Facile Reduction of Methionine Sulfoxide with Sulfur Trioxide/Iodide Ion System[1)]

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A new system for the facile reduction of Met(O) in protected peptides (dimethylformamide–sulfur trioxide complex/iodide ion) was introduced. Met(O) in protected Met–enkephalins and protected leucosulfakinins were reduced to Met within 1 h by this system, and the reducing ability of this new system seemed comparable to or greater than that of the formerly reported dimethylformamide–sulfur trioxide complex/ethanedithiol system. The methylsulfinylbenzyl protecting group of Tyr was also reduced to a methylthiobenzyl group by this system.

Keywords sulfur trioxide; iodide ion; methionine sulfoxide; reduction; peptide synthesis; methionine–enkephalin; leucosulfakinin; leucosulfakinin-II

Met(O)[2)] is a useful derivative for the synthesis of Met-containing peptides to avoid S-alkylation and partial sulfoxide formation during manipulations for peptide chain construction. When Met(O) is used for the protection of Met, the reduction of Met(O) to Met at the final stage of the synthesis is necessary. Recently we reported that the combination of sulfur trioxide, in the form of dimethylformamide complex, with ethanedithiol (EDT) has a great ability to reduce Met(O) in protected peptides to Met.[3)] The usefulness of this reducing system was demonstrated in syntheses of Met–enkephalin[3)] and leucosulfakinin-II.[4)]

Here we wish to report another novel reducing system of Met(O) in protected peptides: this system employs iodide ion (I^-) instead of EDT. The reduction seems to proceed as shown in Fig. 1, where I^- nucleophilically attacks the sulfonium moiety formed by DMF–SO_3 complex.

To compare the reducing ability of the DMF–SO_3 complex/I^- system with that of the DMF–SO_3 complex/EDT system, Z(OMe)–Phe–Met(O)–OMe, a model peptide, in DMF–pyridine (4 : 1) was treated with DMF–SO_3 complex (5 eq) and tetrabutylammonium iodide (TBAI) (2.5 eq) or EDT (1.25 eq). Since EDT has two sulfhydryl groups in one molecule, the molar ratio of EDT was adjusted to half with respect to TBAI. The progress of the reduction was monitored by high-performance liquid chromatography (HPLC). As shown in Fig. 2, the ability of this new reduction system to reduce the model peptide is comparable to that of the DMF–SO_3 complex/EDT system. Moreover, this reduction system does not involve an unpleasant odor like that of EDT in the case of the DMF–SO_3 complex/EDT system.

To characterize the product formed by this reduction system, Z(OMe)–Phe–Met(O)–OMe in DMF–pyridine (4 : 1) was treated with DMF–SO_3 complex (5 eq) and TBAI (5 eq) at 30 °C. The reduction was completed in 20 min as judged by thin layer chromatography (TLC), and the solvent was removed *in vacuo*. After the residue was treated with 5% $Na_2S_2O_3$ and H_2O to remove iodine formed during the reduction, the product was purified by recrystallization from DMF with ether to afford Z(OMe)–Phe–Met–OMe in 72% yield. The *Rf* value in TLC, melting point, $[\alpha]_D$, proton nuclear magnetic resonance (^{1}H-NMR) spectra, and retention time in HPLC were identical with those of authentic Z(OMe)–Phe–Met–OMe.[3b)]

Next, the effectiveness of this reduction system was evaluated through the reduction of Met(O) in two known protected Met–enkephalins.[3b)] The Met(O) in Boc–Tyr(Cl_2Bzl)–Gly–Gly–Phe–Met(O)–OBzl was reduced with DMF–SO_3 complex and TBAI (20 eq each) at 30 °C in 30 min to afford Boc–Tyr(Cl_2Bzl)–Gly–Gly–Phe–Met–OBzl in 94% yield.[5)] The Met(O) in Boc–Tyr(Msib)–Gly–Gly–Phe–Met(O)–OMsib (Msib = methylsulfinylbenzyl) was also reduced with DMF–SO_3 complex and TBAI (60 eq each) at 30 °C within 30 min to afford Boc–Tyr(Mtb)–Gly–Gly–Phe–Met–OMtb (Mtb = methylthiobenzyl) in 95% yield,[6)] where the Msib group was also reduced to the Mtb group together with the reduction of the Met(O). The structures of these two peptides were confirmed by the *Rf* values in TLC, fast atom bombardment mass spectra (FAB-MS) and amino acid analysis after 4 N MSA hydrolysis,[7)] (this hydrolysis system has almost no ability to reduce Met(O)).

