Sulphur Trioxide/Thiol: A Novel System for the Reduction of Methionine Sulphoxide†

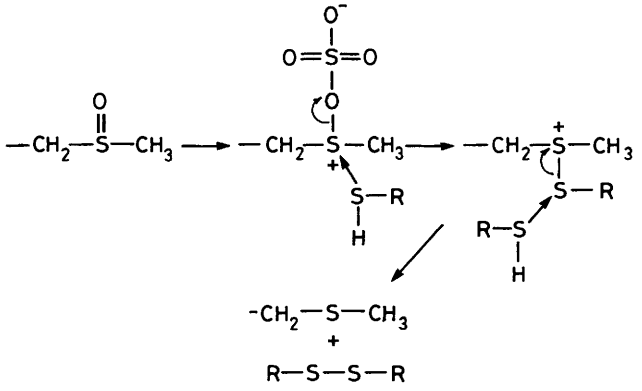
Shiroh Futaki, Takeshi Yagami, Takashi Taike, Tadashi Akita, and Kouki Kitagawa*
Faculty of Pharmaceutical Sciences, The University of Tokushima, Shomachi, Tokushima 770, Japan

A new method for the effective reduction of methionine sulphoxide in protected peptides with sulphur trioxide and thiol has been developed. In practice, a combination of dimethylformamide–sulphur trioxide complex and ethane-1,2-dithiol was found effective, the presence of pyridine in the reaction medium being found necessary for the reduction to proceed. Methionine sulphoxide in two types of protected methionine–enkephalins was efficiently reduced to methionine by this reduction system at 20 °C in an hour. The (*p*-methylsulphinyl)benzyl protecting group was also reduced to (*p*-methylthio)benzyl simultaneously with the reduction of methionine sulphoxide. Subsequent deprotection afforded methionine–enkephalin of high purity in each case.

To avoid *S*-alkylation during the N^{α}-deprotection by acid and partial sulphoxide formation in manipulations during the synthesis of Met containing peptides, an approach in which Met is protected as its sulphoxide form, Met(O), has been developed.[1]‡ When this approach is adopted, it becomes necessary to reduce Met(O) at the final stage of the synthesis. The most popular method now used for this purpose is to incubate Met(O)–peptide after deprotection with a thiol, such as 2-mercaptoethanol, dithiothreitol,[2] and *N*-methylmercaptoacetamide.[3] In this method, however, reaction overnight or for a longer time is necessary for the reduction to go to completion; even so sometimes it is incomplete. To overcome these defects, other approaches have been developed. One is to reduce Met(O) simultaneously with the final deprotection in an acid system with strong reducing ability: TMSBr–thioanisole,[4] TFA–$SiCl_4$,[5] and HF–2-mercaptopyridine[6] or –DMS[7] are listed as examples of this approach. Recently Fujii *et al.*,[8] introduced a new approach: they adopted phenylthiotrimethylsilane[9] as a reducing agent for Met(O)-containing protected peptides. This approach aims at reducing the Met(O) residue prior to the final deprotection in a short time. The usefulness of this method was well demonstrated in the synthesis of human gastrin-releasing peptide,[10] where two Met(O) residues were reduced with the aid of trimethylsilyl trifluoromethanesulphonate in DMF within 30 min.

Here we report another novel system to reduce Met(O) residue prior to the final deprotection. This reduction system is composed of sulphur trioxide, conveniently in the form of its base complex, and a thiol in the presence of pyridine. The reduction seems to proceed *via* initial formation of a sulphonium intermediate and subsequent nucleophilic attack of thiol (Scheme 1). Such an intermediate has been proposed for the oxidation of DMSO by the method of Parikh and Doering[11] in which a pyridine–SO_3 complex initially attacks the oxygen of the DMSO.[12]

First, to evaluate the effect of SO_3 complex, three examples, DMF–SO_3,[13] pyridine–SO_3, and TEA–SO_3[14] were examined (see Figure 1). In the presence of EDT (5 equiv.), Z(OMe)-Phe-Met(O)-OMe,[15] a model peptide, was completely reduced to Z(OMe)-Phe-Met-OMe by DMF– or pyridine–SO_3 complex (5 equiv.) at 20 °C in 30 min. When 2.5 equiv. of SO_3 complex was used, the reduction proceeded most effectively with the DMF–SO_3 complex. The TEA–SO_3 complex proved less effective as compared with the other two agents. Without the SO_3 complex, there was no reduction. The presence of pyridine in the reduction medium was also decisive for the reduction to proceed.



Scheme 1.

Next, the effect of thiol was examined (see Figure 2). Z(OMe)-Phe-Met(O)-OMe was treated with the DMF–SO_3 complex (5 equiv.) in the presence of PhSH, EDT, and EtSH, respectively. When 5 equiv. of thiol was used, the order of effectiveness for the reduction was: PhSH > EDT > EtSH. Since EDT contains two sulphhydryl groups, the molar ratio of EDT was adjusted to half of the others. When 5 equiv. of EDT was used, the reduction was complete within 30 min. DMS (10 equiv.) proved much less effective than the thiols examined, and the progress of the reduction was negligible in the absence of sulphur compounds. Although PhSH proved to be the most effective reagent, EDT was preferred in practice because it avoided the strong and unpleasant odour of PhSH.

† Preliminary communication; S. Futaki, T. Taike, T. Yagami, T. Akita, and K. Kitagawa, *Tetrahedron Lett.*, 1989, **33**, 4411.

