Supplementary Information for

A facile *N*-mercaptoethoxyglycinamide (MEGA) linker approach to peptide thioesterification and cyclization

Patrick M. M. Shelton, Caroline E. Weller, and Champak Chatterjee

Department of Chemistry, University of Washington, Seattle, Washington 98195

General methods

Rink-amide resin (0.30-60 mmol/g substitution) was purchased from Chem-Impex (Wood Dale, IL). Standard Fmoc-L-amino acids were purchased from AGTC Bioproducts (Wilmington, MA) or AnaSpec (Fremont, CA). All other chemical reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Solid phase peptide synthesis (SPPS) was performed manually, or on a Liberty Blue Automated Microwave Peptide Synthesizer (CEM Corporation, Matthews, NC).[1] Analytical reversed-phase HPLC (RP-HPLC) was performed on a Varian (Palo Alto, CA) ProStar HPLC with a Grace-Vydac (Deerfield, IL) C18 column (5 micron, 150 x 4.6 mm) employing 0.1% TFA in water (A) and 90% CH_3CN, 0.1% TFA in water (B) as the mobile phases. Typical analytical gradients were 0-73% B over 30 min at a flow rate of 1 mL/min. Preparative scale purifications were conducted on a Grace-Vydac C18 column (10 micron, 250 x 22 mm) at a flow rate of 9 mL/min. Semi-preparative scale purifications were conducted on a Grace-Vydac C18 column (5 micron, 250 x 10 mm) at a flow rate of 3.5 mL/min. Mass spectrometric analysis was conducted on a Bruker (Billerica, MA) Esquire ESI-MS instrument.

Synthesis of MEGA linked resin

Rink amide resin (0.30-0.60 mmol/g, 0.25 mmol) was allowed to swell in a reaction vessel in 50:50 (v/v) DMF:DCM for 30 min followed by Fmoc-deprotection with 20% piperidine in DMF for 25 min. The resin was thoroughly washed by consecutive 30 sec DMF, DCM and DMF flow washes. Bromoacetic acid (2.5 mmol) and diisopropylcarbodiimide (DIC, 2.5 mmol) were dissolved in 4 mL DMF and added to the resin. The mixture was agitated with $N_{2(g)}$ for 45 min, then an additional 45 min with fresh coupling reagents. The resin was thoroughly washed and dried under vacuum. *O*-(2-(tritylthio)ethyl)hydroxylamine[2] (1.75 mmol) was dissolved in 7 mL of 50:50 (v/v) sieve-dried DMSO:DMF, added to the resin in a 20 mL scintillation vial and shaken for 24-48 h. The resin was filtered, washed and dried under vacuum to give MEGA resin.

Synthesis of Peptide-MEGA

First amino acid coupling: MEGA resin (0.05 mmol) was swelled in 0.5 mL sieve-dried DMF in a 20 mL scintillation vial. 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxid hexafluorophosphate (HATU, 0.49 mmol), Fmoc-amino acid (0.5 mmol) and diisopropylethylamine (DIEA, 0.5 mmol) were dissolved in 2.0 mL dry DMF, added to MEGA resin and mixed for 24 h.

Peptide elongation/purification: Standard Fmoc-SPPS protocols were employed to extend the peptide chain.[3] The peptide was cleaved from the resin in by mixing with 95:2.5:2.5 (v/v)

TFA:H_2O:triisopropylsilane (TIS) for 1-2 h. The crude peptide was precipitated from solution by mixing with 10 volumes of cold diethyl ether and centrifuged for 2 min at 3,500 rpm. The supernatant was discarded and the peptide lyophilized. Preparative scale RP-HPLC was used to purify the crude peptide and provide pure peptide-MEGA after lyopholization.

Microwave-assisted Synthesis of MEGA Peptides: The MEGA resin was loaded with the amino acid of choice as described above for the first amino acid coupling. The pre-loaded MEGA resin was place in a Liberty Blue peptide synthesizer (CEM) and automated peptide synthesis was conducted with 50 °C deprotection steps using a 5% piperazine/0.1 M hydroxybenzotriazole (HOBt) solution in DMF. 50 °C amino acid couplings were used for all coupling steps.[4]

Peptide Thioesterification, Ligation and Cyclization

α-Thioesterification: Peptide-MEGA (0.5 μmol) was dissolved in 500 μL thioesterification buffer consisting of sodium 2-mercaptoethanesulfonate (MESNa, 200-400 mM), sodium phosphate (NaH_2PO_4, 100 mM) and *tris*(2-carboxyethyl)phosphine (TCEP, 25-50 mM) at pH 4-6. The reactions were agitated for 24-72 h at 37-70 °C except where noted in Table 2. The peptide-MES α-thioester was purified by analytical or semi-preparative scale RP-HPLC.

Ligation: Peptide-MEGA thioesterification was performed as described above. The Cys-peptide, CASW (1.25 μmol), was dissolved in 50 μL NaH_2PO_4 (200 mM), TCEP (400 mM) buffer and added directly to the thioesterification reaction vessel. The solution pH was adjusted to 7.5 by litmus and the reaction mixed for 8-24 h. The reaction was analyzed by RP-HPLC after reduction of the assay mixture with additional TCEP (25-50 mM final concentration).

Cyclization: N-terminal Cys-peptide (0.5 μmol) was dissolved in MESNa (200-400 mM), NaH_2PO_4 (100 mM) and TCEP (25-50 mM) buffer at pH 4-6. Reactions were allowed to proceed for 8-72 h at 50-70 °C. Reaction mixtures were analyzed by C18 analytical RP-HPLC with prior treatment with additional TCEP (25-50 mM final concentration).

Synthesis of cyclized and oxidized SFT-1(I10G)

The sequence CFPDGRCTKSIPPG-MEGA was prepared on Rink amide resin (0.05 mmol) via automated peptide synthesis as described above. The crude peptide was cleaved from the resin in a 95:2.5:2.5 (v/v) TFA:H_2O:TIS solution and precipitated with 10 volumes of cold diethyl ether and lyopholized. The linear peptide was purified by C18 preparative RP-HPLC (15-40% B, 60 min) and lyophilized (15.4 mg, 20%).

SFT-1(I10G)-MEGA (5 mg, 3.1 μmol) was cyclized as described above at 70 °C for 24 h. The cyclic peptide was purified by C18 semi-preparative RP-HPLC (10-50% B, 45 min gradient) (1.5 mg, 33%).

Cyclic SFT-1(I10G) was oxidized to form the disulfide bridge by incubating the peptide in 100 mM $(NH_4)HCO_3$ at 25 °C overnight (0.2mg/mL). SFT-1 (I10G) was purified by C18 analytical RP-HPLC (10-50% B, 30 min gradient) with a quantitative yield.

Trypsin inhibition assays

The inhibition of bovine trypsin by the Sunflower Trypsin Inhibitor-1 analog, SFT-1(I10G), was measured spectrophotometrically. The hydrolysis of *N*(α)-benzoyl-L-arginine 4-nitroanilide (BAPNA) by bovine trypsin to generate the yellow colored 4-nitroaniline was followed at 410 nm in the presence of varying concentrations of SFT-1(I10G).[5-6] Briefly, 500 μM BAPNA and 1 nM to 2.5 μM SFT-1(I10G) were incubated for 5 min in a 96-well plate in the presence of assay buffer consisting of 50 mM Tris and 20 mM $CaCl_2$ at pH 8.0. BAPNA hydrolysis was initiated by the addition of 100 nM trypsin and the reaction allowed to proceed for 20 min at 27 °C. The final Abs_{410} was measured using a BioTek Synergy 4 microplate reader and plotted in GraphPad Prism. All experiments were undertaken in triplicate and the average value reported with error being the standard deviation from the mean.

