Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Electronic Supplementary Information for:

Benzeneseleninic acid used as an oxidizing and deprotecting reagent for the synthesis of multi-cyclic peptides constrained by multiple disulfide bonds and thioether bridges

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HPLC elution profiles and mass spectrometric data for the synthesized peptides, 10 supporting tables (Tables S1-S10) and 64 supporting figures (Figures S1-S64) are involved in Electronic Supplementary Information.

Peptides

Linear *a*-conotoxin SI-1: H-IC(Acm)C(SH)NPAC(Acm)GPKYSC(SH)-NH_2. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 25 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 15.8 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{61}H_{100}O_{18}N_{18}S_4$ 750.3173; found 750.3167.

***a*-Conotoxin SI-1:** H-IC(Acm)CNPAC(Acm)GPKYSC-NH_2, disulfide Cys3/Cys13. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 25 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 13.9 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{61}H_{98}O_{18}N_{18}S_4$ 749.3095; found 749.3084.

Linear *a*-conotoxin SI-2 (H-IC(SH)C(Acm)NPAC(SH)GPKYSC(Acm)-NH_2). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 25 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 14.2 min.

***a*-Conotoxin SI-2** (H-ICC(Acm)NPACGPKYSC(Acm)-NH_2, Cys2/Cys7). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 25 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 14.8 min. HRMS (ESI) *m/z*: $[M+H^+]^+$ calcd for $C_{61}H_{97}O_{18}N_{18}S_4$ 1497.6128; found 1497.6128.

***a*-Conotoxin SI:** H-ICCNPACGPKYSC-NH_2, disulfides Cys2/Cys7, Cys3/Cys13. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 25 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 17.6 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{55}H_{86}O_{16}N_{16}S_4$; 677.2636, found 677.2635; $[M+H^+]^+$ calcd for $C_{55}H_{85}O_{16}N_{16}S_4$ 1353.5212; found 1353.5230.

***α*-Conotoxin SI**, (ICCNPACGPKYSC-NH_2, two disulfide bonds, Cys2/Cys7, Cys3/Cys13). The elution protocol for analytical HPLC (C18 column) was set as follows: 14-36% B over 25 min, and further to 90% over 5 min; Retention time = 14.4 min; ESI-HRMS *m/z*: $[M+2H^+]^{2+}$ calcd. for $C_{55}H_{86}O_{16}N_{16}S_4$ 677.2646; found 677.2628.

Linear *α*-conotoxin SI-3: H-IC(Acm)C(Acm)NPAC(SH)GPKYSC(SH)-NH_2. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 14.5 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{61}H_{100}O_{18}N_{18}S_4$ 750.3173; found 750.3167.

***α*-Conotoxin SI-3:** H-IC(Acm)C(Acm)NPACGPKYSC-NH_2, disulfide Cys7/Cys13. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 13.2 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{61}H_{98}O_{18}N_{18}S_4$ 749.3095; found 749.3087.

Bead *α*-conotoxin SI: H-ICCNPACGPKYSC-NH_2, disulfides Cys2/Cys3, Cys7/Cys13. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 15.4 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{55}H_{86}O_{16}N_{16}S_4$; 677.2636, found 677.2639.

Linear *α*-conotoxin SI-4: H-IC(Acm)C(SH)NPAC(SH)GPKYSC(Acm)-NH_2. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 15.4 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{61}H_{100}O_{18}N_{18}S_4$ 750.3173; found 750.31707.

***a*-Conotoxin SI-4:** H-IC(Acm)CNPACGPKYSC(Acm)-NH_2, disulfide Cys3/Cys7. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 14.1 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{61}H_{98}O_{18}N_{18}S_4$ 749.3095; found 749.3089..

Ribbon *a*-conotoxin SI: H-ICCNPACGPKYSC-NH_2, disulfides Cys2/Cys13, Cys3/Cys7. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min and further to 60% B over 8 min, and then to 90% over 4 min, retention time = 15.9 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{55}H_{86}O_{16}N_{16}S_4$; 677.2636, found 677.2640.

Linear apamin (H-C(Acm)NC(SH)KAPETALC(Acm)ARRC(SH)QQH-NH_2). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-50% B over 25 min, and then to 90% over 5 min, retention time = 11.3 min.

Apamin-1 (H-C(Acm)NCKAPETALC(Acm)ARRCQQH-NH_2, disulfide Cys3/Cys15). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-50% B over 25 min, and then to 90% over 5 min, retention time = 11.9 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{85}H_{145}O_{26}N_{33}S_4$; 1086.4974, found 1086.4972.

Apamin (H-CNCKAPETALCARRCQQH-NH_2, disulfides Cys1/Cys11 and Cys3/Cys15). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-50% B over 25 min, and then to 90% over 5 min, retention time = 10.1 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{79}H_{133}O_{24}N_{31}S_4$; 1014.4524, found 1014.4508.

Linear *a*-conotoxin IMI: H-GC(SH)C(Acm)SDPRC(SH)AWRC(Acm)-NH_2. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-60% B over

25 min and further to 90% B over 5 min, retention time = 13.1 min.

***a*-conotoxin IMI-1**: H-GCC(Acm)SDPRCAWRC(Acm)-NH_2, disulfide Cys2/Cys8. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-60% B over 25 min and further to 90% B over 5 min, retention time = 11.9 min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{58}H_{92}O_{17}N_{22}S_4$ 748.2947; found. 748.2939.

***a*-conotoxin IMI**: H-GCCSDPRCAWRC-NH_2, disulfides Cys2/Cys8, Cys3/Cys12. The elution protocol for analytical HPLC (C8 column) was set as follows: 10-60% B over 25 min and further to 90% B over 5 min, retention time = 12.1min; HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd For $C_{52}H_{80}O_{15}N_{20}S_4$ 676.2498; found 676.2491.

Linear M-peptide, (H-GC(SH)C(Acm)SDPFC(SH)NMNNPDYC(Acm)-NH_2). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-23% B over 10 min, further to 48% within 12 min, and then to 90% over 10 min, retention time = 17.1 min.

M-peptide-1, (H-GCC(Acm)SDPFCNMNNPDYC(Acm)-NH_2, disulfide Cys2/Cys8). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-23% B over 10 min, further to 48% within 12 min, and then to 90% over 10 min, retention time = 17.6 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{76}H_{110}O_{27}N_{22}S_5$; 961.3257, found 961.3248.

M-peptide, (GCCSDPFCNMNNPDYC-NH_2, disulfides Cys2/Cys8, Cys3/Cys16). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-23% B over 10 min, further to 48% within 12 min, and then to 90% over 10 min, retention time = 17.8 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{70}H_{98}O_{25}N_{20}S_5$ 889.2808; found 889.2798.

M(O)-peptide, (GCCSDPFCNM(O)NNPDYC-NH_2, disulfides Cys2/Cys8, Cys3/Cys16). The elution protocol for analytical HPLC (C8 column) was set as

follows: 10-23% B over 10 min, further to 48% within 12 min, and then to 90% over 10 min, retention time = 14.5 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{70}H_{98}O_{26}N_{20}S_5$ 897.2782; found 897.2779.

XCBP-1, The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min, further to 60% within 8 min, and then to 90% over 4 min, retention time = 25.6 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{102}O_{16}N_{16}S_4$ 781.3271; found 781.3262.

XCBP-2, The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min, further to 60% within 8 min, and then to 90% over 4 min, retention time = 26.9 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{102}O_{16}N_{16}S_4$ 781.3271; found 781.3261.

XCBP-3, The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min, further to 60% within 8 min, and then to 90% over 4 min, retention time = 27.0 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{102}O_{16}N_{16}S_4$ 781.3271; found 781.3258.

XCBP-4, The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min, further to 60% within 8 min, and then to 90% over 4 min, retention time = 27.2 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{102}O_{16}N_{16}S_4$ 781.3271; found 781.3261.

XCBP-5, The elution protocol for analytical HPLC (C8 column) was set as follows: 10-25% B over 20 min, further to 60% within 8 min, and then to 90% over 4 min, retention time = 27.0 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{102}O_{16}N_{16}S_4$ 781.3271; found 781.3261.

Reduced *α*-conotoxin SI, (H-IC(SH)C(SH)NPAC(SH)GPKYSC(SH)-NH_2). The elution protocol for analytical HPLC (C18 column) was set as follows: 14-36% B

over 25 min, and further to 90% over 5 min; Retention time = 13.5 min.

Isomer of *α*-conotoxin SI, (H-ICCNPACGPKYSC-NH_2, two disulfide bonds, Cys2/Cys3, Cys7/Cys13). The elution protocol for analytical HPLC (C18 column) was set as follows: 14-36% B over 25 min, and further to 90% over 5 min; Retention time = 12.5 min.

Isomer of *α*-conotoxin SI, (H-ICCNPACGPKYSC-NH_2, two disulfide bonds, Cys2/Cys13, Cys3/Cys7). The elution protocol for analytical HPLC (C18 column) was set as follows: 14-36% B over 25 min, and further to 90% over 5 min; Retention time = 12.9 min.

Reduced conotoxin mr3e (H-VC(SH)C(SH)PFGGC(SH)HELC(SH)YC(SH)C(SH)-D-NH_2). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-30% B over 20 min and further to 60% B over 5 min, and then to 90% over 5 min, retention time = 25.9 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{105}O_{21}N_{19}S_6$; 875.8028, found 875.8014.

Conotoxin mr3e (H-VCCPFGGCHELCYCCD-NH_2 Cys2/Cys14, Cys3/Cys12, Cys8/Cys15). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-30% B over 20 min and further to 60% B over 5 min, and then to 90% over 5 min, retention time = 20.5 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{99}O_{21}N_{19}S_6$; 872.7794, found 872.7775.

Isomer of conotoxin mr3e (H-VCCPFGGCHELCYCCD-NH_2, three disulfide bonds). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-30% B over 20 min and further to 60% B over 5 min, and then to 90% over 5 min, retention time = 21.8 min. HRMS (ESI) *m/z*: $[M+2H^+]^{2+}$ calcd for $C_{71}H_{99}O_{21}N_{19}S_6$; 872.7794, found 872.7776.

Reduced enterotoxin STp, (H-C(SH)C(SH)ELC(SH)C(SH)NPAC(SH)AGC(SH)Y-

OH). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-23% B over 10 min and further to 48% B over 12 min, and then to 90% over 10 min, retention time = 18.2 min. HRMS (ESI) *m/z*: $[M+H^+]^+$ calcd for $C_{55}H_{86}N_{15}O_{19}S_6$; 1452.4549, found 1452.4517.

Oxidized enterotoxin STp, (H-CCELCCNPACAGCY-OH, disulfides Cys1/Cys6, Cys2/Cys10, Cys5/Cys13). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-23% B over 10 min and further to 48% B over 12 min, and then to 90% over 10 min, retention time = 17.3 min. HRMS (ESI) *m/z*: $[M-H^+]^-$ calcd for $C_{55}H_{78}N_{15}O_{19}S_6$; 1444.3923, found 1444.3959.

Reduced μ-conotoxin KIIIA, (H-C(SH)C(SH)NC(SH)SSKWC(SH)RDHSRC(SH)C(SH)-NH_2). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-20% B over 25 min, and then to 90% over 10 min, retention time = 20.6 min.

KIIIA-T, (H-CCNCSSKWCRDHSRCC-NH_2, Cys1/Cys15, Cys2/Cys9, Cys4/Cys16). The elution protocol for analytical HPLC (C8 column) was set as follows: 10-20% B over 25 min, and then to 90% over 10 min, retention time = 9.6 min. HRMS (ESI) *m/z*: $[M+3H^+]^{3+}$ calcd for $C_{70}H_{109}N_{28}O_{22}S_6$; 628.5531, found 628.5512.

Reduced linaclotide, (H-C(SH)C(SH)EYC(SH)C(SH)NPAC(SH)TGC(SH)Y-NH_2). The elution protocol for analytical HPLC (C18 column) was set as follows: 10-30% B over 10 min, and further to 60% over 15 min, finally to 90% over 5; Retention time = 15.2 min.

Linaclotide, (H-CCEYCCNPACTGCY-NH_2, three disulfide bonds, Cys2/Cys10, Cys5/Cys13 and Cys1/Cys6). The elution protocol for analytical HPLC (C18 column) was set as follows: 10-30% B over 10 min, and further to 60% over 15 min, finally to 90% over 5; Retention time = 15.0 min; ESI-HRMS *m/z*: $[M+2H^+]^{2+}$ calcd. for $C_{59}H_{81}N_{15}O_{21}S_6$ 763.7028, found 763.7007.

Reduced ziconotide, (H-C(SH)KGKGAKC(SH)SRLMYDC(SH)C(SH)TGSC-(SH)RSGKC(SH)-NH_2). The elution protocol for analytical HPLC (C18 column) was set as follows: 10-30% B over 20 min, and further to 35% over 10 min, finally to 90% over 7 min; Retention time = 18.8 min.

Ziconotide, (H-CKGKGAKCSRLMYDCCTGSCRSGKC-NH_2, three disulfide bonds, Cys1/Cys16, Cys8/Cys20, Cys15/Cys25). The elution protocol for analytical HPLC (C18 column) was set as follows: 10-30% B over 20 min, and further to 35% over 10 min, finally to 90% over 7 min; Retention time = 15.8 min. ESI-HRMS *m/z*: $[M+3H^+]^{3+}$ calcd. for $C_{102}H_{175}N_{36}O_{32}S_7$ 880.3748, found 880.3728.

Table S1. Linear *a*-conotoxin SI-1 (0.5 mM) reaction with BSA at room temperature.

Entry	Solvent	Amount of BSA (Equiv.)[a]	Time (min)	Conversion [b] (%)	Yield [c] (%)
i	*p*H = 7.0 phosphate buffer	0.3	50	92	78
ii	*p*H = 7.0 phosphate buffer	0.5	20	100	89
iii	*p*H = 7.0 phosphate buffer	1.0	20	80	85
iv	H_2O	0.5	50	65	75
v	*p*H = 7.0 Tris buffer	0.5	20	87	86
vi[d]	*p*H = 7.0 phosphate buffer	0.5	20	88	84
vii	*p*H = 6.0 phosphate buffer	0.5	120	75	60
viii	*p*H = 8.0 phosphate buffer	0.5	20	80	85

[a] Equivalent of BSA to peptide; [b] Conversion was calculated by the equation: Conversion = (peak area of *a*-conotoxin SI-1)/(peak area of linear *a*-conotoxin SI-1) × 100%; [c] The yield was calculated by the peak areas of *a*-conotoxin SI-1; [d] 1.0 mM of linear *a*-conotoxin SI-1.

Table S2. *a*-Conotoxin SI-1 (0.5 mM) reaction with BSA at room temperature.

Entry	Solvent	Amount of BSA (Equiv.)[a]	Time (min)	Conversion [b] (%)	Yield [c] (%)
i	1.0 M TFA, 0.5 M NaBr	1.0	15	93	85
ii	0.8 M TFA, 0.5 M NaBr	0.5	20	75	78
iii	0.8 M TFA, 0.5 M NaBr	1.0	20	100	87
iv	0.8 M TFA, 0.5 M NaBr	1.5	20	87	87
v	0.5 M TFA, 0.5 M NaBr	1.0	60	45	67
vi	0.8 M TFA, 0.3 M NaBr	1.0	50	81	77

[a] Equivalent of BSA to peptide; [b] Conversion was calculated by the equation: Conversion = (peak area of *a*-conotoxin SI)/(peak area of linear *a*-conotoxin SI-1) × 100%; [c] The yield was calculated by the peak areas of *a*-conotoxin SI.

Table S3. Oxy(Acm) (0.25 mM) reaction with BSA (0.125 mM) in various solvents at room temperature.

Entry	Solvents	Time (min)	Conversion [a] (%)	Yield [b] (%)
i	TFA	60	79	78
ii	1.0 M TFA	60	-	-
iii	2.0 M TFA	60	-	-
iv	1.0 M HCl	60	-	-
v	1.0 M HBr	10	74	84
vi	0.8 M HBr	10	78	93
vii	0.5 M HBr	60	70	92
viii	0.1 M HBr	60	3	2
ix	1.0 M NaBr	5 h	-	-
x	0.1 M NaBr, 1.0 M TFA	60	10	12
xi	0.5 M NaBr, 1.0 M TFA	60	75	92
xii	1.0 M NaBr, 1.0 M TFA	40	71	93
xiii	2.0 M NaBr, 1.0 M TFA	10	78	93
xiv	1.0 M NaBr, 0.5 M TFA	60	80	92
xv	1.0 M NaBr, 2.0 M TFA	10	74	90

xvi	0.5 M NaBr, 0.5 M TFA	3 h	75	85

[a] Conversion was calculated by the equation: Conversion = (peak area of generated oxytocin)/(peak area of oxy(Acm)) × 100%; "-" : no reaction occurred.

