

Electronic Supplementary Information (ESI)

One-pot native chemical ligation by combination of two
orthogonal thioester precursors

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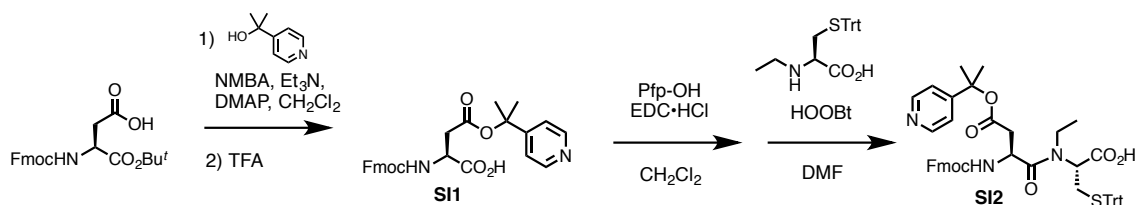
Table of contents

1. General experimental information	3
2. Preparation of dipeptide SI2	3
3. Preparation of the N-terminal peptide segments 2	6
4. Preparation of the middle peptide segment 3	7
5. Preparation of C-terminal peptide segment 4	9
6. One-pot ligation, deprotection, and desulfurization	10
7. HPLC profiles of deprotection and desulfurization (Fig. SI1)	11
8. ^1H and ^{13}C NMR Spectra	12
9. References	16

1. General experimental information

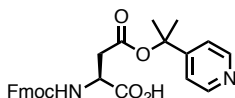
The optical rotation values were determined using a SEPA-300 polarimeter (HORIBA, Kyoto). The NMR spectra were recorded using an Ascend 500 spectrometer (Bruker, MA) at 500 MHz. The chemical shifts are expressed in ppm downfield from the signal for the internal Me₄Si for solutions in the deuterated solvent. Microwave-assisted peptide elongation was carried out using a Liberty Blue peptide synthesizer (CEM, NC) via high-efficiency solid phase peptide synthesis.¹ MALDI-TOF mass spectra were recorded using an Autoflex (Bruker, MA). ESI mass spectra were recorded using an LCQ DECA XP^{plus} (Thermo Fisher Scientific, MC). The amino acid composition was determined using a LaChrom amino acid analyzer (Hitachi, Tokyo) after hydrolysis with 6 M HCl at 180 °C for 25 min in an evacuated sealed tube. The peptide content in the powders was estimated based on the amino acid analysis.

2. Preparation of dipeptide **SI2**



Scheme SI1 Synthetic route of dipeptide **SI2**.

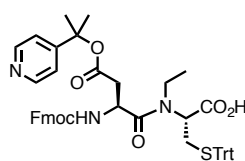
N-(9-Fluorenylmethoxycarbonyl)-*O*^t-[2-(4-pyridyl)-2-propyl]-L-aspartic acid **SI1**



N-(9-Fluorenylmethoxycarbonyl)-L-aspartic acid *tert*-butyl ester (Fmoc-Asp-OBu^t) (0.41 g, 1.0 mmol), 2-methyl-6-nitrobenzoic anhydride (MNBA) (380 mg, 1.1 mmol), and 4-(dimethylamino)pyridine (DMAP) (12 mg, 0.10 mmol) were dissolved in CH₂Cl₂ (2.5 ml) under an Ar atmosphere. Et₃N (0.31 ml, 2.2 mmol) was added, and the mixture was then stirred for 5 min at room temperature. After 2-(4-pyridyl)-2-propanol (160 mg, 0.12 mmol) was added, the mixture was stirred for 18 h at the same temperature. The mixture was concentrated under reduced pressure, and the residue was then dissolved in EtOAc. The organic layer was successively washed with sat. NaHCO₃ aq, H₂O, and

brine, dried over MgSO_4 , filtrated, and concentrated under reduced pressure. The crude was purified by silica gel chromatography (toluene/2-propanol 49:1 to 19:1) to give the ester. The obtained ester was dissolved in trifluoroacetic acid (TFA) (3.0 ml), and the mixture was then stirred for 1.5 h at room temperature. After concentration under reduced pressure, the residue was dissolved in EtOAc. The organic layer was successively washed with 10% citrate aq (pH adjusted to ca. 6 by NaOH) twice, and brine, dried over MgSO_4 , filtrated, and concentrated under reduced pressure. The residue was purified by silica gel chromatography ($\text{CHCl}_3/\text{MeOH}$ 19:1, 1% AcOH). After the collected fractions were condensed, the obtained product was dissolved in 1,4-dioxane and then lyophilized to give aspartic acid **SI1** (284 mg, 60%) in two steps from Fmoc-Asp-OBu^t. $[\alpha]_{\text{D}}^{+23.5}$ (CHCl_3 , c 1.0). Anal. calcd for $\text{C}_{27}\text{H}_{26}\text{N}_2\text{O}_6 + 1/2\text{H}_2\text{O}$: C, 67.07; H, 5.63; N, 5.79. Found: C, 66.73; H, 5.60; N, 5.41. ^1H NMR (CDCl_3): δ 8.53 (d, 2H, $J = 4.5$ Hz, ArH), 7.79 (d, 2H, $J = 7.4$ Hz, ArH), 7.67-7.32 (d, 8H, ArH), 6.04 (d, 1H, $J = 8.2$ Hz, -NH), 4.66-4.65 (m, 1H, αH), 4.49-4.45 (m, 1H, $\text{Ar}_2\text{CH-CH}_2$ -), 4.38-4.34 (m, 1H, $\text{Ar}_2\text{CH-CH}_2$ -), 4.30-4.28 (m, 1H, $\text{Ar}_2\text{CH-}$), 3.31 (br dd, 1H, $J = 3.3, 14.2$ Hz, βH), 5.83 (br dd, 1H, $J = 3.3, 14.2$ Hz, βH), 1.93 (s, 3H, Me), 1.64 (s, 3H, Me). ^{13}C NMR (CDCl_3): δ 50.6 (αC), 37.1 (βC), 31.4 (- CH_3), 25.2 (- CH_3).

