*Supporting Information*

# Regioselective formation of multiple disulfide bonds with the aid of post-synthetic *S*-tritylation

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**1. General Information……..**

**a. Materials**

All reagents and solvents were obtained from Peptide Institute, Inc. (Osaka, Japan), Wako Chemical (Osaka, Japan), Tokyo Chemical Industry (Tokyo, Japan) and Watanabe Chemical Industries (Hiroshima, Japan). Human hepcidin (PI 4392s) was obtained from Peptide Institute, Inc.

**b. HPLC**

Analytical HPLC was performed on a Shimadzu liquid chromatograph Model LC-10AT (Kyoto, Japan) with a YMC-ODS AA12S05-1546WT (4.6 x 150 mm) using a flow rate of 1 mL/min at 40 ºC and the following solvent systems: 0.1% TFA in $H_2O$ (A), 0.1% TFA in $CH_3CN$ (B). Preparative HPLC was performed on a Shimadzu liquid chromatograph Model LC-8A (Kyoto, Japan) with a YMC-ODS AA12305-2530WT (250 x 30 mm) using a flow rate of 20 mL/min and the following solvent systems: 0.1% TFA in $H_2O$ (A), 0.1% TFA in $CH_3CN$ (B).

**c. Mass spectrometry**

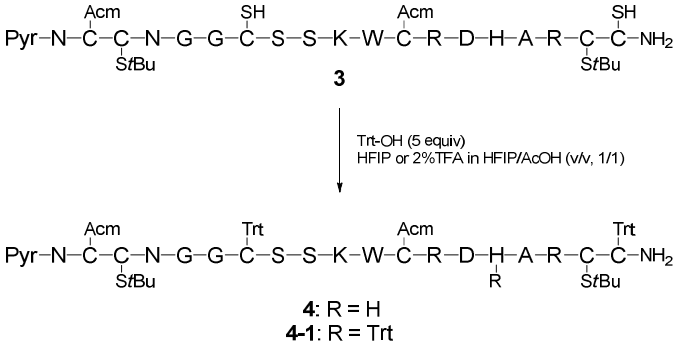
Exact mass spectra were measured on an Agilent G1956B LC/MSD detector using an Agilent 1100 series HPLC system.

**d. Automated solid-phase synthesis**

Automated peptide synthesis was performed on an ABI433A (Foster City, CA, USA) peptide synthesizer. The peptide chain assembly was carried out using the protocol of 30-min coupling with Fmoc-amino acid/DIC/Oxyma (4/4/4 equiv with respect to the peptide resin) in DMF. The acetyl capping was performed using acetic anhydride/NMP in the presence of HOBt/DIEA after each coupling step. The following side-chain-protected amino acids were employed: Asn(Trt), Asp(*t*Bu), Arg(Pbf), Cys(Acm), Cys(MeBzl), Cys(S*t*Bu), Cys(Trt), His(Trt), Lys(Boc), Ser(*t*Bu), Thr(*t*Bu), Trp(Boc), Tyr(*t*Bu). Fmoc deprotection was carried out with 20% morpholine/NMP (5 min x 4) for μ-SIIIA or 20% piperidine/NMP (2.5 min x 4) for human hepcidin.

## 2. Experimental Section……

### a. Post-synthetic tritylation of the His-containing peptide




To a solution of **3** in HFIP/AcOH/TFA (v/v/v, 25/25/2) or HFIP was added Trt-OH (5 equiv). The reaction mixture was stirred at room temperature for 1 h and analyzed by HPLC.

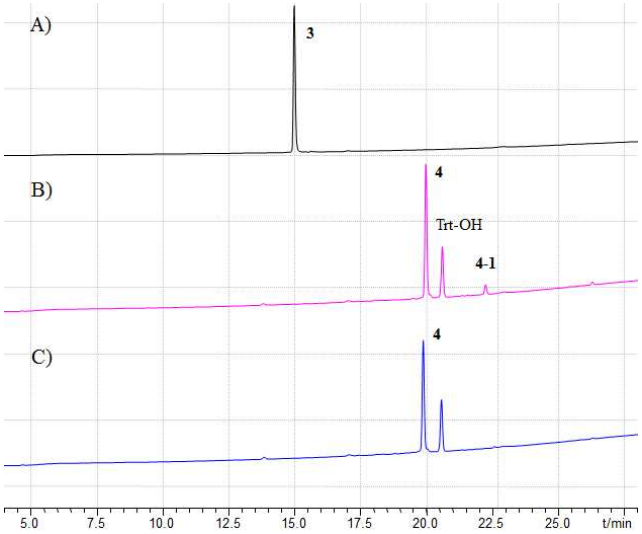
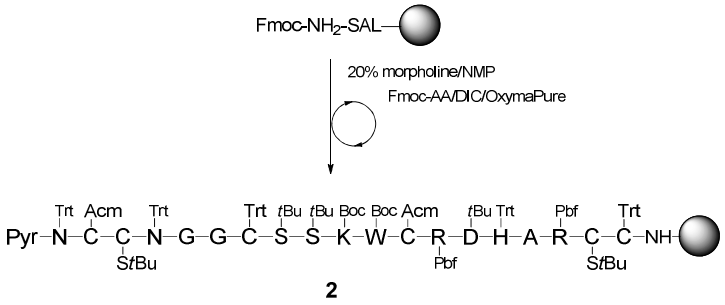



Figure S1. (A) **3**. (B) tritylation of **3** in HFIP. (C) tritylation of **3** in HFIP/AcOH/TFA (v/v/v, 25/25/2). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-80% $CH_3CN$ in 0.1% TFA (25 min) at 40 °C; flow rate, 1.0 mL/min; detection, 220 nm.

