Supporting Information

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○ Preparation of peptide thioester \textbf{S1} for preparation of \textit{Stichodactyla} toxin/zinc-finger fusion

\textbf{H-RSCDTPKPSRCTAFQ-S(CH}_2\text{)CO-L-NH}_2

\textbf{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\textsubscript{2}O (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\textsubscript{2}O (5.0 equiv) and DIPEA (2.0 equiv) in DMF at room temperature for 2 h followed by Trt removal by TFA/Et\textsubscript{3}SiH (95:5, 10 min) gave HSCH\textsubscript{2}CH\textsubscript{2}CO-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\textsubscript{2}CH\textsubscript{2}CO-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 \textit{in situ} neutralization Boc SPPS (Acylation: Boc amino acid (5.0 equiv), DIPCDI (5.0 equiv), HOBt·H\textsubscript{2}O (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 \textbf{S1}.\textsuperscript{1} The resulting completed resin was treated with 1 M TMSOTf–thioanisole in TFA, m-cresol (100/5 (v/v) and 1,2-ethanediethiol (100/5 (v/v) at 4 °C for 2 h. After filtration of the resin, cooled Et\textsubscript{2}O was added to the filtrate to give precipitate. The formed precipitate was collected by centrifugation and thoroughly washed with Et\textsubscript{2}O to afford crude peptide thioester \textbf{S1}. The crude peptide was purified by preparative HPLC to give the purified peptide \textbf{S1} in 7% isolated yield.

\textbf{S1}: Analytical HPLC conditions: Cosmosil 5C\textsubscript{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) \textit{m/z} calcd ([M+2H]\textsuperscript{2+}) 1013.5, found 1013.1.

○ Preparation of N-terminal cysteinyl peptide \textbf{S2} for preparation of \textit{Stichodactyla} toxin/zinc-finger fusion and TsTxV/zinc-finger fusion

\textbf{H-CDICGRKFARSDERKRHTKIHLRQKD-NH}_2

\textbf{S2}

Protected peptide resins corresponding to the title peptides were constructed on NovaSyn\textsuperscript{®} 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–H₂O–1,2-ethanedianthiol (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 5C₁₈-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]⁺) 1065.9, found 1065.6.

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

H-KKDGYPVEGDNCAF-X-(CH₂)₂CO-L-NH₂
S₃a, b, c

H-KKDGYPVEGDNCA-X-(CH₂)₂CO-L-NH₂
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 HSCH₂CH₂CO-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 5C₁₈-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 5C₁₈-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 5C₁₈-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 5C18-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-KKDGPVEGDNCAF-XDICGRKFARSDERKHTKIH-LRQKDK-NH2
S4b, c

H-KKDGPVEGDNCAAA-DICGRKFARSDERKHTKIH-LRQKDK-NH2
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 5C18-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 ZnSO₄ 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 CH₃CN), 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-cyanlation. 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_{2}NH_{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 calc (\([M+2H]^{2+}\)) 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 calc (\([M+2H]^{2+}\)) 919.9, found 919.7.

**S7d (0.52 mg, 0.24 μmol, 38%):** 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, 15% to 30% over 30 min. MS (ESI-TOF) m/z calc (\([M+2H]^{2+}\)) 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 calc (\([M+2H]^{2+}\)) 982.7, found 982.1.

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

![Reaction Diagram]

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(Ph3)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 (CHCl3/MeOH = 10:1) to give the desired compound S9 (1.15 g, 1.31 mmol, 85%) as a pale yellow amorphousness: \([\alpha]^{21}_D\) 27.5 (1.00, CHCl3); IR (CHCl3, KBr): 1163, 1240, 1446, 1505, 1600, 1670, 1707, 1724, 2928, 2976, 3020, 3058, 3411 cm\(^{-1}\); 1H-NMR (CDCl3, 400 MHz) \(\delta = 1.31\) (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) $\delta$ = 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, 129.3, 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$_2$NaO$_6$S [M + Na]$^+$ 903.3444, found 903.3439.

Reference