of methyl 6-chloroanthranilate with furoyl chloride in the presence of pyridine, followed by hydrolysis of the ester. N-Thenoyl-6-chloroanthranilic acid (**27**) was prepared by a similar method using thionyl chloride.

Triazene derivatives (**28**—**30**) were prepared from the corresponding anthranilic acids. To a solution of anthranilic acid or chloroanthranilic acid (10 mmol) in 1 N HCl (40 mmol), a solution of $NaNO_2$ (20 mmol) in water (8 ml) was added with stirring at −5—0°. The resulting mixture was added portion wise to a solution of morpholine (20 mmol) and $NaCO_3$ (20 mmol) in water (50 ml) and this was stirred for 20 min. Next, 1 N HCl was added to the mixture and the precipitated solid was collected and crystallized from a suitable solvent, affording the triazene compounds (**28**—**30**).

The structures of these compounds were confirmed by elemental analyses (C, H, N).

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A Deblocking Method using Thioether–Sulfonic Acid Systems. Application to the Synthesis of Met-Enkephalin[1]

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Thioanisole–trifluoromethanesulfonic acid and thioanisole–methanesulfonic acid systems were found to be useful as deblocking methods in the synthesis of a methionine-containing peptide, Met-enkephalin, without any side reaction.

Keywords——peptide synthesis; thioanisole-trifluoromethanesulfonic acid; thioanisole-methanesulfonic acid; Met-enkephalin; deblocking method

Deblocking methods using trifluoromethanesulfonic acid (TFMSA)–trifluoroacetic acid (TFA)–anisole[3] and methanesulfonic acid (MSA)–anisole[4] were first described by Yajima and associates. Such procedures are complicated by the acidolytic cleavage of anisole, which, in the presence of methionine can result in the transfer of a methyl group from anisole to the sulfur atom of methionine.[5] This side reaction is usually prevented by conversion of methionine to the corresponding sulfoxide.[5,6]

We reasoned that replacement of anisole with a scavenger that was more acid-stable and a better cation acceptor might prevent the transmethylation reaction and therefore eliminate

1) Amino acids, peptides and their derivatives were all of L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Bzl=benzyl, Cl_2Bzl=2,6-dichlorobenzyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, DCC=dicyclohexylcarbodiimide, ONP=*p*-nitrophenyl ester.

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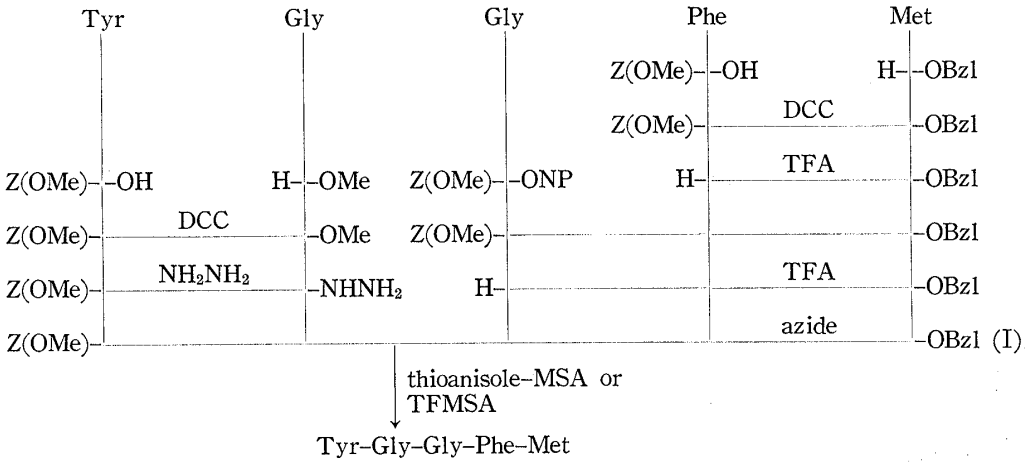
the need to derivatize methionine. Since thioether bonds are generally more acid-stable than ether bonds, the utility of thioanisole, dimethyf sulfide, di-*n*-butyl sulfide, and tetrahydrothiophene was investigated. We note that dimethyl sulfide and anisole were used as scavengers with HF respectively by Arnon *et al.*[7] in the synthesis of nonadecapeptide antigen and Sakakibara *et al.*[8] in the synthesis of mating factor. Also, thioanisole has been employed by Bauer and Pless[9] in the synthesis of somatostatin with boron tris(trifluoroacetate), by Yajima *et al.*[10] in the cleavage of Lys(Z) with TFMSA–TFA, and by us[11] in the cleavage of Tyr(Bzl) and Tyr(Cl_2Bzl) with TFMSA, MSA, and HF.