This reduction system was further applied to the synthesis of leucosulfakinin (LSK). LSK is a Tyr(SO_3H)-containing peptide isolated from head extracts of the cockroach *Leucophaea maderae*,[8)] and has a structural similarity to peptides of the cholecystokinin (CCK)/gastrin family. Recently another peptide, termed LSK-II, was isolated from the same source.[9)] The C-terminal seven amino acid residues of LSK-II are identical with those of LSK (Fig. 3).

The synthesis of LSK was conducted according to the route shown in Fig. 4. The protected LSK was prepared from a TFA-treated sample of Boc–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2,[4)] the intermediate heptapeptide amide used for the synthe-

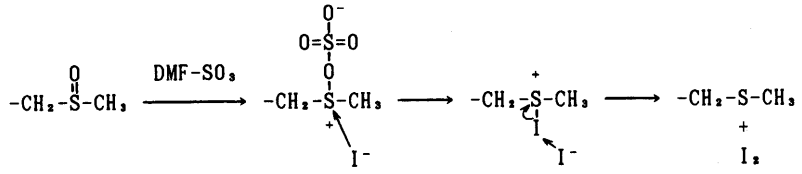

Fig. 1 Plausible Mechanism of Met(O) Reduction by DMF–SO_3 Complex/I^- System

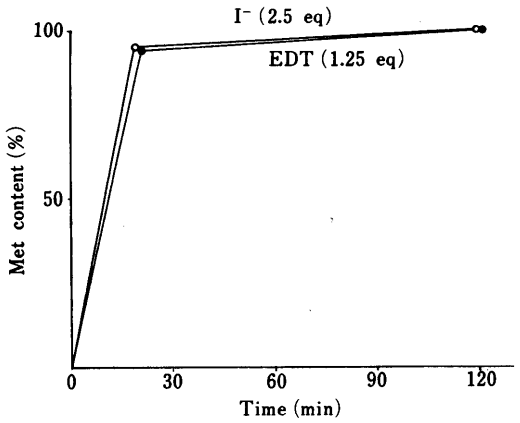

Fig. 2. Effect of EDT and I^- on Met(O) Reduction
Z(OMe)–Phe–Met(O)–OMe/DMF–pyridine + DMF–SO_3 (5 eq).

sis of LSK-II. Z(OMe)–Glu(OBzl)–ONp, Boc–Phe–OSu, Z(OMe)–Gln–ONp, and Z(OMe)–Glu(OBzl)–ONp were successively incorporated onto the heptapeptide by the Np[10] or Su[11] active ester method. The Met(O) in the protected undecapeptide thus obtained was reduced to Met by treatment with DMF–SO_3 complex and TBAI (20 eq each) at 25 °C for 1 h in 94% yield. Its structure was confirmed by amino acid analysis after 4 N MSA hydrolysis and by FAB-MS. Thus the Met(O) was effectively reduced by this system in a short time before final deprotection. When DMF–SO_3 complex/EDT system was employed for the reduction, it took 2 h for the reduction to be completed. Then the reduced peptide was deprotected with 1 M TFMSA–thioanisole/TFA[12] in an ice-bath for 3 h, which deprotection system is known to have adequate ability to prevent S-alkylation and air-oxidation of Met residues.

gastrin-II: pGlu-----Glu-Glu-Glu-Glu-Glu-Ala-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH_2