Table S4. Reactions of reduced *a*-conotoxin SI (0.2 mM) with BSA at room temperature.

Entry	Solvent	Amount of BSA (Equiv.)[a]	Time	Yield [b] (%)	Conversion [c] (%)
i	pH 5.0 Phosphate buffer	1	50	35	15
ii	pH 6.0 Phosphate buffer	1	30	70	62
iii		0.5	30	70	49
iv	pH 7.0 Phosphate buffer	1	30	90	110
v		1.5	45	86	98
vi	pH 8.0 Phosphate buffer	1	45	73	88
vii[d]	pH 7.0 Phosphate buffer	1	50	65	77
viii[e]	pH 7.0 Phosphate buffer	1	50	84	85
ix[f]	pH 7.0 Phosphate buffer	1	50	82	72
x	10 mM HCl	0.5	60	-	-
xi	pH 7.0 Gn.HCl	1	60	32	33

[a] Equivalent of BSA to peptide; [b] Yield was calculated by the peak area of *a*-conotoxin SI and its isomers; [c] Conversion was calculated by the equation: Conversion = (peak area of *a*-conotoxin SI)/(peak area of reduced *a*-conotoxin SI) × 100%; [d] Reaction of 0.1 mM *a*-conotoxin SI with 0.1 mM BSA; [e] Reaction of 0.5 mM *a*-conotoxin SI with 0.5 mM BSA; [f] Reaction of 1.0 mM *a*-conotoxin SI with 1.0 mM BSA; "-" : No *a*-conotoxin SI was generated.

Table S5. The reaction of reduced conotoxin mr3e (0.2 mM) with BSA in pH 7.0 phosphate buffer solution at 25 ℃.

Entry	Amount of BSA (equiv.[a])	Time (min)	Yield [b] (%)	Conversion [c] (%)
i	0.2	240	83	129
ii	0.3	150	83	128
iii	0.5	45	81	115
iv		150	83	140
v	1.0	45	83	142
vi		150	82	141
vii	1.5	45	69	95
viii		150	70	104

[a] Equivalent of BSA to peptide; [b] Yield of conotoxin mr3e was calculated by the peak areas of the generated conotoxin mr3e and its isomer; [c] Conversion was calculated by the equation: Conversion = (peak area of conotoxin mr3e)/(peak area of reduced conotoxin mr3e) × 100%.

Table. S6 Oxidation folding of reduced conotoxin mr3e (0.2 mM) by GSH/GSSG in pH 8.0 Tris buffer solution at 25 ℃.

Entry	Reduced conotoxin mr3e : GSSG : GSH	Time (h)	Yield [a] (%)	Conversion [b] (%)
i	1:10:100	1	82%	114
ii		2	83%	128
iii		4	84%	130
iv		23	84%	130
v	1:10:10	1	84%	164
vi		2	84%	173
vii		3	86%	172
viii		19	86%	175

[a] Yield of conotoxin mr3e was calculated by the peak areas of the generated conotoxin mr3e and its isomer; [b] Conversion was calculated by the equation: Conversion = (peak area of conotoxin mr3e)/(peak area of reduced conotoxin mr3e) × 100%.

Table S7. 0.2 mM reduced STp reaction with BSA in pH 7.5 phosphate buffer solution at 25 ℃.

Entry	Amount of BSA (equiv.)	Time (min)	Yield [a] (%)	Conversion [b] (%)
i	0.5	45	94	63
ii		3 h	91	74
iii		6 h	91	75
iv	1	45	96	85
v		90	94	85
vi	1.5	45	9	4.3

[a] Yield was calculated by the peak area of oxidized STp; [b] Conversion was calculated by the equation: Conversion = (peak area of oxidized STp)/(peak area of reduced STp) × 100%.

Table S8. 0.4 mM reduced μ-conotoxin KIIIA reaction with BSA in pH 7.0 phosphate buffer solution at 25 ℃.

Entry	Amount of BSA (equiv.)	Time (min)	Yield [a] (%)	Conversion [b] (%)
i	0.5	45	78	53
ii		2.5 h	80	88
iii		9 h	80	88
iv	1	45	82	93
v		1.5 h	82	97
vi		2.5 h	82	105
vii	1.5	45	80	96
viii		1.5	80	105
ix[c]	1	1.5	80	105

[a] Yield was calculated by the peak areas of the generated μ-conotoxin KIIIA and its isomer; [b] Conversion was calculated by the equation: Conversion = (peak area of reduced μ-conotoxin KIIIA)/(peak area of μ-conotoxin KIIIA) × 100%; [c] [reduced μ-conotoxin KIIIA] = 0.2 mM.

Table S9. The reaction of reduced linaclotide (0.2 mM) with BSA at 25 ℃.

Entry	Solvent	Amount of BSA (Equiv.)[a]	Time	Yield [b] (%)	Conversion [c] (%)
i		0.8	50	60	34
ii	PH 7.0 phosphate buffer	1	50	88	91
iii		1.5	50	28	21
iv	PH 8.0 phosphate buffer	1	50	40	33
v	PH 7.00 6MGn.HCl	1	50	-	-

[a] Equivalent of BSA to peptide; [b] Yield was calculated by the peak area of the generated linaclotide; [c] Conversion was calculated by the equation: conversion = (peak area of generated linaclotide/ peak area of reduced linaclotide) × 100%; "-" : no linaclotide was generated.

Table S10. The reaction of reduced ziconotide (0.2 mM) with BSA (0.2 mM) for 60 min at 25 ℃.

Entry	Solvent	Yield [a] (%)	Conversion [b] (%)
i	H_2O	-	-
ii	pH 7.0 CH_3COONH_4 solution	-	-
iii	Tris pH 7.0	61	43
iv[c]	Phosphate buffer	88	65
v[d]	Phosphate buffer	80	53
vi[e]	Phosphate buffer	95	140
vii[f]	Phosphate buffer	86	81

[a] Yield was calculated by the equation: Yield = (peak area of generated linaclotide/ peak areas of all peptide contents) × 100%; [b] Conversion was calculated by the equation: conversion = (peak area of generated linaclotide/ peak area of reduced linaclotide) × 100%; [c] Ziconotide in H_2O (0.4 mM, 0.4 mL) mixed with BSA in pH 7.0 phosphate buffer (0.4 mM, 0.4 mL); [d] Ziconotide in H_2O (0.2 mM, 0.4 mL) mixed with BSA in pH 7.0 phosphate buffer (0.2 mM, 0.4 mL); [e] Ziconotide in H_2O (0.4 mM, 0.4 mL) mixed with 0.4 mL pH 7.0 phosphate buffer, followed by treatment with BSA in 7.0 phosphate buffer (4.0 mM, 40 □L); [f] Ziconotide in H_2O (0.2 mM, 0.4 mL) mixed with 0.4 mL pH 7.0 phosphate buffer, followed by treatment with BSA in 7.0 phosphate buffer (4.0 mM, 20 □L); "-" : No linaclotide was generated.

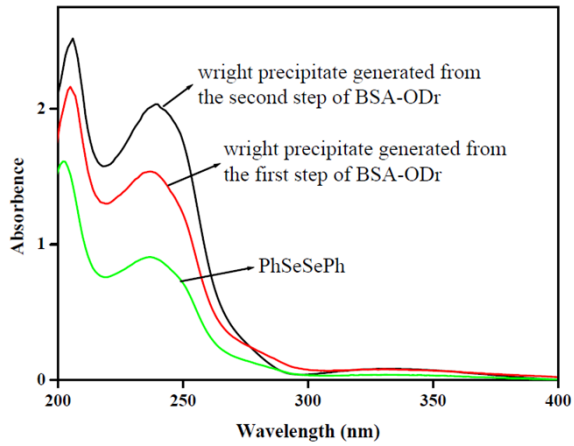


Fig. S1 The precipitate was characterized as diphenyl diselenide by use of UV-Vis spectrometer.

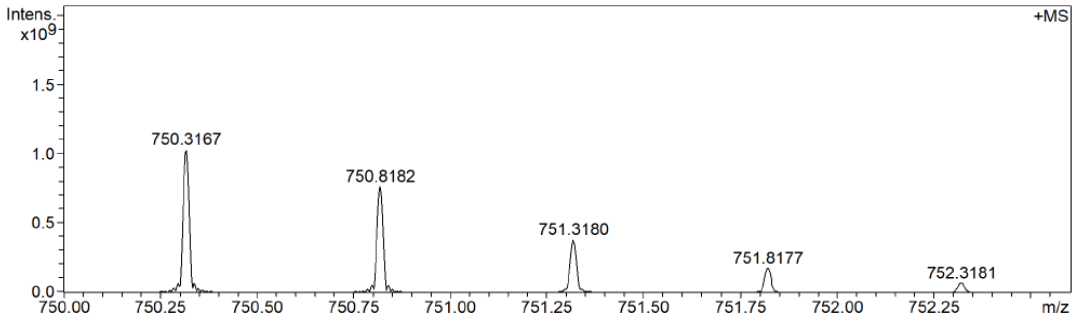


Fig. S2 Mass spectrum of linear *a*-conotoxin SI-1.

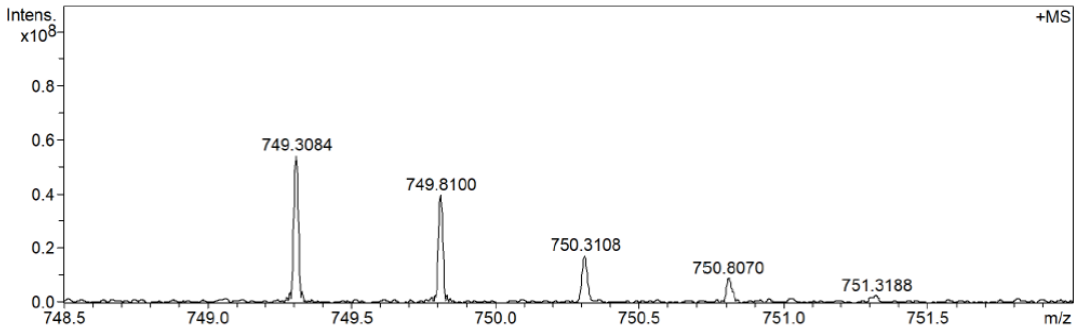


Fig. S3 Mass spectrum of *a*-conotoxin SI-1.

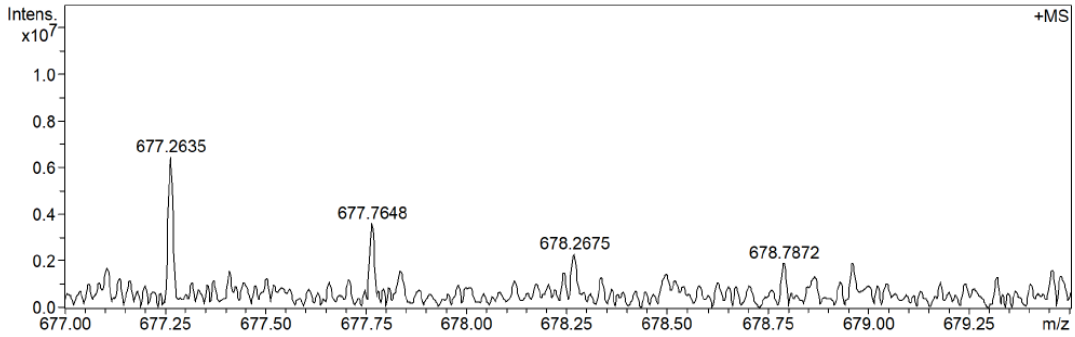


Fig. S4 Mass spectrum of *a*-conotoxin SI.

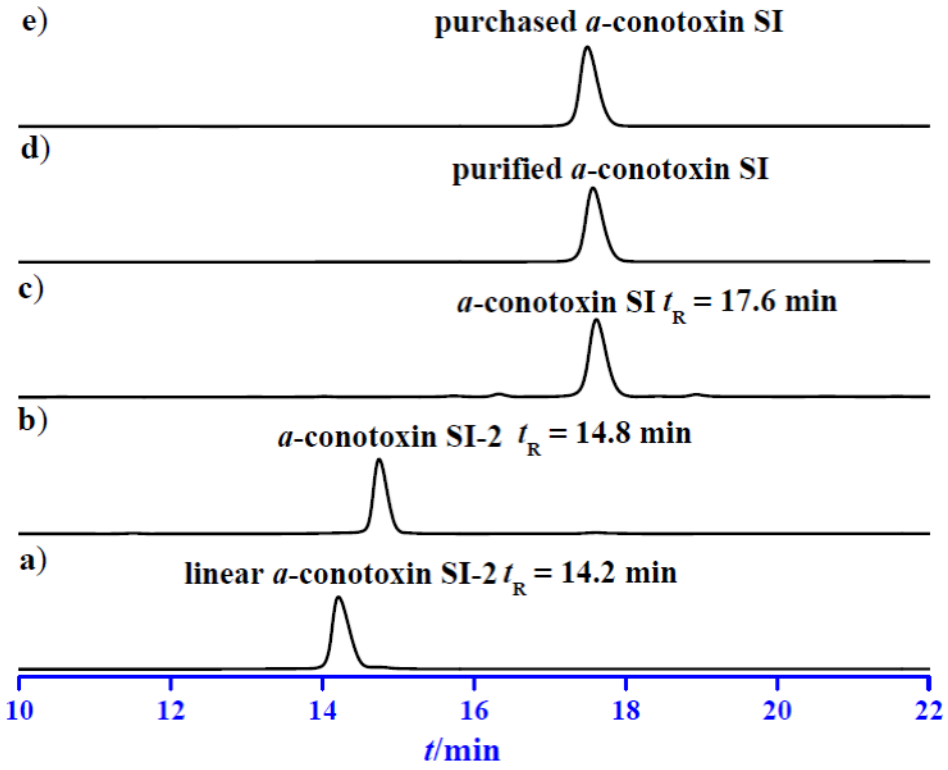


Fig. S5 HPLC chromatograms of a) linear *a*-conotoxin SI-2; b) the generated *a*-conotoxin SI-2 from the reaction of linear *a*-conotoxin SI-2 with BSA in pH 7.0 phosphate buffer; c) the generated *a*-conotoxin SI from the reaction of *a*-conotoxin SI-2 with BSA in 0.8 M TFA and 0.5 M HBr; d) the purified *a*-conotoxin SI; e) the purchased *a*-conotoxin SI.

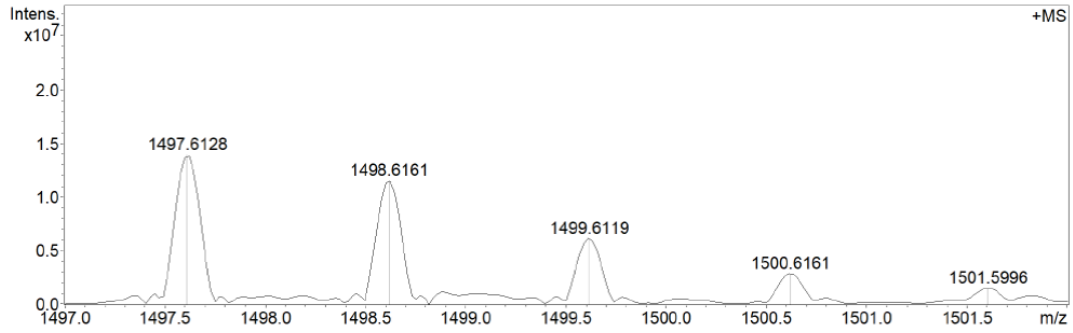


Fig. S6 Mass spectrum of *a*-conotoxin SI-2.

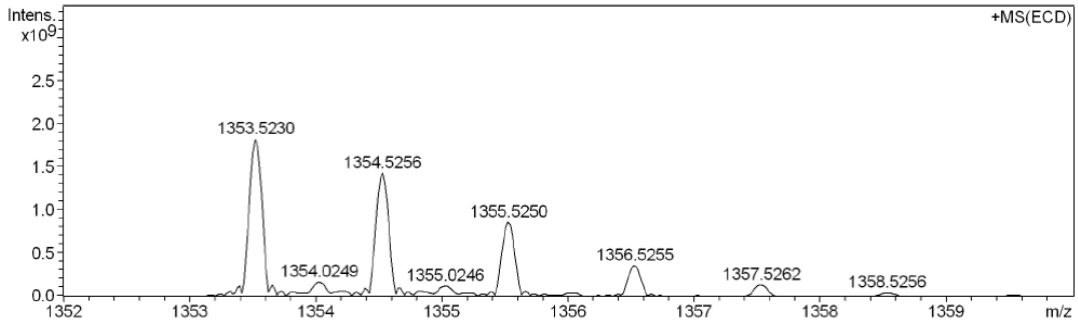


Fig. S7 Mass spectrum of *a*-conotoxin SI.

