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Studies on Peptides. CXXI.[1,2] N^{in}-Mesitylenesulfonyltryptophan, a New Derivative for Peptide Synthesis

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The mesitylenesulfonyl (Mts) group was introduced at the N^{in} of tryptophan by Illi's method. This group is stable under various conditions required for practical peptide synthesis, such as treatments with dil. alkali, hydrazine hydrate, TFA and even 4 N HCl–dioxane or 25% HBr–AcOH. HF is not able to remove this protecting group, but it can be smoothly cleaved by 1 M trifluoromethanesulfonic acid/TFA or methanesulfonic acid. The usefulness of this new tryptophan derivative, Trp(Mts), for practical peptide synthesis was demonstrated by the synthesis of cholecystokinin-heptapeptide as an example.

Keywords——mesitylenesulfonyl group for Trp protection; electron-withdrawing protecting group; Illi's reaction; ethanedithiol as scavenger; 3,5-dimethylanisole as scavenger; cholecystokinin-heptapeptide

Recently, various N^{in}-protecting groups for tryptophan (Trp) have been investigated in order to suppress indole-alkylation[3] or dimerization[4] during the N^{α}-TFA deprotection in peptide synthesis. For the synthesis of small Trp-containing peptides, N^{in}-protection is not an absolute requirement, since side reaction can be largely suppressed by the use of sulfur compounds, such as ethanedithiol,[5] dimethylsulfide,[6] or thioanisole.[7] However, for the synthesis of large peptides, especially peptides containing the Trp residue near the C-terminal portion, a suitable N^{in}-protected Trp derivative, which can survive multiple TFA treatments, is desirable, since the possibility cannot be excluded that the sulfur compounds mentioned above may accelerate the cleavage of side-chain protecting groups during multiple TFA treatments.[8,9] Such N^{in}-protecting groups should in turn be cleavable under mild conditions in the last step of the synthesis.

Besides the formyl,[10] benzyloxycarbonyl,[11] and 2,4-dichlorobenzyloxycarbonyl[11] groups, several sophisticated N^{in}-arylsulfonyl groups were examined by Fukuda *et al.*[12] and of these, the 2,4,6-trimethoxybenzenesulfonyl (Mtb) or 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group was recommended for practical use in peptide synthesis. In particular, the Mtr group is reported to be readily cleaved by HF[13] or MSA.[14] Alternatively, as an electron-withdrawing protecting group, the N^{in}-2,2,2-trichloroethyloxycarbonyl (Troc) group was introduced by Kiso *et al.*[15] This group is removable by treatment with Cd dust in acetic acid[16] and also under basic conditions, *i.e.*, hydrazine hydrate or sodium hydroxide treatment.

We wish to report that the mesitylenesulfonyl (Mts) group introduced by us as an N^{G}-protecting group for arginine[17,18] can also be used for N^{in}-protection of Trp.

Illi's method,[19] employed by Fukuda *et al.*[12] and Kiso *et al.*[15] is also the best method for introduction of the Mts group at the N-indole function of Trp. Mesitylenesulfonyl chloride was allowed to react with Z(OMe)–Trp–OBzl in the presence of pulverized NaOH and a catalytic amount of cetyltrimethylammonium chloride in CH_2Cl_2 to give Z(OMe)–Trp(Mts)–OBzl as an oily product, from which the Bzl group was removed by saponification as shown in

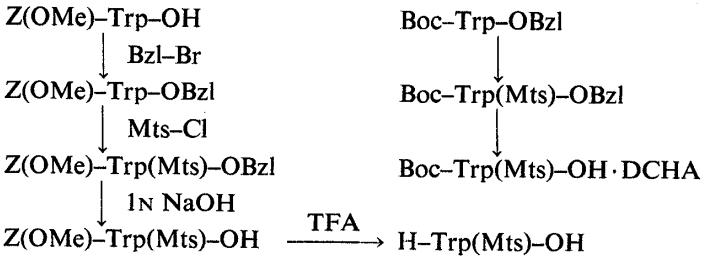

Fig. 1. Scheme for the Preparation of H–Trp(Mts)–OH

TABLE I. Recovery of Trp after Treatment of H–Trp(Mts)–OH under Various Acid Conditions (0 °C, 60 min)