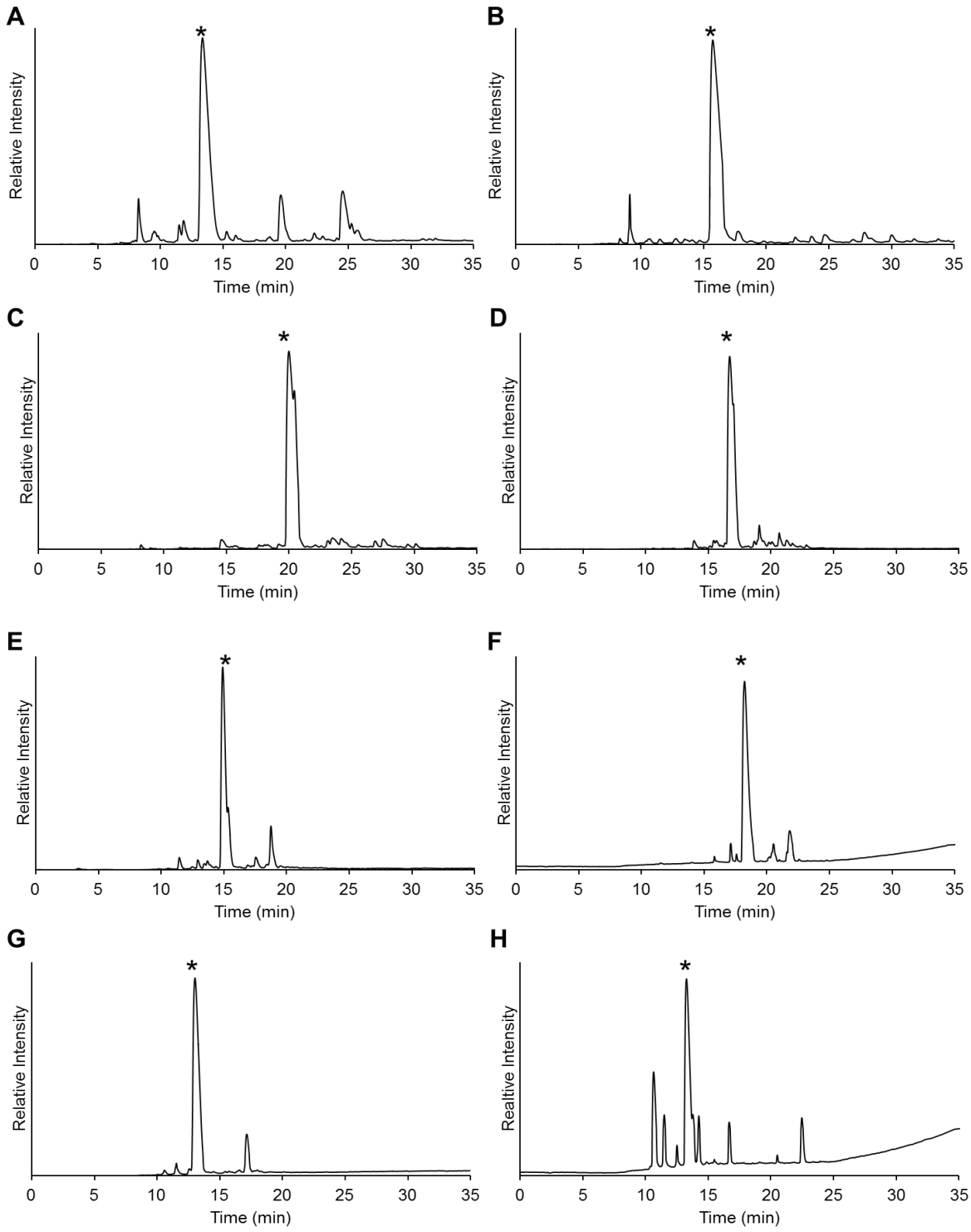
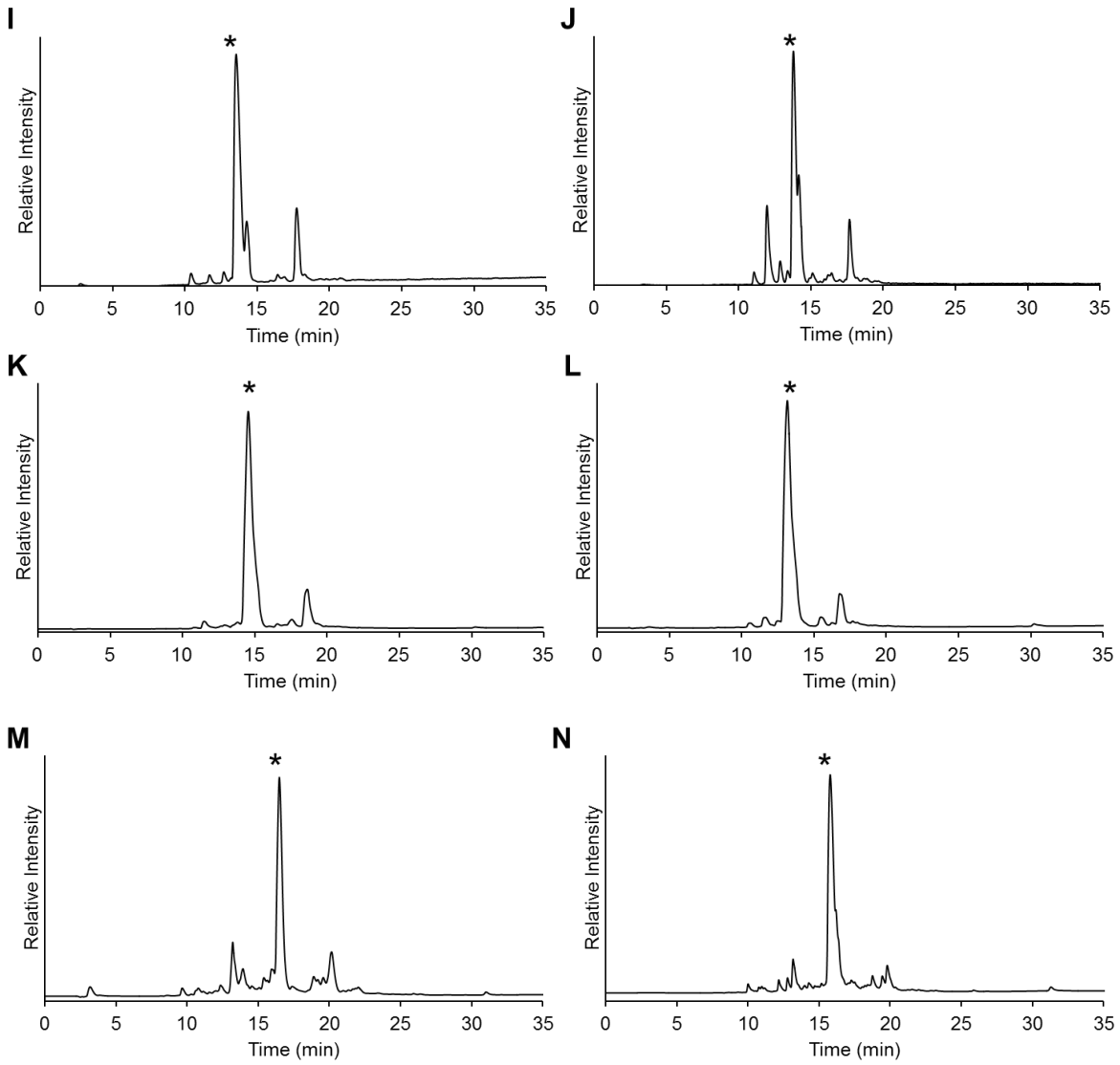


Figure S1. RP-HPLC spectra of crude AWKX-MEGA peptides after TFA-cleavage from resin. (A) AWK**G**-MEGA (B) AWK**A**-MEGA (C) AWK**L**-MEGA (D) AWK**V**-MEGA (E) AWK**D**-MEGA (F) AWK**F**-MEGA (G) AWK**Q**-MEGA (H) AWK**R**-MEGA. RP-HPLC performed on C18 analytical column, 0-73% B, 30 min gradient. * = AWK**X**-MEGA peptide.



Continued Figure S1. RP-HPLC spectra of crude AWKX-MEGA peptides after TFA-cleavage from resin. (I) AWK**S**-MEGA (J) AWK**T**-MEGA (K) AWK**(D-A)**-MEGA (L) AWK**K**-MEGA (M) AWK**C**-MEGA (N) AWK**(D-C)**-MEGA. RP-HPLC performed on C18 analytical column, 0-73% B, 30 min gradient. * = AWK**X**-MEGA peptide.