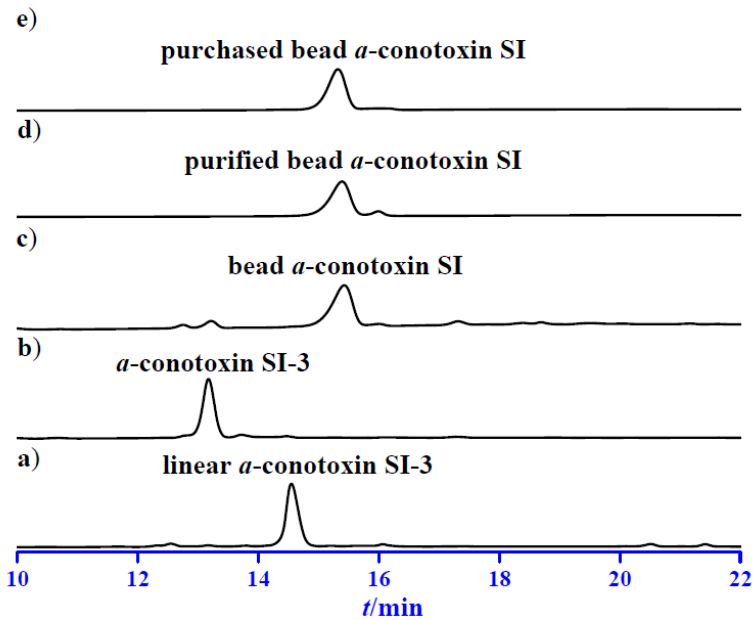


Fig. S8 HPLC chromatograms of a) linear *a*-conotoxin SI-3; b) the generated *a*-conotoxin SI-3 from the reaction of linear *a*-conotoxin SI-3 with BSA in pH 7.0 phosphate buffer; c) the generated bead *a*-conotoxin SI from the reaction of *a*-conotoxin SI-3 with BSA in 0.8 M TFA and 0.5 M HBr; d) the purified bead *a*-conotoxin SI; e) the purchased bead *a*-conotoxin SI.

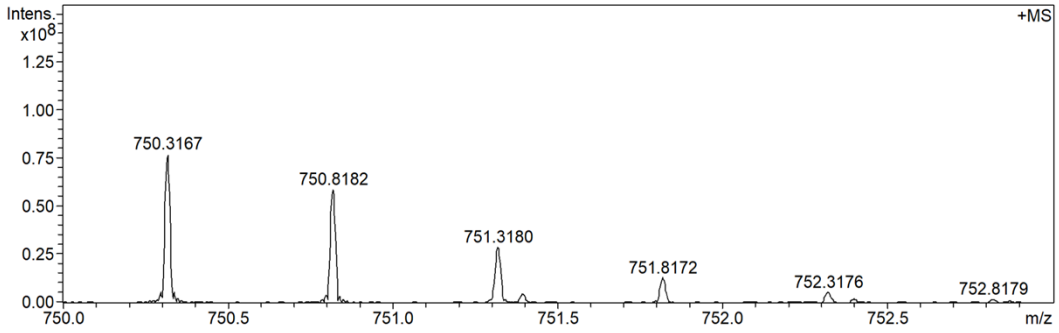


Fig. S9 Mass spectrum of linear *a*-conotoxin SI-3.

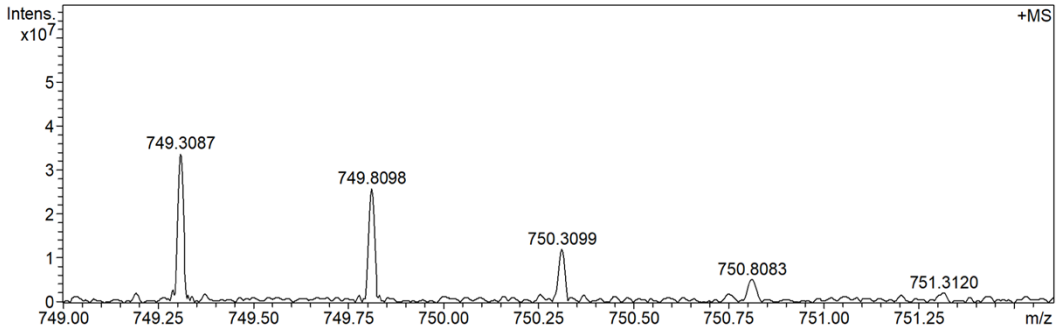


Fig. S10 Mass spectrum of *a*-conotoxin SI-3.

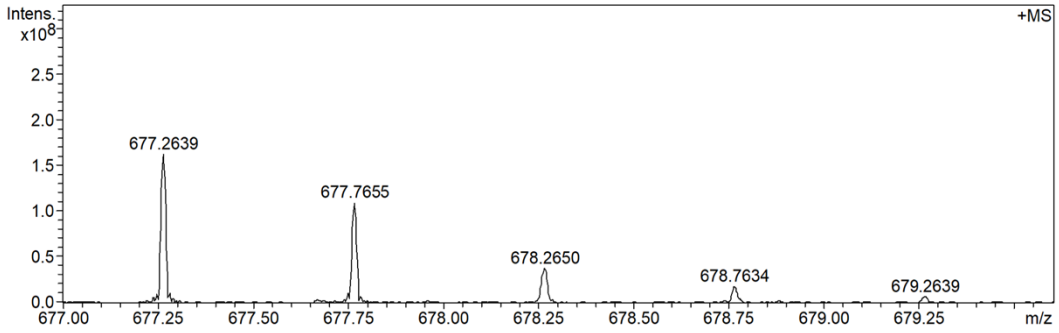


Fig. S11 Mass spectrum of bead *a*-conotoxin SI.

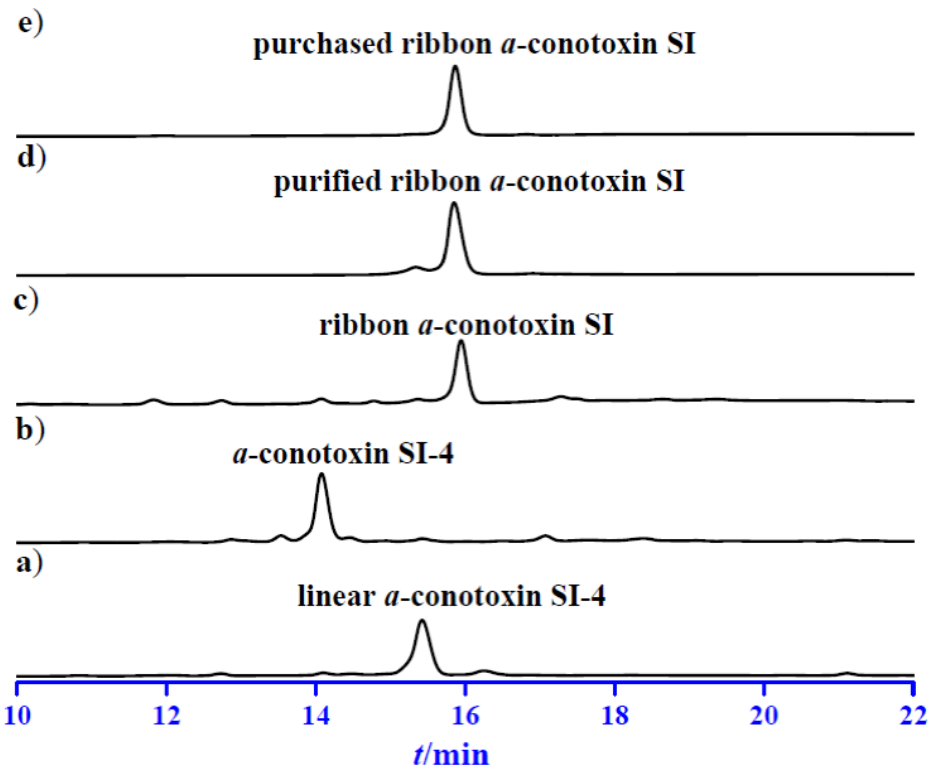


Fig. S12 HPLC chromatograms of a) linear *a*-conotoxin SI-4; b) the generated *a*-conotoxin SI-4 from the reaction of linear *a*-conotoxin SI-4 with BSA in pH 7.0 phosphate buffer; c) the generated ribbon *a*-conotoxin SI from the reaction of *a*-conotoxin SI-4 with BSA in 0.8 M TFA and 0.5 M HBr; d) the purified ribbon *a*-conotoxin SI; e) the purchased ribbon *a*-conotoxin SI.

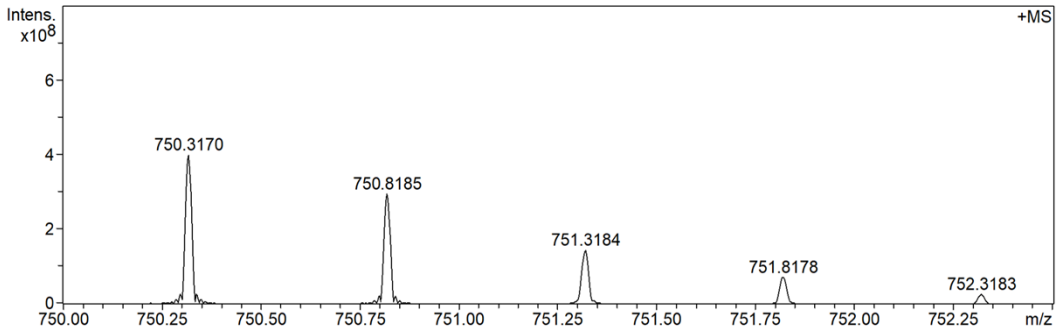


Fig. S13 Mass spectrum of linear *a*-conotoxin SI-4.

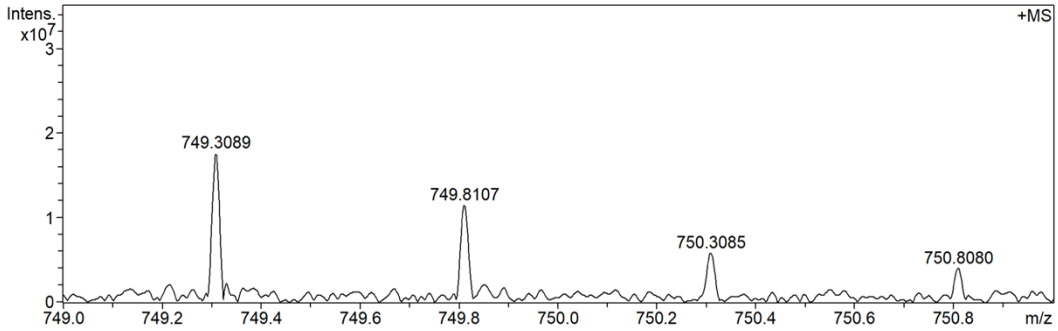


Fig. S14 Mass spectrum of *a*-conotoxin SI-4.

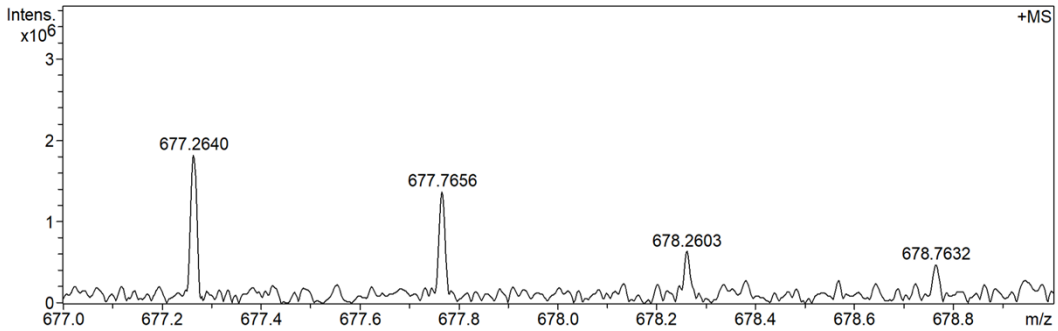


Fig. S15 Mass spectrum of ribbon *a*-conotoxin SI.

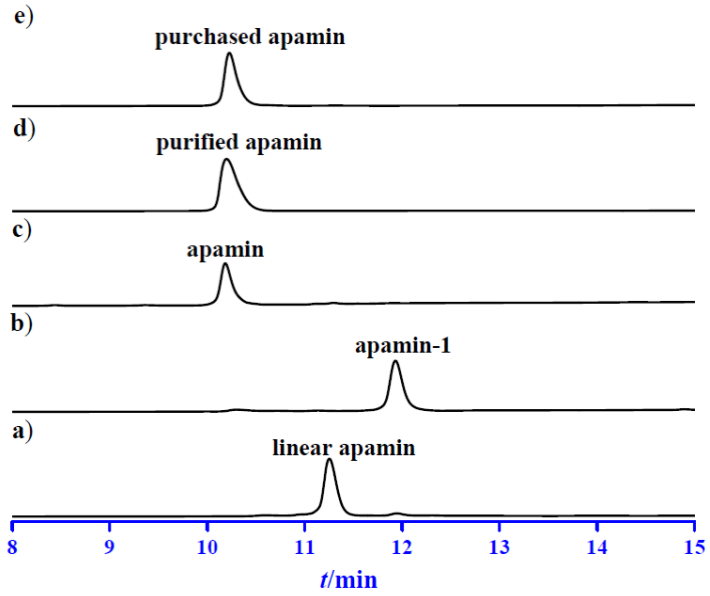


Fig. S16 HPLC chromatograms of a) linear apamin; b) apamin-1 obtained was generated from the reaction between linear apamin reacted and BSA; c) apamin obtained was generated by reaction of apamin-1 with BSA; d) the purified apamin; e) the commercially available apamin.

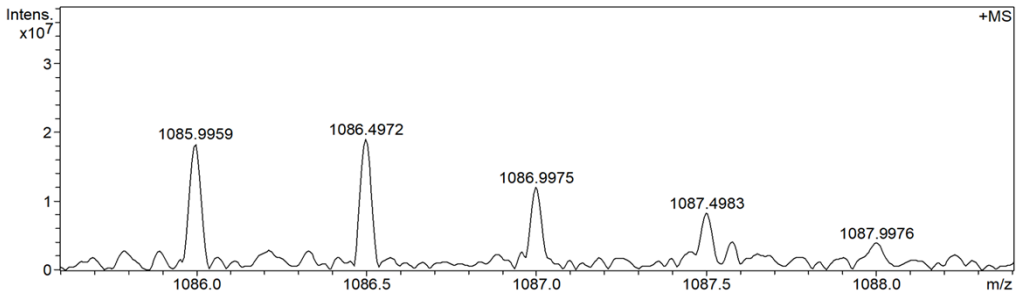


Fig. S17 Mass spectrum of apamin-1.

