.5, DMF). *Anal.* Calcd for $C_{61}H_{89}N_{15}O_{18}S_2\cdot 2H_2O$: C, 51.57; H, 6.60; N, 14.79; S, 4.51. Found: C, 51.54; H, 6.41; N, 14.27; S, 4.45.

Boc-Gly-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-His-Leu-Met-NH_2 (26)——Compound **25** (2.24 g, 1.62 mmol) was treated with TFA (20 ml) as described above. The resulting TFA salt was dissolved in DMF (15 ml) together with Boc–Gly–ONB (653 mg, 1.94 mmol) and Et_3N (0.46 ml), and the mixture was stirred at room temperature for 15 h. To this was added CH_3CN to give a precipitate, which was reprecipitated from 85% CH_3CN: 1.92 g (81.2%) mp 251°C, $[\alpha]_D^{24}$ −17.9° (c=0.5, DMF). *Anal.* Calcd for $C_{63}H_{92}N_{16}O_{19}S_2\cdot 3H_2O$: C, 50.58; H, 6.60; N, 14.98; S, 4.57. Found: C, 50.61; H, 6.30; N, 14.67; S, 4.57.

Boc-Leu-Gly-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-His-Leu-Met-NH_2 (27)——Compound **26** (1.90 g, 1.32 mmol) was treated with TFA as described above. The resulting TFA salt was dissolved in DMF (10 ml) together with Boc–Leu–ONp (558 mg, 1.58 mmol) and Et_3N (0.37 ml), and the mixture was stirred for 15 h. To this was added CH_3CN to give a precipitate, which was reprecipitated from 85% CH_3CN: 1.78 g (86.7%) mp 254°C, $[\alpha]_D^{24}$ −23.6° (c=0.5, DMF). *Anal.* Calcd for $C_{69}H_{103}N_{17}O_{20}S_2\cdot 3H_2O$: C, 51.50; H, 6.87; N, 14.80; S, 3.99. Found: C, 51.73; H, 6.64; N, 14.58; S, 4.08.

Boc-Arg(Tos)-Leu-Gly-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-His-Leu-Met-NH_2 (28)——Compound **27** (1.09 g, 0.7 mmol) was treated with TFA as described above. The resulting TFA salt was dissolved in DMF (5 ml) together with Boc–Arg(Tos)–OH (372 mg, 0.84 mmol), HOBT[33] (123 mg, 0.91 mmol), and Et_3N (0.2 ml). To this was added 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (381 mg, 0.81 mmol) at 0°C, and the mixture was stirred at 0°C for 2 h and at room temperature for 48 h. To this was added CH_3CN to give a precipitate, which was reprecipitated from 85% CH_3CN: 942 mg (70 0%) mp 196—199°C (dec.), $[\alpha]_D^{24}$ −18.5° (c=0.5, DMF). *Anal.* Calcd for $C_{82}H_{121}N_{21}O_{23}S_3\cdot H_2O$: C, 52.29; H, 6.58; N, 15.62; S, 5.11. Found: C, 52.37; H, 6.69; N, 15.49; S, 5.37.

Boc-Gln-Arg(Tos)-Leu-Gly-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-His-Leu-Met-NH_2 (29)——Compound **28** (920 mg, 0.49 mmol) was treated with TFA as described above. The resulting TFA salt was dissolved in DMF (10 ml) together with Boc–Gln–ONB (345 mg, 0.6 mmol) and Et_3N (0.14 ml), and the mixture was stirred for 15 h at room temperature. To this was added CH_3CN to give a precipitate, which was reprecipitated from 85% CH_3CN: 670 mg (68.6%) mp 245°C (dec.), $[\alpha]_D^{21}$ −24.1° (c=0.5, DMF). *Anal.* Calcd for $C_{87}H_{129}N_{23}O_{25}S_3\cdot 2H_2O$: C, 51.49; H, 6.51; N, 15.88; S, 4.74. Found: C, 51.45; H, 6.71; N, 15.84; S, 5.19.

