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Studies on Peptides. CXXIX.[1,2] Application of N^{in}-Mesitylenesulfonyltryptophan for the Syntheses of Neuromedin B and Neuromedin C

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Two Trp containing peptides, neuromedins B and C, were synthesized using a new Trp derivative, N^{in}-mesitylenesulfonyltryptophan, [Trp(Mts)]. The thioanisole-mediated deprotection with trifluoromethanesulfonic acid in trifluoroacetic acid was employed at the final steps of these syntheses to remove the Mts group together with the N^{α}-protecting group. In this deprotecting step, the use of an additional scavenger, ethanedithiol, was recommended to suppress possible indole modification. When smooth-muscle contractile activity in guinea pig ileum was examined, the relative potencies of neuromedins B and C with respect to synthetic substance P (taken as 1) were 0.46 and 0.37, respectively.

Keywords——Trp protecting group; neuromedin B solution synthesis; neuromedin C solution synthesis; thioanisole-mediated deprotection; Trp side-reaction; scavenger ethanedithiol; trifluoromethanesulfonic acid deprotection; smooth-muscle contractile activity

Indole alkylation, a side reaction of Trp observable during the N^{α}-deprotection of the Boc[3,4] or the Z(OMe) group[5] by TFA, can be suppressed to some extent by selecting a suitable cation scavenger, such as 2-methylindole[6] or dimethylsulfide[4] or anisole containing EDT.[7] However, for the synthesis of large Trp-containing peptides, it seems advantageous to protect the indole moiety of Trp by introducing a suitable electron-withdrawing substituent, since multiple TFA treatments are required for chain elongation. To meet this need, we recently introduced a new Trp derivative, N^{in}-mesitylenesulfonyltryptophan, [Trp(Mts)][8] and preliminarily demonstrated that the TFA deprotection of the Boc or the Z(OMe) group can be performed in the presence of a usual scavenger, anisole, without formation of any detectable by-products, and this protecting group can be removed by 1 M TFMSA–thioanisole in TFA[9] in an ice-bath within 60 min.

We applied this new Trp derivative for the syntheses of two newly found porcine spinal cord peptides, neuromedins B[10,11] and C,[12] in order to examine its usefulness in practical peptide syntheses. Neuromedin C is a decapeptide, the sequence of which was found to be identical with that of the C-terminal portion of porcine gastrin-releasing peptide (GRP).[13] Incidentally, the same decapeptide was found in canine intestinal extracts.[14] Neuromedin B is another decapeptide, the sequence of which differs from that of neuromedin C at three positions [3,6 and 9] as shown in Fig. 1. Their solid phase syntheses have been reported, but unprotected Trp was employed.[10,12]

H–Gly–Asn–[3]–Trp–Ala–[6]–Gly–His–[9]–Met–NH_2

	3	6	9
neuromedin B	Leu	Thr	Phe
neuromedin C	His	Val	Leu

Fig. 1. Structures of Neuromedins B and C

We synthesized neuromedin C by the solution phase method (Fig. 2). First, Boc–Trp(Mts)–OH was introduced by the Su active ester procedure[15] onto a TFA-treated sample of Z(OMe)–Ala–Val–Gly–His–Leu–Met(O)–NH_2, a fragment used for our previous synthesis of porcine GRP.[16] The TFA deprotection of the Boc group from the resulting protected heptapeptide, Boc–Trp(Mts)–Ala–Val–Gly–His–Leu–Met(O)–NH_2, proceeded smoothly without producing any pink color in the solution or any extra spot on thin-layer chromatography (TLC). Next, this TFA-treated heptapeptide was condensed *via* the azide[17] with Z(OMe)–Gly–Asn–His–$NHNH_2$ prepared by the stepwise Np method.[18] The protected neuromedin C was obtained in a homogeneous form by simple precipitation from DMSO with MeOH.