Acid	Adduct	Recovery (%)
1 M TFMSA/TFA	—	83.8
1 M TFMSA/TFA	Thioanisole	≒100
MSA	—	94.6
MSA/TFA (1 : 1)	—	95.0
MSA/TFA (1 : 1)	Thioanisole	≒100
HF	—	18.3
HF	Thioanisole	63.4

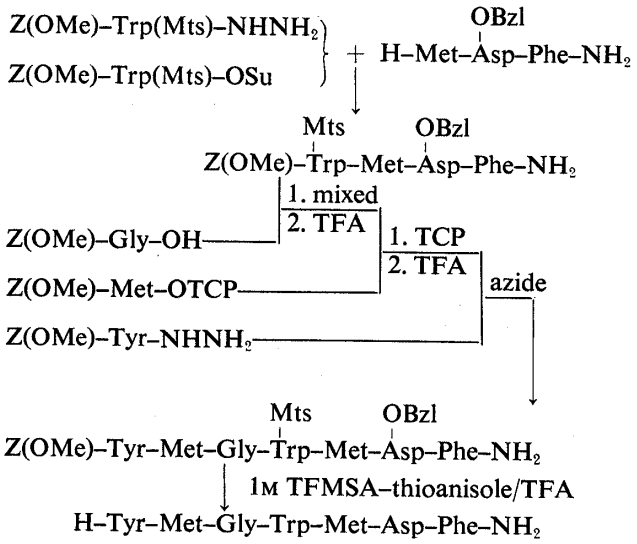

Fig. 2. Synthetic Scheme for CCK-Heptapeptide Amide

Fig. 1. Z(OMe)–Trp(Mts)–OH, thus obtained in nearly quantitative yield, was fully characterized by elemental analysis. Boc–Trp(Mts)–OH was similarly prepared from Boc–Trp–OBzl[20] and characterized as its DCHA salt. TFA treatment of the former compound afforded H–Trp(Mts)–OH as a crystalline compound.

The Mts group attached at the N^{in} of Trp was found to be stable to various treatments required for peptide synthesis; *i.e.*, saponification with 1 N NaOH, hydrazinolysis, and acid treatment with TFA or 4 N HCl–dioxane or even 25% HBr–AcOH. It is also stable under catalytic hydrogenation and even treatment with TFA–thioanisole,[8,9] but is readily cleaved by 1 M TFMSA–thioanisole/TFA[21] or MSA (Table I).[14] The N^{in}-Mts group of Trp was found to be rather more stable than the N^{G}-Mts group of arginine to HF treatment.

In order to examine the usefulness of this new Trp-derivative in practical peptide

synthesis, the desulfated form of cholecystokinin (CCK)-heptapeptide amide, H–Tyr–Met–Gly–Trp–Met–Asp–Phe–NH_2,[22] was synthesized according to the scheme illustrated in Fig. 2. We were interested in examining its analgesic activity at this stage, because of its sequence homology to Met-enkephalin, H–Tyr–Gly–Gly–Phe–Met–OH.[23] Recently, Faris *et al.*[24] reported that several actions of CCK-octapeptide are opposite to those of the opiates under certain conditions.

Z(OMe)–Trp(Mts)–OH was coupled to H–Met–Asp(OBzl)–Phe–NH_2 [derived from the corresponding Z(OMe)-derivative[25]] by two alternative methods; the Su ester method[26] and the azide method.[27] In the latter case, Z(OMe)–Trp(Mts)–$NHNH_2$ was prepared by the usual hydrazine treatment of Z(OMe)–Trp(Mts)–OBzl. The identity of the product, Z(OMe)–Trp(Mts)–Met–Asp(OBzl)–Phe–NH_2, obtained by the azide method with that obtained by the Su method was confirmed by comparison of their *Rf* values on thin layer chromatography (TLC) and their amino acid analyses. This new derivative thus seems to provide a way of synthesizing Trp-containing peptides by the azide fragment condensation procedure.