Z-pGlu-Gln-Arg(Tos)-Leu-Gly-Asn-Gln-Trp(Mtb)-Ala-Val-Gly-His-Leu-Met-NH_2 (30)——Compound **29** (650 mg, 0.36 mmol) was treated with TFA as described above. The resulting TFA salt was dissolved in DMF (10 ml) together with Z–pGlu–ONB (139 mg, 0.42 mmol) and *N*-ethylmorpholine (0.083 ml), and the mixture was stirred at room temperature for 15 h. To this was added CH_3CN to give a precipitate, which was reprecipitated from 85% CH_3CN: 540 mg (77.5%) mp 247°C, $[\alpha]_D^{24}$ −27° (c=0.5, DMF). *Anal.* Calcd for $C_{95}H_{132}N_{24}O_{27}S_3\cdot 4H_2O$: C, 51.61; H, 6.38; N, 15.20; S, 4.35. Found: C, 51.40; H, 6.14; N, 15.26; S, 5.00.

pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (Bombesin, 31)——Compound **30** (150 mg, 0.07 mmol) was treated with HF (3 ml) in the presence of 1,2-ethanedithiol (0.21 ml) and anisole (0.21 ml) at 0°C for 1 h. After evaporation of HF, water (20 ml) and Et_2O (20 ml) were added to the residue, and the insolubles were filtered off. The aqueous layer was taken and washed with Et_2O, then passed through a column (1×10 cm) of Amberlite IRA-410 (acetate form). The eluate and washings were combined and lyophilized. The resulting crude product was purified by partition chromatography on a column (1.5×45 cm) of Sephadex G-25 with *n*-BuOH–AcOH–H_2O (4: 1: 5) as an eluent. The fractions containing the aimed product were collected and lyophilized. The resulting powder was purified by preparative HPLC.[24] After evaporation of organic solvent, the residual aqueous solution was lyophilized to give the pure product: 54 mg (47%) $[\alpha]_D^{22}$ −53.5° (c=0.5, 5% AcOH), Rf^1=0.57 (silica gel), Rf^4=0.20 (cellulose). Amino acid analysis: His 0.96(1); Arg 1.06(1); Trp 0.92(1); Asp 1.06(1); Glu 3.23(3); Gly 1.99(2); Ala 1.00(1); Val 0.93(1); Met 0.92(1); Leu 1.96(2) (average recovery: 67.3%). *Anal.* Calcd for $C_{71}H_{110}N_{24}O_{18}S\cdot 2AcOH\cdot 3H_2O$: C, 47.53; H, 6.97; N, 18.14; S, 1.79. Found: C, 47.65; H, 7.03; N, 17.64; S, 2.21.

Z-Ser-Tyr-D-Leu-Leu-Arg(Mbs)-Pro-NH-C_2H_5 (32)——Z–Leu–Arg(Mbs)–Pro–NH–C_2H_5 (2.15 g, 3 mmol)

was hydrogenated in EtOH (20 ml) over palladium black at room temperature for 5 h. After filtration, the solvent was evaporated, and the residue was dissolved in CH_3CN (10 ml)–DMF (5 ml) together with Z–Ser–Tyr-D-Leu–OH (1.55 g, 3 mmol) and HONB (650 mg, 3.6 mmol). To this solution was added DCC (742 mg, 3.6 mmol) at 0°C, and the mixture was stirred at 0°C for 2 h and at room temperature for 15 h. After filtration, the filtrate was concentrated, and the residue was purified by column chromatography on silica gel (50 g) with MeOH–$CHCl_3$ (1: 19) as an eluent. The fractions containing the desired product were collected and the solvent was evaporated. The residue was triturated with ether to give a solid: 2.00 g (61.8%) mp 120—122°C, $[\alpha]_D^{22}$ −29.1° (c=0.5, DMF). *Anal.* Calcd for $C_{52}H_{74}N_{10}O_{13}S$: C, 57.87; H, 6.91; N, 12.98. Found: C, 57.82; H, 6.93; N, 12.36.