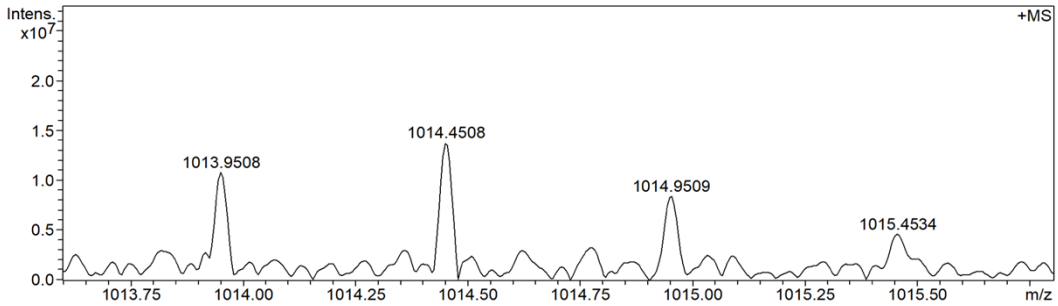
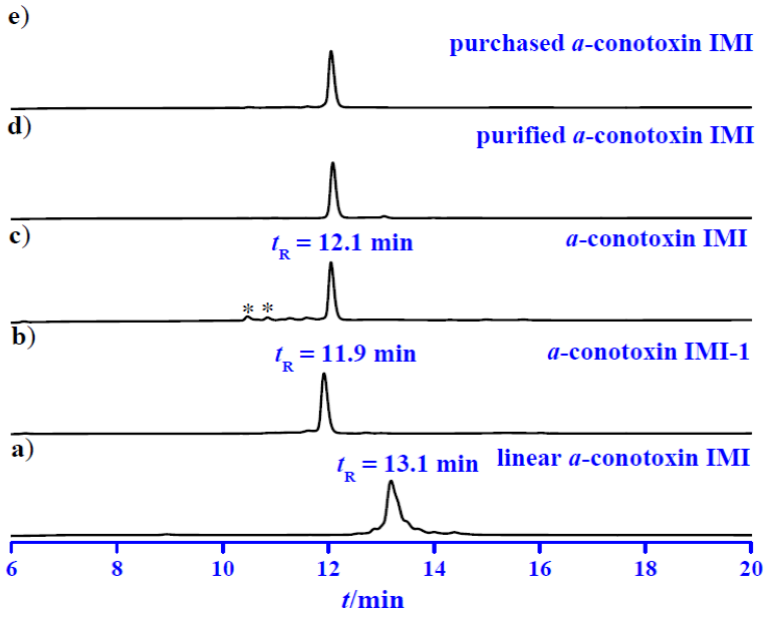
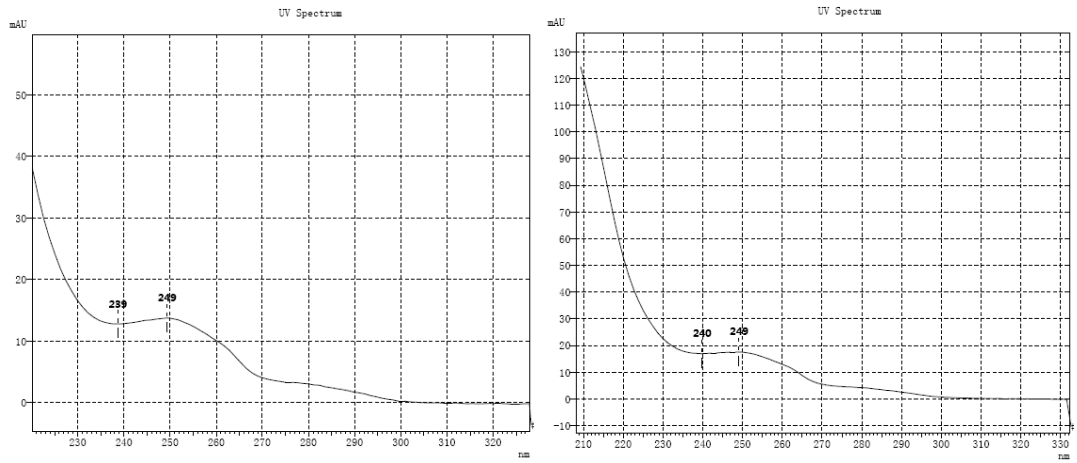


Fig. S18 Mass spectrum of apamin.





Side product, t_R = 10.4 min Side product, t_R = 10.8 min

Fig. S19 HPLC chromatograms of a) linear *a*-conotoxin IMI; b) the generated *a*-conotoxin IMI-1 from the reaction of linear *a*-conotoxin IMI with BSA; c) the generated *a*-conotoxin IMI from the reaction of *a*-conotoxin IMI-1 with BSA; d) the purified *a*-conotoxin IMI; e) the purchased *a*-conotoxin IMI. The * signs in the chromatograms indicate the side products generated from the oxidation of the tryptophan residue. The two side products with an absorption band around 250 nm are all *β*-oxindolyalanine characterized by UV-vis spectrometer [1].

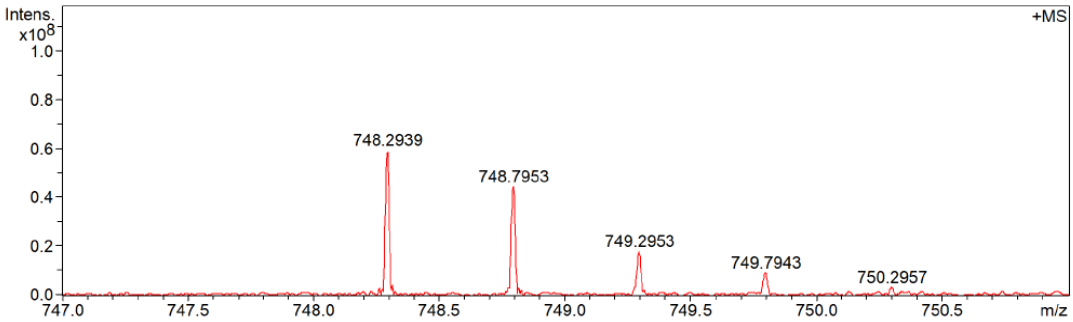


Fig. S20 Mass spectrum of the generated *a*-conotoxin IMI-1.

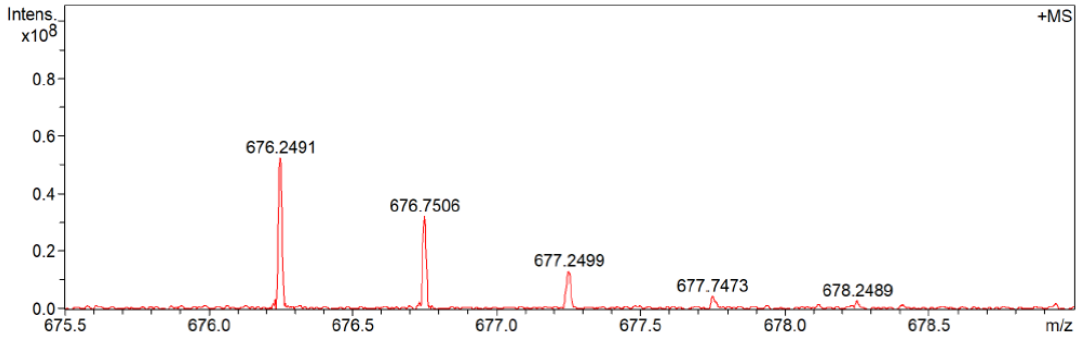


Fig. S21 Mass spectrum of the generated *a*-conotoxin IMI.

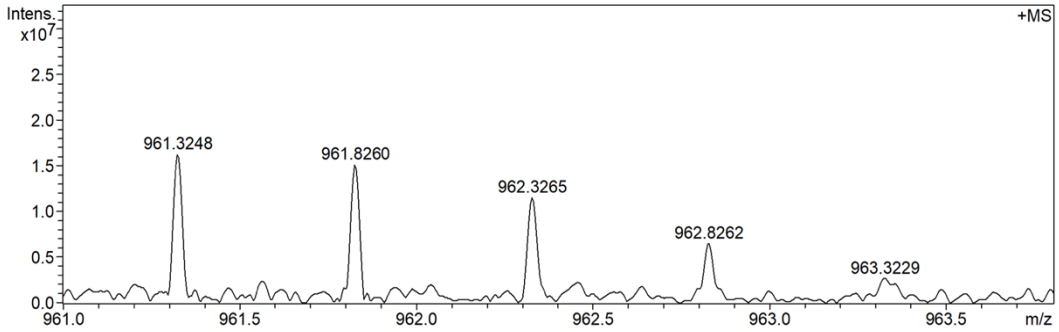


Fig. S22 Mass spectrum of M-peptide-1.

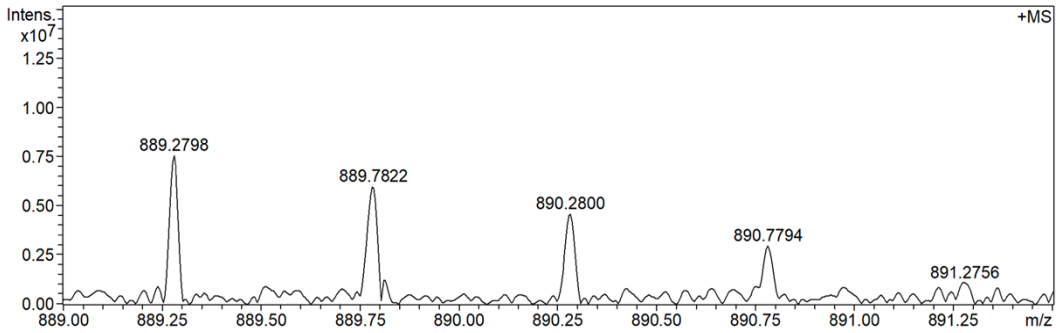


Fig. S23 Mass spectrum of M-peptide.

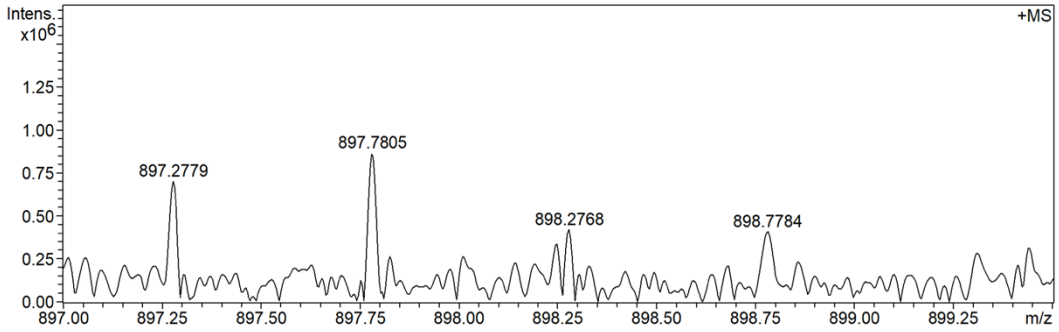


Fig. S24 Mass spectrum of M(O)-peptide.

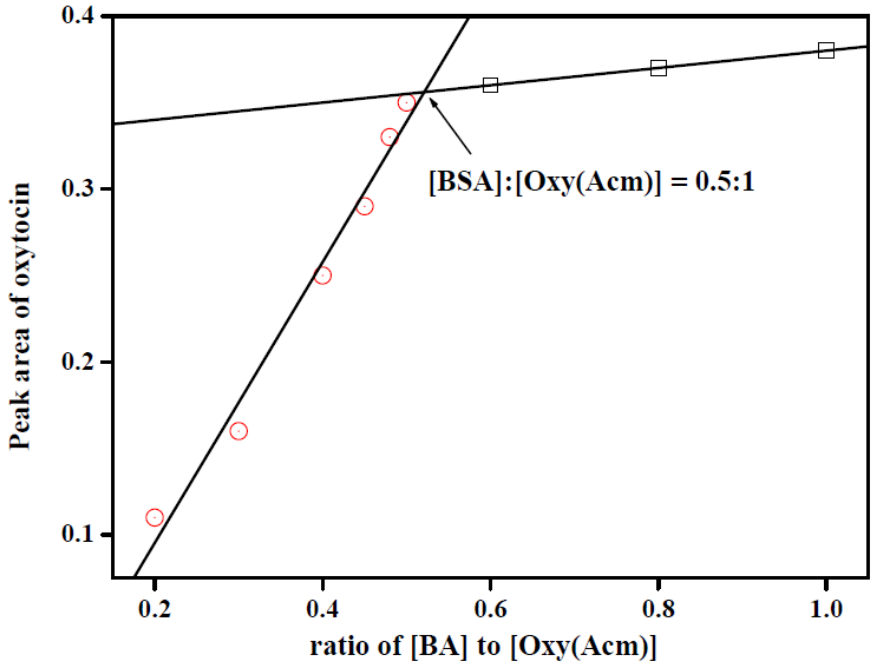


Fig. S25 The stoichiometric ratio of the deprotection of two Acm groups to generate oxytocin by BSA.

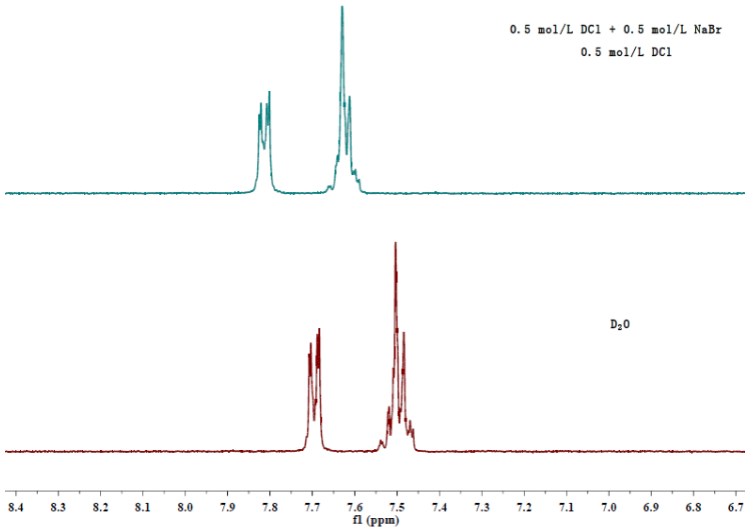


Fig. S26 NMR spectra of BSA.

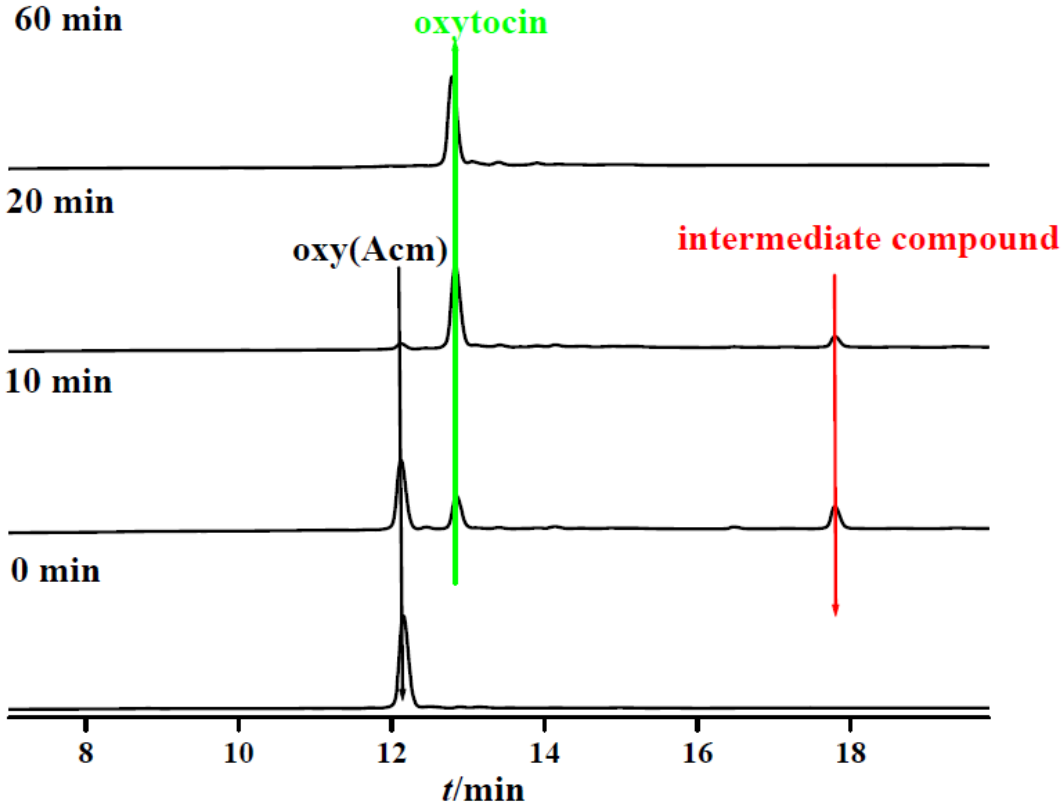


Fig. S27 BSA (0.125 mM) reaction with oxy(Acm) (0.25 mM) in 0.5 M HBr.

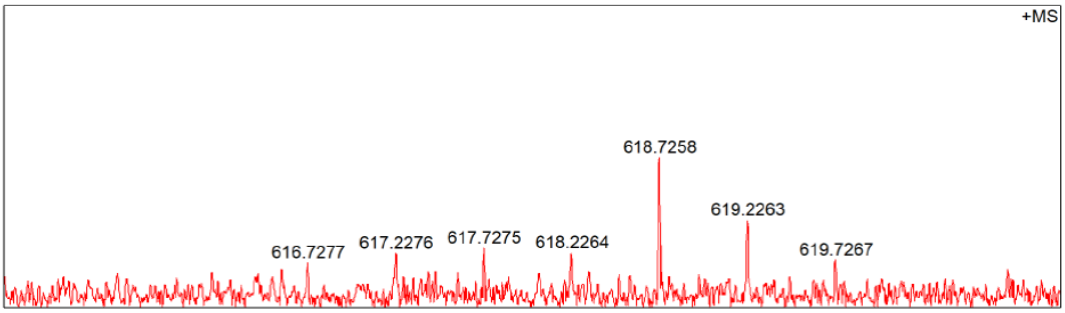


Fig. S28 Mass spectrum of intermediate compound **6**.

