Supporting Information

Deprotection of S-Acetamidomethyl Cysteine with Copper (II) and 1,2-Aminothiols under Aerobic Conditions

Daishiro Kobayashi, Naoto Naruse, Masaya Denda, Akira Shigenaga[†] and Akira Otaka[*]

Insutitute of Biomedical Sciences and Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan.

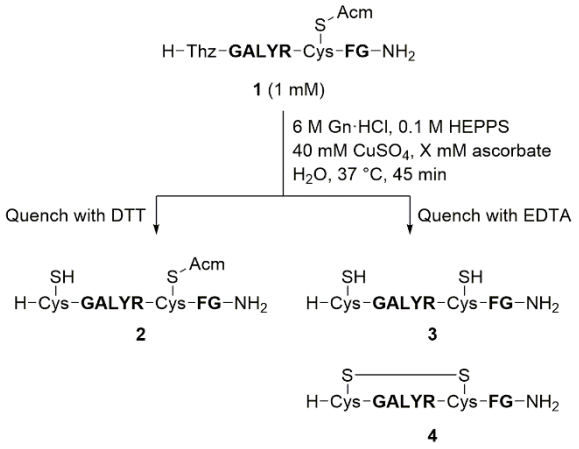
[†]Present address (AS): Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, Hiroshima 729-0292, Japan.