LSK: H-Glu-Gln-Phe-Glu-Asp-Tyr(SO_3H)-Gly-His-Met-Arg-Phe-NH_2

LSK-II: pGlu-Ser-Asp-Asp-Tyr(SO_3H)-Gly-His-Met-Arg-Phe-NH_2

CCK-12: H-Ile-Ser-Asp-Arg-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

Fig. 3. Structures of Leucosulfakinins

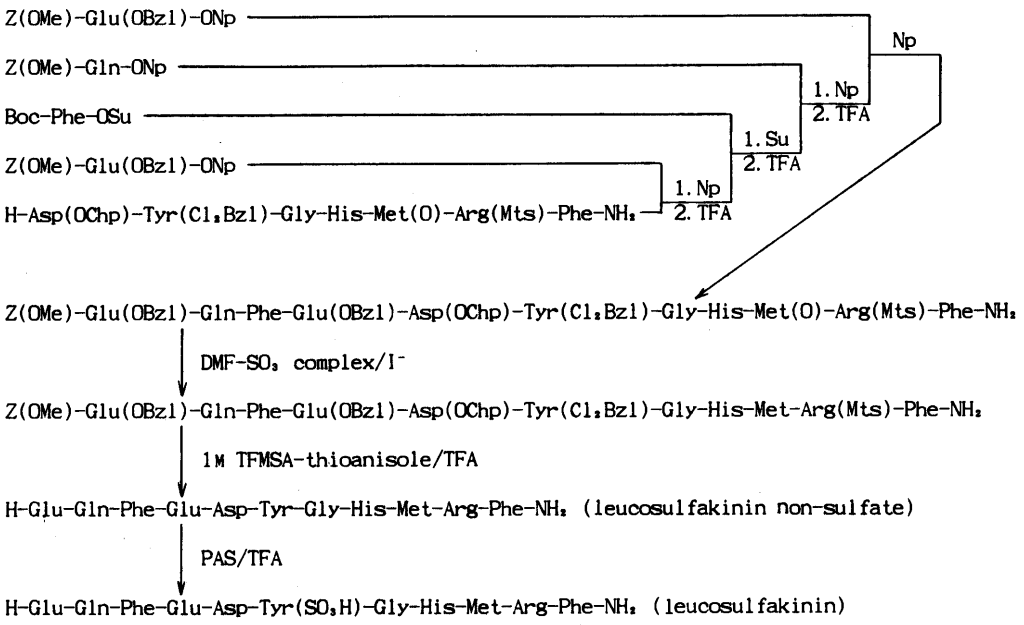

Fig. 4. Synthetic Scheme for Leucosulfakinin

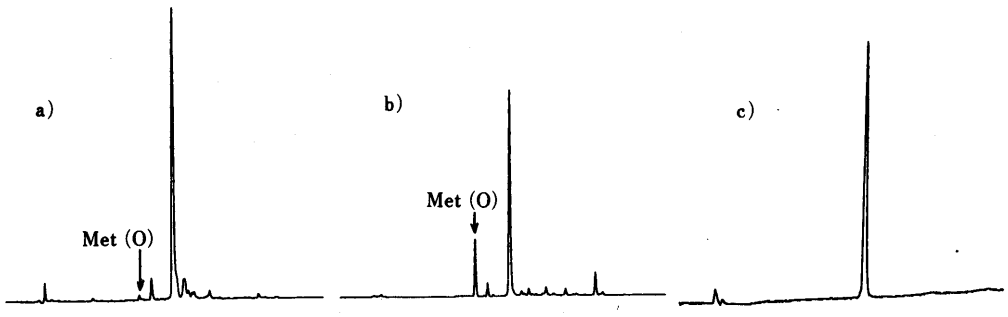

Fig. 5. HPLC of Leucosulfakinin Non-sulfate

a) Gel-filtered sample after Met(O) reduction with DMF–SO_3 complex/I^- system followed by deprotection with TFMSA. b) Gel-filtered sample after deprotection with TFMSA followed by Met(O) reduction with thiol. c) HPLC-purified sample of a). Chromatography was carried out on a YMC AM-312 column (6 × 150 mm) with elution using a gradient of 15—45% CH_3CN in 0.1% TFA over a period of 30 min, flow rate = 1.0 ml/min. The eluate was monitored at 275 nm.