**b. Synthesis of μ-conotoxin SIIIA**

**b-1. Regioselective strategy**

*Protected μ-conotoxin SIIIA (**2**)*

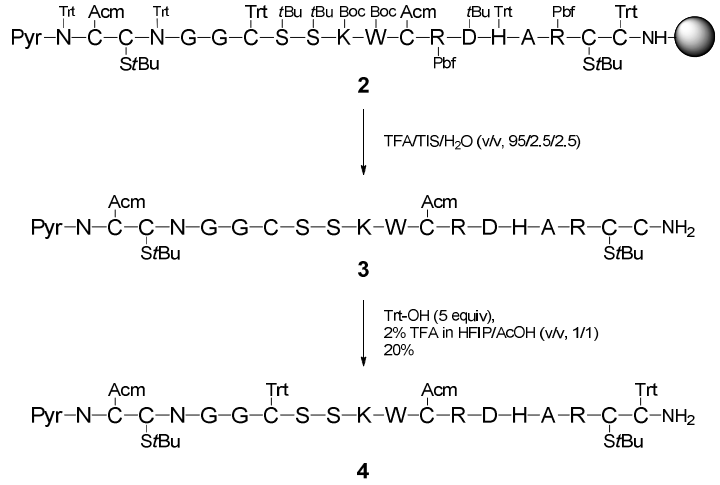



**2**

The peptide was assembled using an ABI 433A peptide synthesizer on an Fmoc-NH-SAL resin (555 mg, 0.25 mmol) according to the general automated SPPS procedure.

*[Cys(Acm)$^{3,13}$, Cys(Trt)$^{8,20}$, Cys(StBu)$^{4,19}$]-μ-SIIIA (**4**)*

*(Deprotection and reintroduction of the Trt groups: route A)*

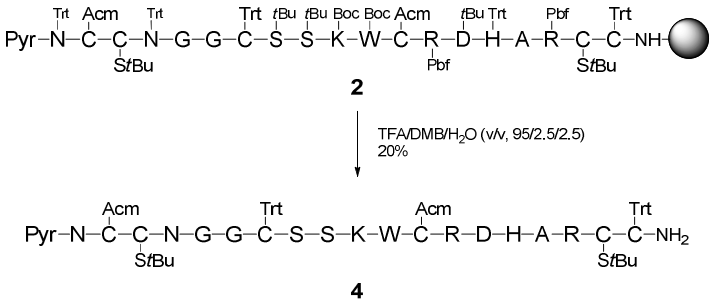



The peptide resin **2** (300 mg, 45.3 μmol) was treated by TFA/TIS/$H_2O$ (10 ml, v/v/v: 95/2.5/2.5) at room temperature. After 1h, the resin was filtered and washed with TFA. The combined filtrates were concentrated under the reduced pressure. The residue was precipitated with $Et_2O$. The resulting powders were used for the next step without further purification. To a solution of the product in HFIP/AcOH/TFA (4 ml, v/v/v: 25/25/2) was added Trt-OH (58.9 mg, 226.5 μmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was directly subjected to preparative HPLC to

obtain the title compound (27.8 mg, 19%). Analytical HPLC: Rt, 19.4 min (10-80% $CH_3CN$/0.1% TFA for 25 min, 220 nm); LRMS (ESI) calcd 3016.6, found 3016.2.

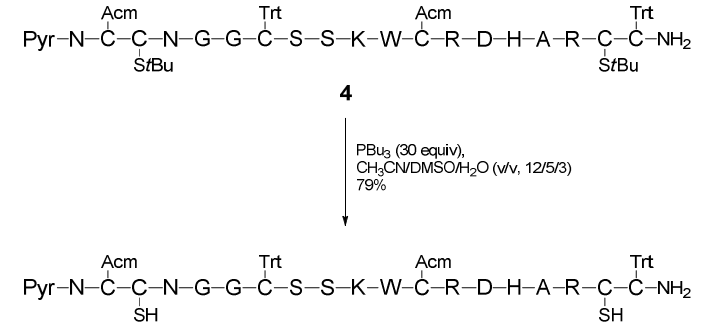
*[Cys(Acm)$^{3,13}$, Cys(Trt)$^{8,20}$, Cys(StBu)$^{4,19}$]-μ-SIIIA (**4**)*

*(Deprotection and retrapping of the Trt groups: route B)*




The peptide resin **2** (300 mg, 45.3 μmol) was treated by TFA/DMB/$H_2O$ (10 ml, v/v/v: 95/2.5/2.5) at room temperature. After 1h stirring, the resin was filtered and washed with TFA. The combined filtrates were concentrated under the reduced pressure. The residue was precipitated with $Et_2O$. The resulting powders was subjected to preparative HPLC to obtain the title compound (28.6 mg, 20%). Analytical HPLC: Rt, 19.4 min (10-80% $CH_3CN$/0.1% TFA for 25 min, 220 nm); LRMS (ESI) calcd 3016.6, found 3016.2.

*[Cys(Acm)$^{3,13}$, Cys(Trt)$^{8,20}$, Cys$^{4,19}$]-μ-SIIIA*




To a solution of **4** (44.8 mg, 13.5 μmol) in $CH_3CN$/DMSO/$H_2O$ (4 ml, v/v/v: 12/5/3) was added $PBu_3$ (100 μl, 405 μmol). The mixture was stirred at room temperature for 2 h and then directly subjected to preparative HPLC to obtain the title compound (33.5 mg, 79%). Analytical HPLC: Rt, 17.2 min (10-80% $CH_3CN$/0.1% TFA for 25 min, 220 nm); LRMS (ESI) calcd 2840.3, found 2840.2.