‡ All amino acids used in this paper are of L-configuration. Abrreviations used are those recommended by IUPAC–IUB Commission on Biochemical Nomenclature (Pure Appl. Chem., 1984, **56**, 595): Z(OMe) = *p*-methoxybenzyloxycarbonyl, Boc = t-butoxycarbonyl, Bzl = benzyl, Cl_2Bzl = 2,6-dichlorobenzyl, Su = *N*-hydroxysuccinimidyl, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulphonic acid, MSA = methanesulphonic acid, TMSBr = trimethylsilyl bromide, AcOH = acetic acid, DMA = 3,5-dimethylanisole, EDT = ethane-1,2-dithiol, DMS = dimethyl sulphide, NMM = *N*-methylmorpholine, TEA = triethylamine, DCHA = dicyclohexylamine, DMF = dimethylformamide, DMSO = dimethyl sulphoxide, AcOEt = ethyl acetate, MeOH = methanol, BuOH = butan-1-ol, THF = tetrahydrofuran, LAP = leucine aminopeptidase.

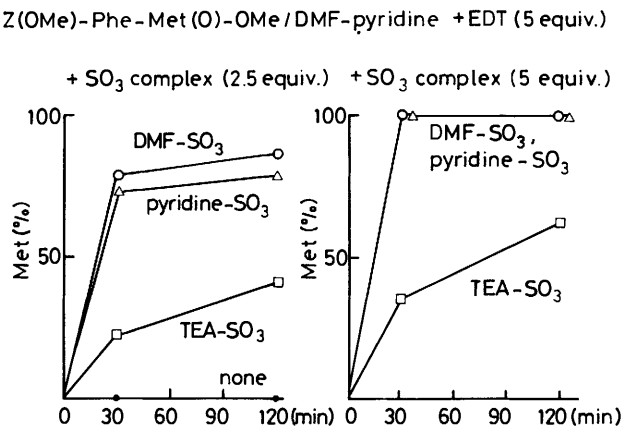
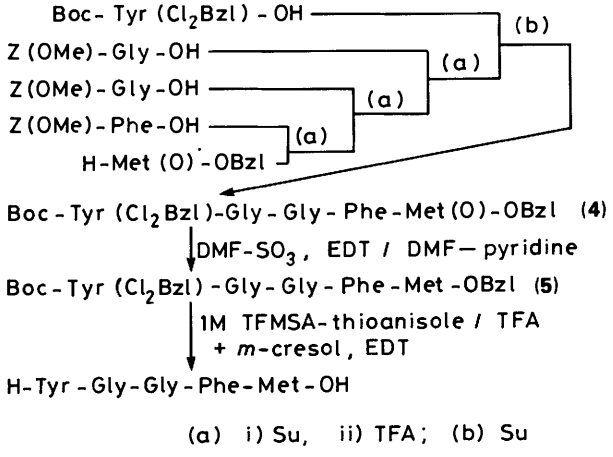


Figure 1. Effect of SO_3 complex on Met(O) reduction.

From these model experiments, we concluded that for practical purposes the combination of the DMF–SO_3 complex with EDT is the most suitable reduction system. To ascertain the homogeneity of the reduced product, Z(OMe)-Phe-Met(O)-OMe in DMF–pyridine (4:1) was treated with DMF–SO_3 complex and EDT (5 equiv., each) at 20 °C for 30 min. The product was isolated in 85% yield after a brief work-up. The R_F value on TLC, m.p., and the optical rotation of the product were identical with those of the authentic Z(OMe)-Phe-Met-OMe. The homogeneity was also confirmed by ^{1}H NMR spectrometry and high performance liquid chromatography (HPLC).

Based on the results thus obtained, we next applied this method to the synthesis of methionine (Met)-enkephalin.[16] Two types of protected pentapeptides were prepared as shown in Schemes 2 and 3.

One was Boc-Tyr(Cl_2Bzl)-Gly-Gly-Phe-Met(O)-OBzl (**4**), which was prepared by successive introduction of Z(OMe)-Phe-OH, Z(OMe)-Gly-OH, and Boc-Tyr(Cl_2Bzl)-OH[17] by the Su[18] active ester method into H-Met(O)-OBzl (Scheme 2). The



Scheme 2.

Met(O) in (**4**) was reduced with DMF–SO_3 complex and EDT (10 equiv., each) in DMF–pyridine (4:1) at 20 °C in 60 min. Completion of the reduction was monitored on TLC and the reduced product (**5**) was isolated in 90% yield after a brief work-up. Its structure was also ascertained by fast atom bombardment mass spectrometry (FABMS) and amino acid analysis after 4M MSA hydrolysis[19] which has almost no ability to reduce Met(O) during hydrolysis. Following the procedure of Kiso *et al.*,[20] we deprotected the product with 1M TFMSA–thioanisole/TFA[21] in the presence of EDT and *m*-cresol; purification was achieved by chromatography on DEAE-Sephadex

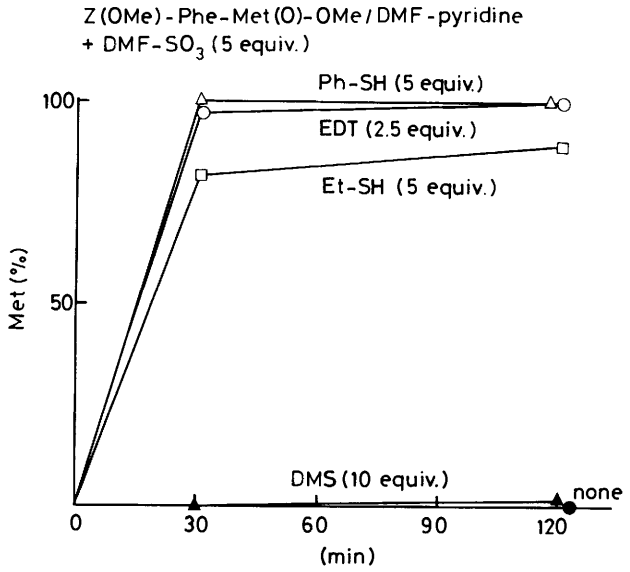
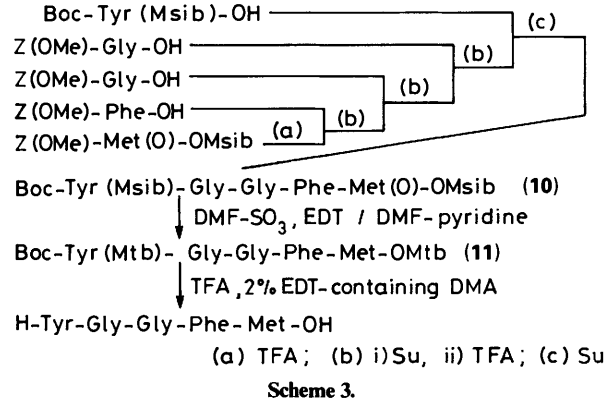


Figure 2. Effect of thiol on Met(O) reduction.