E-mail: aotaka@tokushima-u.ac.jp

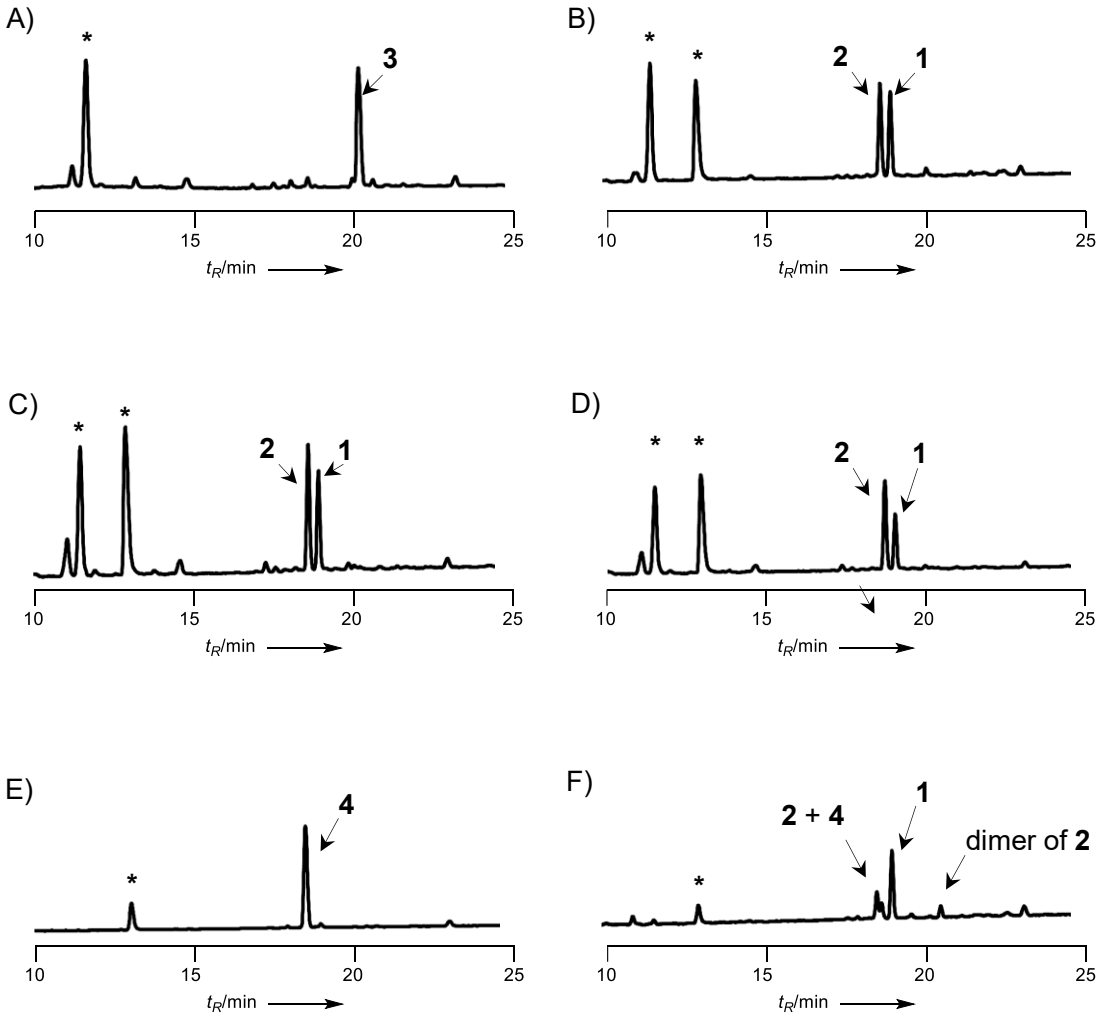
Contents

Examination of ratio of $CuSO_4$ and ascorbate S2
Evaluation of contribution of N-terminal amino acid for deprotection of Acm group S4
Examination of deprotection of *S*-Acm with deaerated solvent..... S5
Evaluation of stability of other *S*-protecting group in the presence of $CuSO_4$ and cysteamine..... S6
Examination of stability of Met-containing peptide in the presence of $CuSO_4$ and cysteamine or not S7
Fe(III) salt-mediated deprotection of Acm group S8
Examination of deprotection of S-Acm with H_2O_2..... S9
Verification of synthetic Apamin by comparison with possible three isomers S10
$CuSO_4$-cysteamine-mediated disulphide bond formation between Thz1 and Cys(Acm)11 in the presence of Cys(MBzl) residues for the alternative attempt at the synthesis of Apamin.....S11

Examination of ratio of $CuSO_4$ and ascorbate



Entry	X (mM)	Quench[a]	Conversion[b]	LC chart
1	0	DTT[c]	**3** (>95)	A
2	40	DTT	**1** (47), **2** (53)	B
3	80	DTT	**1** (43), **2** (57)	C
4	120	DTT	**1** (38), **2** (62)	D
5	0	EDTA	**4** (>95)	E
6[d]	40	EDTA	**1** (72), **2** + **4** (28)	F
7[d]	80	EDTA	**1** (81), **2** + **4** (19)	G
8	120	EDTA	**1** (7), **2** (93)	H



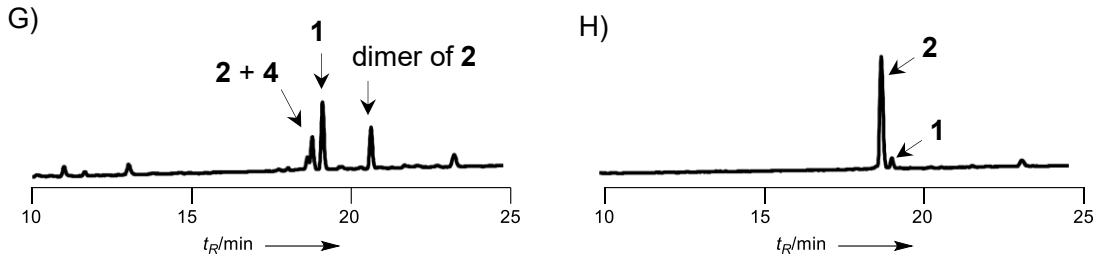
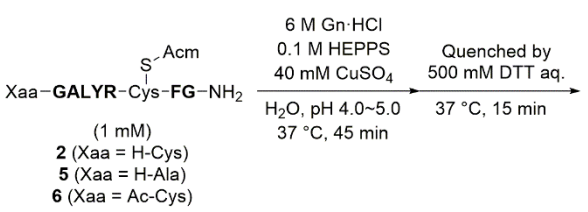


Figure S1. Analytical HPLC charts of crude reaction material. [a]Addition of 100 mM EDTA aq. or addition of 500 mM DTT followed by stirring at 37 ℃ for 15 min; [b]Conversion (%) (or remaining (%)) were determined by HPLC analyses with UV detection at 220 nm and calculated using the equation percent formation = 100 [(integ. **1** or **2** or **3** or **4** or **2** + **4**)/(integ. **1** + integ. **2** + integ. **3** + integ. **4**)]: integ. = integration of peak area of the UV absorption; [c]Stirring for 30 min instead of 15 min; [d]A small amount of dimer of **2** was detected. Analytical HPLC conditions: linear gradient of 0.1% TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 45% over 30 min. *Non-peptide impurity.