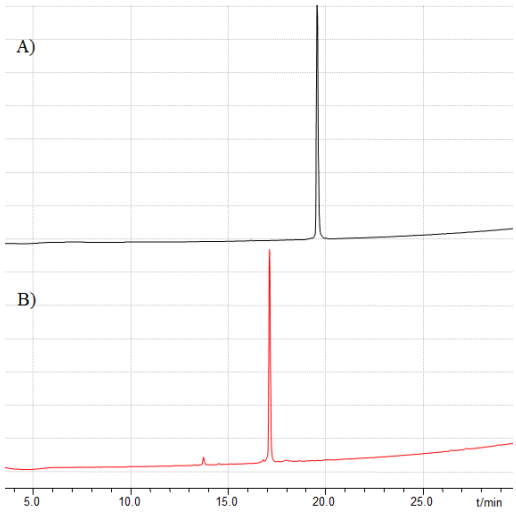
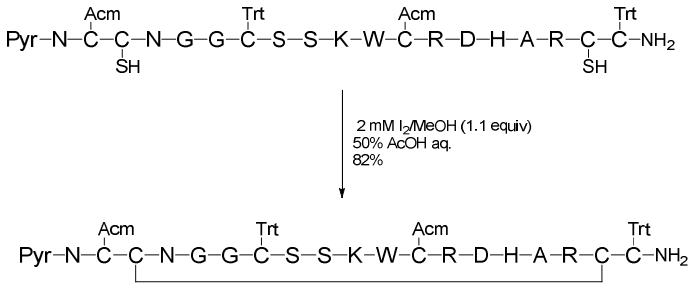



Figure S2. Deproteciton of Cys(S*t*Bu)[4,19]. (A) [Cys(Acm)[3,13], Cys(S*t*Bu)[4,19], Cys(Trt)[8,20]]-μ-SIIIA (**4**). (B) [Cys(Acm)[3,13], Cys[4,19], Cys(Trt)[8,20]]-μ-SIIIA (the reaction mixture, 2 h). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-80% $CH_3CN$ in 0.1% TFA (25 min) at 40 °C; flow rate, 1.0 mL/min; detection, 220 nm.

*[Cys(Acm)[3,13], Cys[4]-Cys[19], Cys(Trt)[8,20]]-μ-SIIIA (**5**)*




**5**

To a solution of [Cys(Acm)[3,13], Cys[4,19], Cys(Trt)[8,20]]-μ-SIIIA (39.0 mg, 12.5 μmol) in 50% AcOH aq. was added 2 mM $I_2$/MeOH (6.9 ml) dropwise. After 5 min, the reaction was quenched by adding 1 M ascorbic acid (125 μl). The mixture was directly subjected to preparative HPLC to obtain the title compound (32.0 mg, 82%). Analytical HPLC: Rt, 14.3 min (30-60% $CH_3CN$/0.1% TFA for 25 min, 220 nm); LRMS (ESI) calcd 2838.3, found 2838.0.

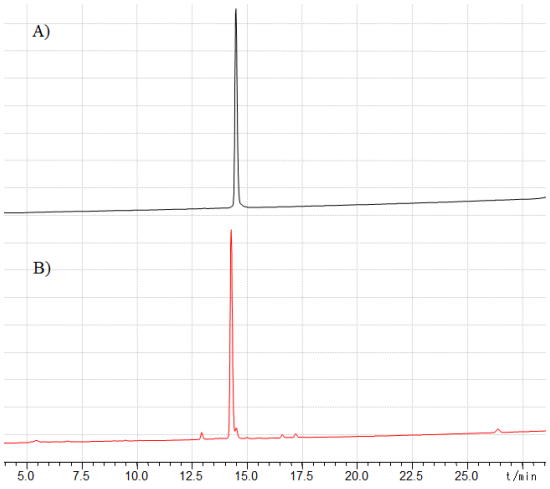
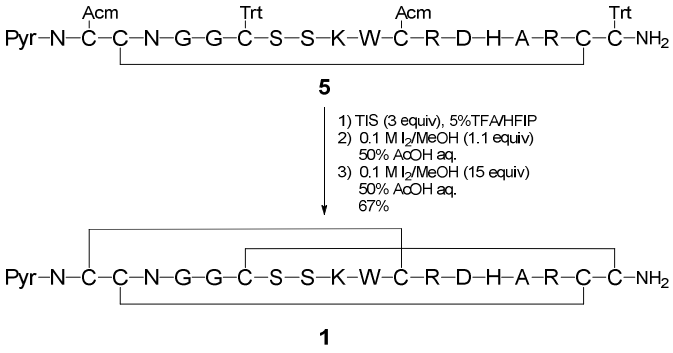



Figure S3. Disulfide formation of $Cys^{4,19}$. (A) [$Cys(Acm)^{3,13}$, $Cys^{4,19}$, $Cys(Trt)^{8,20}$]-μ-SIIIA (B) [$Cys(Acm)^{3,13}$, $Cys^{4}$-$Cys^{19}$, $Cys(Trt)^{8,20}$]-μ-SIIIA (**5**). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 30-60% $CH_3CN$ in 0.1% TFA (25 min) at 40 °C; flow rate, 1.0 mL/min; detection, 220 nm.

*μ-Conotoxin SIIIA (**1**)*




To a solution of **5** (26.5 mg, 8.47 μmol) in 5% TFA/HFIP (3 ml) was added TIS (5.2 μl, 25.4 μmol). The mixture was stirred at room temperature for 10 min and then diluted with 50% AcOH/$H_2O$ (14 ml). After 0.1 M $I_2$/MeOH (86 μl) was added dropwise, the reaction mixture was stirred for 5 min. And then 0.1 M $I_2$/MeOH (1.29 ml) was added and stirred for 1 h. The reaction was quenched by adding 1 M ascorbic acid (260 μl). The reaction mixture was washed with hexane and the aqueous layers were directly subjected to preparative HPLC to obtain the title compound (14.1 mg, 67%). Analytical HPLC: $R_t$, 9.5 min (1-60% $CH_3CN$/0.1% TFA for 20 min, 220 nm); LRMS (ESI) calcd 2207.5, found 2206.9.