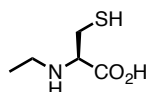
N-(9-Fluorenylmethoxycarbonyl)-*O*^d-[2-(4-pyridyl)-2-propyl]-L-aspartyl-*N*-ethyl-*S*-trityl-L-cysteine **SI2**



To a solution of Fmoc-Asp(OPyP)-OH **SI1** (820 mg, 1.7 mmol) and pentafluorophenol (Pfp-OH) (380 mg, 2.1 mmol) in CH_2Cl_2 (8.0 ml) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDC•HCl) (500 mg, 2.6 mmol) and then stirred for 1 h at room temperature. After the mixture was concentrated under reduced pressure, the residue was dissolved in EtOAc. The organic layer was successively washed with H_2O and brine, dried over MgSO_4 , filtrated, and concentrated under reduced pressure. The obtained Pfp ester was used for the next reaction without further purification. The Pfp ester, *N*-ethyl-*S*-trityl-L-cysteine² (670 mg, 1.7 mmol) and 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HOObt) (282 mg, 1.7 mmol) were dissolved in DMF (5.0 ml),

and the mixture was then stirred for 7 h at 50 °C. After the mixture was concentrated under reduced pressure, the residue was dissolved in EtOAc. The organic layer was successively washed with sat. NaHCO₃ aq twice and brine, dried over MgSO₄, filtrated, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CHCl₃/MeOH 19:1, 1% Et₃N). The product was re-purified by reverse-phase (RP) HPLC (MeCN/H₂O, 0.1% TFA). The collected fraction was concentrated under reduced pressure until 1/3 of the volume remained. The suspension solution was neutralized by 10% citrate aq (pH adjusted to ca. 6 NaOH) and then extracted thrice with EtOAc. The extract was successively washed with brine, dried over MgSO₄, filtrated, and concentrated under reduced pressure. The product was dissolved in 1,4-dioxane, and the solution was filtrated through a short pad of silica gel. The filtrate was lyophilized to give dipeptide **SI2** (0.98 g, 66%). $[\alpha]_D$ -56.5 (CHCl₃, c 1.0). Anal. calcd for C₅₁H₄₉N₃O₇S: C, 72.23; H, 5.82; N, 4.96; O, 13.21; S, 3.78. Found: C, 72.24; H, 5.94; N, 4.87. ¹H NMR (CD₃CD): δ 8.47-8.38 (m, 2H, ArH), 7.79-7.60 (m, 4H, ArH), 7.42-7.19 (m, 23H, ArH), 4.39-4.15 (m, 3H, Ar₂CHCH₂-), 3.46-3.39 (m, 1H, CH₃CH₂N-), 3.09-2.57 (m, 6H, Cys- α H, Cys- β H, CH₃CH₂N-, Asp- β H), 1.72-1.64 (m, 6H, PyC(CH₃)₂-), 1.09-0.96 (m, 3H, CH₃CH₂N-). ¹³C NMR (CD₃OD): δ 60.2 (Cys- α C), 44.0 (CH₃CH₂N-), 37.5 (Asp- β C), 30.3 (Cys- β C).

N-Ethyl-L-cysteine **SI3**



N-Ethyl-*S*-trityl-L-cysteine² (390 mg, 0.10 mmol) was dissolved in 50% TFA/CH₂Cl₂ (5.0 ml) containing Et₃SiH (0.24 ml, 1.5 mmol). The mixture was stirred for 15 min at room temperature and then concentrated under reduced pressure. The residue was dissolved in 1,4-dioxane (6.0 ml) and then lyophilized. The powder was washed with ether thrice and then dried. The residue was dissolved in 50% MeCN aq and then lyophilized to give **SI3** (80 mg, 53%). The product was directly used without further purification.