Evaluation of contribution of N-terminal amino acid for deprotection of Acm group



Entry	**Xaa**	LC chart
1[a]	Cys	A
2[a,b]	Cys	B
3	Ala	C
4	Ac-Cys	D

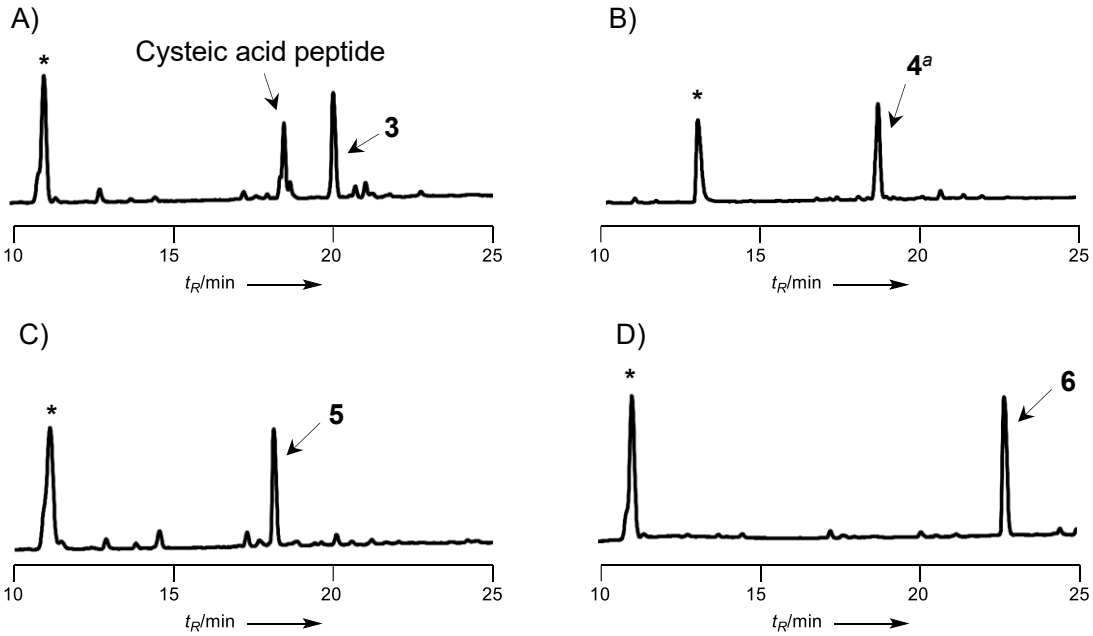
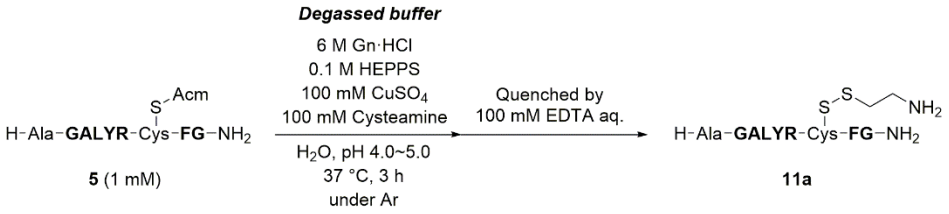


Figure S2. Analytical HPLC charts of crude reaction material. [a]A small amount of N-terminal cysteic acid peptide was detected; [b]Quenched by 100 mM EDTA aq. instead of 500 mM DTT. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 45% over 30 min. *Non-peptide impurity.

Examination of deprotection of *S*-Acm with deaerated solvent



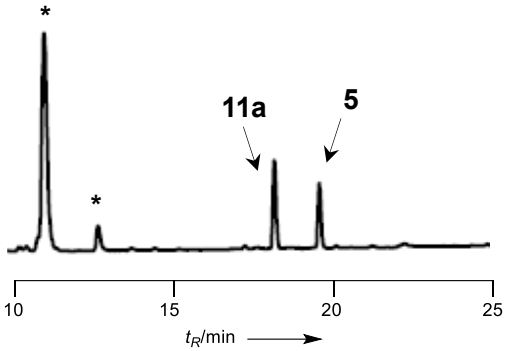


Figure S3. Analytical HPLC charts of crude reaction material. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 45% over 30 min. *Non-peptide impurity.