A-25 and Diaion HP-20 column to afford Met-enkephalin in 49% yield (Figure 3a).

As a further example in order to evaluate our reduction system, the protected Met-enkephalin (**10**) with a (*p*-methylsulphinyl)benzyl (Msib)[22] group at the phenolic hydroxy function of Tyr and the C-terminus carboxy function was prepared (Scheme 3). This protecting group was originally introduced as a carboxy protecting group by Samanen and Brandeis, who reported that the Msib ester is stable to TFA and that its reduced form, (*p*-methylthio)benzyl (Mtb) ester, is cleavable by TFA (20 °C, 30 min).



Scheme 3.

Boc-Tyr(Msib)-OH was prepared from Boc-Tyr-OH with Msib-Br in the presence of NaH. Msib-Br was prepared by H_2O_2 treatment of Mtb-Br, which was conveniently prepared from Mtb-OH and TMSBr according to the method of Jung and Hatfield.[23] Tyr(Msib) was found to be reduced to Tyr(Mtb) with DMF–SO_3 complex and EDT (10 equiv., each) at 20 °C in 30 min, and to have almost the same properties to acid as the Msib ester. Namely, Tyr(Msib) was stable to TFA (0 °C, 3 h), and Tyr(Mtb) was cleavable by TFA (0 °C, 2 h). Employment of a Msib group for protection of a phenolic hydroxy function is, we believe, unprecedented.

As shown in Scheme 3, Boc-Tyr(Msib)-Gly-Gly-Phe-Met(O)-OMsib (**10**) was synthesized in a stepwise manner starting with a TFA-treated sample of Z(OMe)-Met(O)-OMsib, which was prepared from Z(OMe)-Met(O)-OH with Msib-Br and DCHA. The Su method was also employed to lengthen the peptide chain

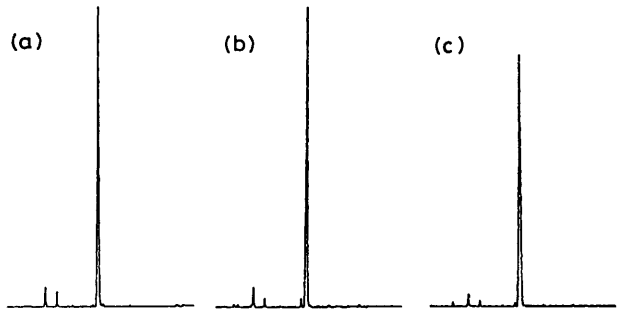


Figure 3. HPLC of synthetic methionine-enkephalin.

compound (**10**) thus obtained was treated with DMF–SO_3 complex and EDT (100 equiv., each) at 20 °C for 60 min to afford Boc-Tyr(Mtb)-Gly-Gly-Phe-Met-OMtb (**11**) in 88% yield. This reduced peptide was deprotected with TFA–DMA containing 2% EDT,[24] a deprotection system which was reported to cause little alkylation of Met. Subsequent purification was conducted as described above to afford Met-enkephain in 57% yield (Figure 3b). In the syntheses accomplished as shown in Scheme 2 and 3, the final products were both identical with authentic Met-enkephalin on TLC and HPLC (Figure 3c).

Through these model experiments, this reduction system was estimated to be useful for the reduction of Met(O)-containing peptides. In addition, this novel system will be applicable to various sulphoxides, as demonstrated in the case of the Msib protecting group.

Experimental

M.p.s were determined with a Yanagimoto micro apparatus and uncorrected. Optical rotations were determined with a Union PM-201 polarimeter. HPLC was conducted with a Hitachi L-6200 model. Amino acid compositions in acid hydrolysates (6M HCl in the presence of phenol, or 4M MSA; 110 °C; 24 h) and LAP digests[25] (Sigma, No. L-6007) were determined with a Hitachi 835 model amino acid analyser. ^1H NMR spectra were taken on a JEOL JNM-PMX 60 SI (60 MHz), or a JEOL JNM-FX 200 (200 MHz) spectrometer with tetramethylsilane as an internal standard. Fast atom bombardment mass spectra were recorded on a JEOL JMS-D 300 spectrometer. Silica gel 60 (Merck, 70–230 mesh) was used for silica gel column chromatography. R_F Values on TLC (pre-coated Silica gel 60 F_{254}, 0.25 mm thickness, Merck) refer to the following solvent systems: R_{F1} $CHCl_3$–MeOH–H_2O (8:3:1), R_{F2} $CHCl_3$–MeOH (10:0.5), R_{F3} $CHCl_3$, R_{F4} $CHCl_3$–MeOH–AcOH (9:1:0.5), R_{F5} BuOH–AcOH–AcOEt–H_2O (1:1:1:1), R_{F6} BuOH–AcOH–pyridine–H_2O (4:1:1:2).

DMF–SO_3 and TEA–SO_3 complexes were purchased from Fluka, and pyridine–SO_3 complex was purchased from Tokyo Chemical Industry Co., Ltd.