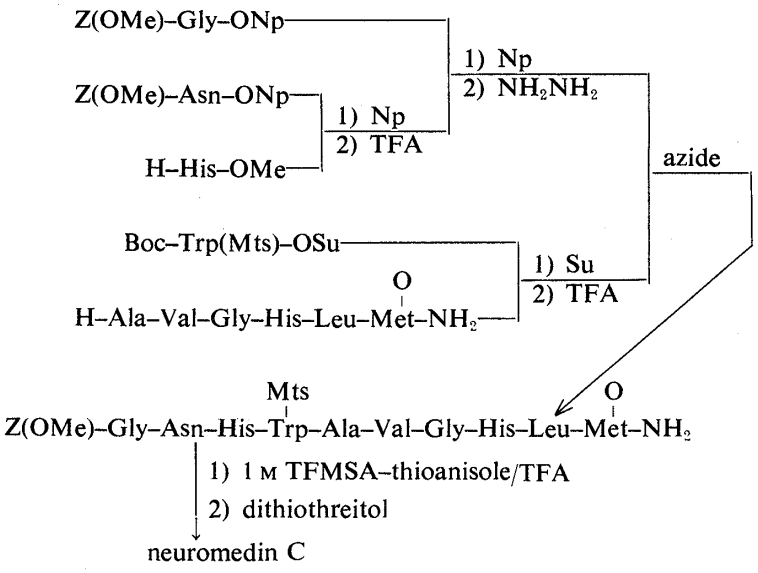

Fig. 2. Synthetic Scheme for Neuromedin C

The protected decapeptide thus obtained was treated with 1 M TFMSA–thioanisole in TFA in an ice-bath for 2 h to remove the Mts group together with the Z(OMe) group. An additional scavenger, EDT, was found to be effective to minimize indole-modification under these acidic conditions. The deprotected peptide was incubated with dithiothreitol[19] to ensure the complete reduction of the Met(O) residue to Met, though this thioanisole-mediated deprotection has the ability to reduce the sulfoxide.[9,20] The reduced peptide was purified by gel-filtration on Sephadex G-15, followed by high-performance liquid chromatography (HPLC). The purity of the product was ascertained by HPLC and the presence of the intact Trp residue in the synthetic peptide was confirmed by 4 M MSA hydrolysis[21] and leucine aminopeptidase digestion.

Next, the synthesis of neuromedin B was carried out as shown in Fig. 3. Three dipeptide units, Z(OMe)–Phe–Met(O)–NH_2, Z(OMe)–Gly–His–$NHNH_2$ and Z(OMe)–Ala–Thr–$NHNH_2$, served to construct the C-terminal hexapeptide backbone of neuromedin B, then Boc–Trp(Mts)–OH was similarly introduced by the Su method. The N^{α}-deprotection with TFA from the resulting heptapeptide, Boc–Trp(Mts)–Ala–Thr–Gly–His–Phe–Met(O)–NH_2, proceeded smoothly as in the case of neuromedin C synthesis. The final condensation of Z(OMe)–Gly–Asn–Leu–$NHNH_2$ with the resulting heptapeptide afforded the protected neuromedin B in nearly quantitative yield. Deprotection and subsequent purifications were carried out in essentially the same manner as described above to obtain a homogeneous product on HPLC. Amino acid ratios in 4 M MSA hydrolysates of synthetic neuromedin B and its intermediates are listed in Table I.