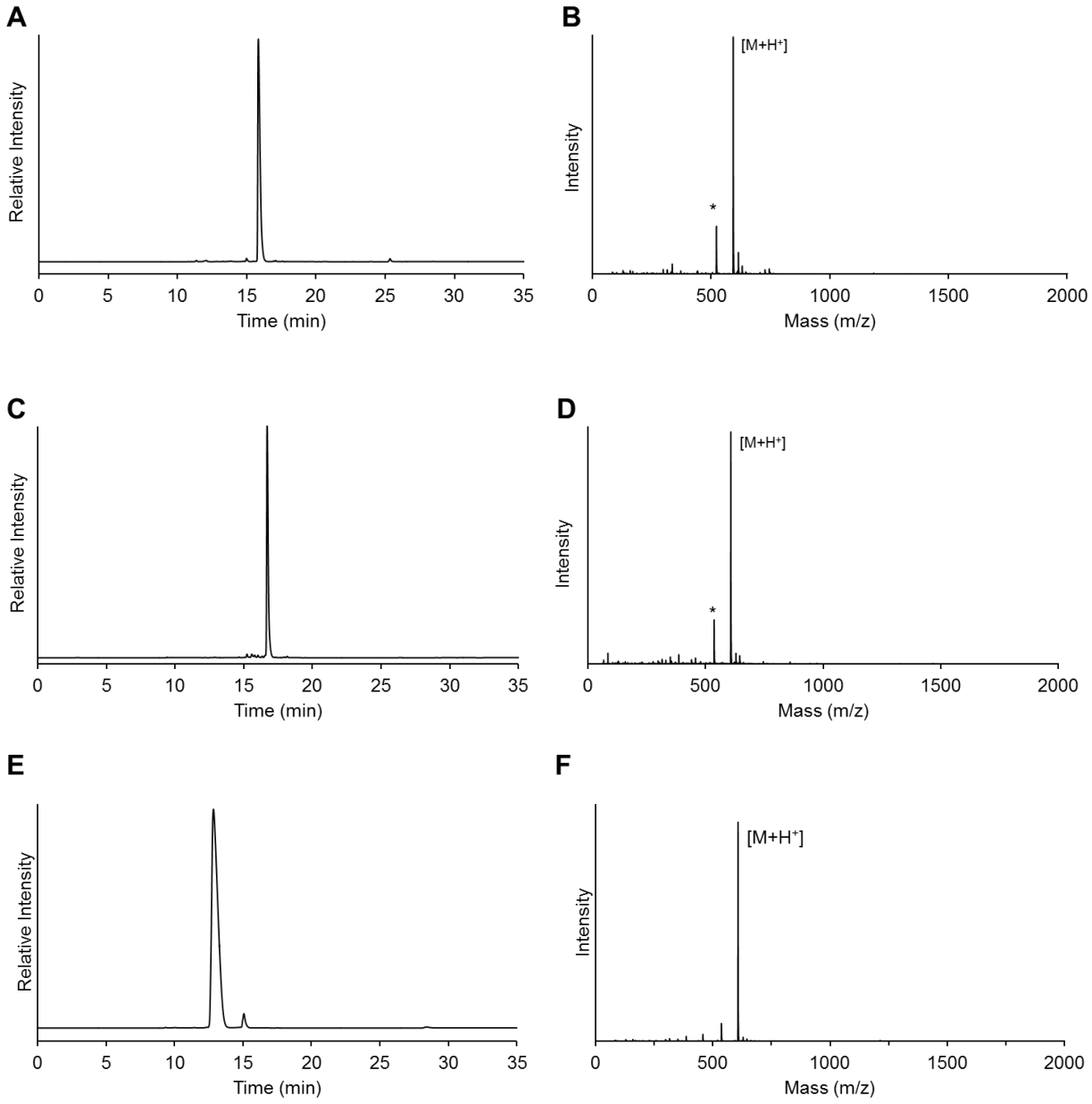
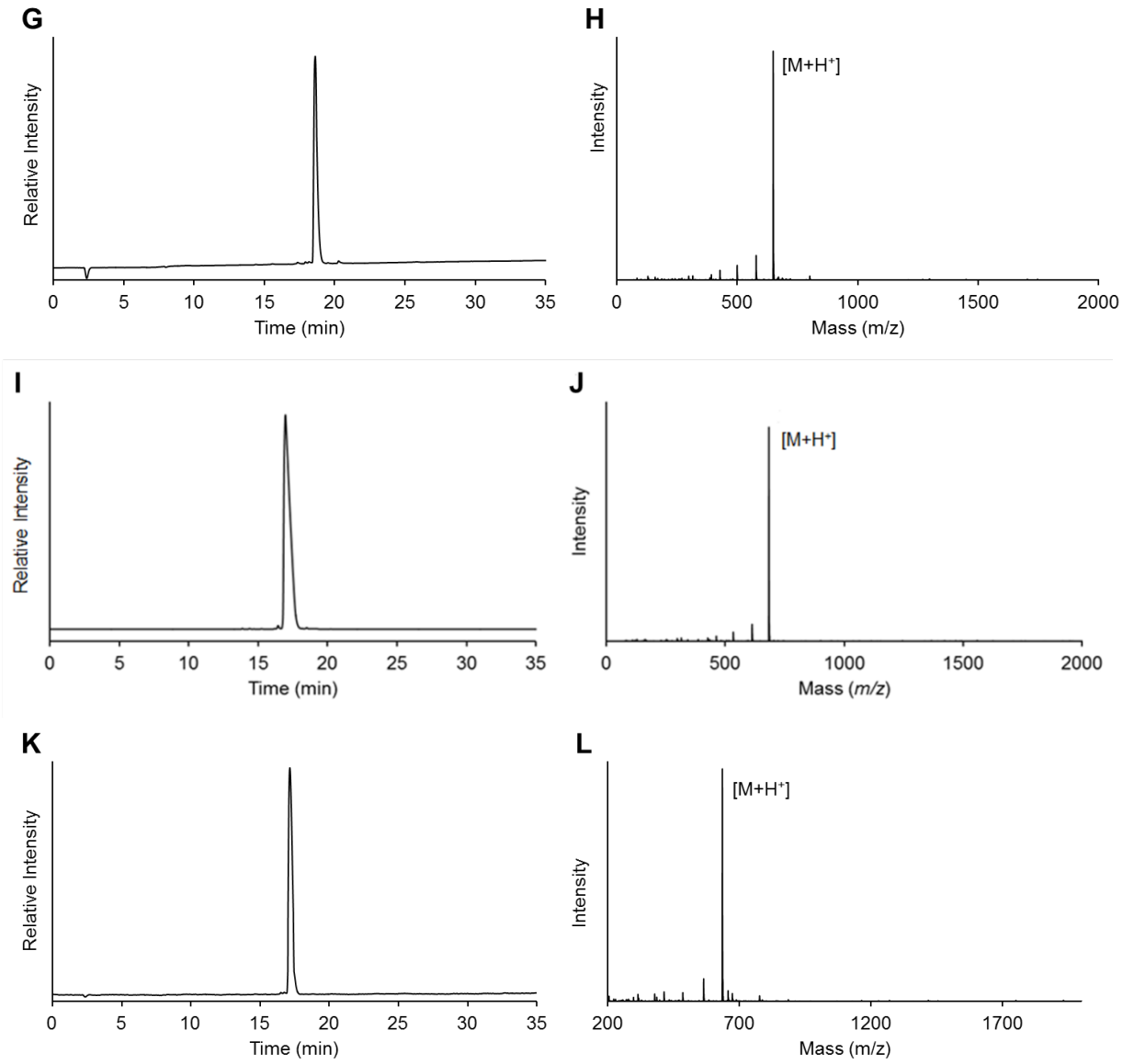
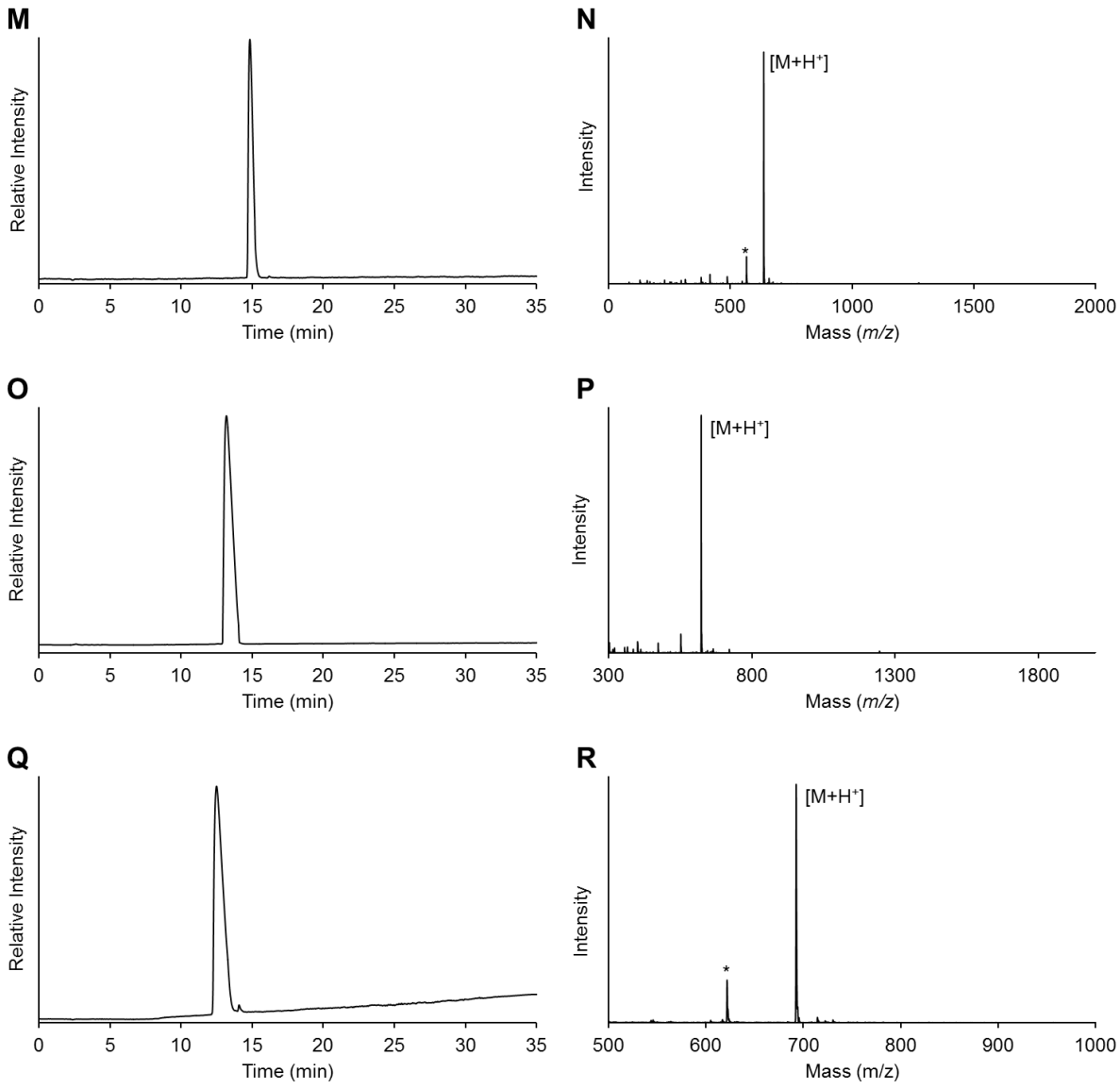


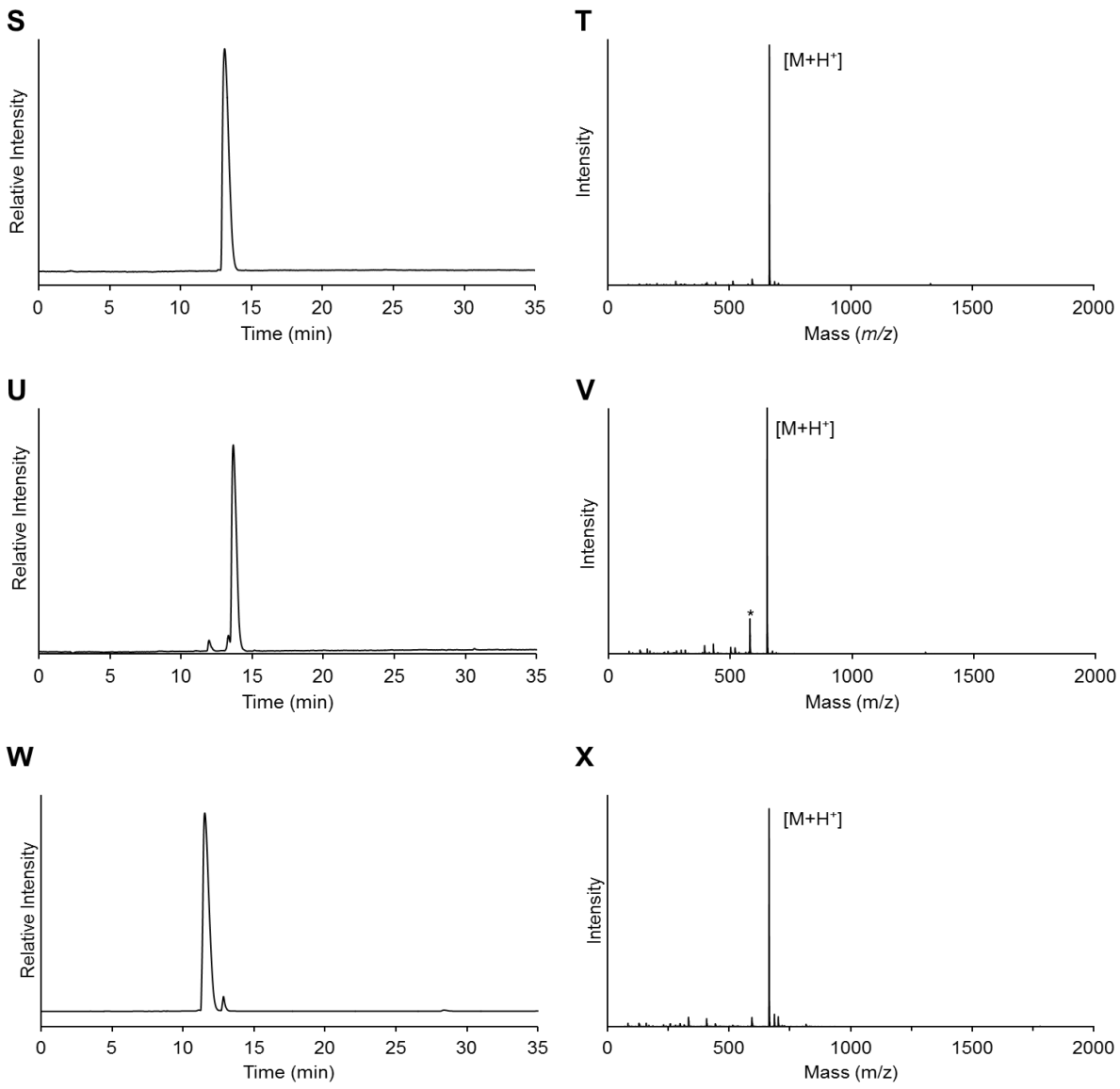
Figure S2. AWKX-MEGA characterization. (A) Purified AWK**G**-MEGA. (B) ESI-MS of purified AWK**G**-MEGA. Calcd. [M+H$^+$] 592.7 Da, obsd. 592.3 Da. (C) Purified AWK**A**-MEGA. (D) ESI-MS of purified AWK**A**-MEGA. Calcd. [M+H$^+$] 606.7 Da, obsd. 606.5 Da. (E) Purified AWK**(D-A)**-MEGA (F) ESI-MS of purified AWK**(D-A)**-MEGA. Calcd. [M+H$^+$] 607.7 Da, obsd. 607.4 Da. * = MS-fragmentation of full-length peptide corresponding to loss of N-terminal Ala. RP-HPLC performed on C18 analytical column, 0-73% B, 30 min gradient.