The peptide chain of this protected form of tetragastrin[28] was elongated by a combination of N^{α}-deprotection with TFA and stepwise addition of appropriate amino acids. 3,5-Dimethylanisole (DMA) containing 2% EDT, instead of anisole, was used as a cation scavenger to suppress S-alkylation at the unprotected Met residues, during the TFA treatment.[29] Every protected product was easily purified by simple precipitation from DMF with ether, and no impurities due to side reactions usually observable at unprotected Trp residues, as well as at unprotected Met residues, were detected by TLC. The protected heptapeptide amide thus obtained was treated with 1 M TFMSA–thioanisole/TFA in the presence of *m*-cresol to remove all protecting groups, then the product was purified by chromatography on silica gel, followed by gel-filtration on Sephadex LH-20. The purity of the product was confirmed by TLC, high performance liquid chromatography (HPLC) and enzymatic digestion.

In terms of the tail-pinch test,[30] this synthetic desulfated form of CCK-heptapeptide amide was found to have analgesic activity comparable to that of Met-enkephalin, when administered with a J-shaped needle into the visternal magna of unanesthetized mice.

Through these preliminary experiments, we have obtained evidence that this new Trp-protecting group should be effective for the synthesis of large Trp-containing peptides.

Experimental

Rf values in TLC (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 $CHCl_3$–MeOH (9 : 1), Rf_4 $CHCl_3$–MeOH–H_2O (18 : 3 : 1). HPLC was conducted with a Waters 204 compact model equipped with a Cosmosil $5C_{18}$ column (0.46 × 15 cm).

Z(OMe)–Trp–OBzl——Starting with Z(OMe)–Trp–OH (20.0 g, 54.4 mmol), the Bzl ester was prepared in the same manner as described for the preparation of the corresponding Boc-derivative[20] and purified by recrystallization from AcOEt and *n*-hexane; yield 21.86 g (88%), mp 94—95 °C, $[\alpha]_D^{22}$ −16.1 ° (*c* = 0.6, DMF), Rf_2 0.71. *Anal.* Calcd for $C_{27}H_{26}N_2O_5$: C, 70.73; H, 5.72; N, 6.11. Found: C, 70.80; H, 5.92; N, 6.21.

Z(OMe)–Trp(Mts)–OH——A solution of Mts–Cl (32.85 g, 150 mmol) in CH_2Cl_2 (300 ml) was added dropwise to an ice–NaCl chilled mixture of Z(OMe)–Trp–OBzl (27.51 g, 60 mmol), NaOH powder (6.0 g, 150 mmol) and cetyltrimethylammonium chloride (0.19 g, 0.6 mmol) in CH_2Cl_2 (300 ml), and the mixture, after being stirred in an ice-bath for 16 h, was acidified with 1 N HCl. The organic phase was washed with H_2O–NaCl, 5% $NaHCO_3$ and H_2O, then dried over Na_2SO_4 and concentrated. The residue was dissolved in EtOH (600 ml) and treated with 1 N NaOH (90 ml) in an ice-bath for 2.5 h. The solvent was evaporated off *in vacuo* and the residue was dissolved in H_2O (400 ml). The aqueous phase, after being washed with ether, was acidified with 1 N HCl and the resulting oily precipitate was extracted with AcOEt. The organic phase was washed with H_2O–NaCl, dried over Na_2SO_4 and concentrated. Treatment of the residue with *n*-hexane afforded a powder; yield 32.4 g (98%), Rf_1 0.79. For elemental analysis, a part of the sample was purified by silica-gel chromatography using $CHCl_3$–MeOH (40 : 1) as an eluant. mp 77—79 °C, $[\alpha]_D^{18}$ −10.1 ° (*c* = 1.8, DMF). *Anal.* Calcd for $C_{29}H_{30}N_2O_7S$: C, 63.26; H, 5.49; N, 5.09. Found: C, 63.21; H, 5.42; N, 5.22.

H–Trp(Mts)–OH——Z(OMe)–Trp(Mts)–OH (0.55 g, 1.0 mmol) was treated with TFA–anisole (1.5 ml–0.3 ml) in an ice-bath for 60 min, then TFA was removed by evaporation. Treatment of the residue with ether afforded a

powder, which was recrystallized from MeOH and ether in the presence of a few drops of Et_3N; yield 0.34 g (87%), mp 211—213 °C, $[\alpha]_D^{25}$ −30.0° (c=0.6, DMF), Rf_1 0.44. *Anal.* Calcd for $C_{20}H_{22}N_2O_4S$: C, 62.16; H, 5.74; N, 7.25. Found: C, 61.89; H, 5.74; N, 7.11.