Our results were that while anisole yielded a by-product under the same conditions, the thioethers were not cleaved by MSA–TFA or TFMSA–TFA in the presence of methionine. Carbobenzoxymethionine was completely deprotected without any side reaction by thioanisole–TFMSA–TFA and thioanisole–MSA–TFA. However, treatment of Z-Met with MSA–TFA or TFMSA–TFA in the presence of the other thioethers gave a small amount of white precipitate. The reasons for the superiority of thioanisole as a scavenger may be as follows. (i) Thioethers are more acid-stable than ethers because H^+ (a hard acid) has no affinity for sulfur (a soft base[12]). (ii) Thioethers are potent cation acceptors because carbonium cations (soft acids) show affinity for sulfur. (iii) Thioanisole is a better cation acceptor than other alkyl thioethers because the resulting sulfonium ion is stabilized by resonance with π-electron of the benzene ring. We therefore concluded that the thioanisole–sulfonic acid system may be used as a deblocking agent in the synthesis of methionine-containing peptides.



Synthetic Scheme of Met–Enkephalin

To demonstrate the applicability of this method, a biologically active peptide, Met-enkephalin,[13] has been synthesized. Z(OMe)-Tyr-Gly-Gly-Phe-Met-OBzl (I) was prepared as shown in the scheme. I was deblocked by acidolysis in thioanisole, TFA, and TFMSA at 0° for 60 min then at room temperature for 30 min. Purification of the deprotected material on DEAE–Sephadex (acetate form)[14] gave pure Met-enkephalin. The synthetic product

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was shown to be homogeneous by thin-layer chromatography, elemental analysis and amino acid ratios after acidic hydrolysis. The biological activity was determined by inhibition of electrically evoked contraction of the guinea pig ileum to give an ED_{50} of 2.8×10^{-8} M, in reasonable agreement with published literature values.[13,15] It was also possible to synthesize Met-enkephalin with the same procedure outlined above using MSA in place of TFMSA.

Experimental

Thin-layer chromatograms (TLC) were run on Merck silica gel $60F_{254}$ plates in the following systems: 1, chloroform–methanol–water (8:3:1, lower layer); 2, chloroform–methanol (20:1); 3, *n*-butanol–acetic acid–water (3:1:1); 4, *n*-butanol–acetic acid–pyridine –water (4:1:1:2). Compounds were revealed by irradiation with ultraviolet (UV) light or by treatment with ninhydrin, iodine or seric sulfate. Optical rotations were determined with a Union automatic digital polarimeter PM-201 or a JASCO optical rotatory dispersion records OPD/UV-5. Melting points are uncorrected.

Treatment of Met with Anisole–TFMSA or Anisole–MSA——Met (15 mg) was treated with anisole (0.1 ml)–TFMSA (0.05 ml)–TFA (2 ml) for 1 hr at room temperature. TLC of the reaction mixture showed another spot (Rf_1 0.02) in addition to that at Rf_1 0.18 (Met) (ninhydrin-positive). In the case of MSA (0.5 ml)–TFA (1.5 ml), the same result was obtained.