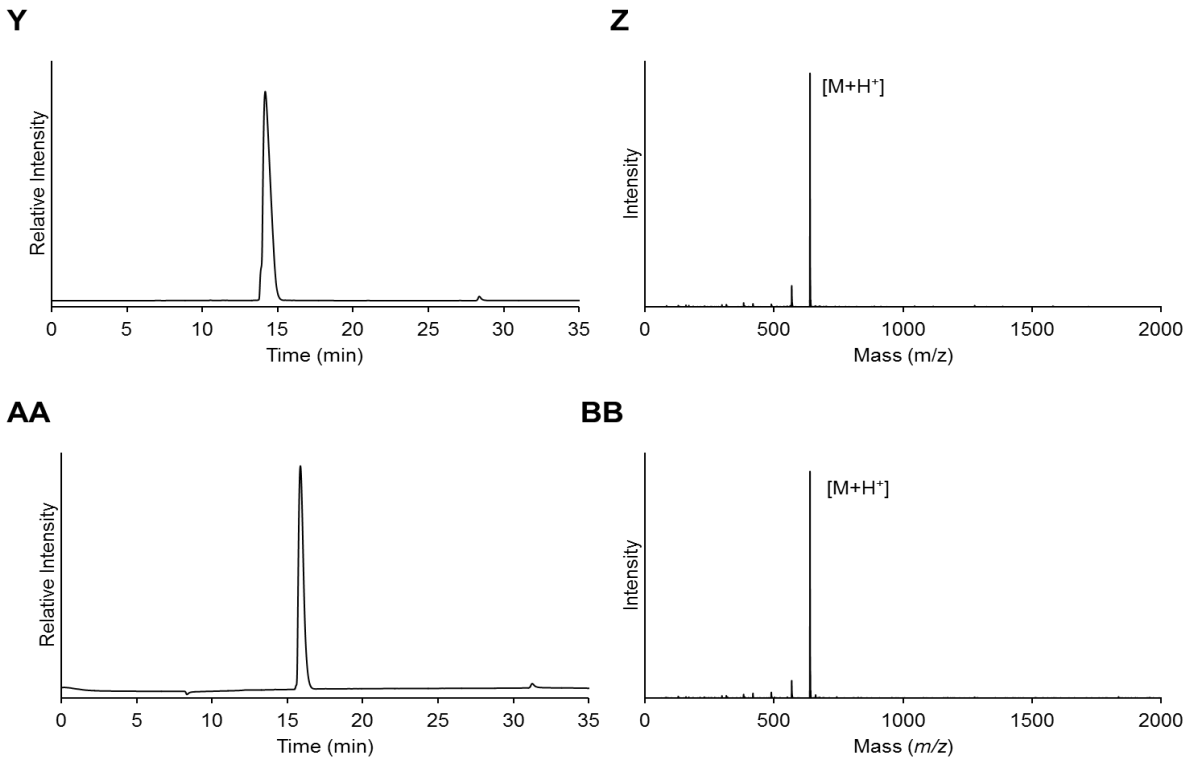
Continued Figure S2. AWKX-MEGA characterization. (G) Purified AWK**L**-MEGA. (H) ESI-MS of purified AWK**L**-MEGA. Calcd. [M+H$^+$] 648.8 Da, obsd. 648.5 Da. (I) Purified AWK**F**-MEGA. (J) ESI-MS of purified AWK**F**-MEGA. Calcd. [M+H$^+$] 682.8 Da, obsd. 682.6 Da. (K) Purified AWK**V**-MEGA. (L) ESI-MS of purified AWK**V**-MEGA. Calcd. [M+H$^+$] 634.8 Da, obsd. 634.4 Da.



Continued Figure S2. AWKX-MEGA characterization. (M) Purified AWK**T**-MEGA. (N) ESI-MS of purified AWK**T**-MEGA. Calcd. [M+H$^+$] 636.8 Da, obsd. 636.5 Da. (O) Purified AWK**S**-MEGA. (P) ESI-MS of purified AWK**S**-MEGA. Calcd. [M+H$^+$] 622.7 Da. obsd. 622.6 Da. (Q) Purified AWK**R**-MEGA. (R) ESI-MS of purified AWK**R**-MEGA. Calcd. [M+H$^+$] 691.9 Da, obsd. 691.5 Da. * = MS-fragmentation of full-length peptide corresponding to loss of N-terminal Ala. RP-HPLC spectra performed on C18 analytical column, 0-73% B, 30 min gradient.



Continued Figure S2. AWKX-MEGA characterization. (S) Purified AWK**Q**-MEGA. (T) ESI-MS of purified AWK**Q**-MEGA. Calcd. [M+H$^+$] 663.8 Da, obsd. 663.8 Da. (U) Purified AWK**D**-MEGA. (V) ESI-MS of purified AWK**D**-MEGA. Calcd. [M+H$^+$] 650.8 Da, obsd. 650.4 Da. (W) Purified AWK**K**-MEGA. (X) ESI-MS of purified AWK**K**-MEGA. Calcd. [M+H$^+$] 664.8 Da, obsd. 664.6 Da * = MS-fragmentation of full-length peptide corresponding to loss of N-terminal Ala. RP-HPLC spectra performed on C18 analytical column, 0-73% B, 30 min gradient.



Continued Figure S2. AWKX-MEGA characterization. (Y) Purified AWK**C**-MEGA. (Z) ESI-MS of purified AWK**C**-MEGA. Calcd. [M+H$^+$] 639.8 Da, obsd. 639.5 Da. (AA) Purified AWK**(D-C)**-MEGA. (BB) ESI-MS of purified AWK AWK**(D-C)**-MEGA. Calcd. [M+H$^+$] 639.8 Da, obsd. 639.5 Da. RP-HPLC spectra performed on C18 analytical column, 0-73% B, 30 min.

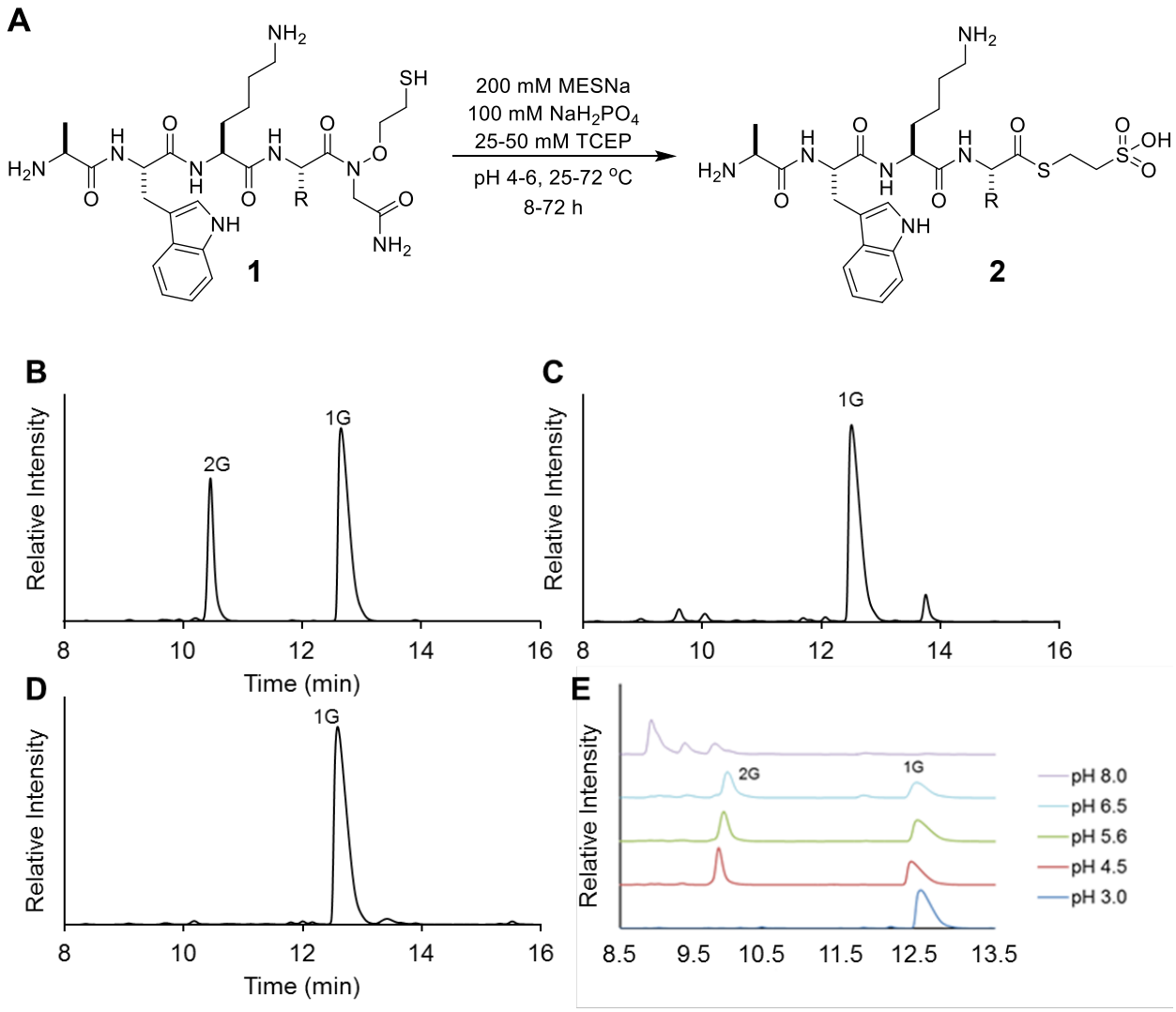


Figure S3. Optimization of thioesterification conditions with AWKG-MEGA. (A) General scheme for peptide-MEGA thioesterification. (B) AWK**G**-MEGA thioesterification, 200 mM MESNa, pH 5.6, 25 °C. (C) AWK**G**-MEGA thioesterification, 200 mM 2,2,2-trifluoroethanethiol, pH 5.6, 25 °C. (D) AWK**G**-MEGA thioesterification, 200 mM 3-mercaptopropionic acid, pH 5.6, 25 °C. (E) 24 h time-points of AWK**G**-MEGA thioesterification with MESNa at varying pH and 25 °C.