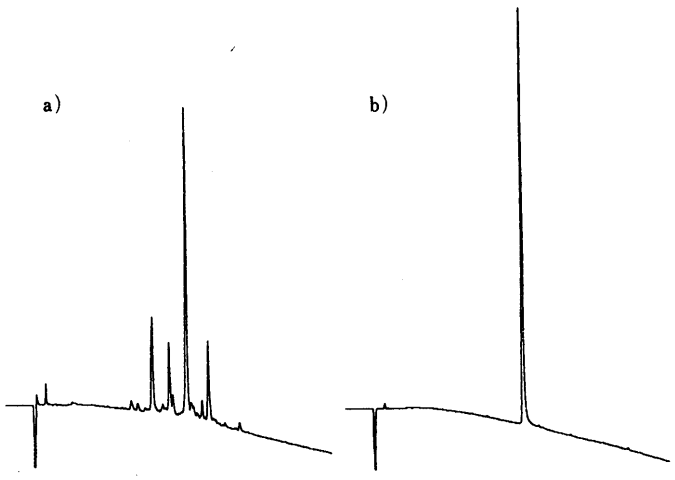

Fig. 6. HPLC of Leucosulfakinin

a) Gel-filtered sample after sulfation. b) HPLC-purified sample. Chromatography was carried out on a YMC AM-312 column (6 × 150 mm) with elution using a gradient of 10—40% CH_3CN in 0.1 M $AcONH_4$ (pH 6.5) over a period of 30 min, flow rate = 1.0 ml/min. The eluate was monitored at 215 nm.

Subsequent purification by gel-filtration on Sephadex G-10 afforded LSK non-sulfate in 70% yield (Fig. 5a). For the comparison of this convenient approach with the conventional TFMSA-deprotection procedure,[13] the Met(O)-form of the protected LSK was treated with 1 M TFMSA–thioanisole/TFA in an ice-bath for 3 h, incubated with 2-mercaptoethanol at 37 °C for 20 h, then purified by gel-filtration. The HPLC profile of the gel-filtered sample is shown in Fig. 5b. In this case, the 20-h incubation afforded incomplete reduction. This result suggested that the former approach was able not only to decrease the time needed for the reduction but also to insure the completion of reduction. For identification, the gel-filtered sample of Fig. 5a was purified by HPLC on a column of YMC AM-323 to afford a pure LSK non-sulfate (Fig. 5c). The purity of this product was confirmed by amino acid analysis after acid hydrolysis and leucine aminopeptidase (LAP) digestion, and by FAB-MS. The gel-filtered sample of Fig. 5a was sulfated with pyridinium acetyl sulfate (PAS)[14] in TFA in an ice-bath for 2 h. Subsequent purification by gel-filtration on Sephadex G-10 and HPLC on a column of YMC AM-323 afforded LSK in 10% yield (Fig. 6). The presence of $Tyr(SO_3H)$ was confirmed by Fourier-transform infrared spectrometry (FT-IR), FAB-MS, and amino acid analysis after LAP digestion.

This reduction system was also applied to the reduction of the Met(O)-form of the protected LSK-II, pGlu–Ser(Bzl)–Asp(OChp)–Asp(OChp)–$Tyr(Cl_2Bzl)$–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2.[4] The Met(O) of the above protected decapeptide amide was reduced to Met by treatment with DMF–SO_3 complex and TBAI (20 eq each) at 30 °C for 30 min.[15] Its structure was confirmed by amino acid analysis after 4 N MSA hydrolysis and FAB-MS.

The results of these experiments indicated the usefulness of this new reduction system for the synthesis of Met-containing peptides. For the reduction of Met(O) in protected peptides, DMF–SO_3 complex/I^- system is preferable to DMF–SO_3 complex/EDT system: it offers equally rapid or faster completion of the reduction, and easy handling owing to the absence of unpleasant odor. This new system would be applicable not only for the reduction of Met(O) in protected peptides but also for the reduction of various other sulfoxides, as was shown in the case of the Msib group.

Experimental

The *Rf* values in TLC, performed on silica gel (precoated Silica gel 60 F_{254}, Merck), refer to the following solvent systems: Rf_1, $CHCl_3$–MeOH–H_2O (8 : 3 : 1), Rf_2, *n*-BuOH–AcOH–AcOEt–H_2O (1 : 1 : 1 : 1). The melting points are uncorrected. The optical rotation was determined with a Union PM-201 polarimeter. HPLC was conducted with a Hitachi L-6200 chromatograph. Amino acid analysis was performed on a Hitachi 835 amino acid analyzer. ^{1}H-NMR spectra were taken on a JEOL JNM-FX 200 (200 MHz) spectrometer with tetramethylsilane as an internal standard. FT-IR spectra were obtained on a Perkin Elmer 1720 spectrometer. Fast atom bombardment mass spectra were recorded on a JEOL JMS-D 300 spectrometer or a VG Analytical ZAB SE spectrometer equipped with a FAB ion source. LAP was purchased from Sigma (No. L-6002). DMF–SO_3 complex was purchased from Fluka.