Prior to condensation, the N^{α}-Z(OMe) or Boc group was removed by TFA treatment in the presence of anisole. The Su active ester reaction was performed at room temperature, and the mixed anhydride (MA) reaction[26] in an ice-bath. Unless otherwise mentioned, products were purified by one of the following procedures. Procedure A: for purification of a product soluble in AcOEt, the extract was washed with 5% aqueous citric acid, 5% aqueous $NaHCO_3$, and brine, and then dried over Na_2SO_4 and concentrated. The residue was recrystallized or precipitated from appropriate solvents. Procedure B: for purification of a product less soluble in AcOEt, the crude product was triturated with 5% aqueous citric acid. The resulting powder was washed with 5% aqueous citric acid, 5% aqueous $NaHCO_3$ and water, and then recrystallized or precipitated from appropriate solvents.

Evaluation of the Effect of SO_3 *Complex on the* SO_3 *Complex/Thiol Reduction System.*—Z(OMe)-Phe-Met(O)-OMe (25 mg, 0.05 mmol) was dissolved in DMF–pyridine (4:1; 0.5 ml), and EDT (23.5 μl, 5 equiv.) and a SO_3 complex (5 or 2.5 equiv.) were added. The mixture was stirred at 20 °C. The progress of the reduction was monitored by HPLC on a Nucleosil 7C8 column (4.6 × 250 mm), which was eluted with a gradient of MeOH (55 to 76% in 28 min) in 0.1% aqueous TFA at a flow rate of 1.0 ml/min (retention time of Z(OMe)-Phe-Met(O)-OMe: 11.3 min, Z(OMe)-Phe-Met-OMe: 18.2 min). In the absence of pyridine, only 2% of Z(OMe)-Phe-Met(O)-OMe (25 mg) in DMF (0.5 ml) was reduced with DMF–SO_3 complex and EDT (5 equiv. each) at 20 °C in 120 min.

Evaluation of the Effect of Thiol on the SO_3 *Complex/Thiol Reduction System.*—Z(OMe)-Phe-Met(O)-OMe (25 mg, 0.05 mmol) was dissolved in DMF–pyridine (4:1; 0.5 ml), where DMF–SO_3 complex (39 mg, 5 equiv.) and a thiol (or sulphide) were added respectively. The mixture was stirred at 20 °C. The progress of the reduction was monitored by HPLC as mentioned above.

Reduction of Z(OMe)-Phe-Met(O)-OMe.—Z(OMe)-Phe-Met(O)-OMe (491 mg, 1.00 mmol) in DMF–pyridine (4:1; 5 ml) was treated with DMF–SO_3 complex (766 mg, 5 equiv.) and EDT (0.47 ml, 5 equiv.). The mixture was stirred at 20 °C. After 30 min, the solvent was evaporated at 40 °C. The residue was treated with water to afford a powder, which was recrystallized from DMF with diethyl ether to afford crystals (402 mg, 85%), m.p. 118–120 °C, $[\alpha]_D^{20}$ −16.5° (*c* 1.0, in DMF), R_{F1} 0.87 (Found: C, 60.4; H, 6.3; N, 5.8. $C_{24}H_{30}N_2O_6S$ requires C, 60.7; H, 6.4; N, 5.9%); δ_H(200 MHz; $CDCl_3$) 1.98 (2 H, m, $CHCH_2CH_2$), 2.04 (3 H, s, SMe), 2.38 (2 H, t, *J* 7 Hz, CH_2S), 3.08 (2 H, m, CH_2Ph), 3.71 (3 H, s, PhO*Me*), 3.81 (3 H, s, MeOCO), 4.42 (1 H, m, CH), 4.60 (1 H, m, CH), 5.02 (2 H, s, CH_2O), 5.26 (1 H, br d, *J* 4 Hz, NH), 6.52 (1 H, br d, *J* 4 Hz, NH), 6.88 (2 H, d, *J* 8.8 Hz, OPh), and 7.12–7.34 (7 H, m, Ph); HPLC: single peak with a retention time 18.2 min eluted as mentioned above. These data are identical with those of the authentic Z(OMe)-Phe-Met-OMe which was synthesized by the MA method from H-Met-OMe (prepared from its HCl salt) and Z(OMe)-Phe-OH (84%), m.p. 118–120 °C, $[\alpha]_D^{20}$ −16.6° (*c* 1.0, in DMF), R_{F1} 0.87 (Found: C, 60.4; H, 6.5; N, 5.8. $C_{24}H_{30}N_2O_6S$ requires C, 60.7; H, 6.4; N, 5.9%).

Z(OMe)-Phe-Met(O)-OBzl (**1**).—A mixture of Z(OMe)-Met(O)-OH (1.70 g, 5.16 mmol), DCHA (1.13 ml, 5.67 mmol), and benzyl bromide (0.97 ml, 8.16 mmol) in DMF (30 ml) were stirred overnight. The product was purified by procedure A to afford Z(OMe)-Met(O)-OBzl (2.10 g, 97%) as an oil, R_{F1} 0.87, R_{F2} 0.31. This oil was treated with TFA in the usual fashion and the resulting oil was dissolved in DMF (20 ml) containing TEA (0.72 ml, 5.16 mmol). Z(OMe)-Phe-OSu (2.20 g, 5.16 mmol) and NMM (0.57 ml, 5.16 mmol) were added to the solution, and the mixture was stirred overnight. The product was purified by procedure A, followed by recrystallization from MeOH with AcOEt–ether to afford the dipeptide (**1**) (2.37 g, 81%). Physical constants and analytical data are listed in Table 1, together with those of other intermediates.

Z(OMe)-Gly-Phe-Met(O)-OBzl (**2**).—A TFA-treated sample of (**1**) (2.35 g, 4.15 mmol) was dissolved in DMF (20 ml) containing TEA (0.58 ml, 4.15 mmol). Z(OMe)-Gly-OSu (1.54 g, 4.57 mmol) and NMM (0.46 ml, 4.15 mmol) were added to the solution, and the mixture was stirred overnight. The product was purified by procedure A, followed by recrystallization from MeOH with AcOEt–ether to afford the tripeptide (**2**) (2.18 g, 84%).