3. Preparation of the N-terminal peptide segments 2

H-Histone H4 (1-37)-Cys-Pro-OCH₂CO-Tle-NH₂ 2



Fmoc-Rink amide MBHA resin (290 mg, 0.10 mmol) was treated with 20% piperidine/*N*-methylpyrrolidone (NMP) for 1 min at room temperature. The resin was repeatedly treated with 20% piperidine/NMP for 2 min at room temperature. An activated *tert*-leucine, which was prepared by mixing (S)-2-[(9-fluorenylmethoxycarbonyl)-amino]-3,3-dimethylbutyric acid (Fmoc-Tle-OH) (140 mg, 0.40 mmol), 0.45 M *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU)/DMF (0.84 ml), and *N,N*-diisopropylethylamine (DIEA) (0.14 ml, 0.80 mmol) for 3 min at room temperature, was added to the resin. The resin was vortexed for 12 min at 50 °C. After the deprotection of Fmoc group, an activated glycolic acid, which was prepared by mixing glycolic acid (30 mg, 0.40 mmol), *N,N'*-diisopropylcarbodiimide (DIC) (62 μL, 0.40 mmol), and 1-hydroxybenzotriazole (HOBT) (65 mg, 0.40 mmol) in NMP (1.0 ml) for 5 min at room temperature, was added to the resin. The resin was vortexed for overnight at room temperature. An activated proline, which was prepared by mixing of Fmoc-Pro-OH (140 mg, 0.40 mmol), 0.45 M HBTU/DMF (0.84 ml), and DIEA (0.14 ml, 0.80 mmol) for 3 min at room temperature, was added to the resin. The resin was vortexed for 30 min at 50 °C. After deprotection of Fmoc, Fmoc-Leu-Cys(Trt)-OH³ (140 mg, 0.20 mmol) and HOBT (65 mg, 0.40 mmol) in NMP (1.0 ml) were added to the resin. After DIC (0.12 ml, 0.40 ml) was added to the mixture, the resin was vortexed for 1 h at room temperature. The resin was treated with 10% Ac₂O and 5% DIEA in NMP for 5 min, and the peptide chain was then elongated by microwave-assisted peptide synthesis using CEM Liberty Blue. The resulting resin was successively washed with NMP, CH₂Cl₂, and MeOH and dried under reduced pressure to give the H-Ser(*t*Bu)-Gly-Arg(Pbf)-Gly-Lys(Boc)-Gly-Gly-Lys(Boc)-Gly-Leu-Gly-Lys(Boc)-Gly-Gly-Ala-Lys(Boc)-Arg(Pbf)-His(Trt)-Arg(Pbf)-Lys(Boc)-Val-Leu-Arg(Pbf)-Asp(O*t*Bu)-Asn(Trt)-Ile-Gln(Trt)-Gly-Ile-Thr(*t*Bu)-Lys(Boc)-Pro-Ala-Ile-Arg(Pbf)-Arg(Pbf)-Leu-Cys(Trt)-Pro-OCH₂CO-Tle-NH-resin (550 mg). A part of the resin (120 mg) was treated with TFA cocktail [TFA:*i*-

Pr₃SiH (TIS):H₂O:1,3-dimethoxybenzene (DMB) 92.5:2.5:2.5:2.5, 3.0 ml] for 2 h at room temperature. After TFA was removed by a N₂ stream, the peptide was precipitated with cold ether. The precipitate was washed thrice with ether and then dried. The crude peptide was dissolved in CH₃CN aq and then filtrated through a short pad of ODS column. After the solution was lyophilized, the peptide was purified by RP HPLC to give peptide segment **2** (2.8 μmol, 13%) from the amino groups on the initial Fmoc-Rink amide MBHA resin. ESI-MASS, found: *m/z* 1078.3, 1437.2, calcd for [M+4H]⁴⁺: 1078.0, [M+3H]³⁺: 1437.1. Amino acid analysis: Asp_{2.00}Thr_{0.76}Ser_{0.78}Glu_{2.01}Pro_{2.26}Gly_{6.94}Ala_{2.00}Val_{1.00}Ile_{2.85}Leu₃Lys_{5.34}His_{1.04}Arg_{5.74}.

4. Preparation of the middle peptide segment **3**

Fmoc-Histone H4 (38-68) [Cys³⁸, Asp(OPyP)⁶⁸]-S-C₆H₄-OH **SI4**



Fmoc-Rink amide HBHA resin (300 mg, 0.10 mmol) was subjected to peptide chain elongation using the microwave-assisted peptide synthesizer to give the H-[Arg(Pbf)]₂-NH-resin. The resin was washed thrice with 1,2-dichloroethane (DCE). An activated dipeptide, which was prepared by mixing Fmoc-Asp(OPyP)-(Et)Cys(Trt)-OH **SI2** (170 mg, 0.20 mmol), HOBt (54 mg, 0.40 mmol), and DIC (62 μl, 0.40 mmol) in DCE (0.50 ml) for 5 min at room temperature, was added to the resin. The resin was vortexed for overnight at room temperature. After acetyl capping by reacting with 10% Ac₂O and 5% DIEA in NMP for 5 min, the resin was subjected to peptide chain elongation using the microwave-assisted peptide synthesizer to give Fmoc-Gly-Leu-Ile-Tyr(*t*Bu)-Glu(*O**t*Bu)-Glu(*O**t*Bu)-Thr(*t*Bu)-Arg(Pbf)-Gly-Val-Leu-Lys(Boc)-Val-Phe-Leu-Glu(*O**t*Bu)-Asn(Trt)-Val-Ile-Arg(Pbf)-Asp(OPyP)-(Et)Cys(Trt)-[Arg(Pbf)]₂-NH-Resin. A part of the resin (33 μmol) was continued to subjected to peptide chain elongation by the microwave-assisted peptide synthesizer. The resulting resin was washed with NMP, CH₂Cl₂, and MeOH and then dried under reduced pressure to give Fmoc-Cys(Trt)-Arg(Pbf)-Arg(Pbf)-Gly-Gly-Val-Lys(Boc)-Arg(Pbf)-Ile-Ser(*t*Bu)-Gly-Leu-Ile-Tyr(*t*Bu)-Glu(*O**t*Bu)-Glu(*O**t*Bu)-Thr(*t*Bu)-Arg(Pbf)-Gly-Val-Leu-Lys(Boc)-Val-Phe-Leu-Glu(*O**t*Bu)-Asn(Trt)-Val-Ile-Arg(Pbf)-Asp(OPyP)-(Et)Cys(Trt)-