Z(OMe)–Trp(Mts)–NHNH$_2$——Z(OMe)–Trp(Mts)–OBzl (0.50 g, 0.78 mmol) in EtOH (20 ml) was treated with 80% hydrazine hydrate (0.19 ml, 5 eq) at room temperature overnight. The solvent was evaporated off and the residue was treated with H_2O to form a powder, which was recrystallized from EtOH and ether; yield 0.34 g (77%), mp 121—123 °C, $[\alpha]_D^{25}$ −41.3° (c=0.8, DMF), Rf_3 0.49. *Anal.* Calcd for $C_{29}H_{32}N_4O_6S$: C, 61.69; H, 5.71; N, 9.92. Found: C, 61.77; H, 5.75; N, 10.16.

Boc–Trp(Mts)–OH·DCHA——Under cooling with ice–NaCl, NaOH powder (0.76 g, 19 mmol) was added to a stirred mixture of Boc–Trp–OBzl[20] (3.0 g, 7.6 mmol) and cetyltrimethylammonium chloride (24.4 mg, 76 μmol) in CH_2Cl_2 (50 ml), then a solution of Mts–Cl (4.16 g, 19.0 mmol) in CH_2Cl_2 (70 ml) was added dropwise during a period of 15 min. Stirring was continued at 4 °C for 24 h and the product was isolated as described in the preparation of the corresponding Z(OMe)-derivative. For characterization, the oily product was converted to the DCHA salt in the usual manner and the salt was recrystallized from THF and *n*-hexane; yield 3.76 g (74%), mp 154—155 °C, $[\alpha]_D^{20}$ +19.9° (c=1.0, MeOH), Rf_1 0.57. *Anal.* Calcd for $C_{25}H_{30}N_2O_6S \cdot C_{12}H_{23}N$: C, 66.53; H, 8.00; N, 6.29. Found: C, 66.73; H, 8.13; N, 6.03.

Stability of H–Trp(Mts)–OH——H–Trp(Mts)–OH (4.0 mg, 10.4 μmol) was treated with i) 1 N NaOH (2 eq) at 0 °C for 60 min, ii) 80% hydrazine hydrate (5 eq in MeOH) at 25 °C for 24 h, iii) 4 N HCl–dioxane (5 eq) at 0 °C for 60 min, iv) TFA–thioanisole (0.1 ml–10 μl) at 50 °C for 120 min, and v) 25% HBr–AcOH (0.1 ml) at 0 °C for 30 min. No change was observed on TLC, in any of these cases.

Treatment of Boc–Trp(Mts)–OH and Z(OMe)–Trp(Mts)–OH with TFA——Boc–Trp(Mts)–OH (10.0 mg, 21 μmol) and Z(OMe)–Trp(Mts)–OH (12.3 mg, 22 μmol) were treated with TFA–anisole (30 μl–6 μl) or TFA–DMA containing 2% EDT[29] (30 μl–6 μl), respectively in an ice-bath for 60 min; no pink color was observed. A single spot (Rf_1 0.44) corresponding to that of Trp(Mts) was detected on TLC in all cases. However, when the treatment was performed in the absence of anisole, besides a spot corresponding to Trp(Mts), four other minor spots were detected on TLC in both cases.

Deprotection of the Mts Group——H–Trp(Mts)–OH (4.3 mg, 11 μmol) and Phe (marker, 1.2 mg, 7.3 μmol) were treated with various deprotecting reagents in the presence of *m*-cresol (23 μl, 220 μmol) in an ice-bath for 60 min. A part of the solution was analyzed in an amino acid analyzer. The results are listed in Table I.