Table 1. Physical constants and analytical data of Boc-Tyr(Cl_2Bzl)-Gly-Gly-Phe-Met(O)-OBzl (**4**) and its intermediates.

Peptide	R_{F1}	M.p. (°C)	$[\alpha]_D^{24}$ (DMF)	Formula	Analyses (%) Found (Required) C	H	N
(**1**)	0.70	133–135	−14.4° (*c* 0.8)	$C_{30}H_{34}N_2O_7S$	63.4 (63.6)	6.2 (6.05)	4.75 (4.9)
(**2**)	0.64	109–111	−10.3° (*c* 0.8)	$C_{32}H_{37}N_3O_8S$	61.6 (61.6)	6.2 (6.0)	6.5 (6.7)
(**3**)	0.61	147–150	−14.6° (*c* 1.0)	$C_{34}H_{40}N_4O_9S\cdot\frac{1}{2}H_2O$	59.3 (59.2)	6.0 (6.0)	7.9 (8.1)
(**4**)	0.63	200–204	−17.7° (*c* 1.0)	$C_{46}H_{53}N_5O_{10}SCl_2\cdot\frac{1}{2}H_2O$	58.35 (58.3)	5.7 (5.7)	7.4 (7.4)

Z(OMe)-Gly-Gly-Phe-Met(O)-OBzl (**3**).—A TFA-treated sample of (**2**) (2.16 g, 3.46 mmol) was dissolved in DMF (15 ml) containing TEA (0.48 ml, 3.46 mmol). Z(OMe)-Gly-OSu (1.28 g, 3.81 mmol) and NMM (0.38 ml, 3.46 mmol) were added to the solution, and the mixture was stirred overnight. The product was purified by procedure A, followed by recrystallization from THF with AcOEt–ether to afford the tetrapeptide (**3**) (2.17 g, 92%).

Boc-Tyr(Cl_2Bzl)-Gly-Gly-Phe-Met(O)-OBzl (**4**).—A TFA-treated sample of (**3**) (1.38 g, 2.03 mmol) was dissolved in DMF (10 ml) containing TEA (0.28 ml, 2.03 mmol). Boc-Tyr(Cl_2Bzl)-OSu (1.42 g, 2.64 mmol) and NMM (0.25 ml, 2.23 mmol) were added to the solution, and the mixture was stirred overnight. The product was purified by procedure A, followed by recrystallization from MeOH to afford the pentapeptide (**4**) (1.23 g, 65%). Amino acid ratios in a 6M HCl hydrolysate: Tyr 1.00, Gly 1.89, Phe 1.00, Met + Met(O) 0.84 (recovery of Phe, 85%).

Boc-Tyr(Cl_2Bzl)-Gly-Gly-Phe-Met-OBzl (**5**).—Compound (**4**) (50 mg, 0.053 mmol) was dissolved in DMF–pyridine (2 ml, 0.5 ml), and then DMF–SO_3 complex (82 mg, 10 equiv.) and EDT (50 μl, 10 equiv.) were added. The mixture was stirred at 20 °C for 1 h and then concentrated. The residue was treated with water to afford a powder, which was precipitated from DMF with diethyl ether to afford the reduced pentapeptide (**5**) (45 mg, 92%); R_{F1} 0.75. Amino acid ratios in a 4M MSA hydrolysate: Tyr 0.89, Gly 1.98, Phe 1.00, Met 0.92 (recovery of Phe, 83%). FABMS: *m/z* 946.5 $(M + Na)^+$.

H-Tyr-Gly-Gly-Phe-Met-OH, *Methionine-enkephalin* [*from* (**5**)].—Compound (**5**) (25 mg, 0.027 mmol) was treated with 1M TFMSA–thioanisole/TFA (2 ml) in the presence of EDT (128 μl, 50 equiv.) and *m*-cresol (147 μl, 50 equiv.) at 0 °C for 90 min. The solvent was concentrated under reduced pressure and the residue was washed with hexane by decantation with cooling and then dissolved in 1M AcOH (2 ml) containing 2-mercaptoethanol (20 μl). The solution was treated with Amberlite IR-400 (acetate form, *ca.* 1 g) at 0 °C for 30 min, filtered, and washed with 1M AcOH. The combined filtrates were lyophilized. The residue was dissolved in 1% pyridine–0.04% AcOH aqueous buffer (4 ml) and applied to a column (2.4 × 27 cm) 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 (8 ml each) were monitored by measuring the absorbance at 275 nm. Fraction Nos. 40–48 were combined and lyophilized. The residue was dissolved in 1M AcOH (2 ml) and applied to a column of Diaion HP-20 (2 × 5 cm), which was eluted by a gradient formed with MeCN through a mixing flask containing 1M AcOH (300 ml). The desired fractions (6 ml each, tube Nos. 22–33) were combined and lyophilized to give a white fluffy powder (7.6 mg, 49%). Amino acid ratios after LAP digestion: Tyr 0.96, Gly 1.85, Phe 1.00, Met 0.92 (recovery of Phe 81%). R_F Values on TLC (R_{F5} 0.68, R_{F6} 0.63) and retention time in HPLC [14.9 min, detected by UV measurement at 275 nm on a YMC-AM312 column (6 × 150 mm) by gradient elution with MeCN (20–35%, 30 min) in 0.1% aqueous TFA at a flow rate of 1 ml/min] were identical with those of the authentic sample (purchased from Peptide Institute Inc., Osaka, Japan).