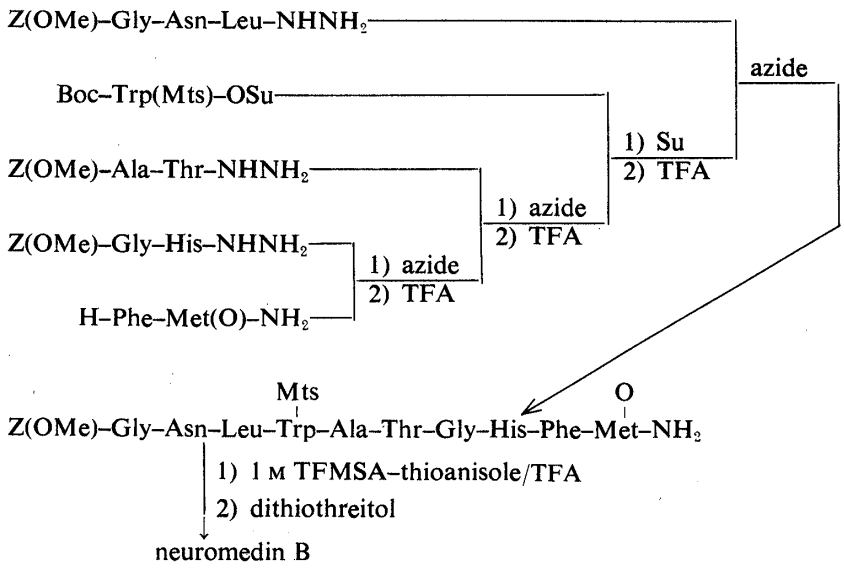

Fig. 3. Synthetic Scheme for Neuromedin B

TABLE I. Amino Acid Ratios in 4 M MSA Hydrolysates

	Protected peptide (positions)				Synthetic neuromedin B
	7—10	5—10	4—10	1—10	
Met	0.94	0.92	1.02	0.97	0.94 (1)
Phe	1.00	1.00	1.00	1.00	1.00 (1)
His	1.03	1.03	1.05	1.05	0.96 (1)
Gly	1.08	1.04	1.12	2.27	2.03 (2)
Thr		1.04	1.08	1.08	0.99 (1)
Ala		1.16	1.13	1.12	1.05 (1)
Trp			1.04	0.84	0.92 (1)
Leu				1.11	1.02 (1)
Asp				1.12	1.02 (1)
Recov. (%)	89	85	87	99	96

We wish to emphasize the role of EDT in the deprotecting step. When Boc–Trp(Mts)–OH was exposed to 1 M TFMSA–thioanisole in TFA at room temperature, a small amount of by-product was detected on TLC and its amount increased with the exposure time. After a 5 h treatment, this by-product was isolated by silica gel chromatography followed by gel-filtration on Sephadex LH-20 and characterized as 2-phenylthiotryptophan by mass and proton nuclear magnetic resonance (^{1}H-NMR) spectral analyses. In the presence of EDT, this side reaction was negligible. These results provide useful information for the application of this new Trp derivative to the solid-phase synthesis of Trp-containing peptide.

Neuromedin B and neuromedin C both exhibited weak smooth muscle contractile activities. When the maximal contractions of guinea pig ileum produced by the synthetic peptides (10^{-6} M each) were examined, the relative potencies of neuromedin B and C with respect to that of synthetic substance P (taken as 1) were 0.46 and 0.37, respectively.

Experimental

The general experimental methods employed here are essentially the same as described in part 88 of the present

series.[22] The DCC condensation[23] and the active ester reactions[15,18] were performed at room temperature. The azide reaction[17] was conducted using isoamyl nitrite. Z(OMe) or Boc group was removed by TFA (*ca.* 2 ml per 1 g of a peptide) in the presence of anisole (2 mol eq or more) at ice-bath temperature for 60 min. The N^{α}-deprotected peptide was precipitated with ether, dried over KOH pellets *in vacuo* for 3 h and then used for the next coupling reaction. *Rf* values on TLC performed on silica gel (Kieselgel G. Merck) refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 $CHCl_3$–MeOH (10:0.5), Rf_3 *n*-BuOH–AcOEt–AcOH–H_2O (1:1:1:1). LAP (leucine aminopeptidase, Lot No. L-6007) was purchased from Sigma. HPLC was conducted with a Waters 204 compact model. ^{1}H-NMR spectra were determined on a JEOL JNM-FX 200 spectrometer and mass spectra were taken on a JEOL JMS-01SG-2 spectrometer with a direct heated inlet system.

Synthesis of Neuromedin C

Z(OMe)–Asn–His–OMe——A mixture of Z(OMe)–Asn–ONp (5.00 g, 12.00 mmol) and H–His–OMe [prepared from 2.90 g (12.00 mmol) of the dihydrochloride] in DMF (60 ml) was stirred for 2 d and the solvent was removed by evaporation. Treatment of the residue with 5% $NaHCO_3$ and ether afforded a powder, which was precipitated from DMF with MeOH; yield 2.90 g (54%), mp 187—190 °C, $[\alpha]_D^{18}$ −8.8° (*c*=1.0, DMF), Rf_1 0.41. *Anal.* Calcd for $C_{20}H_{25}N_5O_7 \cdot H_2O$: C, 51.61; H, 5.85; N, 15.05. Found: C, 51.31; H, 5.68; N, 14.89.

Z(OMe)–Gly–Asn–His–OMe——A TFA-treated sample of Z(OMe)–Asn–His–OMe (2.50 g, 5.59 mmol) was dissolved in DMF (30 ml) together with Et_3N (1.94 ml, 13.98 mmol) and Z(OMe)–Gly–ONp (2.42 g, 6.71 mmol) and the mixture, after being stirred overnight, was concentrated. Trituration of the residue with 5% $NaHCO_3$ and ether afforded a powder, which was precipitated from DMF with AcOEt; yield 2.35 g (83%), mp 192—195 °C, $[\alpha]_D^{18}$ +21.4° (*c*=1.0, DMF), Rf_1 0.35. *Anal.* Calcd for $C_{22}H_{28}N_6O_8$: C, 52.37; H, 5.59; N, 16.66. Found: C, 52.38; H, 5.64; N, 16.45.