Evaluation of stability of other *S*-protecting group in the presence of $CuSO_4$ and cysteamine

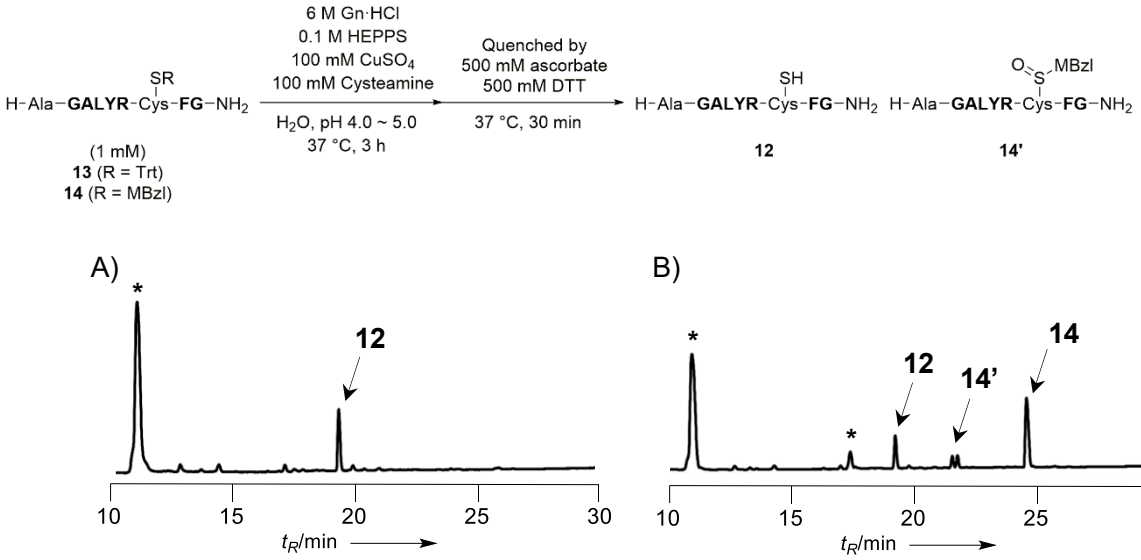
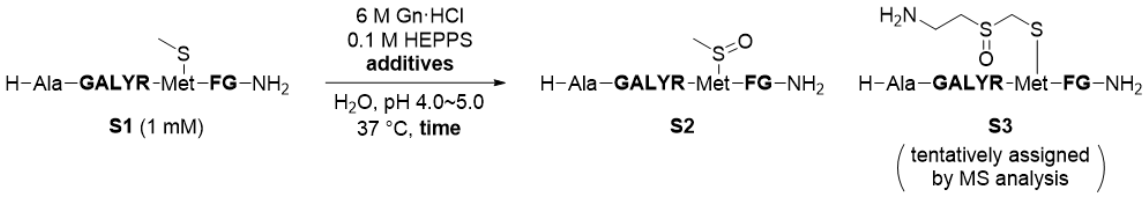


Figure S4. Analytical HPLC charts of crude reaction material. (A) R = Trt; (B) R = MBzl. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 45% over 30 min. *Non-peptide impurity.

Examination of stability of Met-containing peptide in the presence of $CuSO_4$ and cysteamine or not



entry	additives	time
1	40 mM $CuSO_4$	45 min
2	100 mM $CuSO_4$, 100 mM cysteamine	3 h