Z(OMe)–Trp(Mts)–Met–Asp(OBzl)–Phe–NH$_2$——a) By the Active Ester Procedure: Z(OMe)–Met–Asp(OBzl)–Phe–NH_2 (1.40 g, 2.1 mmol) was treated with TFA (5 ml) in the presence of DMA containing 2% EDT[29] (0.8 ml) in an ice-bath for 60 min, then dry ether was added. The resulting powder was dried over KOH pellets *in vacuo* for 3 h and dissolved in DMF (20 ml), together with Et_3N (0.73 ml, 5.3 mmol) and Z(OMe)–Trp(Mts)–OSu (2.05 g, 3.2 mmol, prepared by the usual DCC procedure). After being stirred at room temperature for 20 h, the solution was concentrated and the residue was treated with AcOEt and H_2O to form a powder, which was precipitated from DMF with AcOEt; yield 1.78 g (82%), mp 207—209 °C, $[\alpha]_D^{22}$ −25.8° (c=0.8, DMF), Rf_3 0.67. Amino acid ratios in a 4 N MSA[31] hydrolysate: Trp 0.73, Met 1.09, Asp 1.14, Phe 1.00 (recovery of Phe 79%). *Anal.* Calcd for $C_{54}H_{60}N_6O_{11}S_2$: C, 62.77; H, 5.85; N, 8.13. Found: C, 62.83; H, 6.01; N, 8.17.

b) By the Azide Procedure: Isoamylnitrite (0.4 ml, 3.3 mmol) was added to an ice-chilled solution of Z(OMe)–Trp(Mts)–$NHNH_2$ (1.70 g, 3.0 mmol) in DMF (10 ml) containing 5.0 N HCl–DMF (1.2 ml, 6.0 mmol). After being stirred for 15 min, the solution was neutralized with Et_3N (0.8 ml, 6.0 mmol) and added to an ice-chilled solution of H–Met–Asp(OBzl)–Phe–NH_2 [prepared from 1.80 g (2.7 mmol) of the Z(OMe)-derivative as stated above]. The mixture was stirred at 4 °C for 48 h and H_2O (50 ml) was added. The resulting powder was precipitated from DMF with ether; yield 2.22 g (79%), mp 207—209 °C, $[\alpha]_D^{28}$ −25.3° (c=1.0, DMF), Rf_3 0.67 (identical with the Rf of the sample obtained in a). Amino acid ratios in a 6 N HCl hydrolysate Trp 0.61, Met 1.04, Asp 1.10, Phe 1.00 (recovery of Phe 71%).

Z(OMe)–Gly–Trp(Mts)–Met–Asp(OBzl)–Phe–NH$_2$——Z(OMe)–Trp(Mts)–Met–Asp(OBzl)–Phe–NH_2 (0.60 g, 0.58 mmol) was treated with TFA (2.0 ml) in the presence of DMA containing 2% EDT (0.4 ml) in an ice-bath for 60 min, then dry ether was added. The resulting powder, after being dried over KOH pellets *in vacuo* for 3 h, was dissolved in DMF (10 ml) containing Et_3N (81 μl, 0.58 mmol). A mixed anhydride [prepared from 0.17 g (0.70 mmol) of Z(OMe)–Gly–OH] in THF (10 ml) was added to the above ice-chilled solution and the mixture, after being stirred in an ice-bath for 3 h, was poured into H_2O (80 ml). The resulting powder was precipitated from DMF with ether; yield 0.45 g (71%), mp 190—192 °C, $[\alpha]_D^{35}$ −21.9° (c=0.7, DMF), Rf_3 0.61. *Anal.* Calcd for $C_{56}H_{63}N_7O_{12}S_2$: C, 61.69; H, 5.82; N, 8.99. Found: C, 61.88; H, 5.85; N, 8.75.

Z(OMe)–Met–Gly–Trp(Mts)–Met–Asp(OBzl)–Phe–NH$_2$——The above protected pentapeptide amide (0.30 g, 0.28 mmol) was treated with TFA–DMA containing 2% EDT (1.0 ml–0.2 ml) and the N^{α}-deprotected peptide, isolated as stated above, was dissolved in DMF (3 ml) together with Et_3N (78 μl, 0.56 mmol) and Z(OMe)–Met–OTCP (0.21 g, 0.42 mmol). After being stirred for 24 h, the solution was poured into H_2O (30 ml) and the resulting powder was precipitated from DMF with ether; yield 0.23 g (68%), mp 204—206 °C, $[\alpha]_D^{35}$ −19.8° (c=0.4, DMF), Rf_3 0.58. *Anal.* Calcd for $C_{61}H_{72}N_8O_{13}S_3 \cdot H_2O$: C, 59.11; H, 6.02; N, 9.04. Found: C, 59.31; H, 5.84; N, 8.94.