Z(OMe)–Gly–Asn–His–NHNH$_2$——Z(OMe)–Gly–Asn–His–OMe (2.15 g, 4.26 mmol) in DMF (20 ml) was treated with 80% hydrazine hydrate (1.07 ml, 5 eq) overnight. The solvent was removed by evaporation and the residue was treated with EtOH to form a powder, which was precipitated from DMSO with MeOH; yield 2.12 g (98%), mp 201—203 °C, $[\alpha]_D^{23}$ −25.2° (*c*=1.0, DMSO), Rf_1 0.15, Rf_3 0.52. Amino acid ratios in 6 N HCl hydrolysate: Gly 1.00, Asp 0.98, His 0.96 (recovery of Gly 92%). *Anal.* Calcd for $C_{21}H_{28}N_8O_7 \cdot 1/2H_2O$: C, 49.21; H, 5.69; N, 21.82. Found: C, 49.16; H, 5.62; N, 21.46.

Boc–Trp(Mts)–Ala–Val–Gly–His–Leu–Met(O)–NH$_2$——A TFA-treated sample of Z(OMe)–Ala–Val–Gly–His–Leu–Met(O)–NH_2 (1.00 g, 1.24 mmol) was dissolved in DMF (10 ml) together with Et_3N (0.36 ml, 2.60 mmol) and Boc–Trp(Mts)–OSu (1.09 g, 1.86 mmol) and the mixture, after being stirred overnight, was concentrated. Treatment of the residue with 5% $NaHCO_3$ and ether afforded a powder, which was precipitated from DMF with AcOEt; yield 0.71 g (52%), mp 207—209 °C, $[\alpha]_D^{17}$ +22.4° (*c*=1.0, DMF), Rf_1 0.40. Amino acid ratios in 4 M MSA hydrolysate: Trp 0.83, Ala 1.02, Val 1.03, Gly 1.01, His 0.97, Leu 1.00, Met 0.91 (recovery of Leu 73%). *Anal.* Calcd for $C_{52}H_{75}N_{11}O_{12}S_2 \cdot 2H_2O$: C, 54.48; H, 6.95; N, 13.44. Found: C, 54.36; H, 6.91; N, 13.25.

Z(OMe)–Gly–Asn–His–Trp(Mts)–Ala–Val–Gly–His–Leu–Met(O)–NH$_2$——A TFA-treated sample of the above heptapeptide (0.50 g, 0.45 mmol) was dissolved in DMF (5 ml) containing Et_3N (0.13 ml, 0.90 mmol). The azide [prepared from 0.34 g (0.68 mmol) of Z(OMe)–Gly–Asn–His–$NHNH_2$] in DMF (5 ml) and Et_3N (0.06 ml, 0.45 mmol) were added to the above ice-chilled solution and the mixture, after being stirred at 4 °C for 3 d, was concentrated. The residue was treated with 5% $NaHCO_3$ and ether to form a powder, which was precipitated from DMSO with MeOH; yield 0.57 g (85%), mp 232—235 °C, $[\alpha]_D^{21}$ −25.9° (*c*=0.7, DMSO), Rf_1 0.24. Amino acid ratios in 4 M MSA hydrolysate: Gly 2.02, Asp 1.00, His 1.96, Trp 0.80, Ala 1.00, Val 1.01, Leu 1.00, Met 0.87 (recovery of Leu 92%). *Anal.* Calcd for $C_{68}H_{91}N_{17}O_{17}S_2 \cdot H_2O$: C, 54.42; H, 6.24; N, 15.87. Found: C, 54.40; H, 6.31; N, 15.88.