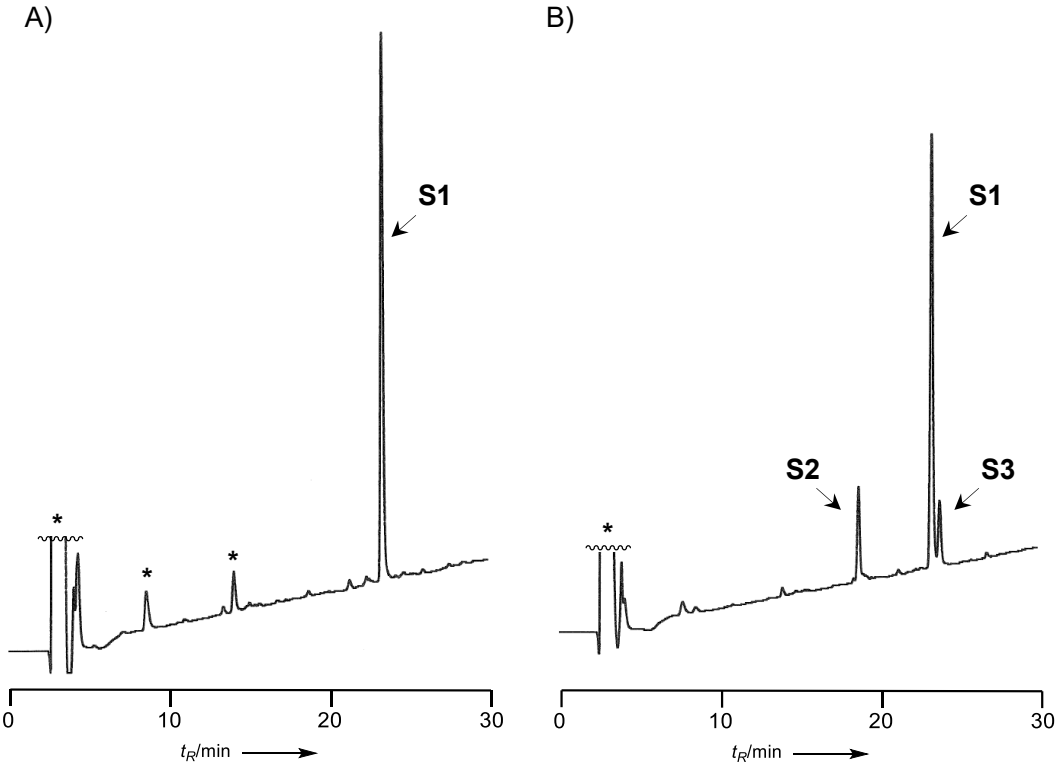


Figure S5. Analytical HPLC charts of crude reaction material. (A) Entry 1; (B) Entry 2. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 45% over 30 min. *Non-peptide impurity.

Fe(III) salt-mediated deprotection of Acm group

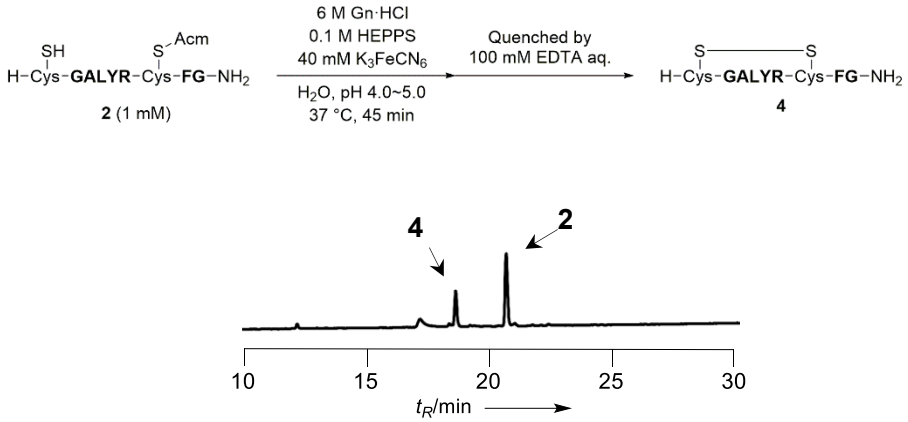
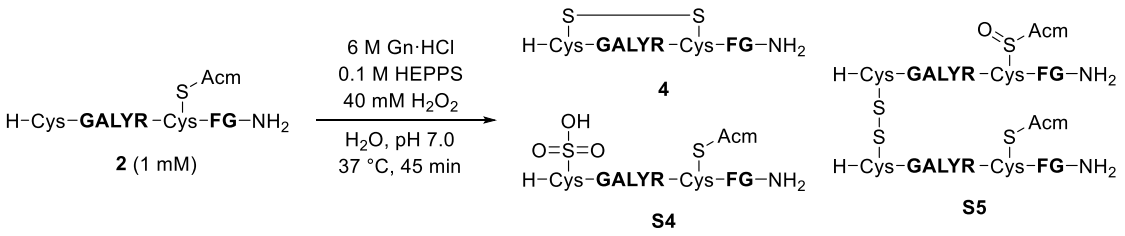


Figure S6. Analytical HPLC charts of crude reaction material. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 45% over 30 min.