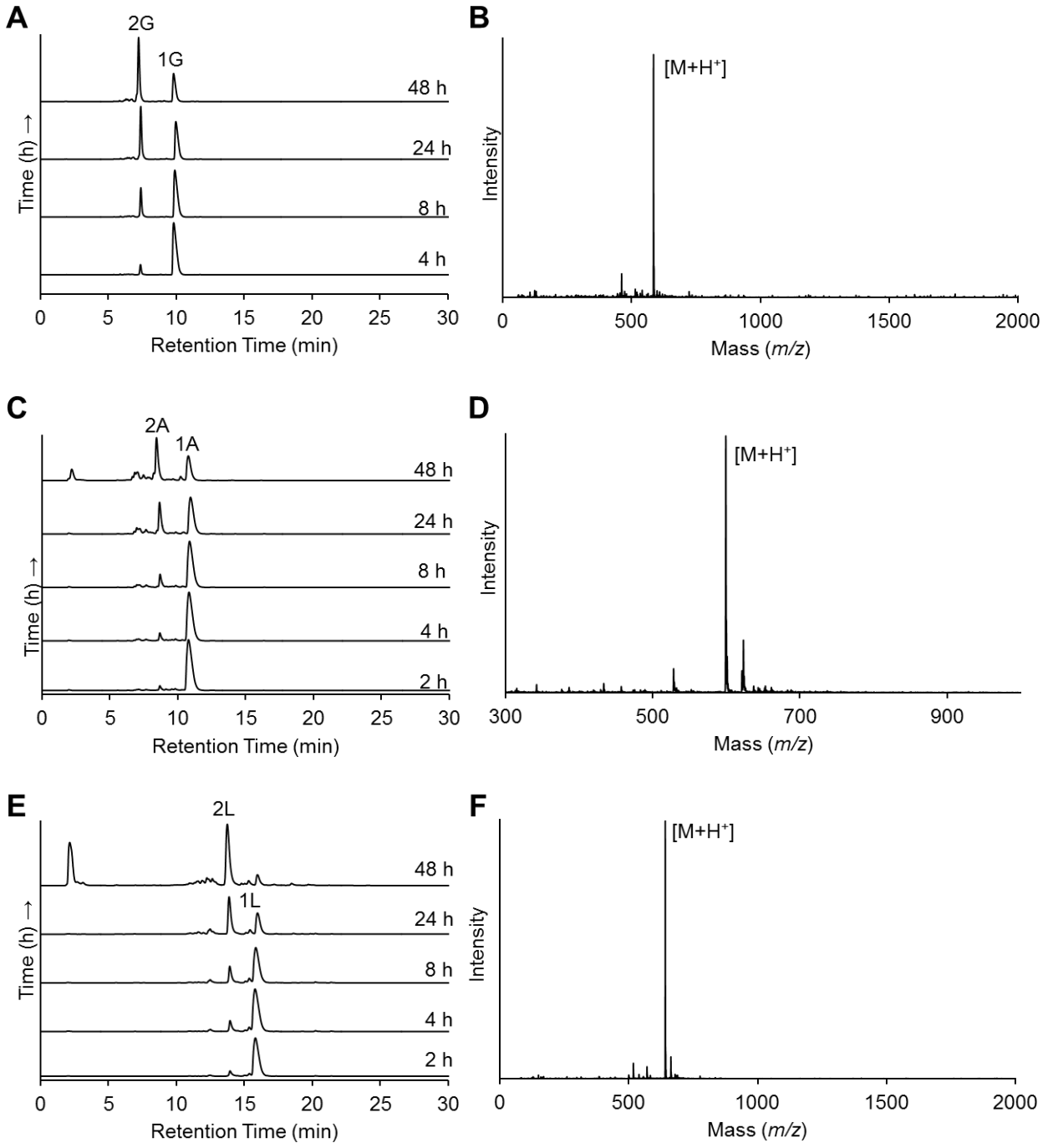
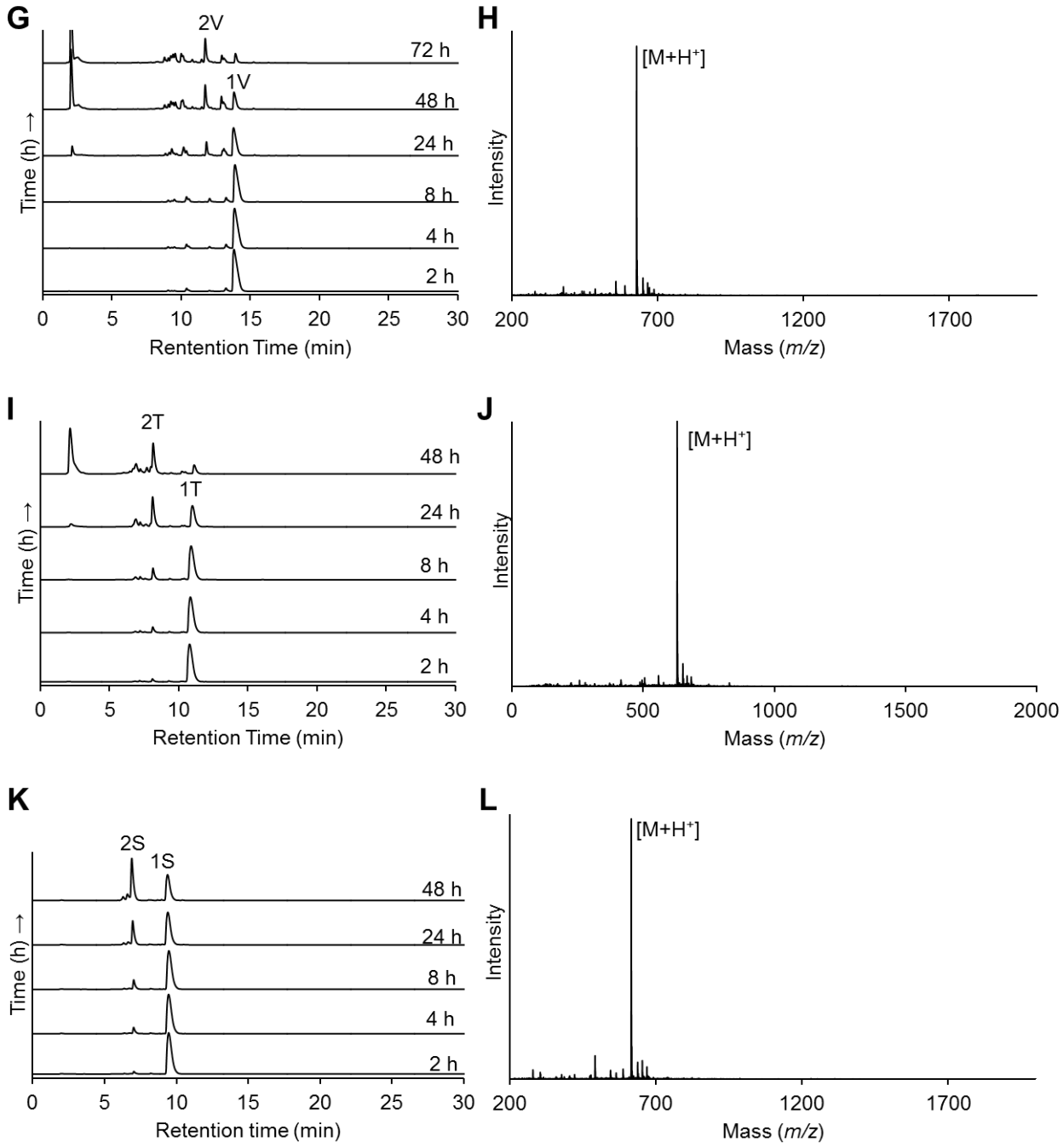
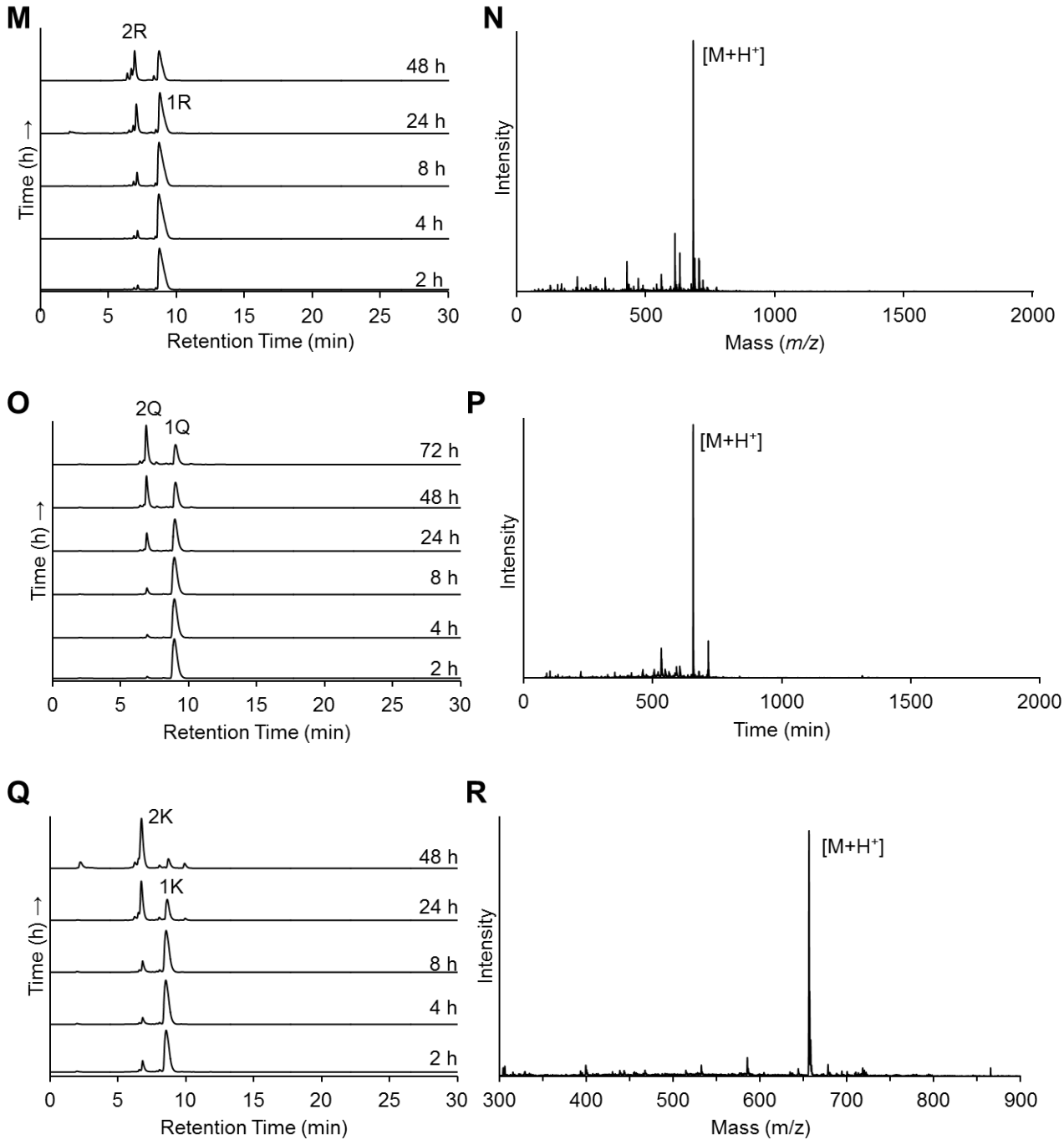