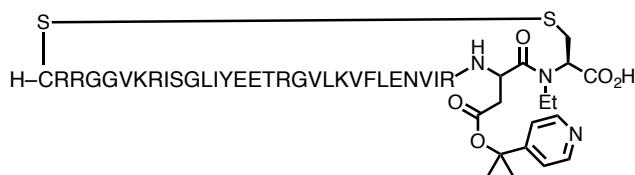
[Arg(Pbf)]₂-NH-Resin (350 mg). A part of the resin (290 mg) was treated with TFA cocktail (TFA:TIS:H₂O:DMB 92.5:2.5:2.5:2.5, 6.0 ml) for 2 h at room temperature. After TFA was removed by a N₂ stream, the peptide was precipitated with cold ether. The precipitate was washed thrice with ether and then dried. The residue was dissolved in a solution (30 ml) of 6 M urea, 5% 4-hydroxythiophenol (4-HTP), and 5% AcOH in 50% MeCN aq. The mixture was vortexed for 24 h at 37 °C. The mixture was acidified by 20% TFA aq (0.75 ml) and then washed with ether to extract an excess amount of thiol. The residual ether was removed by N₂ stream, and the resulting mixture was then purified by RP HPLC to give peptide **SI4** (3.4 μmol, 12%) from the amino groups on the initial Fmoc-Rink amide MBHA resin. ESI-MASS, found: *m/z* 1008.1, 1343.4, calcd for [M+4H]⁴⁺: 1007.7, [M+3H]³⁺: 1343.3. Amino acid analysis: Asp_{1.97}Thr_{0.847}Ser_{0.769}Glu_{3.37}Gly₄Val_{3.65}Ile_{2.57}Leu_{2.89}Tyr_{0.89}Phe_{1.12}Lys_{1.95}Arg_{4.69}.

Fmoc-Histone H4 (38-68) [Cys³⁸, Asp(OPyP)⁶⁸]-*(Et)*Cys-OH **SI5**



Peptide **SI4** (420 nmol) was dissolved in NMP (0.42 ml). The solution was diluted with 6 M Gdn•HCl aq containing 0.10 M phosphate and 10 mM tris(3-carboxyethyl)phosphine (TCEP) hydrochloride (0.38 ml) (pH adjusted to 7.0 by NaOH). *N*-ethyl-L-cysteine **SI3** (0.25 mg, 1.7 μmol) in 50% MeOH/H₂O (5.0 μl) was added to the mixture. The mixture was stored under an Ar atmosphere for 1 h at 37 °C and then additionally reacted for 1.5 h at 50 °C. The resulting mixture was diluted with 50% DMSO/H₂O solution (5.0 ml) containing 5 M Gdn•HCl, 0.5% NH₄HCO₃ and 0.5% acetone at room temperature and then stored for 18 h at the same temperature. After the reaction mixture was acidified by 20% TFA aq, the crude was purified by RP HPLC to give peptide **SI5** (220 nmol, 53%). ESI-MASS, found: *m/z* 1013.1, 1350.3, calcd for [M+4H]⁴⁺: 1013.0, [M+3H]³⁺: 1350.3. Amino acid analysis: Asp_{1.97}Thr_{0.84}Ser_{0.73}Glu_{2.86}Gly₄Val_{3.60}Ile_{2.52}Leu_{2.96}Tyr_{0.90}Phe_{1.39}Lys_{1.98}Arg_{4.94}.

H-Histone H4 (38-68) [Cys³⁸, Asp(OPyP)⁶⁸]-*(Et)*Cys-OH **3**



Peptide **SI5** (800 nmol) was dissolved in 20% Et₂NH/NMP (0.80 ml) and the mixture was vortexed for 15 min at room temperature. The peptide was precipitated with ether. The precipitate was washed twice with ether and then dried. The peptide was dissolved in 50% MeCN containing 1% AcOH and then lyophilized to give peptide **3** (700 nmol, 87%). ESI-MASS, found: m/z 1276.5, 1913.9, calcd for $[M+3H]^{3+}$: 1276.2, $[M+2H]^{2+}$: 1913.8. Amino acid analysis: Asp_{1.87}Thr_{0.79}Ser_{0.82}Glu_{2.75}Gly₄Val_{3.36}Ile_{2.34}Leu_{2.73}Tyr_{0.85}Phe_{1.17}Lys_{1.86}Arg_{4.50}.