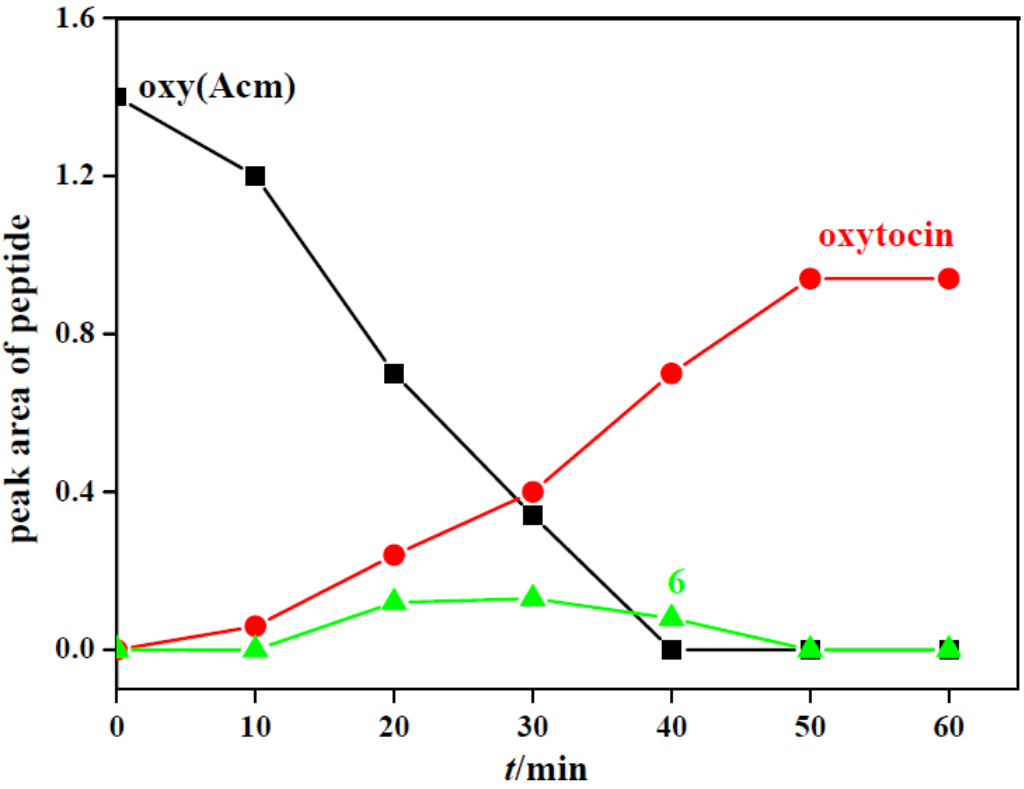


Fig. S29 Kinetic curve of the reaction between BSA (1.0 mM) and oxy(Acm) (0.1 mM) in 0.2 M HBr solution at 25 ℃.

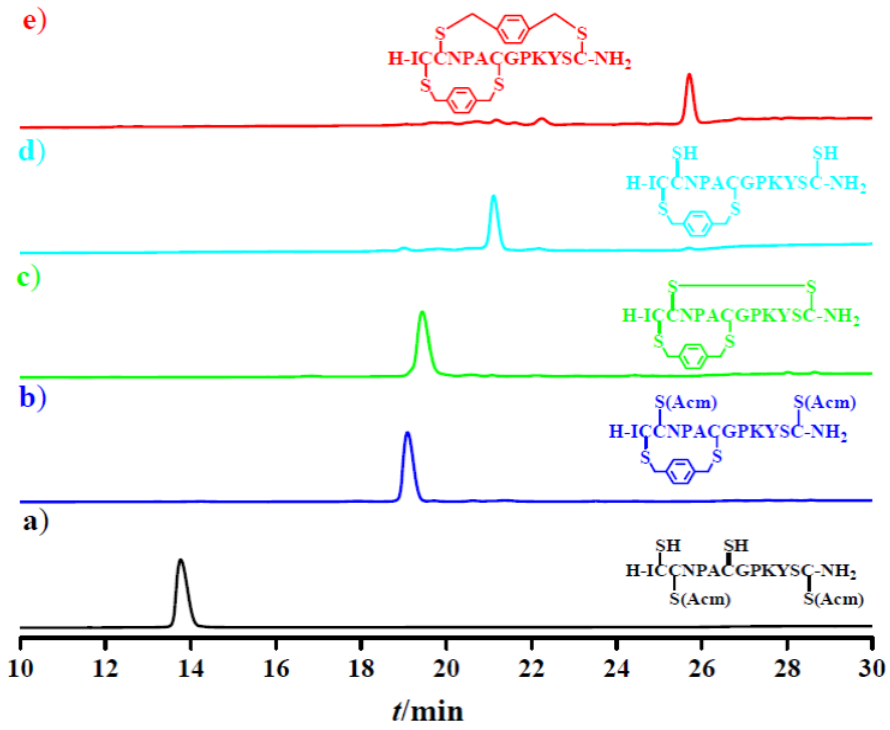


Fig. S30 One-pot synthesis of **XCBP-1**.

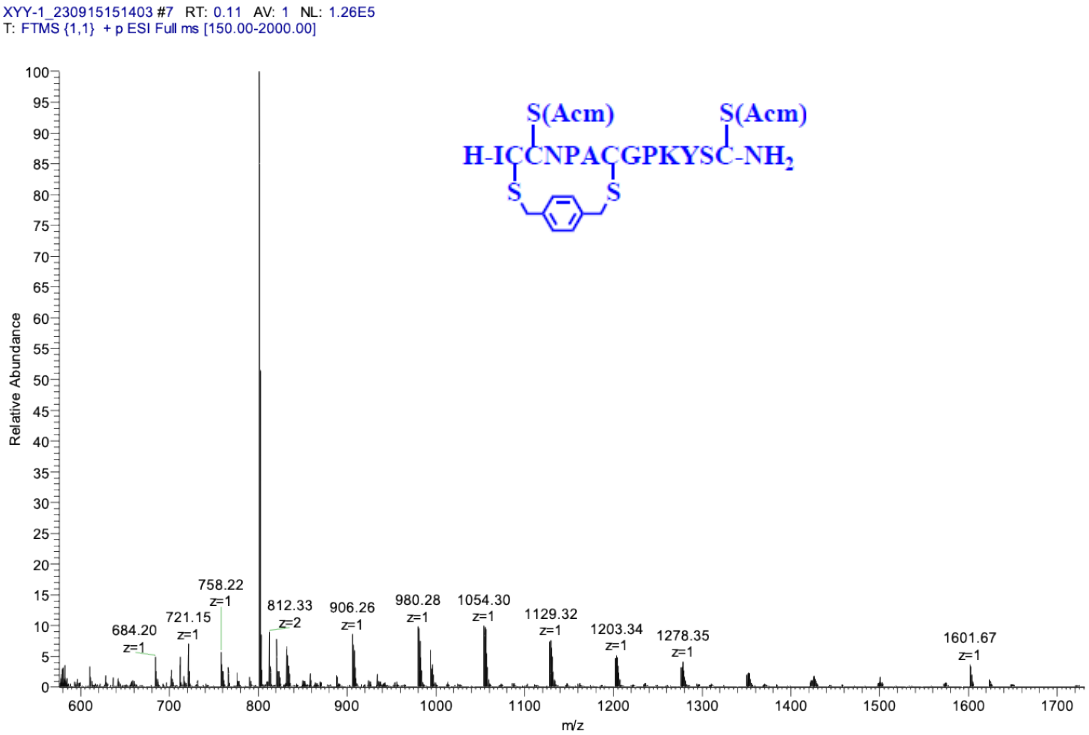


Fig. S31 Mass spectrum of generated peptides as shown in Fig. S29b.

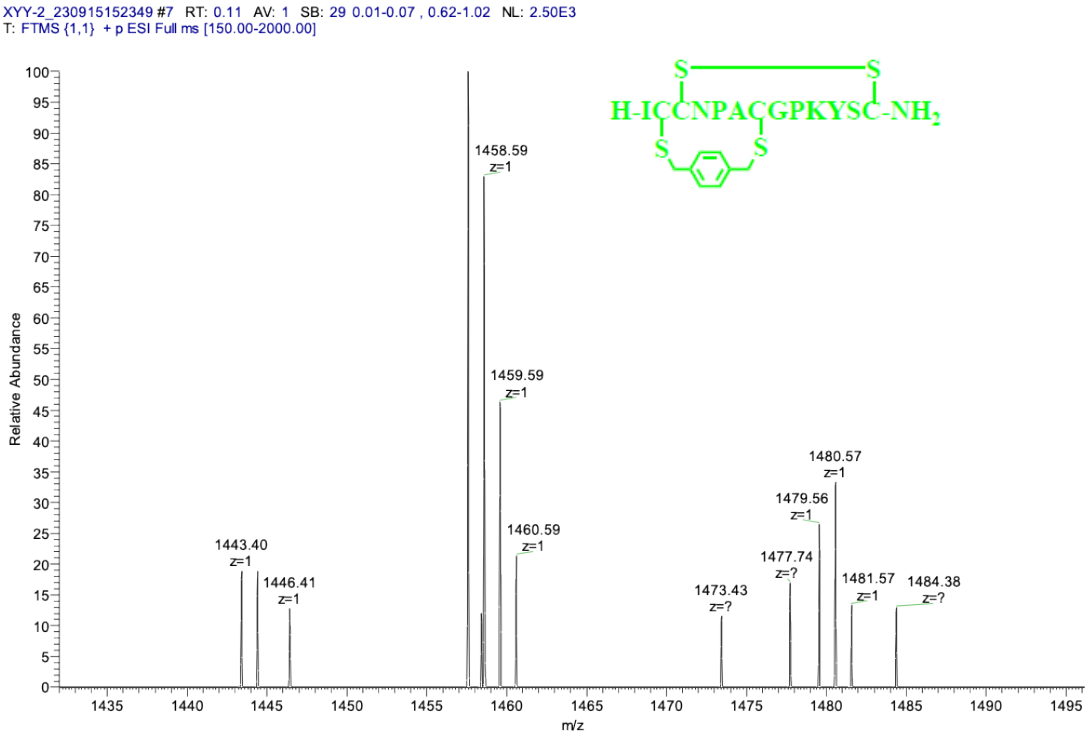


Fig. S32 Mass spectrum of generated peptides as shown in Fig. S29c.

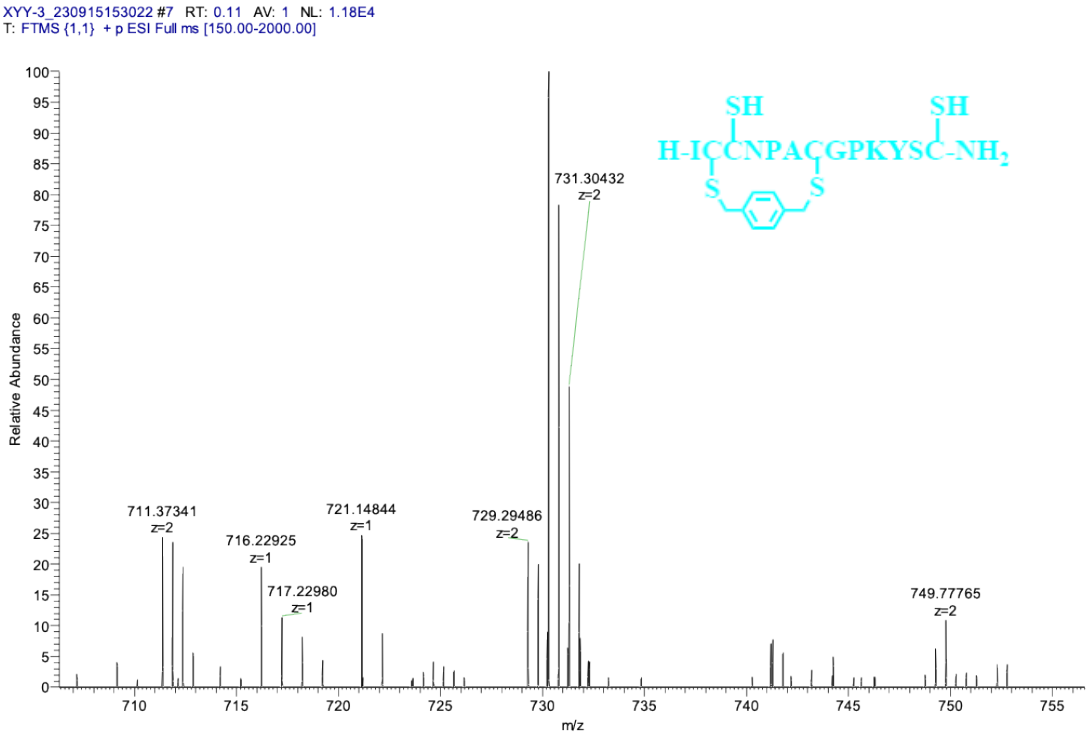


Fig. S33 Mass spectrum of generated peptides as shown in Fig. S29d.

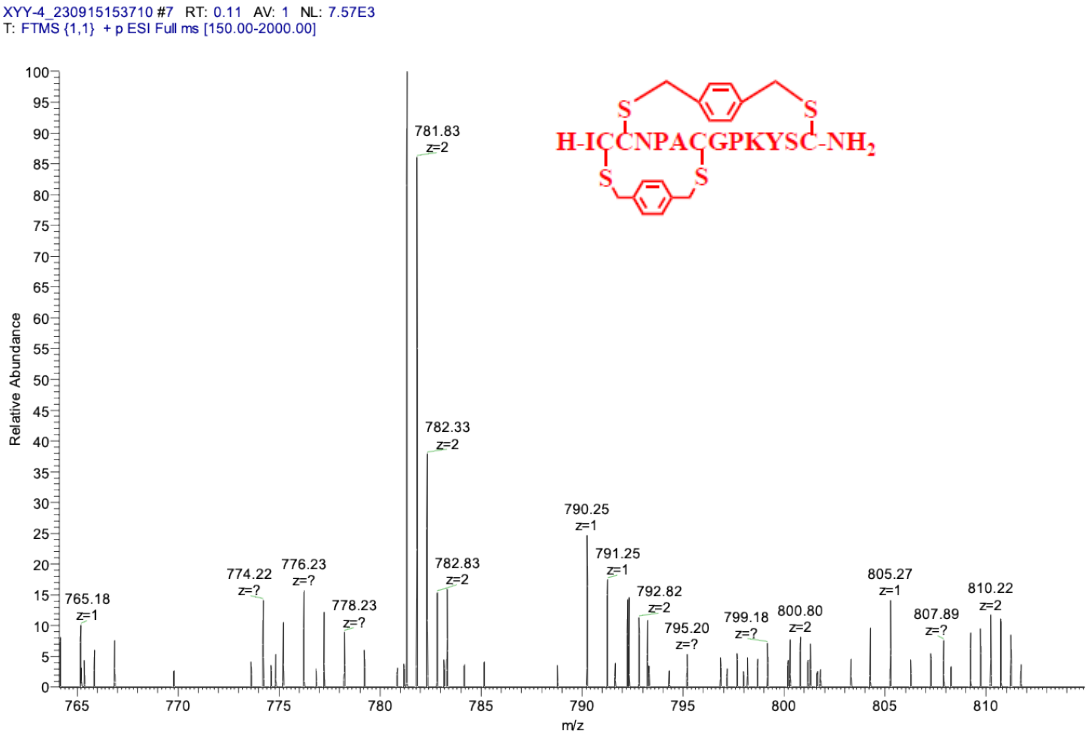


Fig. S34 Mass spectrum of generated peptides as shown in Fig. S29e.