H–Gly–Asn–His–Trp–Ala–Val–Gly–His–Leu–Met–NH$_2$ (Neuromedin C)——The protected neuromedin C (100 mg, 67 μmol) was treated with 1 M TFMSA–thioanisole in TFA (1.35 ml) in the presence of *m*-cresol (0.15 ml, 20 eq) and EDT (0.13 ml, 20 eq) in an ice-bath for 2 h. Dry ether was added and the resulting powder was dissolved in H_2O–MeOH (10 ml–5 ml). The solution was adjusted to pH 7.0 with 1 N NH_4OH under ice-cooling. Dithiothreitol (1.04 g, 100 eq) was added and the solution was incubated at 37 °C for 2 d and then concentrated *in vacuo*. The solution was dissolved in 1 N AcOH (2 ml) and the solution was applied to a column of Sephadex G-15 (2.8 × 86 cm), which was eluted with the same solvent. Individual fractions (8 ml each) were examined by measurement of the ultraviolet (UV) absorption at 280 nm. The fractions corresponding to the front main peak (tube Nos. 26—32) were combined and the solvent was removed by lyophilization to give a powder; yield 38 mg (51% from the protected peptide).

Subsequently a part of the sample (2 mg) was purified by reverse phase HPLC on a Nucleosil $5C_{18}$ column (10 × 250 mm) using isocratic elution with CH_3CN–0.1% TFA (27:73) at a flow rate of 1.4 ml per min. The eluate corresponding to the main peak (retention time 18.5 min) was collected and the solvent was removed by lyophilization to give a white fluffy powder; 1.7 mg (86%). The rest of the sample was similarly purified. The combined sample was dissolved in 1 N AcOH (5 ml), and this, after treatment with Amberlite CG-4B (acetate form, approximately 1 g) for 20 min, was lyophilized to give a white fluffy powder; yield 33 mg (44% from the protected peptide), $[\alpha]_D^{21}$ −80.4° (*c*= 0.1, 1 N AcOH), Rf_3 0.49. Amino acid ratios in a 4 M MSA hydrolysate: 1Asp 0.99, 2Gly 1.95, 1Ala 1.02, 1Met 0.72,

1Val 0.99, 1Leu 1.00, 1Trp 0.74, 2His 1.89 (recovery of Leu 94%). Amino acid ratios in LAP digest: 1Asn ND, 2Gly 2.01, 1Ala 0.95, 1Met 0.96, 1Val 1.02, 1Leu 1.00, 1Trp 0.80, 2His 1.93 (recovery of Leu 75%). *Anal.* Calcd for $C_{50}H_{73}N_{17}O_{11}S \cdot 3CH_3COOH \cdot 2H_2O$: C, 50.32; H, 6.71; N, 17.82. Found: C, 50.57; H, 6.44; N, 18.25.

Synthesis of Neuromedin B

Z(OMe)–Phe–Met(O)–NH_2——A mixture of Z(OMe)–Phe–ONp (5.48 g, 12.20 mmol), Et_3N (1.70 ml 12.20 mmol) and H–Met(O)–NH_2 [obtained from 4.00 g (12.20 mmol) of the Z(OMe)–derivative] in DMF (40 ml) was stirred for 24 h and the solvent was removed by evaporation. The residue was treated with 5% citric acid to afford a powder, which was precipitated from DMF with AcOEt; yield 3.73 g (64%), mp 211—214 °C, $[\alpha]_D^{19}$ −9.4° (c=1.0, DMF), Rf_1 0.65. *Anal.* Calcd for $C_{23}H_{29}N_3O_6S$: C, 58.09; H, 6.15; N, 8.84. Found: C, 57.82; H, 6.14; N, 8.95.

Z(OMe)–Gly–His–OMe——DCC (5.17 g, 25.06 mmol) was added to a mixture of Z(OMe)–Gly–OH (5.00 g, 20.90 mmol) and H–His–OMe [prepared from 5.06 g (20.90 mmol) of the dihydrochloride] in DMF (70 ml) and the mixture was stirred for 24 h. The solution was filtered, the filtrate was concentrated and the residue was dissolved in AcOEt. The organic solution was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, then dried over Na_2SO_4 and concentrated. Trituration of the residue with ether afforded a powder, which was recrystallized from MeOH and ether; yield 6.05 g (74%), mp 106—107 °C, $[\alpha]_D^{18}$ +1.5° (c=2.0, MeOH), Rf_1 0.73. *Anal.* Calcd for $C_{18}H_{22}N_4O_6$: C, 55.38; H, 5.68; N, 14.35. Found: C, 55.58; H, 5.93; N, 14.25.