Mtb-Br.—(*p*-Methylthio)benzyl alcohol (1.90 g, 12.30 mmol) in $CHCl_3$ (40 ml) was treated with TMSBr (2.44 ml, 18.45 mmol) at 0 °C for 1 h and then water was added to the solution. The organic layer was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was stored in a refrigerator to afford crystals (2.64 g, 99%), m.p. 40–42 °C (lit.,[27] 43–44 °C). The ^1H n.m.r. spectroscopic data were identical with those in the literature.

Msib-Br.—Mtb-Br (3.00 g, 13.80 mmol) in AcOH (20 ml) was treated with 30% hydrogen peroxide (2.35 ml, 20.70 mmol) at 20 °C for 20 h. The solvent was evaporated, and the residue was dissolved in AcOEt. The organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was treated with hexane to afford crystals, which were recrystallized from AcOEt with hexane to afford the product (2.40 g, 75%), m.p. 74–77 °C, R_{F3} 0.16 (Found: C, 41.4; H, 3.8. C_8H_9BrOS requires C, 41.2; H, 3.9%); δ_H(60 MHz; $CDCl_3$) 2.73 (3 H, s, S(O)Me), 4.53 (2 H, s, CH_2), and 7.57 (4 H, s, Ph).

Boc-Tyr(Msib)-OH.—NaH (60% oil suspension; 0.32 g, 8.00 mmol) was added to a solution of Boc-Tyr-OH (1.00 g, 3.55 mmol) in DMF (30 ml) at −20 °C in 30 min after which Msib-Br (1.00 g, 4.29 mmol) was added. The mixture was stirred at 0 °C for 3 h after which the solvent was removed by evaporation. The residue was dissolved in 5% aqueous $NaHCO_3$ and washed with AcOEt. The aqueous layer was acidified with citric acid. The product was extracted with AcOEt and the extract washed with water, dried (Na_2SO_4), and concentrated. The residue was treated with ether to afford a powder, which was recrystallized from MeOH with ether. The product was further purified on silica gel column chromatography (3.0 × 15 cm) using $CHCl_3$→$CHCl_3$–MeOH (10:0.5) as eluates. The desired fractions were collected and concentrated. Treatment of the residue with ether afforded crystals (0.75 g, 49%), m.p. 99–101 °C, $[\alpha]_D^{23}$ +4.9° (*c* 1.0, in DMF), R_{F4} 0.47 (Found: C, 60.7; H, 6.6; N, 2.9. $C_{22}H_{27}NO_6S$ requires C, 60.95; H, 6.3; N, 3.2%); δ_H(200 MHz; $CDCl_3$) 1.42 (9 H, s, Bu^t), 2.76 [3 H, s, S(O)Me], 3.07 (2 H, m, $CHCH_2Ph$), 4.54 (1 H, m, CH), 5.07 (2 H, s, OCH_2Ph), 5.52 or 6.08 (1 H, br, NH), 5.52 or 6.08 (1 H, br, CO_2H), 6.87 (2 H, d, *J* 8.6 Hz, PhO), 7.13 (2 H, d, *J* 8.6 Hz, PhO), 7.58 [2 H, d, *J* 8.6 Hz, PhS(O)], and 7.67 [2 H, d, *J* 8.6 Hz, PhS(O)].

Table 2. Physical constants and analytical data of Boc-Tyr(Msib)-Gly-Gly-Phe-Met(O)-OMsib (**10**) and its intermediates.

Peptide	R_{F1}	M.p. (°C)	$[\alpha]_D^{23}$ (c 1, $CHCl_3$)	Formula	Analyses (%) Found (Required) C	H	N
(**6**)	0.61	71–74	+2.9°	$C_{23}H_{27}NO_7S_2 \cdot \frac{3}{2}H_2O$	54.5 (54.3)	5.9 (5.95)	2.9 (2.75)
(**7**)	0.62	84–87	−1.0°	$C_{31}H_{36}N_2O_8S_2$	59.1 (59.2)	6.0 (5.8)	4.4 (4.5)
(**8**)	0.61	84–87	−14.0°	$C_{33}H_{39}N_3O_9S_2 \cdot \frac{1}{2}H_2O$	56.7 (57.0)	5.9 (5.8)	5.8 (6.05)
(**9**)	0.54	108–111	−19.8°	$C_{35}H_{42}N_4O_{10}S_2 \cdot \frac{1}{2}H_2O$	55.8 (55.9)	5.8 (5.8)	7.3 (7.45)
(**10**)	0.63	118–120	−21.5°	$C_{48}H_{59}N_5O_{12}S_3 \cdot 2H_2O$	56.3 (56.0)	5.9 (6.2)	6.7 (6.8)

Boc-Tyr(Mtb)-OH.—NaH (60% oil suspension; 0.32 g, 8.00 mmol) was added to a solution of Boc-Tyr-OH (1.00 g, 3.55 mmol) in DMF (30 ml) at −20 °C during 30 min, after which Mtb-Br (0.93 g, 4.28 mmol) was added. The mixture was stirred at 0 °C for 3 h, and the solvent was removed by evaporation. The product was extracted with AcOEt, and the extract washed with water, dried (Na_2SO_4), and concentrated. Treatment of the residue with ether–hexane afforded a powder, which was recrystallized from MeOH with ether–hexane to afford crystals (1.23 g, 83%), m.p. 146–147 °C, $[\alpha]_D^{28}$ +16.6° (c 1.0, in MeOH), R_{F1} 0.50 (Found: C, 63.1; H, 6.6; N, 3.2. $C_{22}H_{27}NO_5S$ requires C, 63.3; H, 6.5; N, 3.4%); δ_H(200 MHz; $CDCl_3$) 1.42 (9 H, s, Bu^t), 2.48 (3 H, s, SMe), 3.08 (2 H, m, CHCH_2Ph), 4.57 (1 H, m, CH), 4.97 (2 H, s, OCH_2Ph), 5.64 or 6.00 (1 H, br, NH), 5.64 or 6.00 (1 H, br, CO_2H), 6.89 (2 H, d, J 8.6 Hz, PhO), 7.09 (2 H, d, J 8.6 Hz, PhO), 7.25 (2 H, d, J 8.6 Hz, PhS), and 7.33 (2 H, d, J 8.6 Hz, PhS).