5. Preparation of C-terminal peptide segment **4**

H-Histone H4 (69-102) (Cys⁶⁹)-OH **4**



Fmoc-Gly-O-Wang resin (0.10 mmol, 300 mg) was subjected to peptide chain elongation by the microwave-assisted peptide synthesizer to give the H-Cys(Trt)-Val-Thr(*t*Bu)-Tyr(*t*Bu)-Thr(*t*Bu)-Glu(*O**t*Bu)-His(Trt)-Ala-Lys(Boc)-Arg(Pbf)-Lys(Boc)-Thr(*t*Bu)-Val-Thr(*t*Bu)-Ala-Met-Asp(*O**t*Bu)-Val-Val-Tyr(*t*Bu)-Ala-Leu-Lys(Boc)-Arg(Pbf)-Gln(Trt)-Gly-Arg(Pbf)-Thr(*t*Bu)-Leu-Tyr(*t*Bu)-Gly-Phe-Gly-Gly-O-Resin (770 mg). A part of the resin (100 mg) was treated with TFA cocktail [TFA:TIS:H₂O:thioanisole:3,6-dioxo-1,8-octandithiol (DODT) 90:2.5:2.5:2.5:2.5, 3.0 ml] for 2 h at room temperature. After TFA was removed by N₂ stream, the peptide was precipitated by ether. The precipitate was washed thrice with ether and then dried. The residue was purified by RP HPLC to give the peptide **4** (3.9 μmol, 30%) from the amino group of Gly on the initial Wang resin. MALDI-TOF-MASS, found: m/z 3822.0, calcd for $[M+H]^+$: 3822.5. Amino acid analysis: Asp_{1.00}Thr_{4.02}Glu_{1.89}Gly₄Ala_{2.97}Val_{3.58}Met_{0.95}Leu_{2.02}Tyr_{3.10}Phe_{0.99}Lys_{2.99}His_{1.00}Arg_{3.00}.

6. One-pot ligation, deprotection, and desulfurization

H-Histone H4 (1-102) [Cys^{38,69}, Asp(OPyP)⁶⁸]-OH **6**

Segment **3** (500 nmol) and **4** (500 nmol) were dissolved in a buffer (6 M Gdn•HCl, 0.10 M phosphate, 50 mM 4-HTP, and 25 mM TCEP•HCl, pH adjusted to 7.8 by NaOH) (0.50 ml), and the reaction mixture was then stored for 8 h at 37 °C under an Ar atmosphere. Segment **4** (500 nmol) in a buffer (6 M Gdn•HCl, 0.40 M acetate, 50 mM 4-HTP, and 25 mM TCEP•HCl, pH adjusted to 5.0 by NaOH) (0.50 ml) was directly added to the reaction mixture. After the addition of **4** in the solution, the pH of the reaction solution was decreased to 5.5 and stored for 24 h at 37 °C under an Ar atmosphere. The resulting mixture was further acidified by 20% TFA aq. and then washed thrice with ether to remove the extra thiol. The crude was purified by gel filtration chromatography to give the entire polypeptide **6** (290 nmol, 58%). MALDI-TOF-MASS, found: m/z 11299.5, calcd for $[M+2H-PyP]^+$: 11301.3. Amino acid analysis: Asp_{5.12}Thr_{5.47}Ser_{1.60}Glu_{6.54}Gly₁₇Ala_{4.85}Val_{8.15}Met_{0.80}Ile_{5.65}Leu_{8.08}Tyr_{3.66}Phe_{2.31}Lys_{11.2}His_{1.90}Arg_{14.2}.

H-Histone H4 (1-102) (Cys^{38,69}) -OH **7**

Polypeptide **6** (290 nmol) was dissolved in 6 M Gdn•HCl containing 15% 3-mercaptopropionic acid (MPA) (4.0 ml) and Zn (100 mg), which was activated by 1 M HCl aq and then washed thrice with H₂O, was added to the mixture. The reaction mixture was vigorously vortexed for 30 min at room temperature. The resulting mixture was filtrated and then purified by RP HPLC to give polypeptide **7** (190 nmol, 64%). MALDI-TOF-MASS, found: m/z 11301.5, calcd for $[M+H]^+$: 11301.3. Amino acid analysis: Asp_{5.08}Thr_{5.79}Ser_{1.74}Glu_{6.70}Gly₁₇Ala_{5.08}Val_{8.27}Met_{0.80}Ile_{5.59}Leu_{8.16}Tyr_{4.17}Phe_{1.61}Lys_{10.5}His_{2.02}Arg_{13.9}.