Z(OMe)–Gly–His–$NHNH_2$——Z(OMe)–Gly–His–OMe (4.00 g, 10.24 mmol) dissolved in MeOH (50 ml) was treated with 80% hydrazine hydrate (2.56 ml, 5 eq). The solid that formed on standing overnight was precipitated from DMSO with MeOH; yield 3.01 g (75%), mp 216—218 °C, $[\alpha]_D^{18}$ −5.9° (c=1.0, DMSO), Rf_1 0.29. *Anal.* Calcd for $C_{17}H_{22}N_6O_5$: C, 52.30; H, 5.68; N, 21.53. Found: C, 52.10; H, 5.68; N, 21.25.

Z(OMe)–Ala–Thr–OMe——The title compound was prepared by the usual condensation of Z(OMe)–Ala–OH (5.31 g, 20.98 mmol) and H–Thr–OMe [prepared from 3.00 g (25.18 mmol) of the hydrochloride] with DCC (4.76 g, 23.08 mmol) and purified by the extraction procedure as described above followed by recrystallization from MeOH and AcOEt; yield 4.84 g (63%), mp 132—134 °C, $[\alpha]_D^{18}$ −18.3° (c=1.0, MeOH), Rf_1 0.86, Rf_2 0.35. *Anal.* Calcd for $C_{17}H_{24}N_2O_7$: C, 55.42; H, 6.57; N, 7.60. Found: C, 55.68; H, 6.64; N, 7.66.

Z(OMe)–Ala–Thr–$NHNH_2$——The above methyl ester (4.40 g, 11.94 mmol) in MeOH (50 ml) was treated with 80% hydrazine hydrate (2.99 ml, 5 eq) and the solid that formed on standing overnight was precipitated from DMF with ether; yield 4.10 g (93%), mp 168—170 °C, $[\alpha]_D^{17}$ +19.9° (c=1.0, DMF), Rf_1 0.55. *Anal.* Calcd for $C_{16}H_{24}N_4O_6$: C, 52.16; H, 6.57; N, 15.21. Found: C, 51.87; H, 6.71; N, 15.45.

Z(OMe)–Gly–Asn–Leu–OMe——Z(OMe)–Gly–ONp (3.46 g, 9.61 mmol) and Et_3N (1.33 ml, 9.61 mmol) were added to a solution of H–Asn–Leu–OMe [prepared from 3.70 g (8.74 mmol) of the Z(OMe)-derivative[24]] in DMF (30 ml) and the mixture was stirred for 15 h. The solvent was removed by evaporation and the residue was treated with H_2O to form a powder, which was recrystallized from MeOH with ether; yield 2.50 g (60%), mp 168—170 °C, $[\alpha]_D^{18}$ −20.4°(c=1.0, MeOH), Rf_1 0.69. *Anal.* Calcd for $C_{22}H_{32}N_4O_8 \cdot 1/2H_2O$: C, 53.97; H, 6.80; N, 11.45. Found: C, 54.18; H, 6.64; N, 11.54.

Z(OMe)–Gly–Asn–Leu–$NHNH_2$——Z(OMe)–Gly–Asn–Leu–OMe (2.40 g, 4.99 mmol) in MeOH (150 ml) was treated with 80% hydrazine hydrate (1.25 ml, 5 eq) and the solid that formed on standing for 24 h was precipitated from DMF with ether; yield 1.90 g (79%), mp 200—202 °C, $[\alpha]_D^{17}$ −20.6° (c=1.0, DMF), Rf_1 0.33. Amino acid ratios in 6 N HCl hydrolysate: Gly 1.00, Asp 0.99, Leu 1.02 (recovery of Gly 99%). *Anal.* Calcd for $C_{21}H_{32}N_6O_7 \cdot 1/2H_2O$: C, 51.52; H, 6.80; N, 17.17. Found: C, 51.83; H, 6.70; N, 17.36.

Z(OMe)–Gly–His–Phe–Met(O)–NH_2——The azide [prepared from 2.85 g (7.30 mmol) of Z(OMe)–Gly–His–$NHNH_2$] in DMF (30 ml) and Et_3N (1.01 ml, 7.30 mmol) were added to an ice-chilled solution of H–Phe–Met(O)–NH_2 [prepared from 3.47 g (7.30 mmol) of the Z(OMe)-derivative] in DMF (15 ml) and the mixture, after being stirred at 4 °C overnight, was concentrated. Trituration of the residue with 5% $NaHCO_3$ and ether afforded a powder, which was precipitated from DMSO with MeOH; yield 3.35 g (65%), mp 189—191 °C, $[\alpha]_D^{18}$ +2.0° (c=1.0, DMSO), Rf_1 0.25. Amino acid ratios in an acid hydrolysate are shown in Table I, together with those of other intermediates. *Anal.* Calcd for $C_{31}H_{39}N_7O_8S \cdot 1.5H_2O$: C, 53.43; H, 6.08; N, 14.07. Found: C, 53.37; H, 6.08; N, 13.84.