Prior to condensation, the N^a–Z(OMe) or Boc group was removed by TFA treatment in the presence of anisole. The active ester reaction was performed at room temperature. Unless otherwise mentioned, products were purified by one of the following procedures.

A: After evaporation of the solvents at 30 °C, the residue was treated with 5% $Na_2S_2O_3$. The resulting powder was washed with 5% $Na_2S_2O_3$ and water, and precipitated from appropriate solvents.

B: After evaporation of the solvents, the residue was triturated with 5% citric acid. The resulting powder was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, and precipitated from appropriate solvents.

Comparison of Reducing Ability of DMF–SO_3 Complex/I^- System with That of DMF–SO_3 Complex/EDT System Z(OMe)–Phe–Met(O)–OMe (25 mg, 0.05 mmol) was dissolved in DMF–pyridine (4 : 1, 0.5 ml), then DMF–SO_3 complex (39 mg, 5 eq) and TBAI (46 mg, 2.5 eq) [or EDT (6 μl, 1.25 eq)] 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).

Reduction of Z(OMe)–Phe–Met(O)–OMe Z(OMe)–Phe–Met(O)–OMe (123 mg, 0.25 mmol) in DMF–pyridine (4 : 1, 2 ml) was treated with DMF–SO_3 complex (191 mg, 5 eq) and TBAI (462 mg, 5 eq). The mixture was stirred at 30 °C for 20 min. The product was purified by procedure A, followed by recrystallization from DMF with ether; yield 85 mg (72%), mp 117—118 °C, $[\alpha]_D^{28}$ −15.0° (*c* = 1.0, DMF), Rf_1 0.87 {lit.[3b] mp 118—120 °C, $[\alpha]_D^{20}$ −16.6° (*c* = 1.0, DMF), Rf_1 0.87}. *Anal.* Calcd for $C_{24}H_{30}N_2O_6S$: C, 60.74; H, 6.37; N, 5.90. Found: C, 60.41; H, 6.49; N, 5.65. The retention time in HPLC and the ^{1}H-NMR spectrum were identical with those of an authentic sample.[3b]

Reduction of Boc–Tyr(Cl_2Bzl)–Gly–Gly–Phe–Met(O)–OBzl Boc–Tyr(Cl_2Bzl)–Gly–Gly–Phe–Met(O)–OBzl (50 mg, 0.053 mmol) in DMF–pyridine (4 : 1, 2.5 ml) was treated with DMF–SO_3 complex (163 mg, 20 eq) and TBAI (391 mg, 20 eq) at 30 °C for 30 min. The product was purified by procedure A, followed by precipitation from DMF with ether to afford Boc–Tyr(Cl_2Bzl)–Gly–Gly–Phe–Met–OBzl; yield 46 mg (94%), Rf_1 0.75 (lit.[3b] Rf_1 0.75). Amino acid ratios in a 4 N MSA hydrolysate: Tyr 0.81, Gly 2.04, Phe 1.00, Met 0.82 (recovery of Phe, 85%). FAB-MS: 922.0 $(M+H)^+$.

Reduction of Boc–Tyr(Msib)–Gly–Gly–Phe–Met(O)–OMsib Boc–Tyr(Msib)–Gly–Gly–Phe–Met(O)–OMsib (30 mg, 0.030 mmol) in DMF–pyridine (4 : 1, 2.5 ml) was treated with DMF–SO_3 complex (276 mg, 60 eq) and TBAI (665 mg, 60 eq) at 30 °C. After 30 min, the solvent was removed by evaporation, and the product was purified as stated above to afford Boc–Tyr(Mtb)–Gly–Gly–Phe–Met–OMtb; yield 27 mg (95%), Rf_1 0.68 (lit.[3b] Rf_1 0.68). Amino acid ratios in a 4 N MSA hydrolysate: Tyr 0.80, Gly 1.92, Phe 1.00, Met 0.88 (recovery of Phe, 88%). FAB-MS: 946.0 $(M+H)^+$.

