Supporting Information

Rin Miyajima,[1] Yusuke Tsuda,[1] Tsubasa Inokuma,[1] Akira Shigenaga,[1] Miki Imanishi,[2] Shiroh Futaki,[2] and Akira Otaka[1,*]

[1]*Institute of Biomedical Sciences and Graduate School of Pharmaceutical Sciences Tokushima University, Tokushima 770-8505, Japan*
[2]*Institute for Chemical Research, Kyoto University, Kyoto 611-0011, japan*

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○ Preparation of peptide thioester **S1** for preparation of *Stichodactyla* toxin/zinc-finger fusion

H-**RSCIDTIPKSRCTAFQ**-S$(CH_2)_2$CO-**L**-NH_2

S1

On 4-methylbenzhydrylamine (MBHA) resin (0.70 mmol amine/g, 0.57 g, 0.40 mmol), introduction of Boc-Leu-OH (5.0 equiv) in the presence of DIPCDI (5.0 equiv), HOBt·H_2O (5.0 equiv) and DIPEA (2.0 equiv) in DMF at room temperature for 2 h followed by Boc removal by TFA/anisole/toluene (50:2:48 (v/v), 15 min) afforded the Boc-Leu-incorporated resin. Next, treatment of the resulting resin with *S*-Trt mercaptopropionic acid (5.0 equiv), DIPCDI (5.0 equiv), HOBt·H_2O (5.0 equiv) and DIPEA (2.0 equiv) in DMF at room temperature for 2 h followed by Trt removal by TFA/Et3SiH (95:5, 10 min) gave $HSCH_2CH_2CO$-Leu-MBHA resin. Activated Boc-Ala-OH (5.0 equiv) with HATU (4.95 equiv) and DIPEA (10 equiv) in DMF was coupled with $HSCH_2CH_2CO$-Leu- MBHA resin for 2 h, and the resin was subsequently subjected to Boc removal by TFA/anisole/toluene (50:2:48 (v/v), 15 min). On the resulting resin, standard *in situ* neutralization Boc SPPS (Acylation: Boc amino acid (5.0 equiv), DIPCDI (5.0 equiv), HOBt·H_2O (5.0 equiv) and DIPEA (2.0 equiv) in DMF at room temperature for 2 h; Boc removal: TFA/anisole/toluene (50:2:48 (v/v), 15 min)) was performed for chain elongation to give protected peptide resin for peptide thioester **S1**.[1] The resulting completed resin was treated with 1 M TMSOTf–thioanisole in TFA , *m*-cresol (100/5 (v/v) and 1,2-ethanedithiol (100/5 (v/v) at 4 °C for 2 h. After filtration of the resin, cooled Et_2O was added to the filtrate to give precipitate. The formed precipitate was collected by centrifugation and thoroughly washed with Et_2O to afford crude peptide thioester **S1**. The crude peptide was purified by preparative HPLC to give the purified peptide **S1** in 7% isolated yield.

S1: Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 17.9 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 30% over 30 min. MS (ESI-TOF) *m*/*z* calcd ($[M+2H]^{2+}$) 1013.5, found 1013.1.

○ Preparation of N-terminal cysteinyl peptide **S2** for preparation of *Stichodactyla* toxin/zinc-finger fusion and TsTxV/zinc-finger fusion

H-**CDICGRKFARSDERKRHTKIHLRQKD**-NH_2

S2

Protected peptide resins corresponding to the title peptides were constructed on NovaSyn® TGR

resin (Rink amide type: 0.22 mmol amine/g, 0.91 g, 0.20 mmol) using standard Fmoc SPPS. TFA cleavage (TFA–*m*-cresol–thioanisole–H_2O–1,2-ethanedithiol (80:5:5:5:5 (v/v), 50 μL/1 mg resin) of the protected resin at room temperature for 2 h followed by HPLC purification afforded the desired peptide in 54% isolated yield..

S2: Analytical HPLC conditions: Cosmosil $5C_{18}$-AR-II analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 14.7 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 30% over 30 min. MS (ESI-TOF) *m*/*z* calcd ($[M+3H]^{3+}$) 1065.9, found 1065.6.