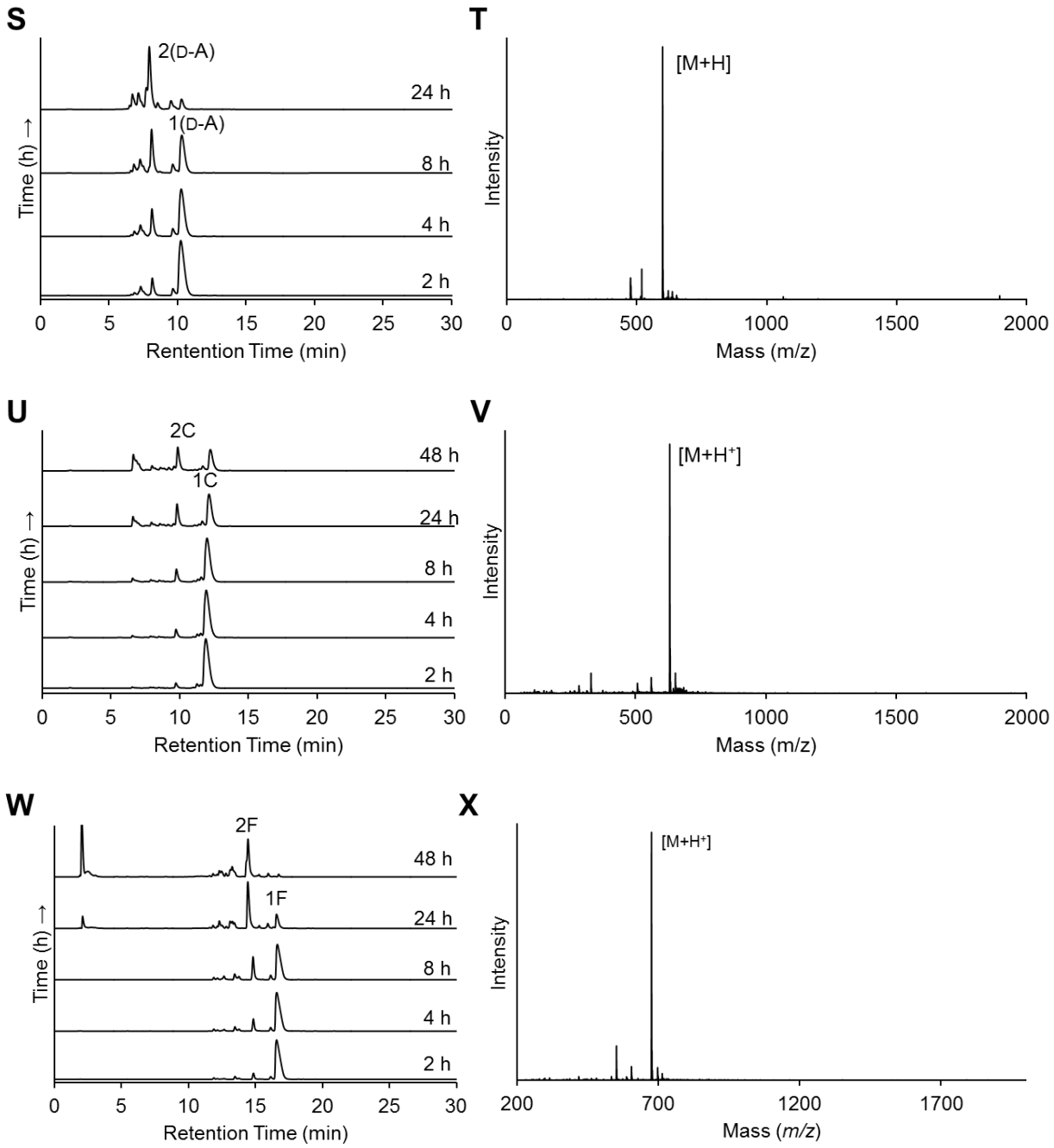
Figure S4. AWKX-MEGA thioesterification. (A) AWK**G**-MEGA thioesterification time-course. (B) ESI-MS of AWK**G**-MES thioester. Calcd. [M+H$^+$] 584.7 Da, obsd. 584.1 Da. (C) AWK**A**-MEGA thioesterification time-course. (D) ESI-MS of AWK**A**-MES thioester. Calcd. [M+H$^+$] 598.7 Da, obsd. 598.3 Da. (E) AWK**L**-MEGA thioesterification time-course. (F) ESI-MS of AWK**L**-MES thioester. Calcd. [M+H$^+$] 640.8 Da, obsd. 640.4 Da. **1** = AWK**X**-MEGA, **2** = AWK**X**-MES thioester. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.



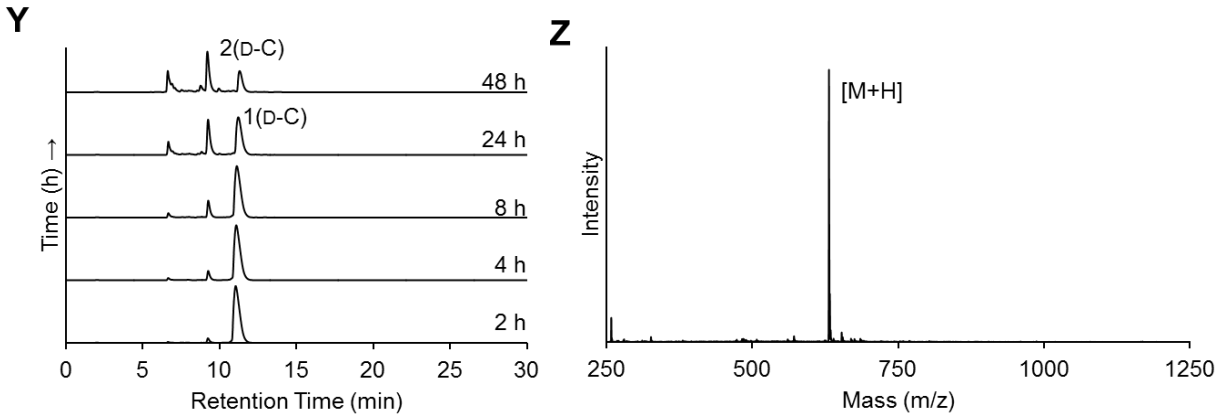
Continued Figure S4. AWKX-MEGA thioesterification. (G) AWK**V**-MEGA thioesterification time-course. (H) ESI-MS of AWK**V**-MES thioester. Calcd. [M+H$^+$] 626.8 Da, obsd. 626.4 Da. (I) AWK**T**-MEGA thioesterification time-course. (J) ESI-MS of AWK**T**-MES thioester. Calcd. [M+H$^+$] 628.8 Da, obsd. 628.7 Da. (K) AWK**S**-MEGA thioesterification time-course. (L) ESI-MS of AWK**S**-MES thioester. Calcd. [M+H$^+$] 614.7 Da, obsd. 614.4 Da. **1** = AWK**X**-MEGA, **2** = AWK**X**-MES thioester. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.



Continued Figure S4. AWKX-MEGA thioesterification. (M) AWK**R**-MEGA thioesterification time-course. (N) ESI-MS of AWK**R**-MES thioester. Calcd. [M+H$^+$] 683.8 Da, obsd. 683.5 Da. (O) AWK**Q**-MEGA thioesterification time-course. (P) ESI-MS of AWK**Q**-MES thioester. Calcd. [M+H$^+$] 655.8 Da, obsd. 655.3 Da. (Q) AWK**K**-MEGA thioesterification time-course. (R) ESI-MS of AWK**K**-MES thioester. Calcd. [M+H$^+$] 656.8 Da, obsd. 656.8 Da. **1** = AWK**X**-MEGA, **2** = AWK**X**-MES thioester. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.