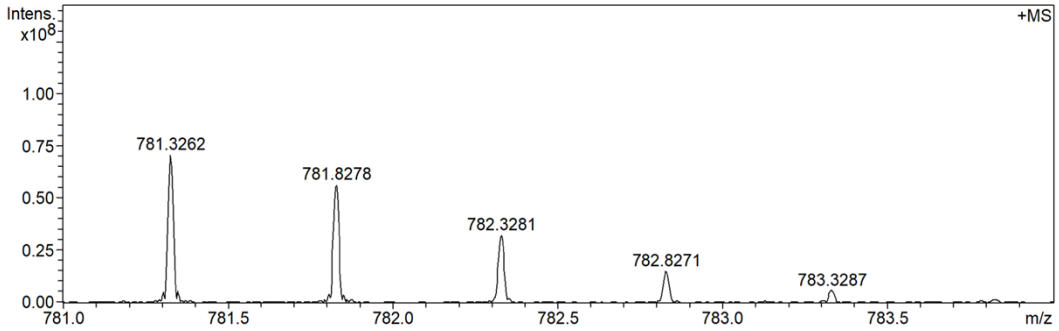


Fig. S35 Mass spectrum of **XCBP-1**.

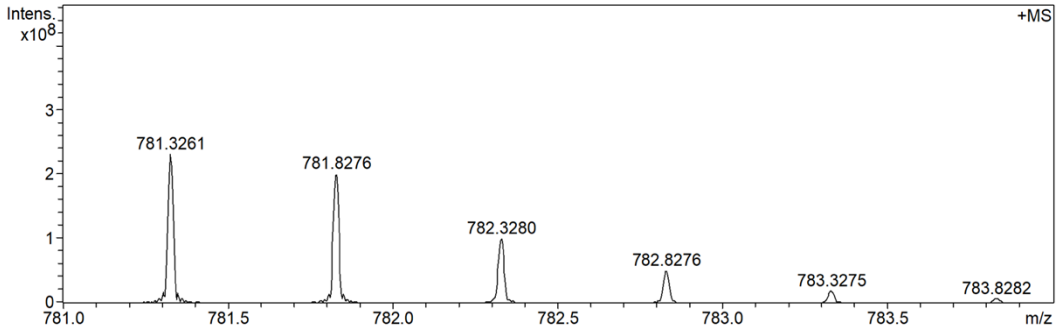


Fig. S36 Mass spectrum of **XCBP-2**.

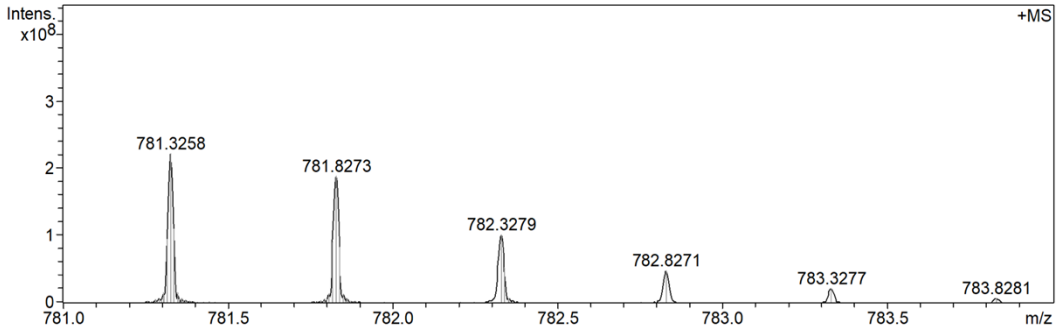


Fig. S37 Mass spectrum of **XCBP-3**.

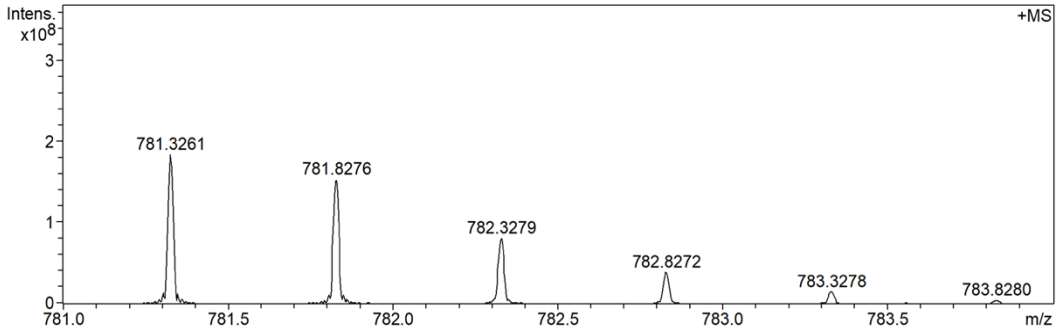


Fig. S38 Mass spectrum of **XCBP-4**.

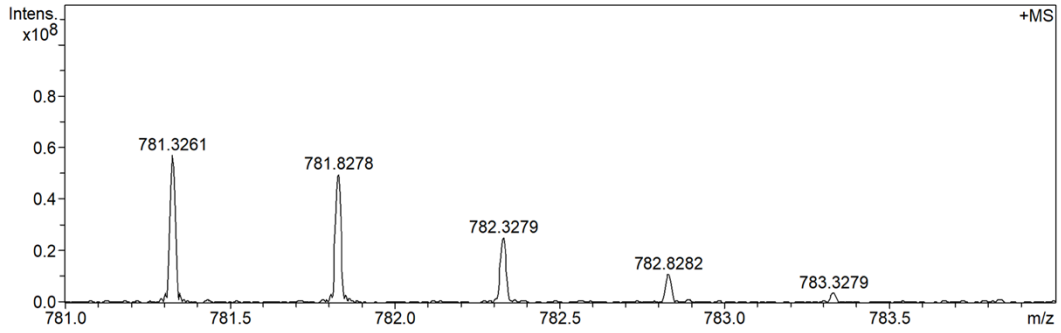


Fig. S39 Mass spectrum of **XCBP-5**.

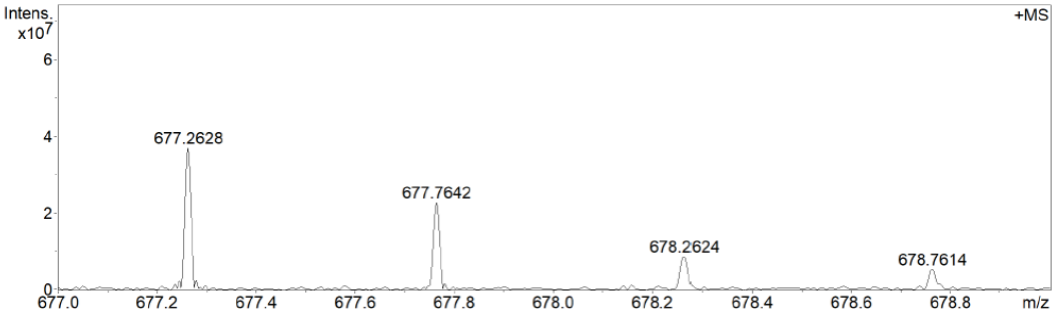


Fig. S40 Mass spectrum of α-conotoxin SI.

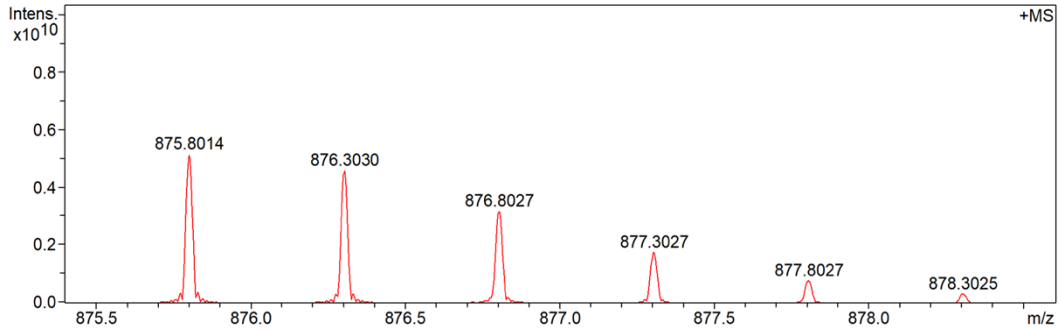


Fig. S41 Mass spectrum of reduced conotoxin mr3e.

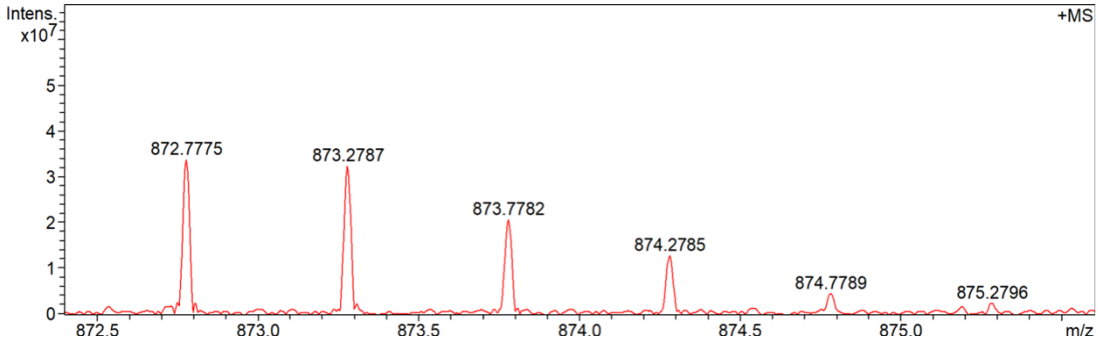


Fig. S42 Mass spectrum of conotoxin mr3e.

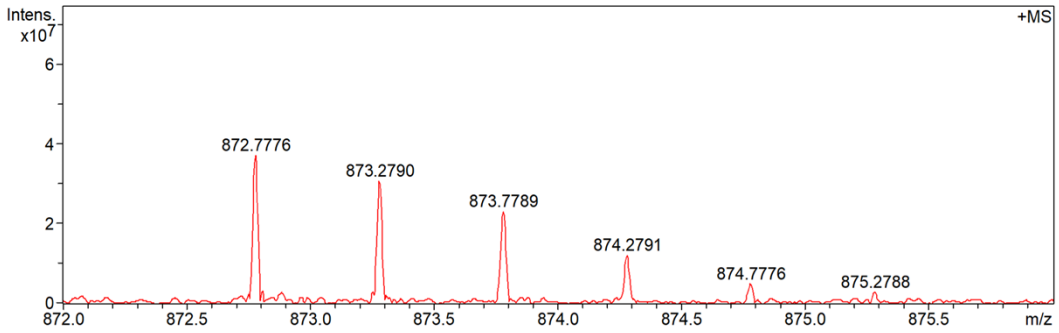


Fig. S43 Mass spectrum of the isomer of conotoxin mr3e.

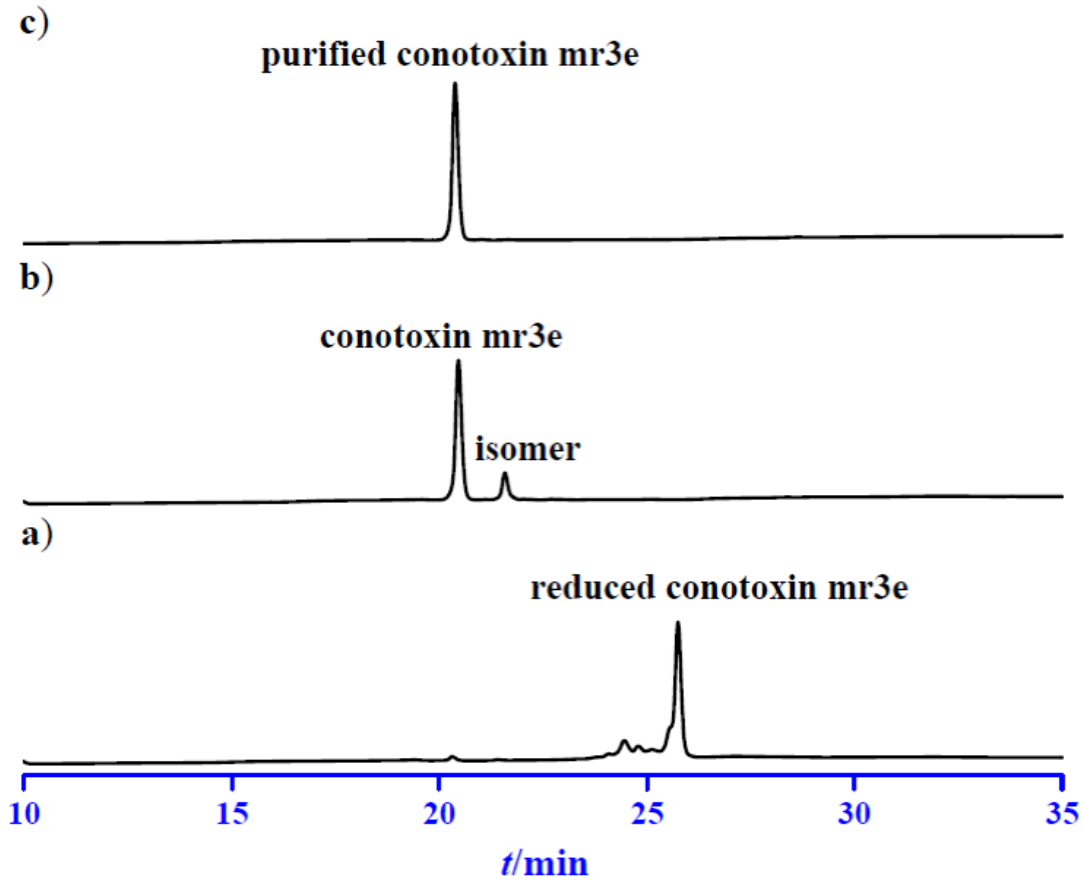


Fig. S44 HPLC chromatograms of a) reduced conotoxin mr3e; b) reduced conotoxin mr3e (0.2 mM) reacted with GSH (2.0 mM)/GSSG (2.0 mM) for 3 h in pH 8.0 Tris buffer solution; c) the purified conotoxin mr3e.

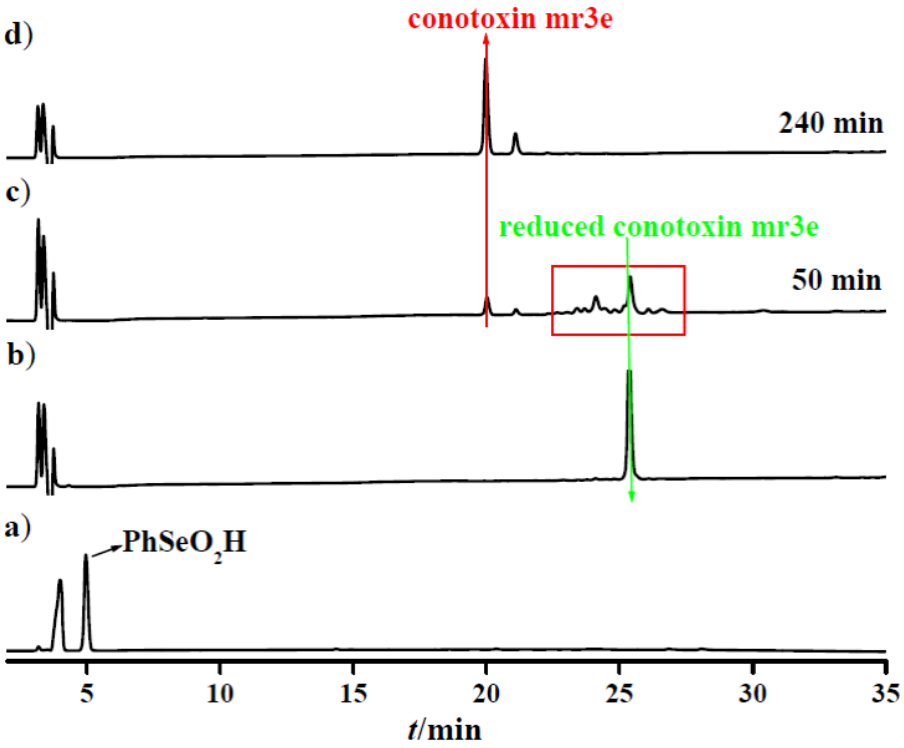


Fig. S45 HPLC chromatograms of a) $PhSeO_2H$; b) reduced conotoxin mr3e; c) reduced conotoxin mr3e (0.2 mM) reacted with BSA (0.04 mM) for 50 min in pH 7.0 phosphate buffer at 25 ℃; d) reduced conotoxin mr3e (0.2 mM) reacted with BSA (0.04 mM) for 240 min in pH 7.0 phosphate buffer at 25 ℃.