Boc-Trp(Mtr)-Ser-Tyr-D-Leu-Leu-Arg(Mbs)-Pro-NH-C_2H_5 (33)——Compound **32** (315 mg, 0.292 mmol) was hydrogenated in MeOH (10 ml) over palladium black at room temperature for 3 h. After filtration, the solvent was evaporated, and the resulting free base was dissolved in a solution of Boc–Trp(Mtr)–ONB [prepared from compound **10** (187 mg, 0.4 mmol)] in DMF (5 ml). The mixture was stirred for 15 h at room temperature and concentrated to dryness. The residue was triturated with Et_2O to give a powder, which was reprecipitated from MeOH–Et_2O; 297 mg (70.0%) mp 151—152°C, $[\alpha]_D^{22}$ −32.4° (c=0.5, DMF). *Anal.* Calcd for $C_{69}H_{96}N_{12}O_{19}S_2 \cdot 2H_2O$: C, 56.81; H, 6.94; N, 11.35; S, 4.33. Found: C, 56.87; H, 6.95; N, 11.60; S, 3.91.

pGlu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NH-C_2H_5 (LH-RH analog, 35)——Compound **33** (280 mg, 0.194 mmol) was treated with TFA (2 ml) at room temperature for 30 min followed by addition of 7 N HCl/dioxane (0.1 ml). To this was added Et_2O to give a precipitate, which was collected by filtration. The resulting HCl salt was dissolved in DMF (2 ml) together with Z–pGlu–His–OH (96 mg, 0.24 mmol), HONB (53 mg, 0.3 mmol), and *N*-ethylmorpholine (0.024 ml). To this was added DCC (49 mg, 0.24 mmol) at 0°C. The mixture was stirred at 0°C for 2 h and at room temperature for 15 h, then filtered. After evaporation of the solvent, the residue was precipitated from MeOH–Et_2O to give the protected nonapeptide ethylamide (**34**): 319 mg (84.0%). Without further purification, compound **34** (180 mg, 0.092 mmol) was treated with MSA (1.5 ml) in the presence of 1,2-ethanedithiol (0.1 ml) and thioanisole (0.15 ml) at room temperature for 1 h. To this was added Et_2O to give an oily precipitate, which was washed with Et_2O by decantation. The product was dissolved in water and passed through a column (0.5×10 cm) of Amberlite IRA-410 (acetate form). The eluate and washings were applied to a column (1.5×30 cm) of CM-cellulose, which was developed with a linear gradient with ammonium acetate buffer (pH 6.8, 0.005—0.15 M, 400 ml each). The fractions containing the desired product were collected and applied to a column (1.2×7 cm) of Amberlite XAD-2, which was developed with a gradient of aqueous EtOH (5—80%, 200 ml each). The fractions containing the desired product was collected and EtOH was evaporated. The residual aqueous solution was lyophilized to give a white powder: 43 mg (38.6%) $[\alpha]_D^{26}$ −33.0° (c=0.5, 5% AcOH), Rf^1=0.65 (silica gel), Rf^2=0.72 (silica gel) (lit.[27] $[\alpha]_D^{24}$ −32.7° (c=0.55, 5% AcOH), Rf^1=0.65, Rf^2=0.72). Amino acid analysis: His 0.92(1); Arg 1.00(1); Trp 0.88(1); C_2H_5NH 0.95(1); Ser 0.91(1); Glu 1.02(1); Pro 1.06(1); Leu 2.03(2); Tyr 1.09(1) (average recovery: 80%). *Anal.* Calcd for $C_{59}H_{84}N_{16}O_{12} \cdot AcOH \cdot 6H_2O$: C, 53.18; H, 7.31; N, 16.27. Found: C, 53.18; H, 6.99; N, 16.94.