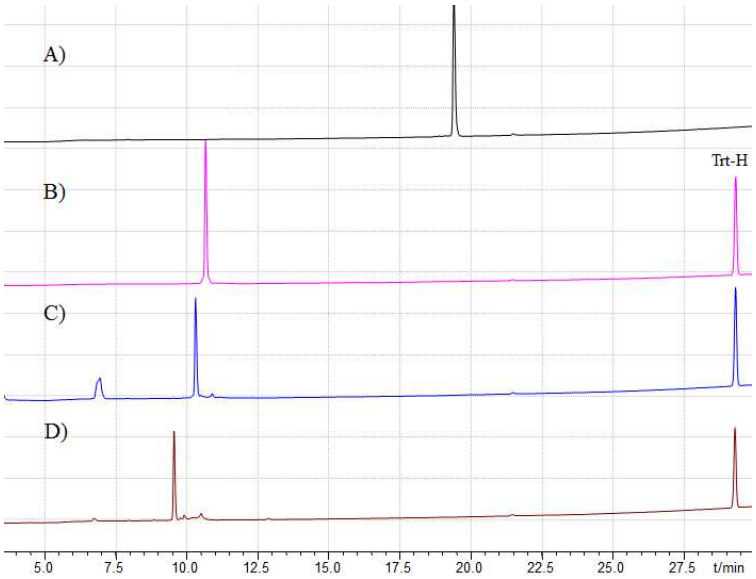



Figure S4. Disulfide formation of $Cys^{8}$-$Cys^{20}$ and $Cys^{3}$-$Cys^{13}$. (A) [$Cys(Acm)^{3,13}$, $Cys^{4}$-$Cys^{19}$, $Cys(Trt)^{8,20}$]-μ-SIIIA (**5**). (B) Deprotection of the Trt groups on $Cys^{8,20}$: [$Cys(Acm)^{3,13}$, $Cys^{4}$-$Cys^{19}$, $Cys^{8,20}$]-μ-SIIIA. (C) $I_2$ oxidation of $Cys^{8,20}$: [$Cys(Acm)^{3,13}$, $Cys^{4}$-$Cys^{19}$, $Cys^{8}$-$Cys^{20}$]-μ-SIIIA. (D) Oxidative deprotection of $Cys(Acm)^{3,13}$: μ-SIIIA (**1**). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 1-60-98% $CH_3CN$ in 0.1% TFA (20-10 min) at 40 °C; flow rate, 1.0 mL/min; detection, 220 nm.

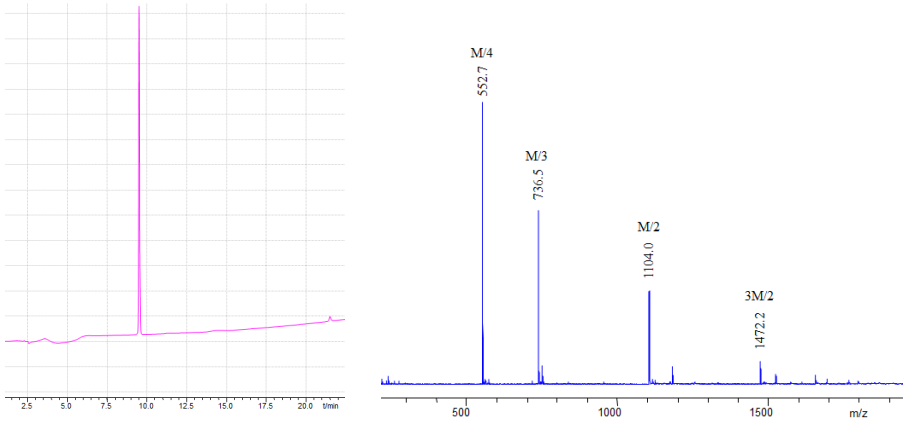



Figure S5. HPLC and ESI-MS profiles of μ-conotoxin SIIIA (**1**). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 1-60% $CH_3CN$ in 0.1% TFA (20 min) at 40 °C; flow rate, 1.0 mL/min; detection, 220 nm.

**b-2. Oxidative folding strategy**

*Reduced μ-conotoxin SIIIA*

The linear μ-SIIIA was synthesized with an ABI 430A peptide synthesizer using Boc strategy on a MBHA resin (0.64 mmol/g, 0.50 mmol). The functional of the side chains were protected as follows: Bzl for Ser, cHx for Asp, ClZ for Lys, Bom for His, Tos for Arg, For for Trp, and Meb for Cys. Z-Pyr was used for *N*-terminal incorporation. The peptide chain was elongated using standard protocols of coupling with performed HOBt ester in NMP (single couplings, acetylation after each coupling step). A four-fold excess of Boc amino acids was used based on the original substitution of MBHA. The peptide resin (2.5 g, 0.30 mmol) was treated with HF/*p*-cresol/butanedithiol (v/v 80/5/15, 50 mL) in the presence of methoxyamine·HCl (0.21 g, 5 equiv) at -2 °C to -5 °C for 1 h to give a crude product, which was purified by RP-HPLC (YMC-Pak ODS column 30 x 250 mm; flow rate 20 mL; detection at 220 nm) using 0.1% TFA as buffer A and 0.1% TFA/$CH_3CN$ as buffer B to obtain 0.30 g (22%). LRMS (ESI) calcd 2213.5, found 2213.1.

*μ-Conotoxin SIIIA (1)*

The reduced μ-SIIIA (0.51 g, 0.23 mmol) was dissolved in 0.1% TFA (40 mL). This solution was added to 5.7 L of 2 M $NH_4OAc$ buffer (pH 7.8) containing GSH (7.1 g) and GSSG (1.4 g). The ratio of peptide and redox reagent was 1/100/10, and the peptide concentration was 4 x $10^{-5}$ M. After adjusting the pH to 7.8 with 1.0 M aqueous $NH_3$, the reaction mixture was gently stirred for 2 h and was allowed to stand for 48 h at 25 ºC. After the mixture was acidified to pH 2 by adding TFA, the folded peptide was desalted by RP-HPLC (YMC-Pak ODS column 30 x 250 mm; flow rate 20 mL/min; detection at 220 nm) using 0.1% TFA/$H_2O$ as buffer A and 0.1% TFA/$CH_3CN$ as buffer B. Further purification was carried out by RP-HPLC (Capcell-Pak ODS column 30 x 250 mm; flow rate 20 mL/min; detection at 220 nm) using 10% $CH_3CN$/0.1 M phosphate buffer (pH 7.0) as buffer A and 60% $CH_3CN$/0.1 M phosphate buffer (pH 7.0) as buffer B. The purified peptide was desalted by YMC-Pak ODS column using 0.1% TFA system as described above to yield 0.14 g (27%). LRMS (ESI) calcd 2207.5, found 2207.3.