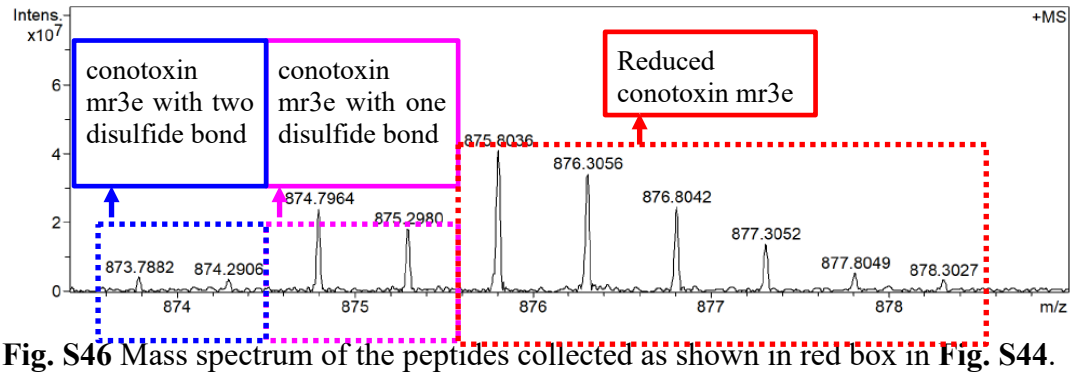


Fig. S46 Mass spectrum of the peptides collected as shown in red box in **Fig. S44**.

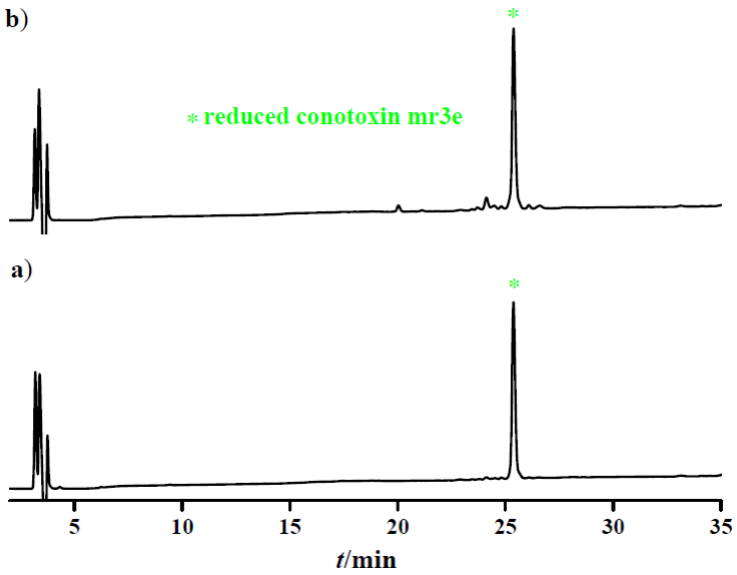


Fig. S47 HPLC chromatograms of a) reduced conotoxin mr3e (0.2 mM) in pH 7.0 phosphate buffer at 25 ℃; b) reduced conotoxin mr3e (0.2 mM) was aged for 5 h in pH 7.0 phosphate buffer at 25 ℃.

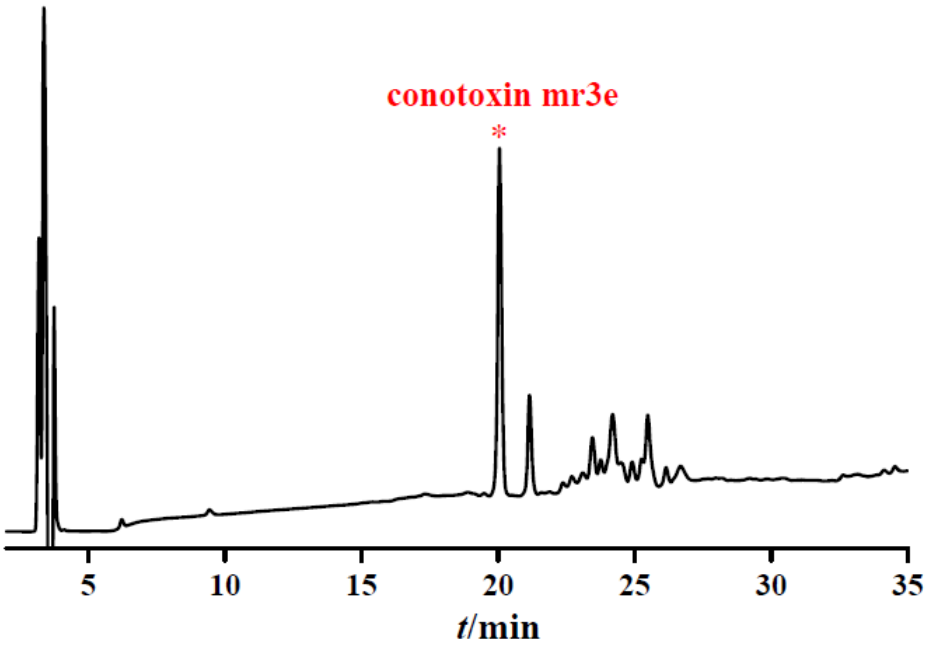


Fig. 48 HPLC chromatogram of reduced conotoxin mr3e (0.2 mM, 1.6 mL) reacted with PhSeSePh (0.02 mg, 65 μL) for 4 h in pH 7.0 phosphate buffer at 25 ℃.

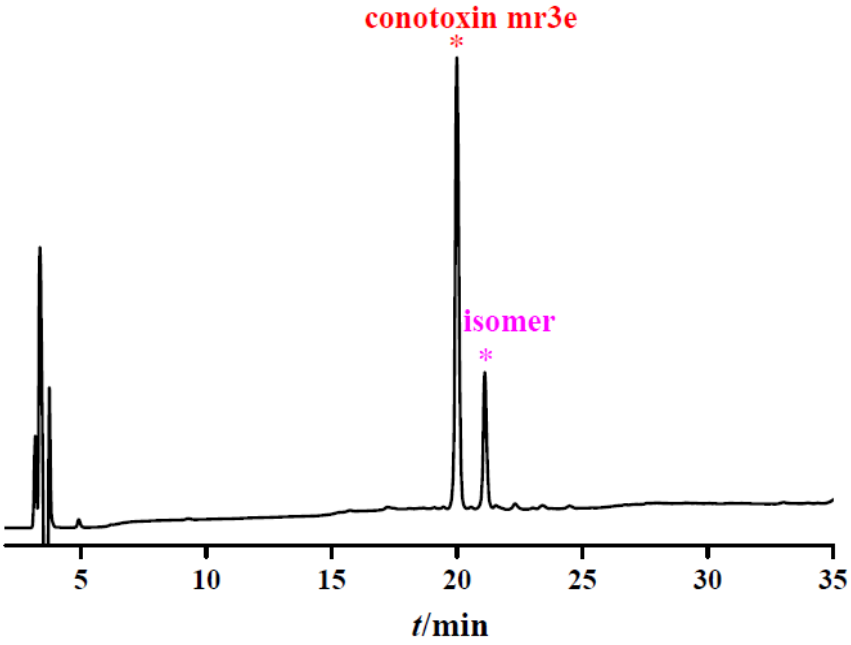


Fig. S49 HPLC chromatogram of reduced conotoxin mr3e (0.2 mM, 2.6 mL) reacted with phenylselenyl chloride (0.02 mg, 105 μL) for 4 h in pH 7.0 phosphate buffer at 25 ℃.

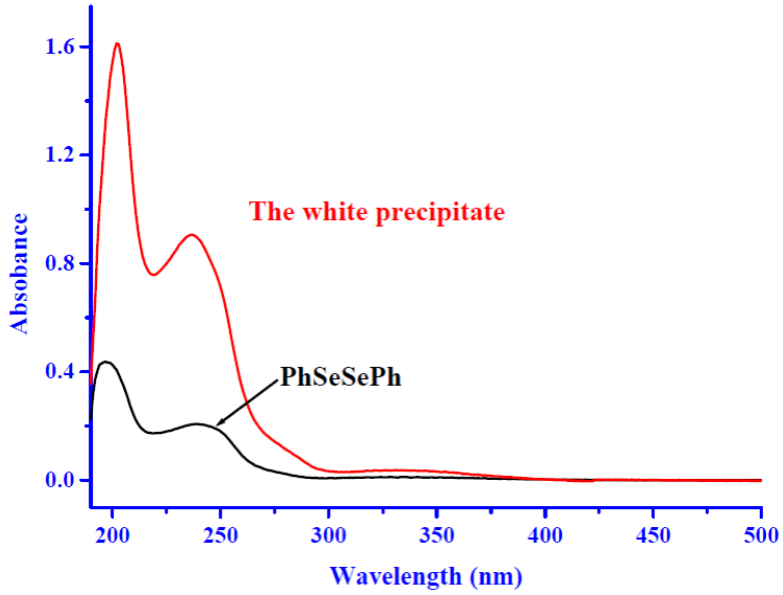


Fig. S50 UV-vis spectra of the white precipitate generated from the reaction between the reduced conotoxin mr3e and phenylselenyl chloride.

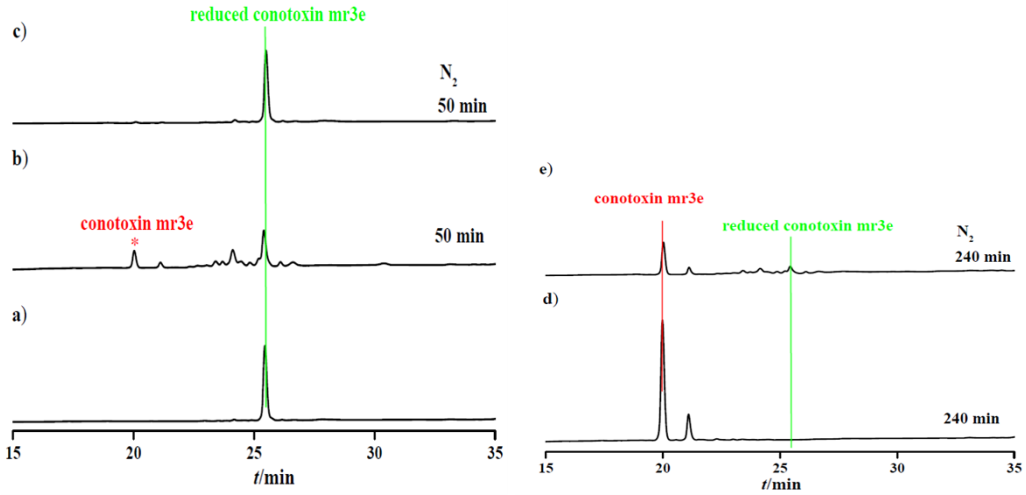


Fig. S51 HPLC chromatograms of a)reduced conotoxin mr3e (0.2 mM); b) reduced conotoxin mr3e (0.2 mM) reacted with BSA (0.04 mM) for 50 min in pH 7.0 phosphate buffer at 25 ℃; c) reduced conotoxin mr3e (0.2 mM); b) reduced conotoxin mr3e (0.2 mM) reacted with BSA (0.04 mM) for 50 min under N_2 in pH 7.0 phosphate buffer at 25 ℃; d) reduced conotoxin mr3e (0.2 mM) reacted with BSA (0.04 mM) for 240 min in pH 7.0 phosphate buffer at 25 ℃; e) reduced conotoxin mr3e (0.2 mM); b) reduced conotoxin mr3e (0.2 mM) reacted with BSA (0.04 mM) for 240 min under N_2 in pH 7.0 phosphate buffer at 25 ℃.

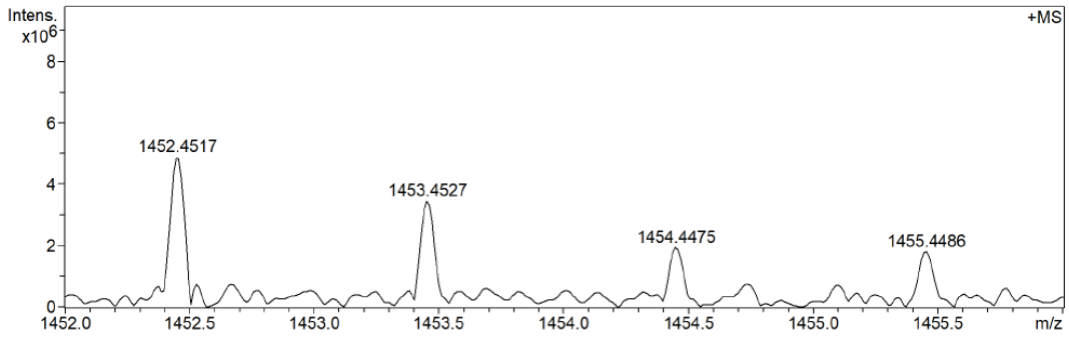


Fig. S52 Mass spectrum of reduced enterotoxin STp.

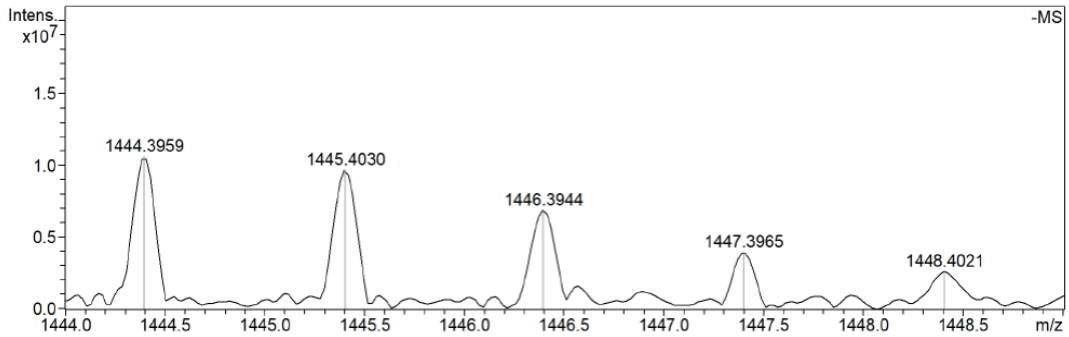


Fig.S53 Mass spectrum of oxidized enterotoxin STp.

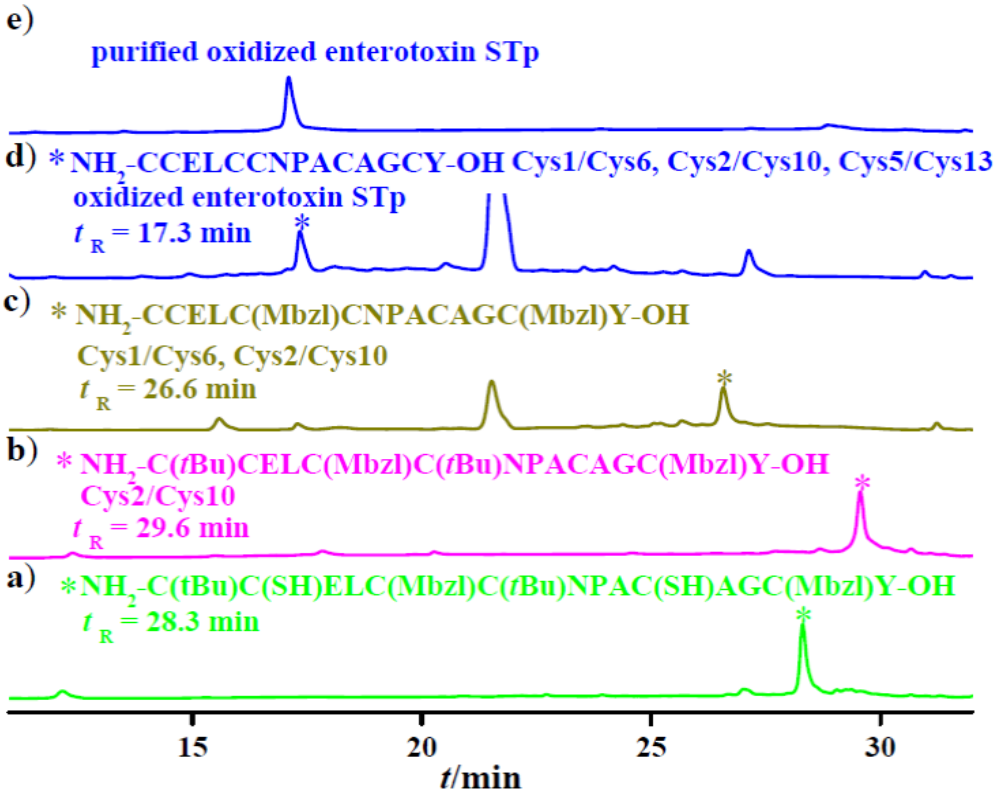


Fig. S54 HPLC chromatograms of a) linear enterotoxin STp; b) linear enterotoxin STp reaction with DMSO to construct the first disulfide bond Cys2/Cys10; c) the one disulfide bond (Cys2/Cys10) containing enterotoxin STp reaction with DMSO in neat TFA to construct the second disulfide bond (Cys1/Cys6); d) the two disulfide bonds (Cys2/Cys10 and Cys1/Cys6) containing enterotoxin STp reaction with DMSO in neat TFA at 60 ℃ to construct the third disulfide bond (Cys5/Cys13); e) the purified oxidized enterotoxin STp with three disulfide bonds (Cys1/Cys6, Cys2/Cys10, and Cys5/Cys13); Peptides were purified on an LC-6AD semi-preparative high performance liquid chromatography system (HPLC) system (Shimadzu, Japan) and were analyzed on an LC-20AB HPLC system (Shimadzu, Japan), respectively. The

UV-vis detector was set at 215 nm. The flow rate was set as 10.0 mL/min with a 250 mm × 20 mm ODS-C18 column and 1.0 mL/min with 250 mm × 20 mm C8 column for semi-preparative HPLC and analytical HPLC, respectively. Two solvent systems consisting of 0.1% TFA in acetonitrile and 0.1% TFA in water (referred to as solvents A and B) were used for peptide elution with a suitable gradient. The elution protocol for analytical HPLC was set as follows: 10-23% B over 10 min and further to 48% B over 12 min, and then to 90% over 10 min.