Treatment of Met with Thioethers–TFMSA or Thioethers–MSA——Met (15 mg) was treated with various thioethers (0.1 ml)–TFMSA (0.05 ml)–TFA (2 ml) for 2 hr at room temperature. TLC of the reaction mixture showed one spot (Rf_1 0.18, Met)(ninhydrin-positive). In the case of MSA (0.5 ml)–TFA (1.5 ml), the same result was obtained.

Reaction of Z-Met with Thioethers–TFMSA or Thioethers–MSA——Z-Met (28 mg) was treated with thioanisole (0.24 ml)–TFMSA (0.05 ml)–TFA (2 ml) for 2 hr at 0°. TLC of the reaction mixture showed one spot (Rf_1 0.18, Met) (HBr/AcOH–ninhydrin positive). The use of another thioether (dimethyl sulfide, di-*n*-butyl sulfide or tetrahydrothiophene) in place of thioanisole gave one spot (Rf_1 0.18, Met) in TLC of the reaction mixture and a small amount of white precipitate. In the case of MSA (0.5 ml)–TFA (1.5 ml), the same result was obtained.

Z(OMe)-Phe-Met-OBzl——DCC (9.5 g) was added to an ice-cold solution of Z(OMe)-Phe-OH (15 g) in AcOEt (350 ml) and the mixture was stirred for 20 min in an ice-water bath. A solution of H-Met-OBzl·tosylate (23.6 g) in dimethylformamide (DMF) (70 ml) containing triethylamine (10.8 ml) was added and the mixture was stirred at room temperature for 24 hr. The solution was filtered, the filtrate was condensed *in vacuo* and the residue was dissolved in AcOEt. The solution was washed with 10% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and condensed. The resulting mass was recrystallized from AcOEt and ether; yield 19 g (75.8%), mp 128—131°, $[\alpha]_D^{25}$ −6.0° (c=0.5, DMF), Rf_2 0.83. *Anal.* Calcd. for $C_{30}H_{34}N_2O_6S$: C, 65.43; H, 6.22; N, 5.09. Found: C, 65.19; H, 6.33; N, 5.19.

Z(OMe)-Gly-Phe-Met-OBzl——Z(OMe)-Phe-Met-OBzl (8.2 g) was dissolved in TFA (25 ml)–anisole (5 ml) at 0° and kept for 1 hr at 0°. The solvent was removed *in vacuo*. The residue was washed with pet.ether by decantation and dried. The product was dissolved in DMF (40 ml) and neutralized by the addition of triethylamine. To this solution was added a solution of Z(OMe)-Gly-ONP (5.6 g) in DMF (30 ml) and the mixture was stirred at room temperature for 24 hr. The solution was evaporated and the residue was dissolved in AcOEt. The solution was washed with 10% citric acid, 5% $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and condensed. The resulting mass was recrystallized from AcOEt and ether; yield 6.0 g (66%), mp 142—147°, $[\alpha]_D^{25}$ −8.0° (c=0.5, DMF), Rf_2 0.49. *Anal.* Calcd. for $C_{32}H_{37}N_3O_7S$: C, 63.24; H, 6.14; N, 6.92. Found: C, 63.30; H, 6.17; N, 6.92.

Z(OMe)-Tyr-Gly-NHNH$_2$——This hydrazide was prepared according to Kubota *et al.*[6d]; mp 217—220° (lit.[6d] 218—222°), Rf_1 0.46. *Anal.* Calcd. for $C_{20}H_{24}N_4O_6$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.43; H, 5.80; N, 13.13.