Reduction of Tyr(Msib) *by* DMF–SO_3 *Complex/EDT System.*—DMF–SO_3 complex (0.36 g, 2.30 mmol) and EDT (217 μl, 2.30 mmol) were added to a solution of Boc-Tyr(Msib)-OH (100 mg, 0.23 mmol) in DMF–pyridine (4:1; 2 ml). The mixture was stirred at 20 °C for 30 min and then concentrated. The product was extracted with AcOEt, and the extract washed with water dried (Na_2SO_4), and concentrated. The residue was treated with hexane to afford a powder, which was recrystallized from MeOH with ether–hexane to afford crystals (70 mg, 73%), m.p. 145–147 °C, $[\alpha]_D^{28}$ −16.8° (c 1.0, in MeOH), R_{F1} 0.50 (Found: C, 63.0; H, 6.5; N, 3.3. $C_{22}H_{27}NO_5S$ requires C, 63.3; H, 6.5; N, 3.4%). The ^1H n.m.r. spectroscopic data were identical with those of authentic Boc-Tyr(Mtb)-OH.

H-Tyr(Msib)-OH.—Boc-Tyr(Msib)-OH (50 mg, 0.12 mmol) was treated with TFA (2 ml) in the presence of anisole (0.2 ml) in an ice-bath for 30 min after which TFA was removed by evaporation. Treatment of the residue with ether afforded a powder, which was recrystallized from MeOH with ether in the presence of a few drops of TEA to afford crystals (30 mg, 86%), m.p. 224–227 °C, $[\alpha]_D^{23}$ +2.0° (c 1.0, in AcOH), R_{F1} 0.17, R_{F5} 0.46 (Found: C, 59.4; H, 5.6; N, 4.0. $C_{17}H_{19}NO_4S \cdot \frac{1}{2}H_2O$ requires C, 59.6; H, 5.9; N, 4.1%).

Stability of Tyr(Msib) *and* Tyr(Mtb) *to* TFA.—H-Tyr(Msib)-OH (10 mg, 0.030 mmol) was treated with TFA (0.45 ml) in the presence of anisole (0.05 ml) in an ice-bath for 3 h. No change was observed on TLC. When Boc-Tyr(Mtb)-OH (10 mg, 0.023 mmol) was treated with TFA (0.45 ml) in the presence of anisole (0.05 ml) in an ice-bath for 2 h, starting material (R_{F1} 0.50) disappeared (TLC) having been fully converted into H-Tyr-OH (R_{F5} 0.51).

Z(OMe)-Met(O)-OMsib (**6**).—Msib-Br (0.85 g, 3.64 mmol) and DCHA (0.73 ml, 3.64 mmol) were added to an ice-chilled solution of Z(OMe)-Met(O)-OH (1.00 g, 3.04 mmol) in DMF (10 ml), and the mixture was stirred overnight; the DCHA·HBr formed was removed by filtration. The product was purified by procedure A, followed by column chromatography (3 × 20 cm) on silica gel using $CHCl_3 \rightarrow CHCl_3$–MeOH (10:0.5) as eluants. The desired fractions were collected and concentrated. The residue was treated with ether to afford a powder (0.90 g, 62%). Physical constants and analytical data are listed in Table 2, together with those of other intermediates.

Z(OMe)-Phe-Met(O)-OMsib (**7**).—A TFA-treated sample of (**6**) (2.93 g, 6.08 mmol) was dissolved in DMF (20 ml) containing TEA (0.85 ml, 6.08 mmol). Z(OMe)-Phe-OSu (2.85 g, 6.68 mmol) and NMM (0.73 ml, 6.68 mmol) were added to the solution, and the mixture was stirred for 3 h. The product was purified by procedure A (extraction solvent: $CHCl_3$), followed by precipitation from $CHCl_3$ with ether to afford the dipeptide (**7**) (3.00 g, 81%).

Z(OMe)-Gly-Phe-Met(O)-OMsib (**8**).—A TFA-treated sample of (**7**) (2.90 g, 4.60 mmol) was dissolved in DMF (30 ml) containing TEA (0.66 ml, 4.60 mmol). Z(OMe)-Gly-OSu (1.75 g, 5.20 mmol) and NMM (0.57 ml, 5.20 mmol) were added to the solution, and the mixture was stirred for 3 h. The product was purified by procedure A (extraction solvent: $CHCl_3$), followed by precipitation from $CHCl_3$ with ether to afford the tripeptide (**8**) (3.10 g, 98%).

Z(OMe)-Gly-Gly-Phe-Met(O)-OMsib (**9**).—A TFA-treated sample of (**8**) (3.00 g, 4.40 mmol) was dissolved in DMF (40 ml) containing TEA (0.62 ml, 4.40 mmol). Z(OMe)-Gly-OSu (1.65 g, 4.90 mmol) and NMM (0.54 ml, 4.90 mmol) were added to the solution, and the mixture was stirred for 3 h. The product was purified by procedure A (extraction solvent: $CHCl_3$), followed by precipitation from $CHCl_3$ with AcOEt to afford the tetrapeptide (**9**) (2.70 g, 83%).