H-Histone H4 (1-102)-OH **1**

Polypeptide **7** (93 nmol) was dissolved in 6 M Gdn•HCl containing 0.20 M TCEP, 0.10 M phosphate, and 0.10 M sodium 2-mercaptoethane sulfonate (MESNa) (pH adjusted to 6.8 by NaOH, 1.0 ml). After 0.10 M 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044) (10 µl) was added, the mixture was stored for 24

h at 37 °C under an Ar atmosphere. The mixture was acidified by 20% TFA aq and then purified by RP HPLC to give final product **1** (73 nmol, 79%). MALDI-TOF-MASS, found: m/z 11236.0, calcd for $[M+H]^+$: 11237.1. Amino acid analysis: Asp_{5.25} Thr_{6.05} Ser_{1.71} Glu_{6.57} Gly₁₇ Ala_{7.04} Val_{8.17} Met_{0.52} Ile_{5.58} Leu_{8.18} Tyr_{4.01} Phe_{2.06} Lys_{11.3} His_{2.11} Arg_{14.3}.

7. HPLC profile of deprotection and desulfurization (Fig. SI1)

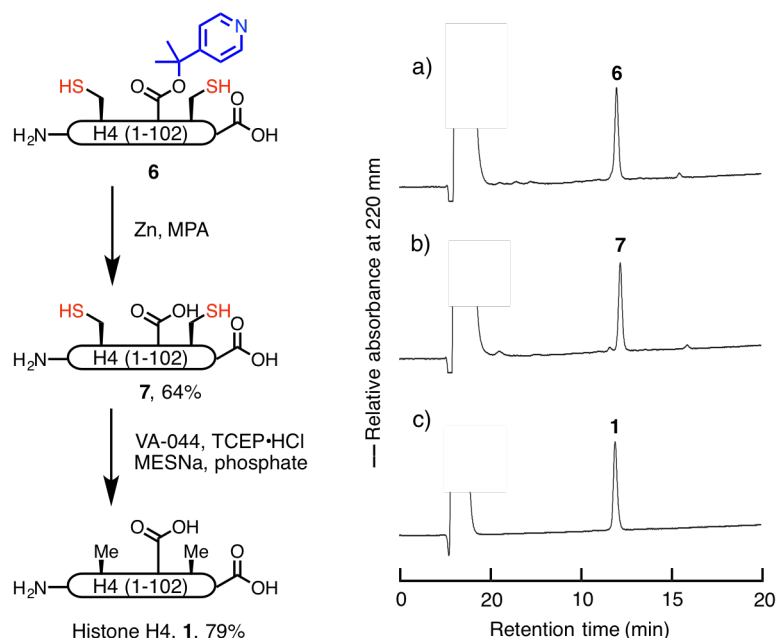
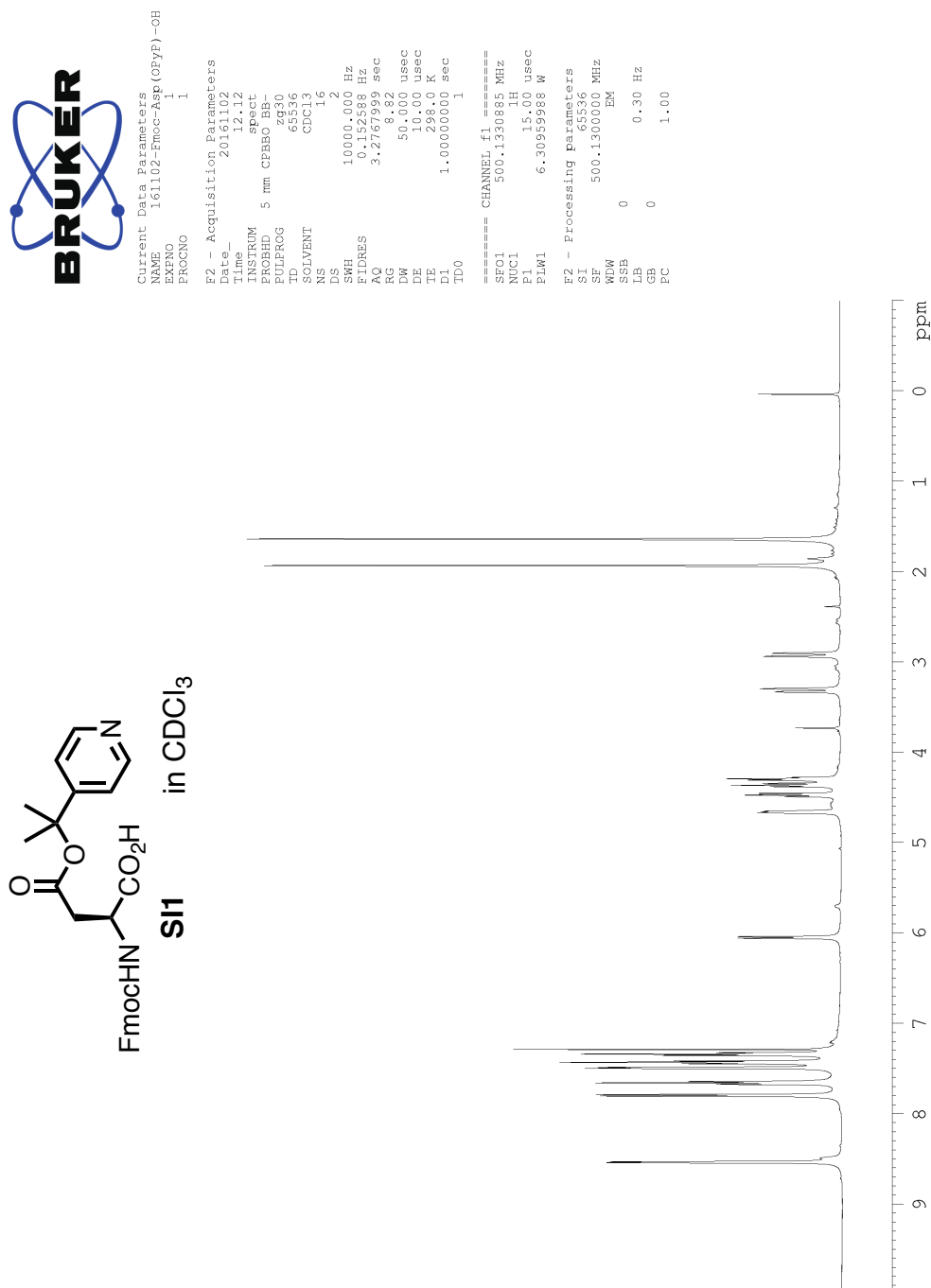