H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH (Dynorphin, 36)——Boc–Gln–resin (2.5 g, 1.35 mmol Gln anchored on 2% divinylbenzene–styrene copolymer) was subjected to the following solid-phase synthetic procedure: (1) washing with CH_2Cl_2 for 1.5 min, 3 times (2) washing with 50% TFA/CH_2Cl_2 for 1.5 min (3) reaction with 50% TFA/CH_2Cl_2 for 20 min (4) washing with CH_2Cl_2 for 1.5 min, twice (5) washing with 25% dioxane/CH_2Cl_2 for 1.5 min, twice (6) repeat step 4 (7) washing with 10% Et_3N/CH_2Cl_2 for 1.5 min (8) reaction with 10% Et_3N/CH_2Cl_2 for 10 min (9) washing with CH_2Cl_2 for 1.5 min, 4 times (10) reaction with 5.4 mmol of the symmetrical anhydride of Boc-amino acid in CH_2Cl_2 for 20 min (11) addition of *N*-ethylmorpholine (0.09 ml) in CH_2Cl_2 to the coupling mixture and continued reaction for 20 min (**12**) washing with CH_2Cl_2 for 1.5 min (13) repeat step 10 (14) repeat step 11 (15) repeat step 4 (16) washing with 33% EtOH/CH_2Cl_2 for 1.5 min, 3 times. Boc group was used for N^{α}-protection of all amino acids. The symmetrical anhydrides were prepared as described by Li *et al.*[30] On completion of the synthetic procedure the peptide resin was washed successively with DMF, MeOH and CH_2Cl_2, then dried to give the protected peptide resin: 3.83 g. A part of this protected peptide resin (1.5 g) was treated with HF (20 ml) at 0°C for 60 min in the presence of 1,2-ethanedithiol (2 ml) and anisole (3 ml). After evaporation of HF, AcOEt (10 ml) was added to the residue and the mixture was stirred for 5 min. To this was added water (15 ml) and the stirring was continued for 10 min. The resin was filtered off and the aqueous layer was taken. The aqueous layer was washed again with AcOEt and passed through a column (2×20 cm) of Amberlite IRA-410 (acetate form). The eluate and washings were collected and lyophilized to give a crude product: 300 mg. This material was purified by ion-exchange chromatography on CM-cellulose, preparative HPLC, and gel-filtration on Sephadex G-25 in the same manner as for the purification of dynorphin synthesized by the solution method:[32] 15 mg (5% based on crude deprotected product) $[\alpha]_D^{24}$ −58.4° (c=0.3, 1% AcOH), Rf^2=0.64 (cellulose), Rf^3=0.56 (cellulose). (lit.[32] $[\alpha]_D^{23}$ −62.1° (c=0.3, 1% AcOH), Rf^2=0.64, Rf^3=0.56). Amino acid analysis: Lys 1.95(2); Arg 2.86(3); Trp 0.98(1); Asp 2.07(2); Glu 1.07(1); Pro 1.15(1); Gly 2.02(2); Ile 0.87(1); Leu 1.97(2); Tyr 1.00(1); Phe 0.98(1) (average recovery, 71%).

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References and Notes

1) Amino acids, peptides and their derivatives described in this paper are of L-configuration unless otherwise noted. Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature in July 1965 and 1966; *Biochemistry*, **5**, 2435 (1966); **6**, 362 (1967). Additional abbreviations: Tos, *p*-toluenesulfonyl; Mbs, *p*-methoxybenzenesulfonyl; Dmb, 2,4-dimethoxybenzenesulfonyl; Mtb, 2,4,6-trimethoxybenzenesulfonyl; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; Boc, *tert*-butoxycarbonyl; Bu^t, *tert*-butyl; Tr, trityl; Bzl, benzyl; Z, benzyloxycarbonyl; ONp, *p*-nitrophenyl ester; TFA, trifluoroacetic acid; HF, hydrogen fluoride; MSA, methanesulfonic acid; DCC, dicyclohexylcarbodiimide; HONB, *N*-hydroxy-5-norbornene-2,3-dicarboximide; HOBT, *N*-hydroxybenzotriazole; DMF, *N*,*N*-dimethylformamide; TLC, thin layer chromatography; HPLC, high-performance liquid chromatography; CM, carboxymethyl; LH-RH, luteinizing hormone-releasing hormone.
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