Examination of deprotection of *S*-Acm with H_2O_2



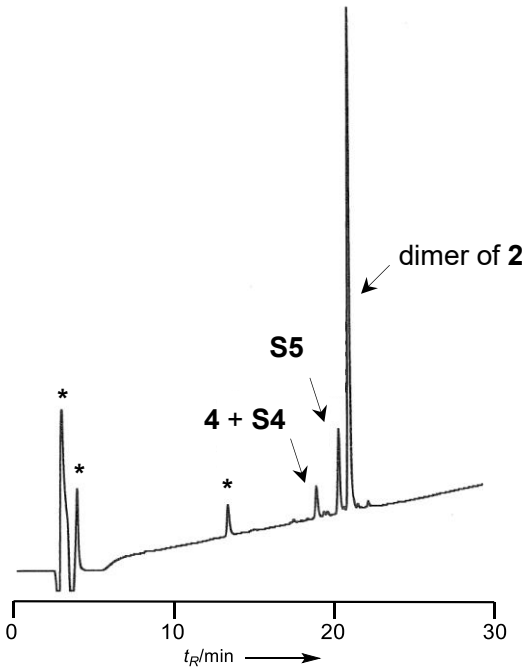


Figure S7. Analytical HPLC charts of crude reaction material. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 45% over 30 min. *Non-peptide impurity.