Fig. SI1 HPLC profiles of deprotection and desulfurization. Deprotection of PyP ester **6** at 0 (a) and 30 min (b). The desulfurization reaction after 24 h (c). A linear gradient starting from 30% MeCN at 0 min to 50 % MeCN at 20 min was applied. The isolated yields were determined by amino acid analysis.

8. ^1H and ^{13}C NMR Spectra



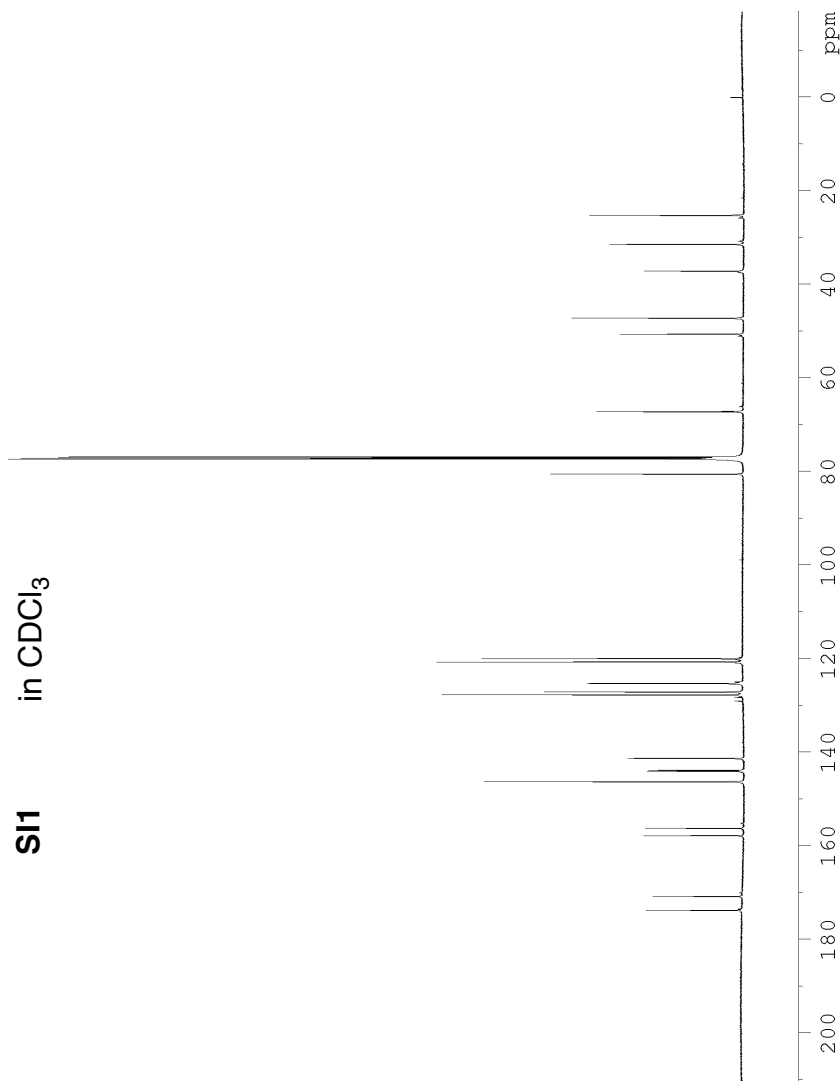
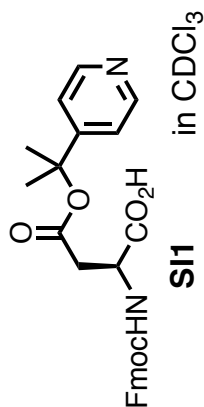