Z(OMe)–Ala–Thr–Gly–His–Phe–Met(O)–NH_2——The azide [prepared from 2.72 g (7.37 mmol) of Z(OMe)–Ala–Thr–$NHNH_2$] in DMF (30 ml) and Et_3N (0.78 ml, 7.37 mmol) were added to an ice-chilled solution of H–Gly–His–Phe–Met(O)–NH_2 [prepared from 3.80 g (5.67 mmol) of the Z(OMe)-derivative] in DMF (20 ml) and the mixture, after being stirred at 4 °C overnight, was concentrated. The product was purified by trituration with 5% $NaHCO_3$ and ether, followed by precipitation from DMF with EtOH; yield 1.65 g (35%), mp 193—195 °C, $[\alpha]_D^{23}$ −28.6° (c=0.2, DMF), Rf_1 0.24. *Anal.* Calcd for $C_{38}H_{51}N_9O_{11}S \cdot H_2O$: C, 53.07; H, 6.21; N, 14.66. Found: C, 53.17; H, 6.15; N, 14.27.

Boc–Trp(Mts)–Ala–Thr–Gly–His–Phe–Met(O)–NH_2——Boc–Trp(Mts)–OSu (1.72 g, 2.94 mmol) and Et_3N (0.27 ml, 1.96 mmol) were added to a solution of H–Ala–Thr–Gly–His–Phe–Met(O)–NH_2 [prepared from 1.65 g (1.96 mmol) of the Z(OMe)-derivative] in DMF (10 ml) and the mixture, after being stirred at room temperature overnight, was concentrated. Trituration of the residue with 5% $NaHCO_3$ afforded a powder, which was purified by column chromatography on silica gel (1.2 × 35 cm) using $CHCl_3$–MeOH–H_2O (8 : 3 : 1) as an eluant, followed by precipitation from DMF with AcOEt; yield 1.12 g (50%), mp 157—159 °C, $[\alpha]_D^{20}$ −9.1° (c=0.8, DMF), Rf_1 0.39.

Anal. Calcd for $C_{54}H_{71}N_{11}O_{13}S_2 \cdot H_2O$: C, 55.70; H, 6.32; N, 13.23. Found: C, 55.82; H, 6.50; N, 13.01.

Z(OMe)–Gly–Asn–Leu–Trp(Mts)–Ala–Thr–Gly–His–Phe–Met(O)–NH_2——The TFA-treated sample of the above heptapeptide (1.00 g, 0.87 mmol) was dissolved in DMF (10 ml) containing Et_3N (0.24 ml, 1.74 mmol). The azide [prepared from 0.63 g (1.31 mmol) of Z(OMe)–Gly–Asn–Leu–$NHNH_2$] in DMF (5 ml) was added to the above ice-chilled solution and the mixture, after being stirred at 4 °C for 3 d, was concentrated. The residue was treated with 5% $NaHCO_3$ and ether, and the resulting powder was precipitated from DMF with MeOH; yield 1.18 g (91%), mp 221—224 °C, $[\alpha]_D^{17}$ −27.8° (c=0.3, DMF), Rf_1 0.30. *Anal.* Calcd for $C_{70}H_{91}N_{15}O_{18}S_2$: C, 56.25; H, 6.14; N, 14.06. Found: C, 55.98; H, 6.36; N, 13.92.

H–Gly–Asn–Leu–Trp–Ala–Thr–Gly–His–Phe–Met–NH_2——The protected neuromedin B (100 mg, 67 μmol) was treated with 1 M TFMSA–thioanisole in TFA (1.34 ml) in the presence of *m*-cresol (0.15 ml, 20 eq) and EDT (0.13 ml, 20 eq) in an ice-bath for 2 h, then dry ether was added to form a powder, which was dissolved in H_2O–MeOH (10 ml–5 ml). The solution was adjusted to pH 8.0 with 1 N NH_4OH under ice-cooling and after 30 min to pH 7.0 with 1 N AcOH. Dithiothreitol (1.04 g, 100 eq) was added and the solution was incubated at 37 °C for 2 d. After evaporation of the solvent, the residue was dissolved in 1 N AcOH (5 ml) and this solution was applied to a column of Sephadex G-15 (2.8 × 86 cm), which was eluted with the same solvent. Each fraction (8 ml each) was examined by measuring the UV absorption at 280 nm, and the fractions corresponding to the front main peak (tube Nos. 48—53) were combined and the solvent was removed by lyophilization to give a powder; yield 70 mg (92% from the protected peptide).