Verification of synthetic Apamin by comparison with possible three isomers

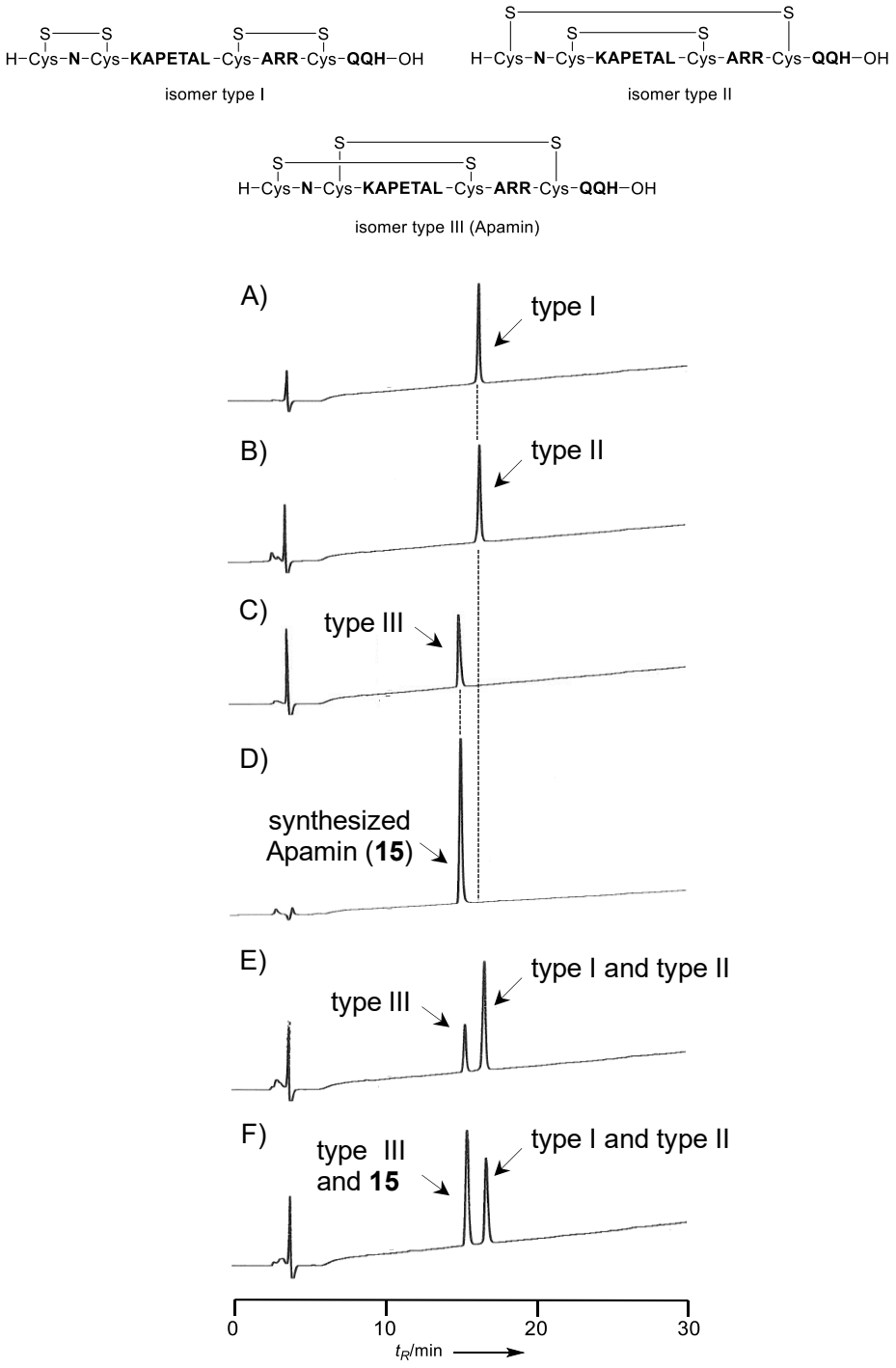


Figure S8. Analytical HPLC charts of Apamin and isomers. (A) Type I; (B) Type II; (C) Type III (Apamin); (D) Synthesized Apamin (**15**); (E) Co-injection of type I and type II and type III; (F) Co-injection of type I and type II and type III and synthesized Apamin. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 40% over 30 min.

$CuSO_4$–cysteamine-mediated disulphide bond formation between Thz1 and Cys(Acm)11 in the presence of Cys(MBzl) residues for the alternative attempt at the synthesis of Apamin

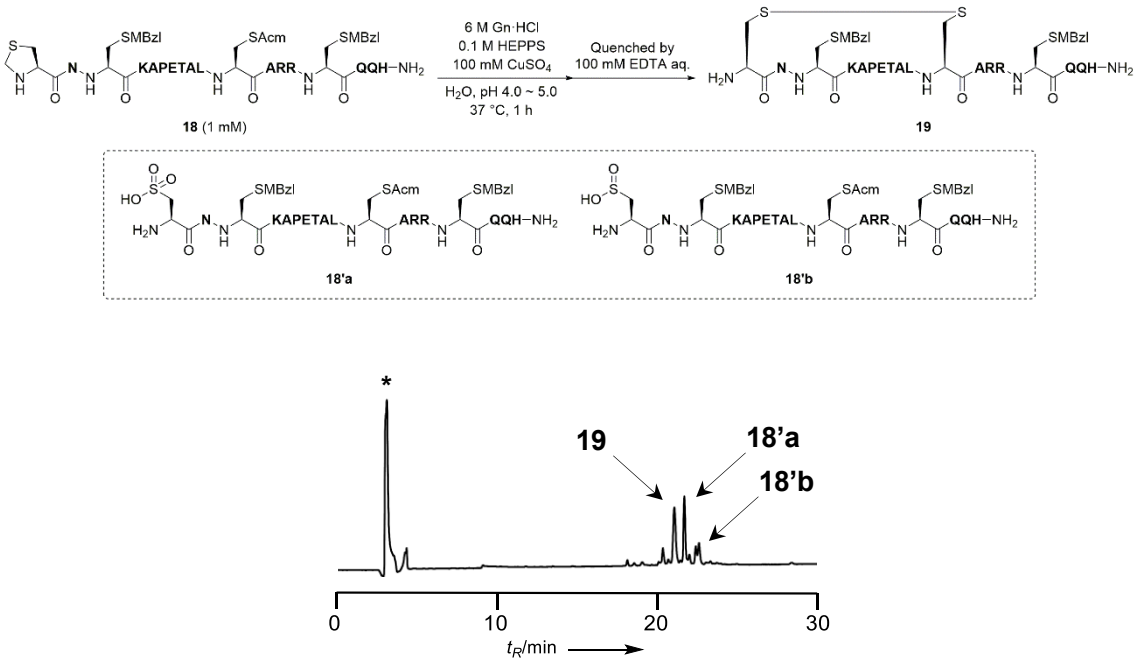


Figure S9. Analytical HPLC charts of crude reaction material. Analytical HPLC conditions: linear gradient of 0.1%TFA/CH_3CN in 0.1%TFA/H_2O, 5% to 40% over 30 min. *Non-peptide impurity.