○ Preparation of peptide **S3a**, **b**, **c** and **d** for preparation of TsTxV/zinc-finger fusion and its derivatives

H-**KKDGYPVEGDNCAFX**-$S(CH_2)_2CO$-**L**-NH_2

S3a, **b**, **c**

H-**KKDGYPVEGDNCAAA**-$S(CH_2)_2CO$-**L**-NH_2

S3d

Representative procedure: Peptide thioester **S3a** (**X = Ala**), **b** (**X = Phe**), **c** (**X = Ser**) and **d** were prepared by Boc SPPS using *in situ* neutralization protocol on $HSCH_2CH_2CO$-Leu-4-methylbenzhydrylamine (MBHA) resin as similar to that employed for preparation of peptide thiester **S1**.

S3a (**X = Ala**) (18% isolated yield): Analytical HPLC conditions: Cosmosil $5C_{18}$-AR-II analytical column with a linear gradient of solvent B in solvent A, 10% to 40% over 30 min, retention time = 20.6 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 20% to 35% over 30 min. MS (ESI-TOF) *m*/*z* calcd ($[M+2H]^{2+}$) 907.4, found 907.1.

S3b (**X = Phe**) (13% isolated yield): Analytical HPLC conditions: Cosmosil $5C_{18}$-AR-II analytical column with a linear gradient of solvent B in solvent A, 15% to 45% over 30 min, retention time = 20.7 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 20% to 35% over 30 min. MS (ESI-TOF) *m*/*z* calcd ($[M+2H]^{2+}$) 945.4, found 945.0.

S3c (**X = Ser**) (8% isolated yield): Analytical HPLC conditions: Cosmosil $5C_{18}$-AR-II analytical column with a linear gradient of solvent B in solvent A, 10% to 40% over 30 min, retention time = 19.4 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 12% to 27% over 30 min. MS (ESI-TOF) *m*/*z* calcd ($[M+2H]^{2+}$) 915.4, found 915.2.

S3d (8% isolated yield): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 17.0 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 12% to 27% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+2H]$^{2+}$) 869.4, found 869.2.

○ NCL for the synthesis of **S4b**, **c**, **d** for examination of effect of N-terminal residues in the zinc-finger sequence

H-**KKDGYPVEGDNCAFX-CDICGRKFARSDERKRHTKIHLRQKD**-NH_2

S4b, **c**

H-**KKDGYPVEGDNCAAA-CDICGRKFARSDERKRHTKIHLRQKD**-NH_2

S4d

Representative procedure: Peptide thioester **S3b** (5.6 mg , 2.5 μmol) and N-terminal cysteinyl peptide **S2** (11.4 mg, 2.5 μmol) were dissolved in 6 M guanidine (Gn)·HCl–0.2 M Na phosphate buffer containing 30 mM 4-mercaptophenyl acetic acid (MPAA) and 30 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl) (pH 7.0, 2.5 mL, 1 mM each peptide). The reaction mixture was incubated at 37 °C for 6 h and reaction progress was monitored by analytical HPLC. After completion of the reaction, the crude material was purified by preparative HPLC to give **S4b** (14.5 mg, 2.3 μmol, 90% isolated yield).

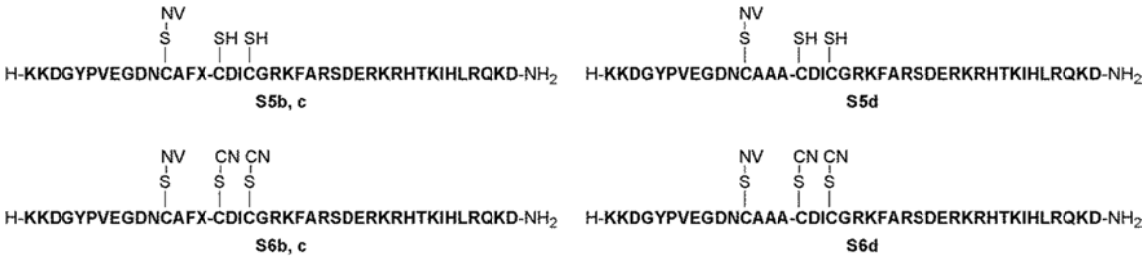
S4b (**X = Phe**): Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 17.4 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 20% to 35% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 974.1, found 973.8.

S4c (**X = Ser**) (14.8 mg, 2.2 μmol, 90%): Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 14.5 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 13% to 28% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 962.1, found 961.8.

S4d (13.4 mg, 2.1 μmol, 85%): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 17.1 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 30%

over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 943.7, found 943.4.