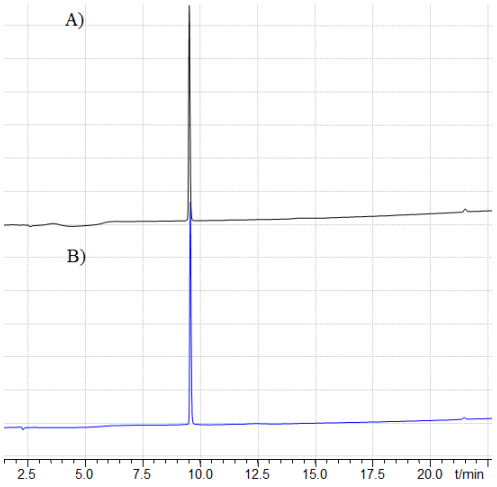
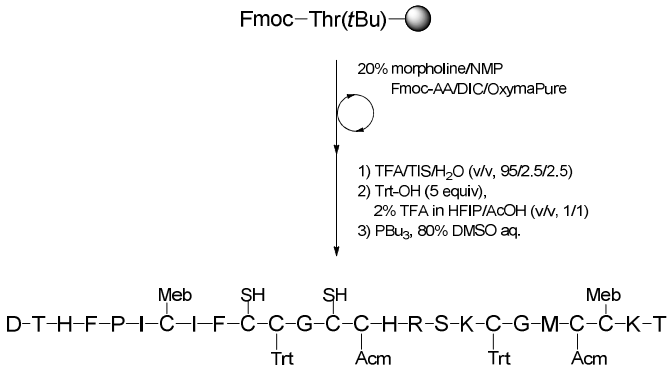



Figure S6. RP-HPLC profiles of the synthetic μ-SIIIA. (A) Regioselectively synthesized μ-SIIIA (**1**). (B) Product obtained by the freely oxidative folding procedure. HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 1-60% $CH_3CN$ in 0.1% TFA (20 min) at 40 °C; flow rate, 1.0 mL/min; detection, 220 nm.

**c**. **Synthesis of human hepcidin**

*[Cys(Meb)$^{7,23}$, Cys$^{10,13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin*

*(Deprotection and reintroduction of the Trt groups: route A)*




The peptide was assembled using an ABI 433A peptide synthesizer on a Fmoc-Thr(*t*Bu) resin (400 mg, 0.22 mmol) according to the general automated SPPS procedure. The peptide resin **7** (800mg, 0.15 mmol) was treated by TFA/TIS/$H_2O$ (10 ml, v/v/v: 95/2.5/2.5) at room temperature. After 1.5 h stirring, the resin was filtered and washed with TFA. The combined filtrates were concentrated under the reduced pressure. The residue was precipitated with $Et_2O$. To a solution of the crude product in

HFIP/AcOH/TFA (4 ml, v/v/v: 25/25/2) was added Trt-OH (195 mg, 0.75 mmol). The reaction mixture was stirred at room temperature for 2 h. The product was precipitated with IPE, and collected by filtration and washed with IPE. The resulting powders were used for the next step without further purification. To a solution of the product in DMSO/$H_2O$ (4 ml, v/v: 4/1) was added $PBu_3$ (500 µl, 2.0 mmol). The reaction mixture was stirred at room temperature for 4 h and then directly subjected to preparative HPLC to obtain the title compound (130 mg, 20%). Analytical HPLC: Rt, 17.4 min (10-95% $CH_3CN$/0.1% TFA for 25 min at 50 °C, 220 nm); LRMS (ESI) calcd 3632.5, found 3631.7.

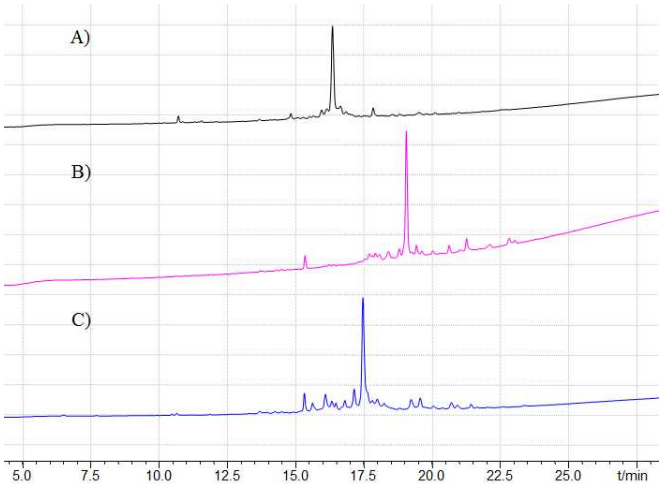
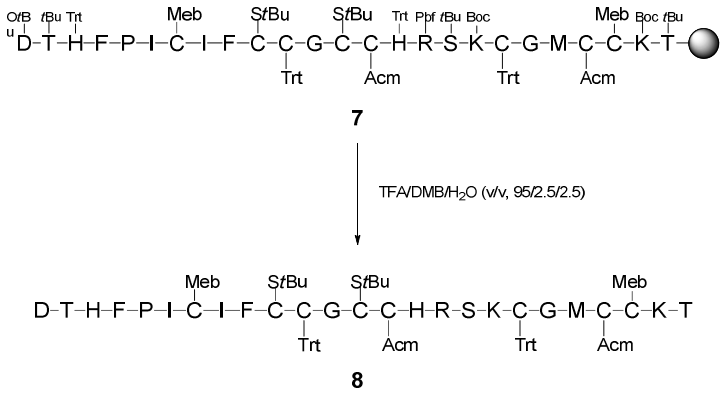



Figure S7. HPLC profiles of the synthetic intermediates of human hepcidin. (A) [Cys(Meb)$^{7,23}$, Cys(S*t*Bu)$^{10,13}$, Cys$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin. (B) [Cys(Meb)$^{7,23}$, Cys(S*t*Bu)$^{10,13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin (**8**). (C) Deprotection of Cys(S$^t$Bu)$^{10,13}$: [Cys(Meb)$^{7,23}$, Cys$^{10,13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin. HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-95% $CH_3CN$ in 0.1% TFA (25 min) at 50 °C; flow rate, 1.0 mL/min; detection, 220 nm.