Z(OMe)–Glu(OBzl)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2 (1) A mixture of Z(OMe)–Glu(OBzl)–ONp (1.31 g, 2.50 mmol), TEA (0.63 ml, 4.50 mmol) and a TFA-treated sample of Boc–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2 (2.96 g, 2.00 mmol) in DMF (20 ml) was stirred for 24 h. The product was purified by procedure B, followed by precipitation from DMF with ether; yield 2.75 g (78%). Physical constants and analytical data are listed in Table I, together with those of other intermediates.

TABLE I. Physical Constants and Analytical Data of the Protected Leucosulfakinin (4) and Its Intermediates

Peptide	Rf_1	mp (°C)	$[\alpha]_D^{23}$ ($c=1$, DMF)	Formula	*Anal.* Calcd (Found) (%)		
					C	H	N
1	0.53	174—179	−9.7°	$C_{85}H_{104}Cl_2N_{14}O_{19}S_2 \cdot 4H_2O$	55.69	6.16	10.70
					(55.29	5.72	10.48)
2	0.52	156—159	−9.1°	$C_{90}H_{113}Cl_2N_{15}O_{19}S_2 \cdot 5H_2O$	55.88	6.41	10.86
					(55.43	5.97	10.67)
3	0.51	214—217	−14.8°	$C_{99}H_{121}Cl_2N_{17}O_{22}S_2 \cdot 3H_2O$	56.88	6.12	11.39
					(56.83	6.12	11.39)
4	0.48	227—230	−9.0°	$C_{111}H_{134}Cl_2N_{18}O_{25}S_2 \cdot 5H_2O$	56.84	6.19	10.75
					(56.81	5.83	10.46)

Boc–Phe–Glu(OBzl)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2 (2) A mixture of Boc–Phe–OSu [prepared from Boc–Phe–OH (0.53 g, 2.00 mmol)], TEA (0.49 ml, 3.50 mmol) and a TFA-treated sample of **1** (2.64 g, 1.50 mmol) in DMF (20 ml) was stirred for 24 h. The product was purified by procedure B, followed by precipitation from MeOH; yield 2.52 g (91%).

Z(OMe)–Gln–Phe–Glu(OBzl)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2 (3) A mixture of Z(OMe)–Gln–ONp (0.65 g, 1.50 mmol), TEA (0.38 ml, 2.70 mmol) and a TFA-treated sample of **2** (2.21 g, 1.20 mmol) in DMF (20 ml) was stirred for 5 h. The product was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 1.92 g (79%).

Z(OMe)–Glu(OBzl)–Gln–Phe–Glu(OBzl)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2 (4) [Met(O)-Form of the Protected Leucosulfakinin] A mixture of Z(OMe)–Glu(OBzl)–ONp (0.42 g, 0.80 mmol), TEA (0.21 ml, 1.50 mmol) and a TFA-treated sample of **3** (1.43 g, 0.70 mmol) in DMF (20 ml) was stirred for 5 h. The product was purified by procedure B, followed by precipitation from DMF with AcOEt; yield 1.36 g (86%). Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.03, Glu 2.87, Gly 0.99, Met + Met(O) 0.78, Tyr 0.98, Phe 2.00, His 0.91, Arg 0.91 (recovery of Phe, 83%).

Z(OMe)–Glu(OBzl)–Gln–Phe–Glu(OBzl)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met–Arg(Mts)–Phe–NH_2 (5) (Reduced Form of the Protected Leuscosulfakinin) (i) Reduction with DMF–SO_3 Complex/I^- System Compound **4** (30 mg, 0.015 mmol) in DMF–pyridine (4 : 1, 0.5 ml) was treated with DMF–SO_3 complex (46 mg, 20 eq) and TBAI (110 mg, 20 eq) at 25 °C for 1 h. The solvent was removed by evaporation, and the product was purified by procedure A, followed by precipitation from DMF with ether; yield 28 mg (94%), Rf_1 0.56. Amino acid ratios in a 4 N MSA hydrolysate: Asp 1.06, Glu 3.05, Gly 1.10, Met 0.81, Tyr 0.89, Phe 2.00, His 0.91, Arg 0.90 (recovery of Phe, 86%). FAB-MS: 2237.9 $(M+H)^+$.