○ Regioselective S-cyanylation of Peptide **S4b**, **c**, **d**



Representative procedure: Peptide **S4b** (13.9 mg , 2.2 μmol) was dissolved in 10 mM Na phosphate buffer (9.3 mL, pH = 7.5). After addition of $ZnSO_4$ solution (3.2 μmol, 0.64 mL of a 5.0 mM solution in deionized water), the reaction mixture was stirred at room temperature for 10 min under argon atmosphere. Following addition of 6-nitroveratryl bromide (3.6 μmol, 0.77 mL of a 4.2 mM solution in CH_3CN), the mixture was stirred under light-blocking conditions at room temperature for 9 h. To the reaction mixture were successively added solution of CDAP (43 μmol, 1.0 mL of a 10 mg/mL solution in 0.1 M AcOH) and 0.1% (v/v) TFA aq. (2.0 mL) to carry out S-cyanylation. After being stirred under light-blocking conditions at room temperature for 1.5 h, the reaction was purified by semi-preparative HPLC to give **6b** (9.1 mg, 1.4 μmol, 63%)

S5b (**X = Phe**): Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 20.0 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 1013.1, found 1012.7.

S6b (**X = Phe**) (9.12 mg, 1.36 μmol, 63%): Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 20.1 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 20% to 35% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 1023.1, found 1022.7.

S5c (**X = Ser**): Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 16.7 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 1001.1, found 1000.7.

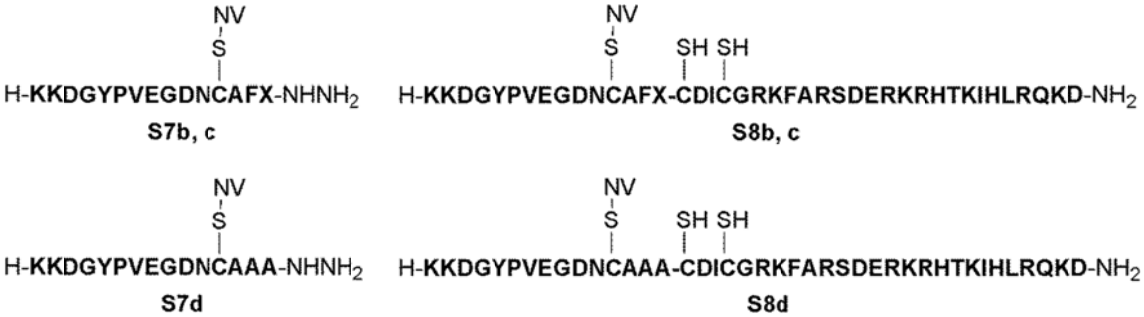
S6c (**X = Ser**) (5.24 mg, 0.79 μmol, 35%): Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 16.9 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 18% to 33% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 1011.1, found 1010.9.

S5d: Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 16.4 min. MS (ESI-TOF) *m*/*z* calcd for ([*M*+5H]$^{5+}$) 982.7, found 982.7.

S6d (5.04 mg, 0.77 μmol, 38%): Analytical HPLC conditions: TSKgel Octadecyl-2PW analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 16.4 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 18% to 33% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 992.7, found 992.4.

○ Hydrazinolysis of peptide **S6b**, **c** and **d**

Representative procedure: Peptide **S6b** (7.1 mg, 1.1 μmol) was dissolved in 33 mM Na phosphate buffer containing 1 M NH_2NH_2–1 M Gn·HCl (0.76 mL, pH = 10.3) at 0 °C. Then the mixture was incubated at room temperature under light-blocking conditions for 8 h, and reaction progress was monitored by analytical HPLC. After disappearance of the **S6b**, the crude material was purified by semi-preparative HPLC to give **S7b** (0.90 mg, 0.38 μmol, 36%) and **S8b** (0.96 mg, 0.14 μmol, 14%).



S7b (**X = Phe**) (0.90 mg, 0.38 μmol, 36%): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 15% to 45% over 30 min, retention time = 19.7 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 20% to 35% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+2H]$^{2+}$) 949.9, found 949.6.

S8b (**X = Phe**) (0.96 mg, 0.14 μmol, 14%): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 15% to 45% over 30 min, retention time = 18.6 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 20% to 35% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 1013.1, found 1012.8.