*[Cys(Meb)$^{7,23}$, Cys(StBu)$^{10,13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin*

*(Deprotection and retrapping of the Trt groups: route B)*




The peptide resin **7** (200 mg, 37.5 μmol) was treated by TFA/DMB/$H_2O$ (10 ml, v/v/v: 95/5/5) at room temperature. After 1.5 h stirring, the resin was filtered and washed with TFA. The combined filtrates were concentrated under the reduced pressure. The residue was precipitated with $Et_2O$. The resulting residue was analyzed by HPLC as shown in Figure S8.

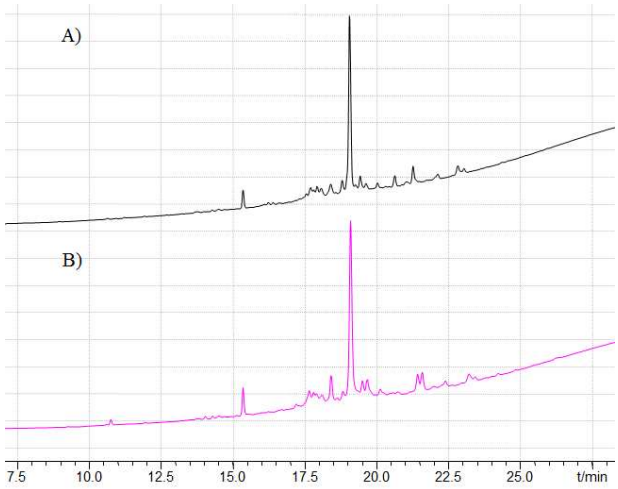
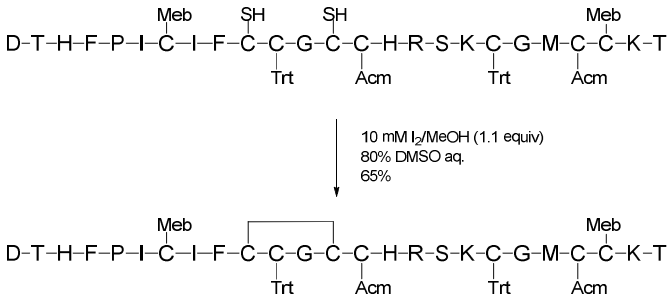



Figure S8. HPLC profiles of the synthetic intermediates of human hepcidin. (A) [Cys(Meb)$^{7,23}$, Cys(S*t*Bu)$^{10,13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin (**8**) obtained by route A. (B) **8** obtained by route B. HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-95% $CH_3CN$ in 0.1% TFA (25 min) at 50 °C; flow rate, 1.0 mL/min; detection, 220 nm.

*[Cys(Meb)$^{7,23}$, Cys$^{10}$-Cys$^{13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin (**9**)*




**9**

To a solution of [Cys(Meb)$^{7,23}$, Cys$^{10,13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin (130 mg, 30.8 μmol) in DMSO/$H_2O$ (30 ml, v/v: 4/1) was added 10 mM $I_2$/MeOH (3.1 ml) was added dropwise. After 5 min, the reaction was quenched by adding 1 M ascorbic acid (308 μl). The reaction mixture was directly subjected to preparative HPLC to obtain the title compound (84.4 mg, 65%). Analytical HPLC: Rt, 17.2 min (10-95% $CH_3CN$/0.1% TFA for 25 min at 50 °C, 220 nm); LRMS (ESI) calcd 3630.5, found 3630.0.

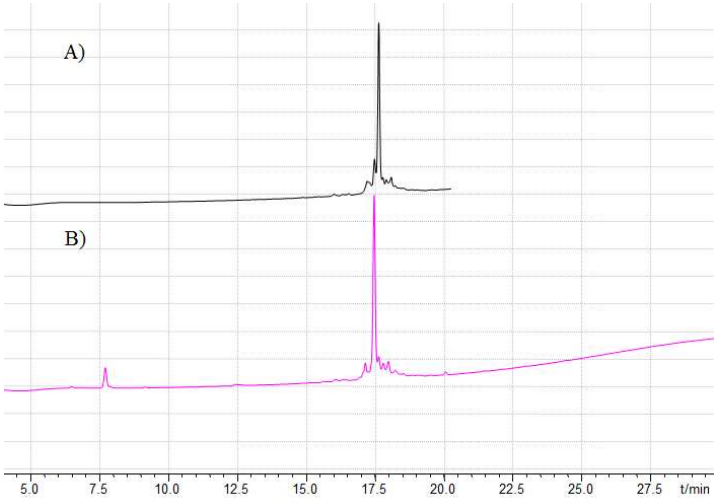
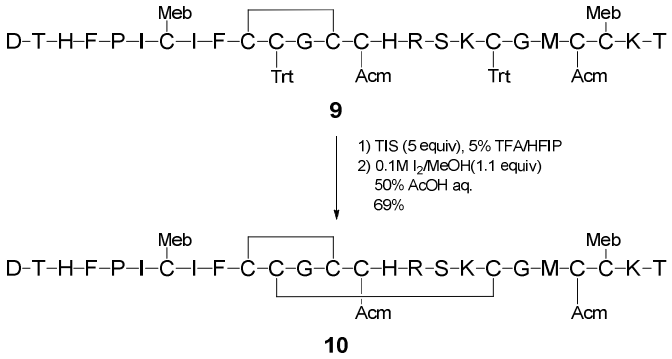



Figure S9. Disulfide formation of Cys$^{10}$-Cys$^{13}$. (A) [Cys(Meb)$^{7,23}$, Cys$^{10,13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin. (B) $I_2$ oxidation of Cys$^{10,13}$: [Cys(Meb)$^{7,23}$, Cys$^{10}$-Cys$^{13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin (**9**). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-95% $CH_3CN$ in 0.1% TFA (25 min) at 50 °C; flow rate, 1.0 mL/min; detection, 220 nm.