Z(OMe)–Tyr–Met–Gly–Trp(Mts)–Met–Asp(OBzl)–Phe–NH_2——The above protected hexapeptide amide (0.20 g, 0.16 mmol) was treated with TFA–DMA containing 2% EDT (0.6 ml–0.1 ml) and the N^{α}-deprotected peptide, isolated as stated above, was dissolved in DMF (1 ml) containing Et_3N (22 μl, 0.16 mmol). The azide [prepared from 86 mg (0.24 mmol) of Z(OMe)–Tyr–$NHNH_2$] in DMF (1 ml) and Et_3N (22 μl, 0.16 mmol) were added to the above ice-chilled solution and the mixture, after being stirred at 4 °C for 48 h, was poured into H_2O (10 ml) to form a powder, which was precipitated from DMF with ether; yield 0.15 g, (68%), mp 200—202 °C, $[\alpha]_D^{35}$ −13.4° (c=0.7, DMF), Rf_3 0.56. Amino acid ratios in a 4 N MSA hydrolysate: Asp 0.98, Gly 1.00, Met 1.77, Tyr 1.02, Phe 0.96, Trp 0.94 (recovery of Gly, 70%). *Anal.* Calcd for $C_{70}H_{81}N_9O_{15}S_3$:C, 60.72; H, 5.90; N, 9.10. Found: C, 60.44; H, 5.88; N, 9.13.

H–Tyr–Met–Gly–Trp–Met–Asp–Phe–NH_2——The above protected heptapeptide amide (200 mg, 145 μmol) was treated with 1 M TFMSA–thioanisole/TFA (5.8 ml) in the presence of *m*-cresol (0.61 ml, 40 eq) and EDT (0.12 ml, 10 eq) in an ice-bath for 120 min, then dry ether was added. The resulting powder was dissolved in MeOH (5 ml). The solution was treated with Amberlite CG-4B (acetate form, approximately 2 g) for 30 min and then filtered. The filtrate was incubated with dithiothreitol (200 mg, 9 eq) at room temperature overnight. Ether was added and the resulting powder (Rf_1 0.83 with some impurities) was purified by column chromatography on silica gel (3.3 × 12 cm) using $CHCl_3$–MeOH–H_2O (8 : 3 : 1) as an eluant. The eluate fractions containing the desired substance were concentrated and the residue was passed through a column of Sephadex LH-20 (1.8 × 60 cm), using MeOH as an eluant. The ultraviolet absorption at 280 nm was determined in each fraction (5 ml) and the desired fractions (tube Nos. 22—28) were combined. After evaporation of the solvent, the residue was treated with ether to afford a powder; yield 63 mg (46%), $[\alpha]_D^{20}$ −35.7° (c=0.3, MeOH), Rf_1 0.83, Rf_4 0.12. Amino acid ratios in a 4 N MSA hydrolysate: Tyr 0.98, Met 1.83, Gly 1.00, Trp 0.91, Asp 0.96, Phe 0.93 (recovery of Gly 77%). Amino acid ratios in a leucine aminopeptidase (Sigma Lot. No. 62F-8000) digest: Tyr 0.89, Met 1.88, Gly 1.00, Trp 1.08, Asp 0.84, Phe 0.87 (recovery of Gly 71%). *Anal.* Calcd for $C_{45}H_{57}N_9O_{10}S_2 \cdot CH_3COOH \cdot 2H_2O$: C, 54.06; H, 6.27; N, 12.07. Found: C, 54.17; H, 6.24; N, 11.55.

The synthetic peptide exhibited a single peak on HPLC (retention time 15.2 min), upon when eluted by gradient elution with acetonitrile (from 40 to 50% in 30 min) in 0.2% TFA at a flow rate of 0.5 ml/min.

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References and Notes

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