(ii) Reduction with DMF–SO_3 Complex/EDT System Compound **4** (30 mg, 0.015 mmol) in DMF–pyridine (4 : 1, 0.5 ml) was treated with DMF–SO_3 complex (46 mg, 20 eq) and EDT (28 μl, 20 eq) at 25 °C for 2 h. The solvent was removed by evaporation. The residue was treated with H_2O to afford a powder, which was precipitated from DMF with ether; yield 28 mg (94%), Rf_1 0.56. Amino acid ratios in a 4 N MSA hydrolysate: Asp 1.07, Glu 3.12, Gly 1.07, Met 0.94, Tyr 1.00, Phe 2.00, His 0.99, Arg 1.05 (recovery of Phe, 82%). FAB-MS: 2237.8 $(M+H)^+$.

H–Glu–Gln–Phe–Glu–Asp–Tyr–Gly–His–Met–Arg–Phe–NH_2 (Leucosulfakinin Non-sulfate) (i) Obtained from 5 Compound **5** (28 mg, 0.014 mmol) was treated with 1 M TFMSA–thioanisole/TFA (5 ml) in the presence of *m*-cresol (200 μl) and EDT (200 μl) in an ice-bath for 3 h, then ether was added to the solution. The resulting precipitate was collected by centrifugation and dissolved in H_2O (2 ml). The solution was applied to a column of Sephadex G-10 (2.4 × 60 cm), which was eluted with 2 N AcOH, and each fraction (6 ml) was monitored by measuring ultraviolet (UV) absorbance at 275 nm. Fractions corresponding to the main peak (Nos. 9—11) were collected and the solvent was removed by lyophilization to afford a fluffy powder; yield 14 mg (70%) (Fig. 5a). This powder was further purified by HPLC on a column of YMC AM-323 (10 × 250 mm). A part of the above crude sample (*ca.* 2 mg each) was applied to the column, which was eluted with a linear gradient of CH_3CN (15—45% in 30 min) in 0.1% TFA at a flow rate of 3 ml/min. The eluate corresponding to the main peak (retention time: 15.2 min, monitored by measuring UV absorbance at 275 nm) was collected and the solvent was removed by lyophilization to give a white fluffy powder; yield 7 mg (34%), $[\alpha]_D^{23}$ −20.0° ($c=0.5$, 0.1% TFA), Rf_2 0.41. Retention time in HPLC was 17.6 min on an analytical YMC AM-312 column (6 × 150 mm) (Fig. 5c). Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.06; Glu 3.05, Gly 1.00, Met 0.93, Tyr 1.00, Phe 2.00, His 1.00, Arg 0.93 (recovery of Phe, 90%). Amino acid ratios in a LAP digest: Asp 1.00, Glu 2.03, Gly 1.00, Met 0.72, Tyr 1.00, Phe 2.00, His 0.88, Arg 0.90, Gln N.D. (recovery of Phe, 81%). FAB-MS: 1457.4 $(M+H)^+$.

(ii) Obtained from 4 by TFMSA-Deprotection Followed by the Reduction of Met(O) with Thiol Compound **4** (15 mg, 0.007 mmol) was treated with 1 M TFMSA–thioanisole/TFA (2.5 ml) in the presence of *m*-cresol (100 μl) and EDT (100 μl) in an ice-bath for 3 h, then ether was added to the solution. The resulting powder was collected by centrifugation and dissolved in H_2O (2 ml) containing 2-mercaptoethanol (200 μl). The solution was incubated at 37 °C for 20 h under an N_2 atmosphere, then applied to a column of Sephadex G-10 (2.4 × 60 cm). The column was eluted with 2 N AcOH, and each fraction (6 ml) was monitored by measuring UV absorbance at 275 nm. Fractions corresponding to the main peak (Nos. 10—13) were collected and the solvent was removed by lyophilization to afford a fluffy powder; yield 7 mg (65%) (Fig. 5b).