Continued Figure S4. AWKX-MEGA thioesterification. (S) AWK**(D-A)**-MEGA thioesterification time-course. (T) ESI-MS of AWK**(D-A)**-MES thioester. Calcd. $[M+H^+]$ 599.7 Da, obsd. 599.8 Da. (U) AWK**C**-MEGA thioesterification time-course. (V) ESI-MS of AWK**C**-MES thioester. Calcd. $[M+H^+]$ 631.8 Da, obsd. 631.4 Da. (W) AWK**F**-MEGA thioesterification time-course. (X) ESI-MS of AWK**F**-MES thioester. Calcd. $[M+H^+]$ 675.8 Da, obsd. 675.3 Da. **1** = AWK**X**-MEGA, **2** = AWK**X**-MES thioester. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.



Continued Figure S4. AWKX-MEGA thioesterification. (Y) AWK**(D-C)**-MEGA thioesterification time-course. (Z) ESI-MS of AWK**(D-C)**-MES thioester. Calcd. [M+H^+] 631.8 Da, obsd. 631.1 Da. **1** = AWK**(D-C)**-MEGA, **2** = AWK**(D-C)**-MES thioester. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.

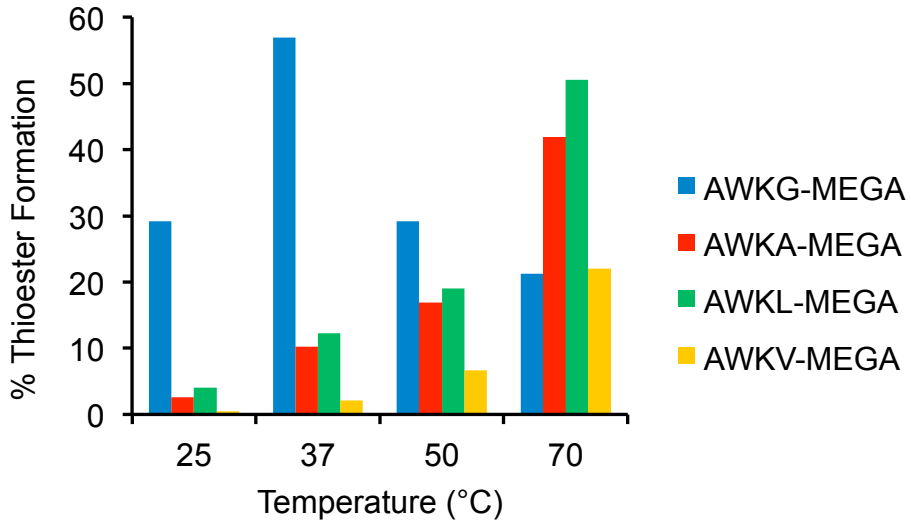


Figure S5. Temperature-dependence of AWKX-MEGA thioesterification. Histogram shows the percentage of AWK**X**-MES thioester formed at 24 h under optimized temperatures and pH 5.6 for the given AWK**X**-MEGA peptides. Percent thioester formation was determined by RP-HPLC peak integration at 280 nm.

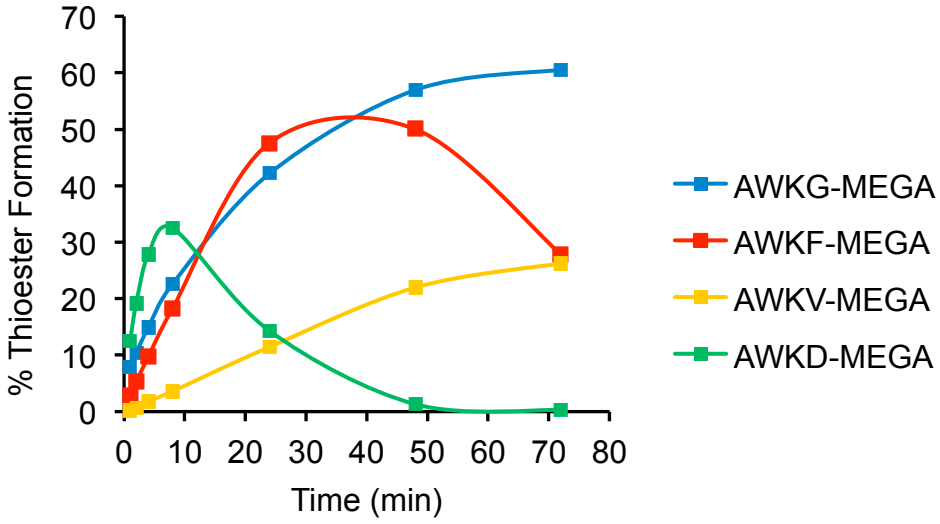


Figure S6. Time-course of thioester formation for AWKX-MEGA peptides. Reactions performed under optimized temperatures at pH 5.6 for each peptide. The final percent thioester formed at each time-point was determined by RP-HPLC peak integration at 280 nm.

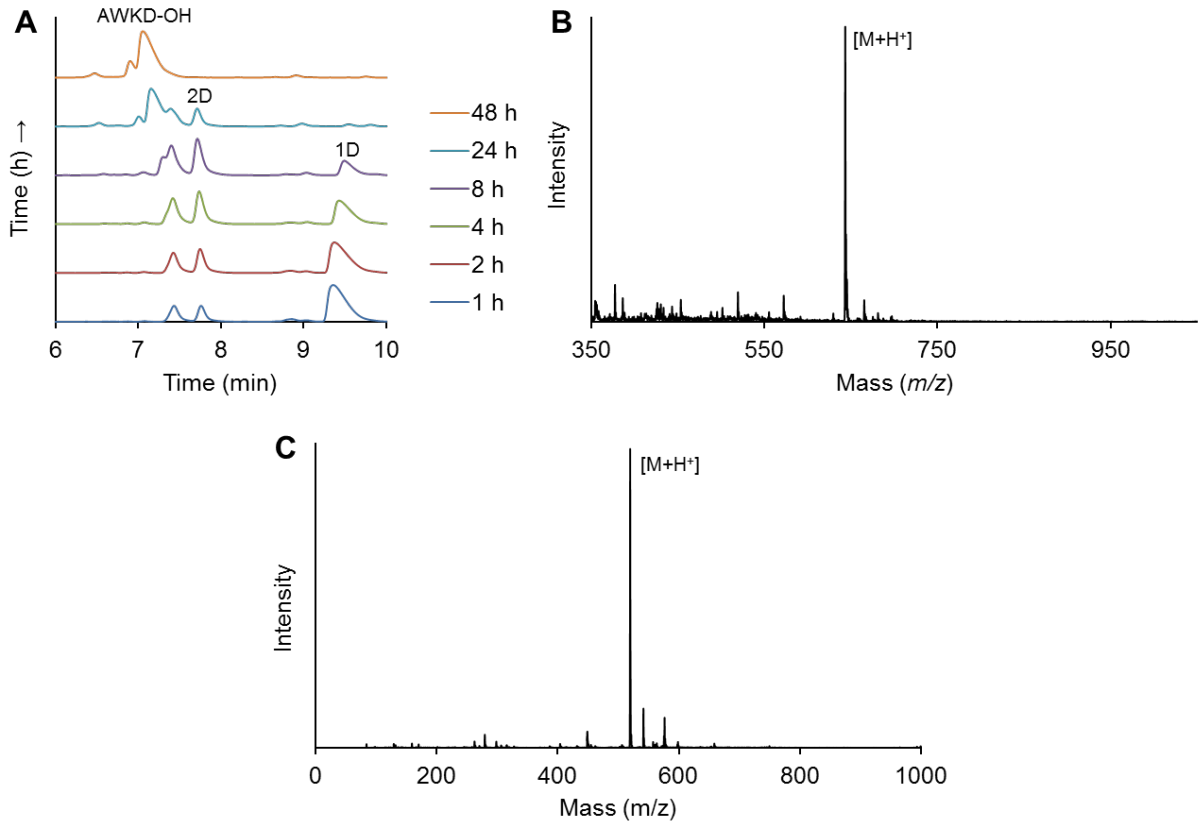


Figure S7. AWKD-MEGA thioesterification (A) AWK**D**-MEGA thioesterification time-course. (B) ESI-MS of AWK**D**-MES thioester. Calcd. [M+H$^+$] 642.7 Da, obsd. 642.3 Da. (C) ESI-MS of AWK**D**-OH by-product. Calcd. [M+H$^+$] 518.5 Da; obsd. 518.4 Da. **1** = AWK**D**-MEGA, **2** = AWK**D**-MES thioester. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.

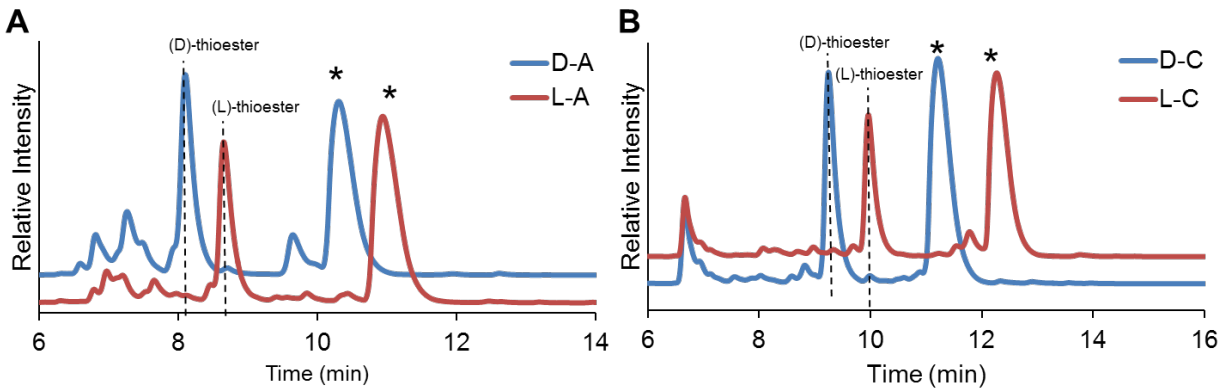


Figure S8. Epimerization during MEGA-mediated thioesterification (A) Overlaid C18 analytical RP-HPLC of AWK**(D-A)**-MES and AWK**A**-MES thioesters formed at 8 h. 0.6% and 1.3% epimerization were observed for AWK**(D-A)**-MEGA and AWK**A**-MEGA, respectively. (B) Overlaid C18 analytical RP-HPLC of AWK**(D-C)**-MES and AWK**C**-MES thioesters formed at 24 h; 0.8% and 1.1% epimerization were observed for AWK**(D-C)**-MEGA and AWK**C**-MEGA, respectively. RP-HPLC: 10-60% B, 30 min gradient. * = AWKX-MEGA starting material.