*[Cys(Meb)$^{7,23}$, Cys$^{10}$-Cys$^{13}$, Cys$^{11}$-Cys$^{19}$, Cys(Acm)$^{14,22}$]-human hepcidin (**10**)*




To a solution of **9** (84.0 mg, 20.0 µmol) in 5% TFA/HFIP (4 ml) was added TIS (20 µl, 100 µmol). The mixture was stirred at room temperature for 10 min and then diluted with 50% AcOH/$H_2O$ (16 ml). After 0.1 M $I_2$/MeOH (210 µl) was added dropwise, the reaction mixture was stirred for 10 min. The reaction was quenched by adding 1 M ascorbic acid (210 µl). The reaction mixture was directly subjected to preparative HPLC to obtain the title compound (51.2 mg, 69%). Analytical HPLC: Rt, 13.6 min (10-95% $CH_3CN$/0.1% TFA for 25 min at 50 °C, 220 nm); LRMS (ESI) calcd 3143.8, found 3143.2.

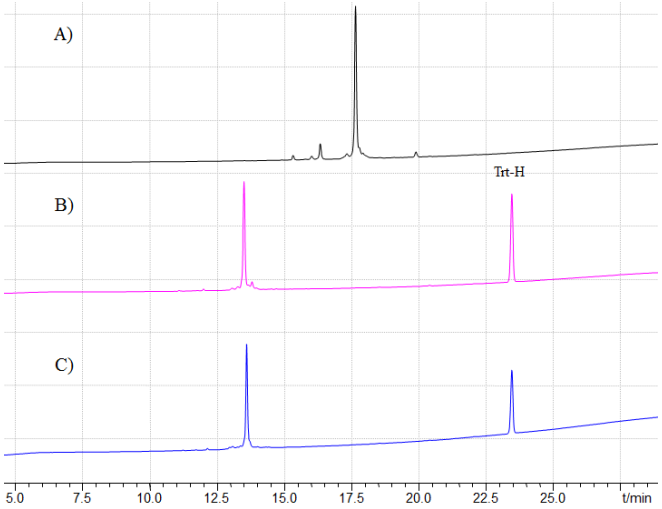
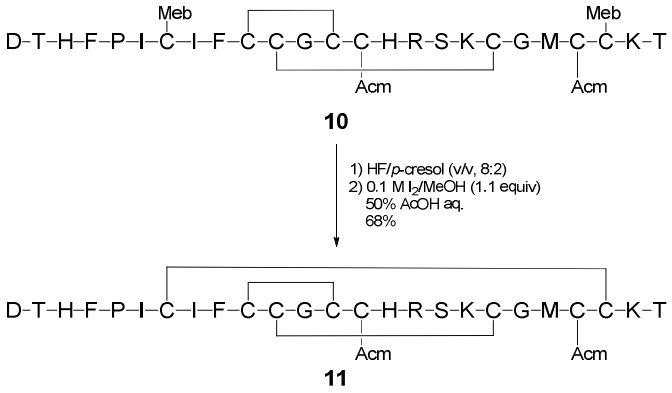



Figure S10. Deprotection and oxidation of Cys(Trt)$^{11,19}$. (A) [Cys(Meb)$^{7,23}$, Cys$^{10}$-Cys$^{13}$, Cys(Trt)$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin (**9**). (B) Deprotection of Cys(Trt)$^{11,19}$: [Cys(Meb)$^{7,23}$, Cys$^{10}$-Cys$^{13}$, Cys$^{11,19}$, Cys(Acm)$^{14,22}$]-human hepcidin. (C) $I_2$ oxidation of Cys$^{11,19}$: [Cys(Meb)$^{7,23}$, Cys$^{10}$-Cys$^{13}$, Cys$^{11}$-Cys$^{19}$, Cys(Acm)$^{14,22}$]-human hepcidin

(**10**). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-95% $CH_3CN$ in 0.1% TFA (25 min) at 50 °C; flow rate, 1.0 mL/min; detection, 220 nm.

*[Cys$^{7}$-Cys$^{23}$, Cys$^{10}$-Cys$^{13}$, Cys$^{11}$-Cys$^{19}$, Cys(Acm)$^{14,22}$]-human hepcidin (**11**)*




**10** (10.0 mg, 2.67 μmol) was treated by HF/*p*-cresol in (4 ml, v/v: 8/2) at -3°C. After 1 h stirring, the reaction mixture were concentrated under the reduced pressure. The mixture was diluted with $H_2O$/AcOH (v/v, 6/4) and washed with hexane. The resulting aqueous layer was used for the next step without further purification. 0.1 M $I_2$/MeOH (30 μl) was added dropwise for 5 min. The reaction was quenched by adding 1 M ascorbic acid (30 μl). The reaction mixture was directly subjected to preparative HPLC to obtain the title compound (6.4 mg, 68%). Analytical HPLC: Rt, 18.0 min (1-60% $CH_3CN$/0.1% TFA for 25 min at 40 °C, 220 nm); LRMS (ESI) calcd 2933.5, found 2933.2.