Z(OMe)-Tyr-Gly-Gly-Phe-Met-OBzl (I)——Z(OMe)-Gly-Phe-Met-OBzl (4.1 g) was dissolved in TFA (30 ml)–anisole (2.7 ml) at 0° and kept for 1 hr at 0°. The solvent was removed *in vacuo*. The residue was washed with pet.ether by decantation and dried. The product was dissolved in DMF (20 ml) and neutralized by the addition of triethylamine. To this ice-chilled solution, the azide (prepared from 2.9 g of Z(OMe)-Tyr-Gly-NHNH$_2$ with 6.8 ml of 2.75 N HCl–DMF, 0.92 ml of isoamylnitrite and 3.8 ml of triethylamine) in DMF (40 ml) was added. After stirring at 4° for 48 hr, the mixture was condensed and the residue was treated with water. The resulting powder was washed with ether, 5% citric acid, 5% $NaHCO_3$ and water. The solid mass was recrystallized from CH_3CN and AcOEt; yield 4.7 g (82.5%), mp 196—198°, $[\alpha]_D^{27}$ −21.5° (c=0.5, DMF), Rf_1 0.74. *Anal.* Calcd. for $C_{43}H_{49}N_5O_{10}S$: C, 62.38; H, 5.97; N, 8.46. Found: C, 62.13; H, 5.99; N, 8.28.

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H-Tyr-Gly-Gly-Phe-Met-OH, (Met-Enkephalin)——(a) TFMSA Method: I (305 mg) was treated with thioanisole (0.65 ml)–TFA (3 ml)–TFMSA (0.3 ml) at 0° for 60 min then at room temperature for 30 min. The solvent was removed *in vacuo*. The residue was washed with *n*-hexane by decantation with cooling and dissolved in water (*ca.* 15 ml). The solution was treated with Amberlite IRA-400 (acetate form, *ca.* 10 g) for 10 min, filtered and washed with 10% AcOH. The combined solution, after washing with ether, was evaporated *in vacuo*. The residue was dissolved in the minimum volume of 1% pyridine–0.04% acetic acid aqueous buffer and applied to a column (23×540 mm) of DEAE-Sephadex A-25 (acetate form) which had previously been equilibrated with the same buffer. The column was eluted with 1% pyridine–0.04% acetic acid and the fractions (15 ml) were monitored by absorbancy at 280 nm and TLC. Fractions 23—26 were pooled and evaporated, and the residue was re-evaporated several times with ethanol to give a white solid. The solid mass was reprecipitated from EtOH–AcOEt; yield 136 mg (64%), mp 195—196° (lit.[14] 196—198°; lit.[16] 195°), $[\alpha]_D^{27}$ +23.4° (c=0.5, 3% AcOH) (lit.[17] +17.7°), Rf_3 0.67, Rf_4 0.70. *Anal.* Calcd. for $C_{27}H_{35}N_5O_7S \cdot H_2O$: C, 54.81; H, 6.30; N, 11.84. Found: C, 54.81; H, 6.16; N, 11.73. Amino acid ratios (after acidic hydrolysis with 6 N HCl at 105° for 24 hr): Tyr 0.96, Gly 2.09, Phe 0.98, Met 0.96.

(b) MSA Method: I (300 mg) was treated with thioanisole (0.64 ml)–TFA (1.5 ml)–MSA (1.5 ml) at 0° for 30 min then at room temperature for 2 hr. The solvent was removed *in vacuo*. The residue was washed with *n*-hexane by decantation with cooling and dissolved in water (*ca.* 25 ml). The solution was treated with Amberlite IRA-400 (acetate form, *ca.* 30 g) for 10 min, filtered and washed with 10% AcOH. The combined solution, after washing with ether, was evaporated *in vacuo*. The residue was dissolved in the minimum volume of 1% pyridine–0.04% acetic acid aqueous buffer and applied to a column (23×520 mm) of DEAE-Sephadex A-25 (acetate form) which had previously been equilibrated with the same buffer. The column was eluted with the same buffer and the fractions (15 ml) were monitored by absorbancy at 280 nm and TLC. Fractions 16—19 were pooled and evaporated, and the residue was reevaporated several times with ethanol to give a white solid. The solid mass was reprecipitated from EtOH–AcOEt; yield 77 mg (37%), mp 195—196°, Rf_3 0.67, Rf_4 0.70.

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