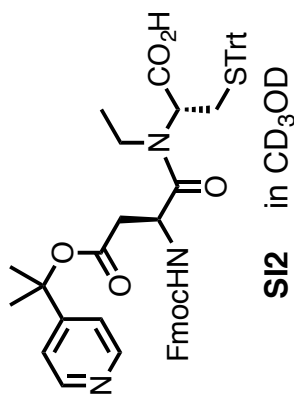
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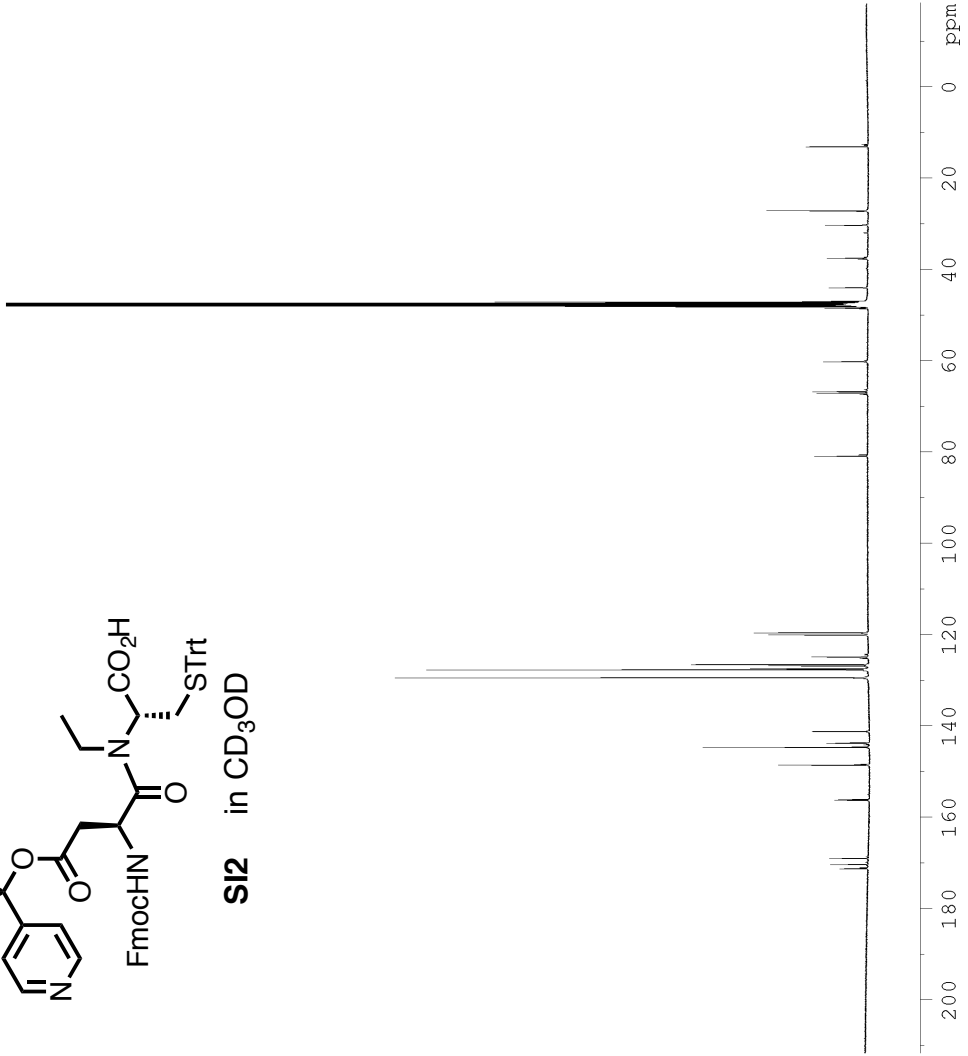
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Current Data Parameters
 NAME 51102-Emc-Sig (GPy)- (Et)Cys (Ttt)-OH
 EXPNO 2
 PROCNO 1
 P2 - Acquisition Parameters
 Date_ 2015.05
 Time 11.12
 INSTRUM spect
 PULPROG zgpg30
 TD 65536
 SFO 400
 NUC1 13C
 NS 768
 DS 4
 FIDRES 0.454131 Hz
 AQ 1.1010048 sec
 EQ 2
 DE 15.800 usec
 DE 18.00 usec
 D1 2.00000000 sec
 D11 0.03000000 sec
 TSD 1
 ===== CHANNEL f1 =====
 SFO1 125.770337 MHz
 NUC1 13C
 P1 1.00 usec
 PL1 0.00 dB
 FWH 38.1735909 W
 ===== CHANNEL f2 =====
 SFO2 500.1350003 MHz
 NUC2 1H
 PULPROG wahtzg16
 PCPD2 6.3095968 W
 FWH2 0.00000000 W
 FWH3 0.1418699 W
 P2 - Processing Parameters
 SI 32768
 SF 125.7577885 MHz
 DSF 0
 ASF 0
 ZF 0
 PC 1.40



9. References

1. J. M. Collins, K. A. Porter, S. K. Singh and G. S. Vanier, *Org. Lett.*, 2014, **16**, 940–943.
2. H. Hojo, Y. Onuma, Y. Akimoto, Y. Nakahara and Y. Nakahara, *Tetrahedron Lett.*, 2007, **48**, 25-28; Y. Asahina, K. Nabeshima and H. Hojo, *Tetrahedron Lett.*, 2015, **56**, 1370-1373.
3. T. Kawakami and S. Aimoto, *Chem. Lett.*, 2007, **36**, 76-77.