S7c (**X = Ser**) (1.16 mg, 0.51 μmol, 65%): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II

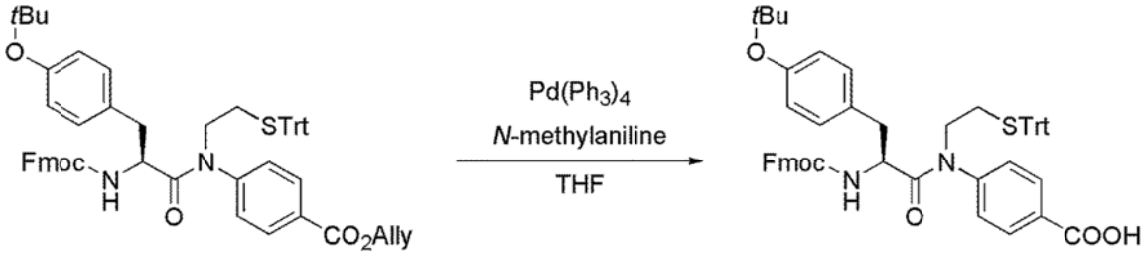
analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 19.3 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 18% to 33% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+2H]$^{2+}$) 919.9, found 919.7.

S8c (X = Ser) (0.47 mg, 0.07 μmol, 9%): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 19.3 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 18% to 33% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$)1001.1, found 1000.7.

S7d (0.52 mg, 0.24 μmol, 38%): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column n with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 17.0 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 30% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+2H]$^{2+}$) 873.9, found 873.5.

S8d (0.46 mg, 0.07 μmol, 11%): Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column with a linear gradient of solvent B in solvent A, 2% to 50% over 30 min, retention time = 18.8 min. Preparative HPLC conditions: A linear gradient of solvent B in solvent A, 15% to 30% over 30 min. MS (ESI-TOF) *m*/*z* calcd ([*M*+5H]$^{5+}$) 982.7, found 982.1.

○ Preparation of *4-[(Fmoc-L-Tyr-(t-Bu)-2-tritylsulfanylethyl)amino]benzoic acid* **S9**



S# **S9**

Allyl 4-[(Fmoc-L-Tyr-(*t*-Bu)-2-tritylsulfanylethyl)amino]benzoate **S#**[2] (1.42 g, 1.54 mmol) in THF (34 mL) was treated with *N*-methylaniline (1.67 mL, 15.4 mmol) and $Pd(PPh_3)_4$ (178 mg, 0.154 mmol). After being stirred at room temperature for 16 h , the solvent was removed in vacuo and the product was purified by silica gel chromatography ($CHCl_3$/MeOH = 10:1) to give the desired compound **S9** (1.15 g, 1.31 mmol, 85%) as a pale yellow amorphousness: $[\alpha]^{31}_D$ 27.5 (1.00, $CHCl_3$); IR ($CHCl_3$, KBr): 1163, 1240, 1446, 1505, 1600, 1670, 1707, 1724, 2928, 2976, 3020, 3058, 3411 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 1.31 (9H, s), 2.29-2.37 (2H, m), 2.71 (1H, dd, *J* = 13.0 and 5.9 Hz), 2.85 (1H, dd, *J* = 13.0 and 9.7 Hz), 3.29-3.37 (2H, m), 4.17 (1H, t, *J* = 6.9 Hz), 4.27-4.37 (3H, m), 5.55 (1H, d, *J* = 9.0 Hz), 6.79 (2H, d, *J* = 8.7 Hz), 6.83 (2H, d, *J* = 8.7 Hz), 7.11-7.32 (19H,

m), 7.39 (2H, t, *J* = 7.4 Hz), 7.57 (2H, d, *J* = 6.8 and 2.4 Hz), 7.76 (2H, d, *J* = 7.6 Hz), 7.91 (2H, d, *J* = 8.1 Hz); ^{13}C-NMR ($CDCl_3$, 75 Hz) δ = 28.9, 29.0, 39.4, 47.2, 49.2, 53.1, 67.2, 67.2, 78.6, 120.1, 124.3, 125.3, 125.3, 126.8, 127.2, 127.9, 128.0, 128.1, 128.3, 128.3, 129.4, 129.5, 129.6, 130.3, 130.7, 131.5, 141.4, 143.9, 143.9, 144.6, 144.9, 154.6, 155.6, 169.0, 171.5; HRMS (ESI-TOF) m/z calcd for $C_{56}H_{52}N_2NaO_6S$ $[M + Na]^+$ 903.3444, found 903.3439.

Reference

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