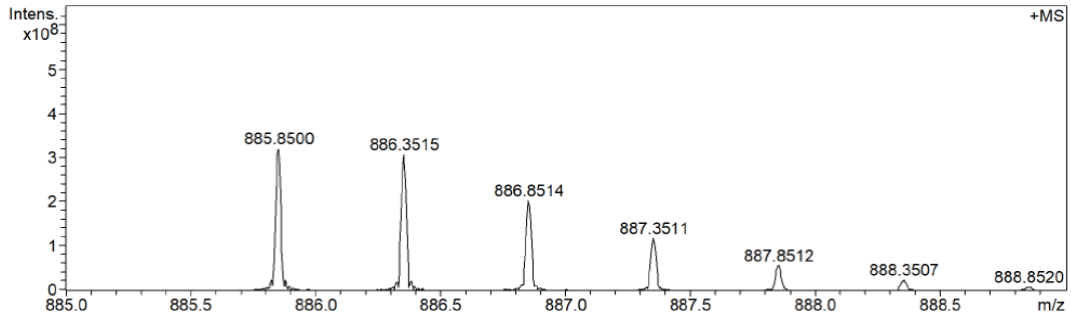


Fig. S55 Mass spectrum of enterotoxin STp with one disulfide bond (NH_2-C(*t*Bu)CELC(Mbzl)C(*t*Bu)NPACAGC(Mbzl)Y-OH, Cys2/Cys10, $C_{79}H_{115}N_{15}O_{19}S_6$).

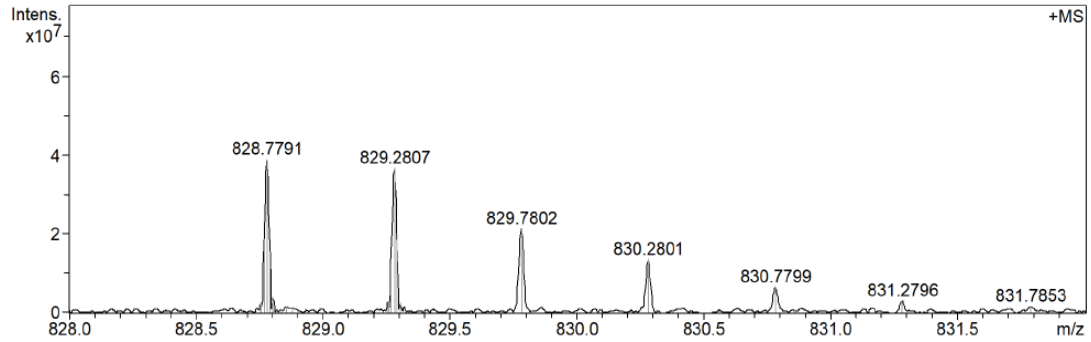


Fig. S56 Mass spectrum of enterotoxin STp with two disulfide bonds (NH_2-CCELC(Mbzl)CNPACAGC(Mbzl)Y-OH, Cys1/Cys6 and Cys2/Cys10, $C_{71}H_{97}N_{15}O_{19}S_6$).

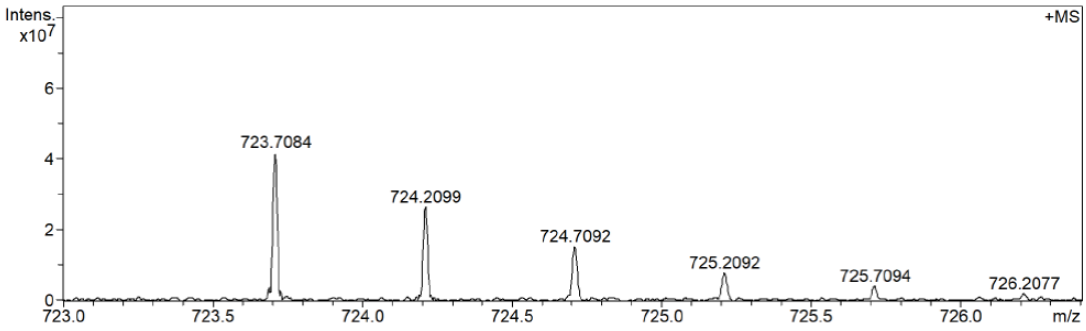


Fig. S57 Mass spectrum of oxidized enterotoxin STp (NH_2-CCELCCNPACAGCY-OH, Cys1/Cys6, Cys2/Cys10 and Cys5/Cys13).

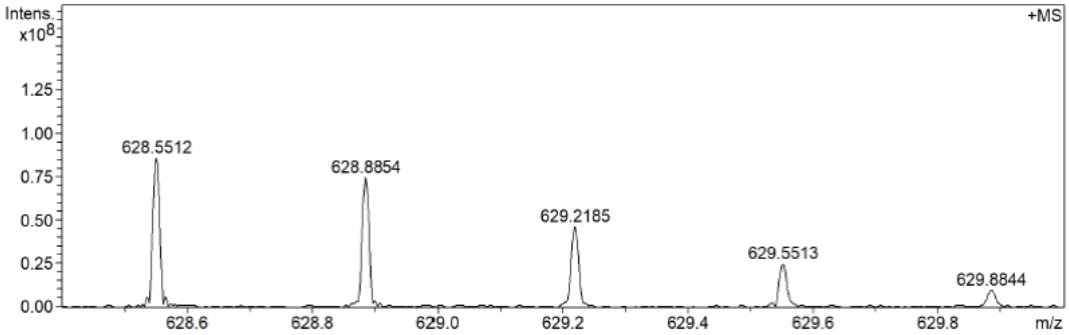


Fig. S58 Mass spectrum of KIIIA-T.

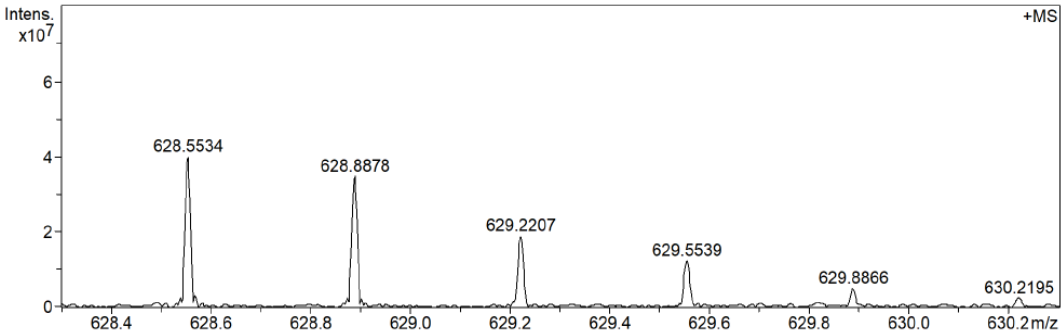


Fig. S59 Mass spectrum of the isomer of KIIIA-T.

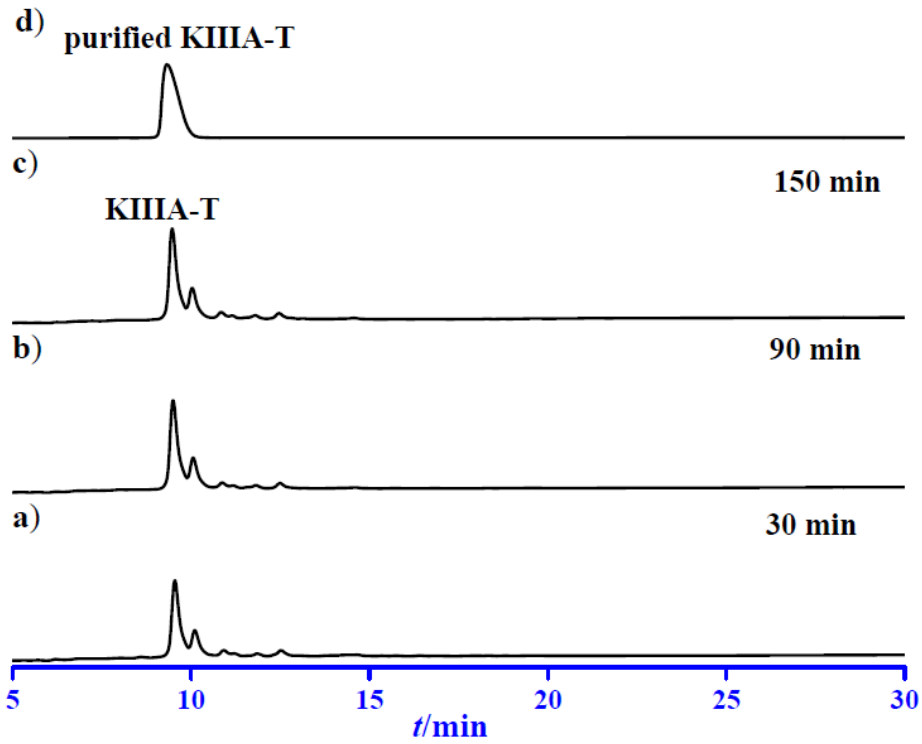


Fig. S60 Reduced μ-conotoxin KIIIA (20 μM) was reacted with GSH (1.0 mM) and with GSSGH (1.0 mM) in pH 7.5 Tris buffer solution containing 1.0 mM of EDTA; the isolated yield of KIIIA-T is 42%.

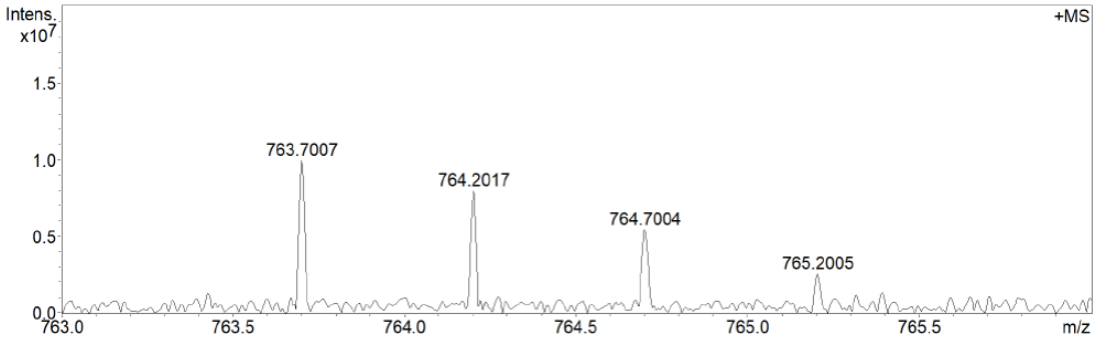


Fig. S61 Mass spectrum of linaclotide.

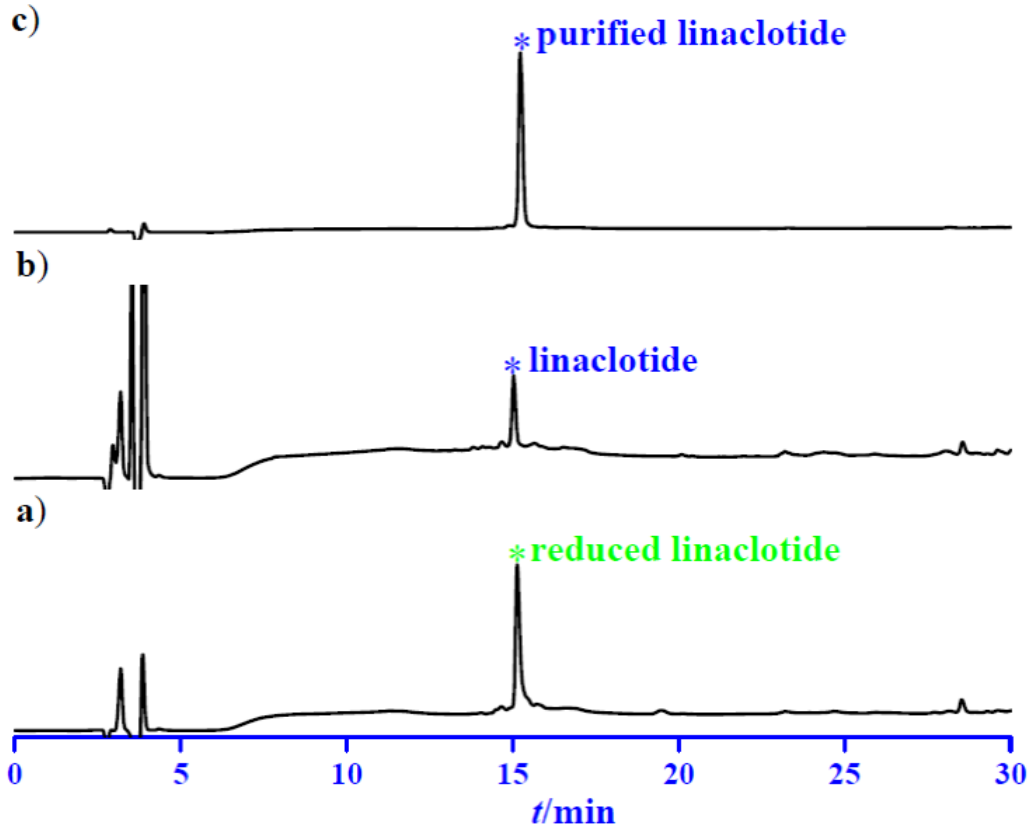


Fig. S62 HPLC chromatograms of the linaclotide generated from the oxidative folding reduced linaclotide by GSSG/GSH.

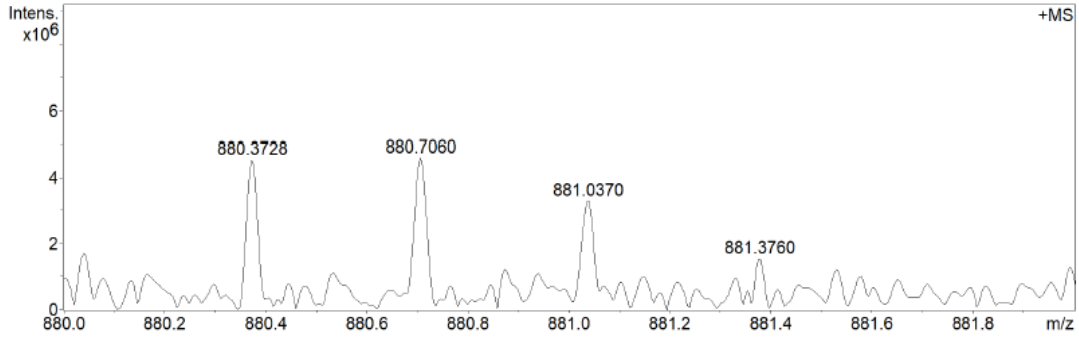


Fig. S63 Mass spectrum of ziconotide.

References

1. D. Ma, J. Sun, S. Shen, H. Chen, W. Xu, Y. Wang, C. Song, T. Shi and S. Huo, Deprotection of S-acetamidomethyl and 1,3-thiazolidine-4-carbonyl protecting groups from cysteine side chains in peptides by *trans*-$[PtX_2(CN)_4]^{2-}$: one-pot regioselective synthesis of disulfide bonds, *J. Org. Chem.*, 2022, **87**, 1470-1476.