Boc-Tyr(Msib)-Gly-Gly-Phe-Met(O)-OMsib (**10**).—A TFA-treated sample of (**9**) (0.50 g, 0.67 mmol) was dissolved in DMF (3 ml) containing TEA (0.09 ml, 0.67 mmol). Boc-Tyr(Msib)-OSu [prepared from Boc-Tyr(Msib)-OH (0.35 g, 0.80 mmol) as usual] and NMM (0.09 ml, 0.80 mmol) were added to the solution, and the mixture was stirred overnight. The product was purified by procedure B, followed by column chromatography (3.0 × 20 cm) on silica gel using $CHCl_3$–MeOH (10:0.5) as an eluant. The desired fractions were collected and concentrated, and the residue was treated with diethyl ether to

afford crystals (0.45 g, 68%). Amino acid ratios in a 6M HCl hydrolysate: Tyr 1.02, Gly 2.09, Phe 1.00, Met + Met(O) 0.89 (recovery of Phe, 85%).

Boc-Tyr(Mtb)-Gly-Gly-Phe-Met-OMtb (**11**).—Compound (**10**) (30 mg, 0.030 mmol) was dissolved in DMF–pyridine (4:1; 2.5 ml), and then DMF–SO_3 complex (462 mg, 100 equiv.) and EDT (284 μl, 100 equiv.) were added. The mixture was stirred at 20 °C for 1 h and then evaporated. The residue was treated with water to afford a powder, which was precipitated from DMF with diethyl ether to afford the reduced pentapeptide (**11**) (25 mg, 88%), R_{F1} 0.68. Amino acid ratios in a 4M MSA hydrolysate: Tyr 0.98, Gly 2.10, Phe 1.00, Met 0.96 (recovery of Phe, 85%). FABMS *m/z*: 946.0 $(M + H)^+$.

H-Tyr-Gly-Gly-Phe-Met-OH, *Methionine-enkephalin* [*from* (**11**)].—Compound (**11**) (25 mg, 0.026 mmol) was treated with TFA (1.8 ml) in the presence of 2% EDT-containing DMA (0.2 ml) at 0 °C for 120 min and then at 20 °C for 60 min. The solvent was removed under reduced pressure. The product was similarly purified as described in the deprotection of (**5**) to afford a white fluffy powder (8.6 mg, 57%). Amino acid ratios in a LAP digest: Tyr 0.93, Gly 1.90, Phe 1.00, Met 1.01 (recovery of Phe, 85%). R_F Values on TLC (R_{F5} 0.68, R_{F6} 0.63) and retention time in HPLC [14.9 min, eluted as described in the deprotection of (**5**)] were identical with those of the authentic sample and Met-enkephalin prepared from (**5**).

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References

1 B. Iselin, *Helv. Chim. Acta*, 1961, **44**, 61.
2 Y. Schecter, Y. Burstein, and A. Patchornik, *Biochemistry*, 1975, **14**, 4497.
3 R. A. Houghten and C. H. Li, *Anal. Biochem.*, 1979, **98**, 36.
4 N. Fujii, A. Otaka, N. Sugiyama, M. Hatano, and H. Yajima, *Chem. Pharm. Bull.*, 1987, **35**, 3880.
5 Y. Kiso, M. Yoshida, T. Fujisaki, T. Mimoto, T. Kimura, and M. Shimokura, in 'Peptide Chemistry 1986,' ed. T. Miyazawa, Protein Research Foundation, Osaka, 1987, p. 205.
6 D. Yamashiro, *Int. J. Pept. Protein Res.*, 1982, **20**, 63.
7 J. P. Tam, W. F. Heath, and R. B. Merrifield, *Tetrahedron Lett.*, 1982, 2939.
8 N. Fujii, S. Kuno, A. Otaka, S. Funakoshi, K. Takagi, and H. Yajima, *Chem. Pharm. Bull.*, 1985, **33**, 4587.
9 T. Numata, H. Togo, and S. Oae, *Chem. Lett.*, 1979, 329.
10 S. Kuno, K. Akaji, O. Ikemura, M. Moriga, M. Aono, K. Mizuta, A. Takagi, and H. Yajima, *Chem. Pharm. Bull.*, 1986, **34**, 2462.
11 J. R. Parikh and W. von E. Doering, *J. Am. Chem. Soc.*, 1967, **89**, 5505.
12 K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, 1965, **87**, 5661.
13 K. K. Kelly and J. S. Matthews, *J. Org. Chem.*, 1971, **36**, 2159.
14 W. B. Hardy and M. Scalera, *J. Am. Chem. Soc.*, 1952, **74**, 5212.
15 N. Fujii, M. Sakurai, S. Kuno, H. Yajima, M. Satoh, M. Matsushita, N. Yamamoto, H. Takagi, Z.-M. Wang, W. Lee, and P.-F. Wang, *Chem. Pharm. Bull.*, 1985, **33**, 4326.
16 J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature*, 1975, **258**, 577.
17 B. W. Erickson and R. B. Merrifield, *J. Am. Chem. Soc.*, 1973, **95**, 3750.
18 G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, 1963, **85**, 3039.
19 R. J. Simpson, M. R. Neuberger, and T.-Y. Liu, *J. Biol. Chem.*, 1976, **251**, 1936.
20 Y. Kiso, K. Ito, S. Nakamura, K. Kitagawa, T. Akita, and H. Moritoki, *Chem. Pharm. Bull.*, 1979, **27**, 1472.
21 H. Yajima and N. Fujii, *J. Am. Chem. Soc.*, 1981, **103**, 5867.
22 J. M. Samanen and E. Brandeis, *J. Org. Chem.*, 1988, **53**, 561.
23 M. E. Jung and G. L. Hatfield, *Tetrahedron Lett.*, 1978, 4483.
24 K. Okamoto, K. Yasumura, K. Fujitani, S. Katakura, K. Akaji, H. Yajima, Y. Nakata, A. Inoue, and T. Segawa, *Chem. Pharm. Bull.*, 1984, **32**, 430.
25 D. H. Spackman, E. L. Smith, and D. M. Brown, *J. Biol. Chem.*, 1955, **212**, 255.
26 J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.*, 1952, **74**, 676.
27 D. D. M. Wayner and D. R. Arnold, *Can. J. Chem.*, 1984, **62**, 1164.

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