Subsequently a part of the resulting powder (2.5 mg) was purified by HPLC on a column of Nucleosil $5C_{18}$ (10 × 250 mm) using isocratic elution with CH_3CN–0.1% TFA (28 : 72) at a flow rate of 1.4 ml per min. The eluate corresponding to the main peak (retention time 27 min) was collected and the solvent was removed by lyophilization to give a white fluffy powder; yield 1.5 mg (58%). The rest of the sample was similarly purified. The combined sample was dissolved in 1 N AcOH (5 ml), and this, after treatment with Amberlite CG-4B (acetate form), was lyophilized to give a white fluffy powder; yield 41 mg (54% from the protected peptide), $[\alpha]_D^{21}$ −142.4° (c=0.1, 1 N AcOH), Rf_3 0.52. Amino acid ratios in a 4 M MSA hydrolysate are listed in Table I. Amino acid ratios in LAP digest: 1Thr 0.98, 1Asn, 0.90, 2Gly 2.03, 1Ala 0.98, 1Met 0.99, 1Leu 1.00, 1Phe 1.00, 1Trp 0.91, 1His 0.98 (recovery of Phe 95%). *Anal.* Calcd for $C_{52}H_{73}N_{15}O_{12}S \cdot 2CH_3COOH \cdot 3H_2O$: C, 51.48; H, 6.40; N, 16.08. Found: C, 51.70; H, 6.21; N, 16.15.

2-Phenylthiotryptophan——Boc–Trp(Mts)–OH [derived from 1.00 g (1.50 mmol) of the dicyclohexylamine (DCHA) salt] was treated with 1 M TFMSA–thioanisole in TFA (15 ml) in the presence of *m*-cresol (0.80 ml, 5 eq) at room temperature for 5 h, then *n*-hexane was added. The main by-product was first isolated by preparative silica gel chromatography (Kieselgel 60 F_{254}, Lot No. 5717, Merk) using $CHCl_3$–MeOH–H_2O (8 : 3 : 1) and then by gel-filtration on Sephadex LH-20 (2.2 × 120 cm) using MeOH as an eluant. Treatment with *n*-hexane afforded a powder; yield 19 mg (4.1%), mp 208 °C (dec.), Rf_1 0.35. MS *m/e*: 312 (M^+ $C_{17}H_{16}N_2O_2S$), 110 (HS-C_6H_5). NMR (CD_3OD) δ: 3.24 (1H, dd, J_1=10.0 Hz, J_2=15.0 Hz, α-CH), 3.61 (1H, dd, J_1=4.2 Hz, J_2=15.0 Hz, $-CH_2-$), 3.88 (1H, dd, J_1=4.2 Hz, J_2=10.0 Hz, $-CH_2-$), 7.04—7.26 (7H, m, aromatic H), 7.36 (1H, d, J=7.6 Hz, indole 4-H), 7.78 (1H, d, J=7.6 Hz, indole 7-H). NMR (DMSO-d_6) δ: 11.48 (1H, s, indole NH).

References and Notes

1) Part CXXVIII: N. Fujii, Y. Hayashi, S. Katakura, K. Akaji, H. Yajima, A. Inouye, and T. Segawa, *Int. J. Pept. Protein Res.*, in press.
2) The terms of neuromedin B and neuromedin C were recommended by IUPHAR, a satellite symposium on substance P, metabolism and biological actions, London, Aug. 6, 1984. Amino acids, peptides and their derivatives are of the L-configuration. Abbreviations used are those recommended by the IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN): *Eur. J. Biochem.*, **138**, 9 (1984). The following abbreviations are used: Boc=*tert*-butoxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Np=*p*-nitrophenyl, Su=*N*-hydroxysuccinimidyl, TFA=trifluoroacetic acid, MSA=methanesulfonic acid, TFMSA=trifluoromethanesulfonic acid, DMF=dimethylformamide, DMSO=dimethylsulfoxide, EDT=ethanedithiol, DCC=dicyclohexylcarbodiimide.
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