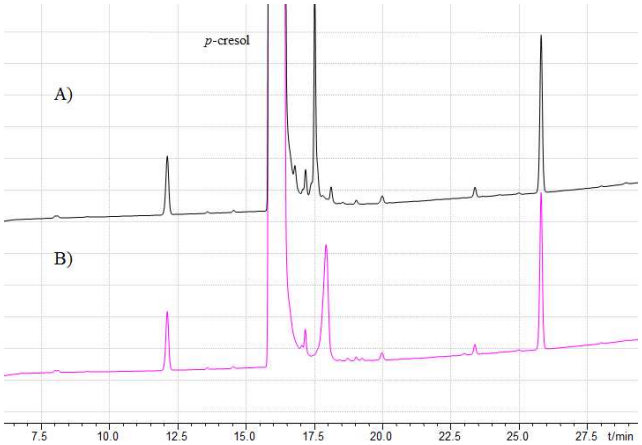
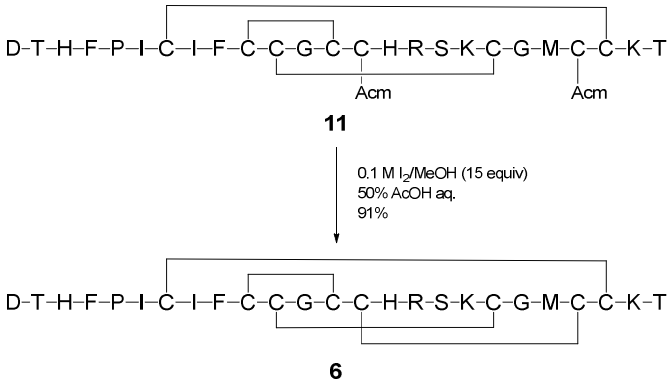



Figure S11. Deprotection and oxidation of Cys(Meb)$^{7,23}$. (A) Deprotection of Cys(Meb)$^{7,23}$: [Cys$^{7,23}$, Cys$^{10}$-Cys$^{13}$, Cys$^{11}$-Cys$^{19}$, Cys(Acm)$^{14,22}$]-human hepcidin. (B) $I_2$ oxidation of Cys$^{7,23}$: [Cys$^{7}$-Cys$^{23}$, Cys$^{10}$-Cys$^{13}$, Cys$^{11}$-Cys$^{19}$, Cys(Acm)$^{14,22}$]-human

hepcidin (**11**). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 1-60% $CH_3CN$ in 0.1% TFA (25 min) at 40 °C; flow rate, 1.0 mL/min; detection, 220 nm.

*Human hepcidin (**6**)*




To a solution of **11** (5.0 mg, 1.42 mmol) in 50% AcOH aq. (6 ml) was added 0.1 M $I_2$/MeOH (213 µl) dropwise. After stirring the reaction mixture for 20 min, the reaction was quenched by adding 1 M ascorbic acid (213 µl). The reaction mixture was directly subjected to preparative HPLC to obtain the title compound (4.4 mg, 91%). Analytical HPLC: Rt, 14.2 min (10-60% $CH_3CN$/0.1% TFA for 25 min at 50 °C, 220 nm); LRMS (ESI) calcd 2789.4, found 2788.8.

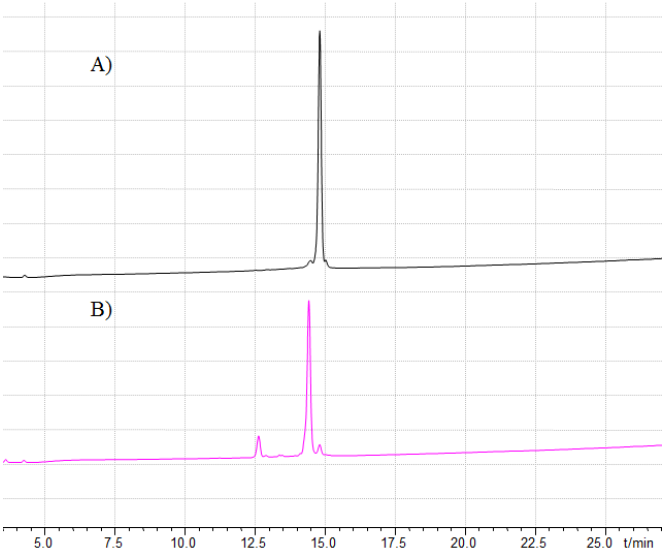



Figure S12. Oxidative deprotection of Cys(Acm)[14,22]. (A) [Cys[7]-Cys[23], Cys[10]-Cys[13], Cys[11]-Cys[19], Cys(Acm)[14,22]]-human hepcidin (**11**). (B) Human hepcidin **6**. HPLC

conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-60% $CH_3CN$ in 0.1% TFA (25 min) at 50 °C; flow rate, 1.0 mL/min; detection, 220 nm.

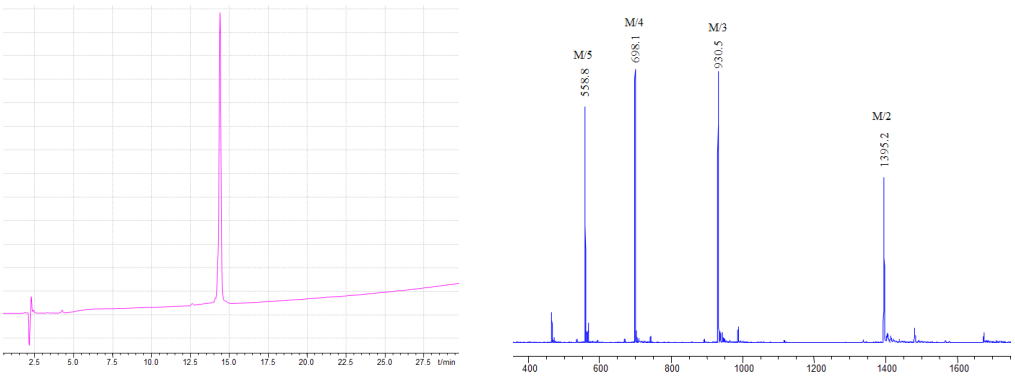



Figure S13. HPLC and ESI-MS profiles of human hepcidin (**6**). HPLC conditions: column, YMC-Pak ODS (4.6 x 150 mm); elution, 10-60% $CH_3CN$ in 0.1% TFA (25 min) at 50 °C; flow rate, 1.0 mL/min; detection, 220 nm.