H–Glu–Gln–Phe–Glu–Asp–Tyr(SO_3H)–Gly–His–Met–Arg–Phe–NH_2 (Leucosulfakinin) The above non-sulfate of Fig. 5a (21 mg, 0.014 mmol) in TFA (1.5 ml) was treated with PAS (158 mg, 50 eq) in an ice-bath for 2 h. Then ether was added to the solution and the resulting powder was collected by centrifugation. The powder was dissolved in 0.5 M NH_4HCO_3 (3 ml) in an ice-bath. The solution was stirred in an ice-bath for 10 min, then applied to a column of Sephadex G-10 (2.0 × 52 cm), which was eluted with 0.1 M NH_4HCO_3. The desired fractions (4 ml each, tube Nos. 18—24, monitored by measuring UV absorbance at 275 nm) were collected and the solvent was removed by lyophilization to afford a powder; yield 21 mg (95%) (Fig. 6a). This powder was further purified by HPLC on a column of YMC AM-323 (10 × 250 mm). A part of the above crude sample (*ca.* 2 mg each) was applied to the column, which was eluted with gradients of CH_3CN (20—25% in 20 min, then 25—40% in 10 min) in 0.1 M $AcONH_4$ (pH 6.5) at a flow rate of 2 ml/min. The eluate corresponding to the main peak (retention time: 14.2 min, monitored by measuring UV absorbance at 275 nm) was collected and the solvent was removed by lyophilization to give a white fluffy powder; yield 2 mg (10%), $[\alpha]_D^{24}$ −24.2° ($c=0.2$, H_2O), Rf_2 0.38. The retention time in HPLC was 17.5 min on an analytical YMC AM-312 column (6 × 150 mm) (Fig. 6b). Amino acid ratios in a 6 N HCl hydrolysate: Asp 1.06, Glu 3.03, Gly 1.07, Met 0.99, Tyr 1.06, Phe 2.00, His 1.00, Arg 1.01 (recovery of Phe, 82%). Amino acid ratios in a LAP digest: Asp 1.01, Glu 2.26, Gly 1.02, Met 0.83, Tyr(SO_3H) 0.80, Phe 2.00, His 0.97, Arg 0.93, Gln N.D. (recovery of Phe, 81%). FAB-MS: 1559.2 $(M+Na)^+$. FT-IR: 1049, 1259 cm^{-1}.

pGlu–Ser(Bzl)–Asp(OChp)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met–Arg(Mts)–Phe–NH_2 (Reduced Form of the Protected Leucosulfakinin-II) pGlu–Ser(Bzl)–Asp(OChp)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2 (20 mg, 0.10 mmol) dissolved in DMF–pyridine (4 : 1, 0.5 ml) was treated with DMF–SO_3 complex (33 mg, 20 eq) and TBAI (79 mg, 20 eq) at 30 °C for 30 min, then evaporated. The product was purified by procedure A, followed by precipitation from DMF with ether; yield 18 mg (90%), Rf_1 0.54 (lit.[4] Rf_1 0.54). Amino acid ratios in a 4 N MSA hydrolysate: Asp 2.03, Ser 0.88, Gly 1.00, Glu 1.00, Met 0.83, Tyr 0.93, Phe 1.00, His 0.89, Arg 0.98 (recovery of Phe, 89%). FAB-MS: 2237.9 $(M+H)^+$.

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References and Notes

1) Amino acids used in this work were of L-configuration. The following abbreviations are used: Z(OMe)=*p*-methoxybenzyloxycarbonyl, Boc=*tert*-butoxycarbonyl, Bzl=benzyl, Cl_2Bzl=2,6-dichlorobenzyl, Chp=cycloheptyl, Mts=mesitylenesulfonyl, (O)=sulfoxide, Su=*N*-hydroxysuccinimidyl, Np=*p*-nitrophenyl, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid, MSA=methanesulfonic acid, EDT=ethanedithiol, NMM=*N*-methylmorpholine, TEA=triethylamine, DMF=dimethylformamide.
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5) The Met(O) in Boc–Tyr(Cl_2Bzl)–Gly–Gly–Phe–Met(O)–OBzl was reduced to Met with DMF–SO_3 complex and EDT (10 eq each) at 20 °C in 1 h (yield 92%) (in ref. 3*b*).
6) Boc–Tyr(Msib)–Gly–Gly–Phe–Met(O)–OMsib was reduced to Boc–Tyr(Mtb)–Gly–Gly–Phe–Met–OMtb with DMF–SO_3 complex and EDT (100 eq each) at 20 °C in 1 h (yield 88%) (in ref. 3*b*).
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15) The Met(O) in pGlu–Ser(Bzl)–Asp(OChp)–Asp(OChp)–Tyr(Cl_2Bzl)–Gly–His–Met(O)–Arg(Mts)–Phe–NH_2 was reduced to Met with DMF–SO_3 complex and EDT (40 eq each) at 30 °C in 2 h (yield 91%) (in ref. 4).