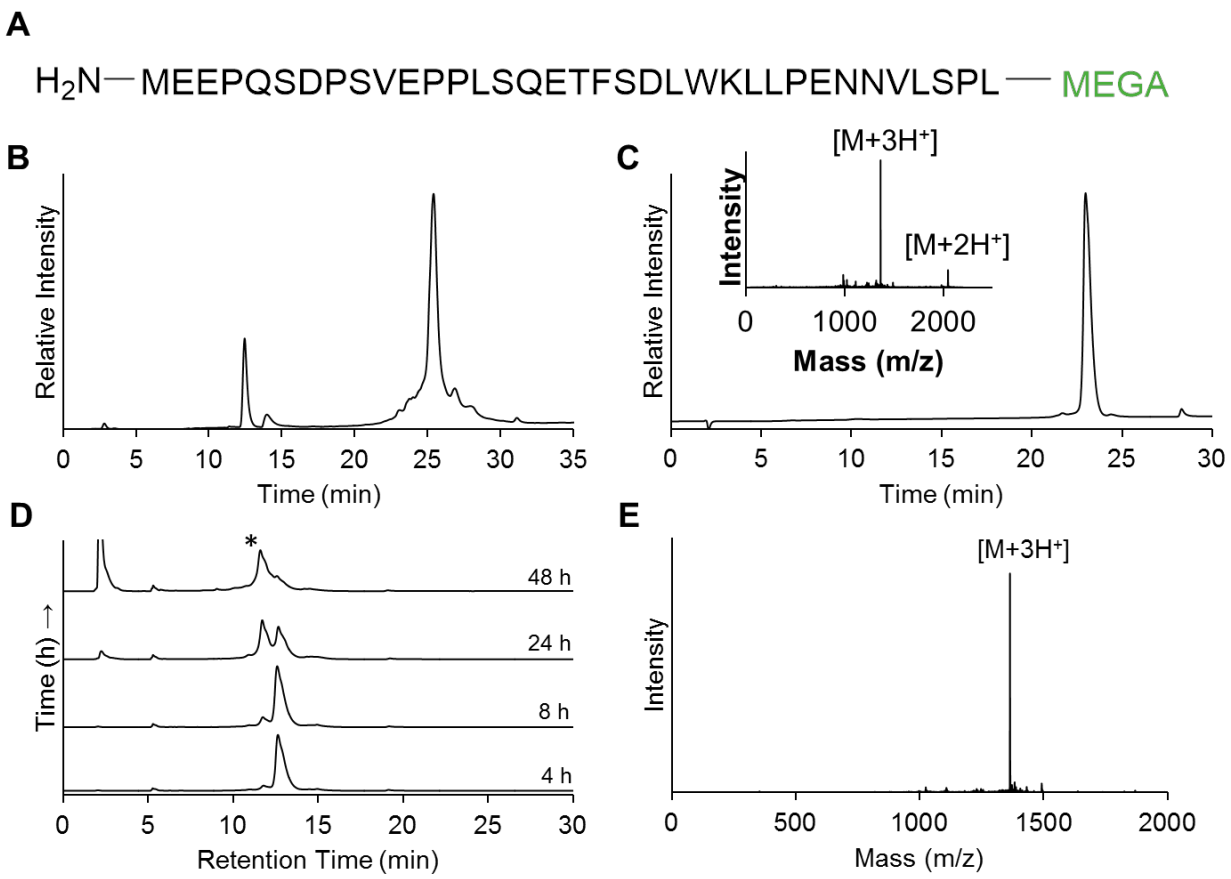


Figure S9. Synthesis and thioesterification of p53(1-35)-MEGA. (A) Amino acid sequence of p53(1-35)-MEGA. (B) RP-HPLC of crude p53(1-35)-MEGA peptide product after TFA cleavage from solid support (0-73% B, 30 min gradient). (C) RP-HPLC of pure p53(1-35)-MEGA (0-73% B, 30 min gradient). Inset is ESI-MS of purified p53(1-35)-MEGA. Calcd. [M+H$^+$] 4,098.6 Da, obsd. 4,097.7 ± 0.7 Da. (D) Thioesterification time-course of p53(1-35)-MEGA (C18 RP-HPLC, 30-80% B, 30 min gradient). Buffer: 400 mM MESNa, 100 mM NaH_2PO_4, 25 mM TCEP, pH 4.5, 70 °C. (E) ESI-MS of isolated p53(1-35) C-terminal thioester; Calcd. [M+H$^+$] 4,091.6 Da, obsd. 4,090.9 ± 2.2 Da. * = p53(1-35)-MEGA thioester.

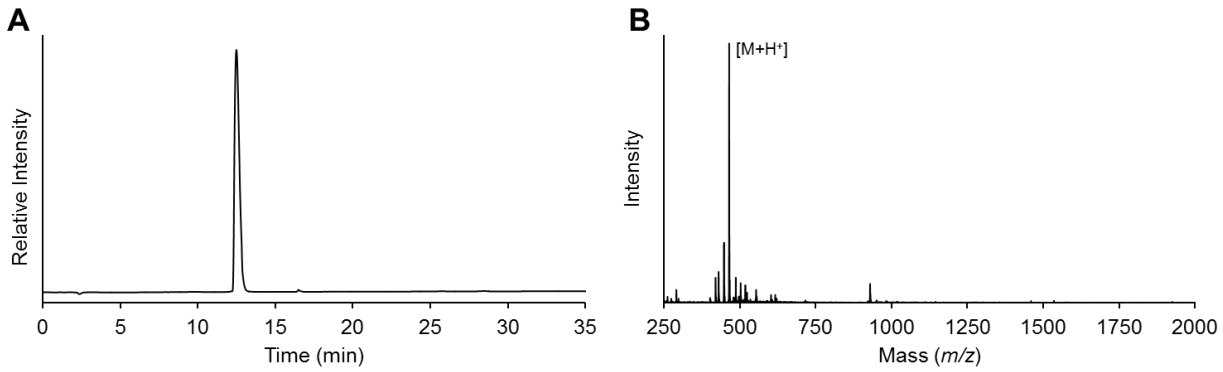


Figure S10. Purified CASW peptide. (A) C18 analytical RP-HPLC chromatogram of purified CASW, 0-73% B, 30 min gradient. (B) ESI-MS of purified CASW. Calcd. [M+H$^+$] 464.2 Da, obsd. 464.2 Da.

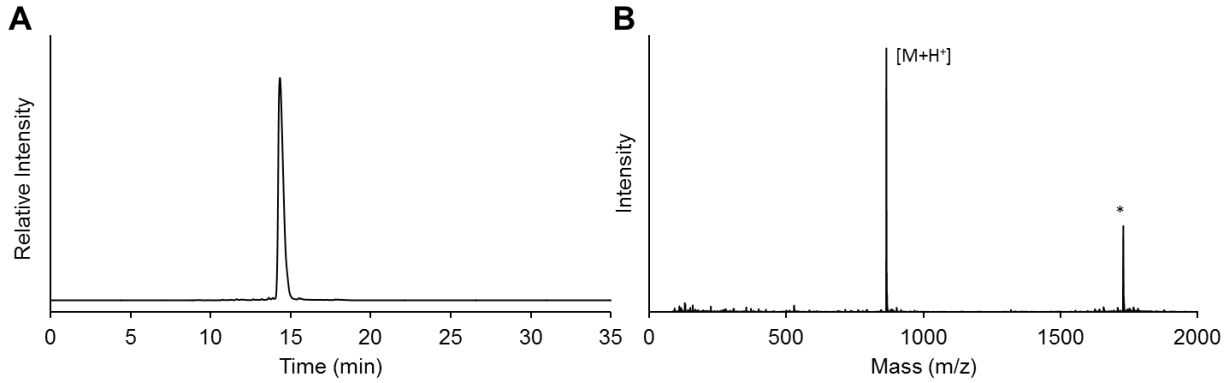


Figure S11. Synthesis of CASHEW-MEGA peptide. (A) C18 analytical RP-HPLC chromatogram of purified CASHE**W**-MEGA peptide, 0-73% B, 30 min gradient. (B) ESI-MS of purified CASHE**W**-MEGA peptide. Calcd. [M+H$^+$] 864.0 Da, obsd. 864.0 Da. * = Symmetric disulfide of CASHE**W**-MEGA.

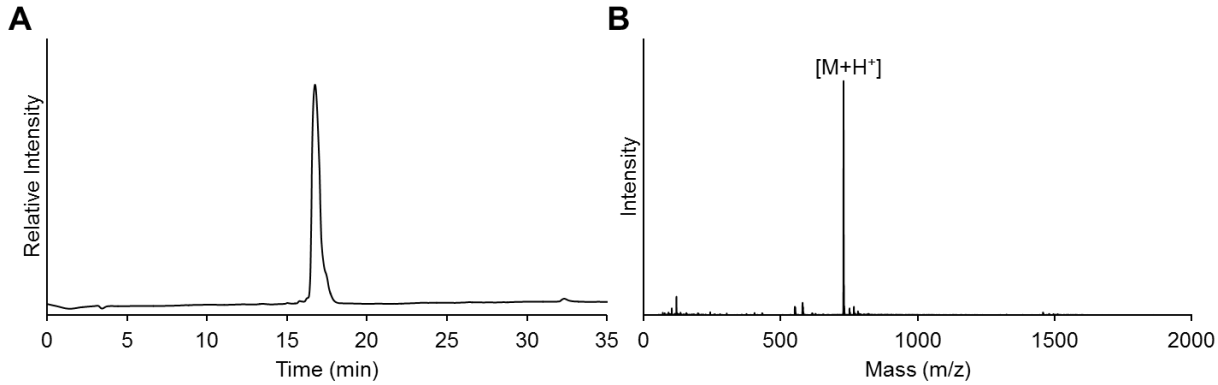


Figure S12. Synthesis of CRGD(D-F)-MEGA peptide. (A) C18 analytical RP-HPLC chromatogram of purified CRGD**(D-F)**-MEGA peptide, 0-73% B, 30 min gradient. (B) ESI-MS of purified CRGD(D-F)-MEGA peptide. Calcd. [M+H$^+$] 728.8 Da, obsd. 728.7 Da.

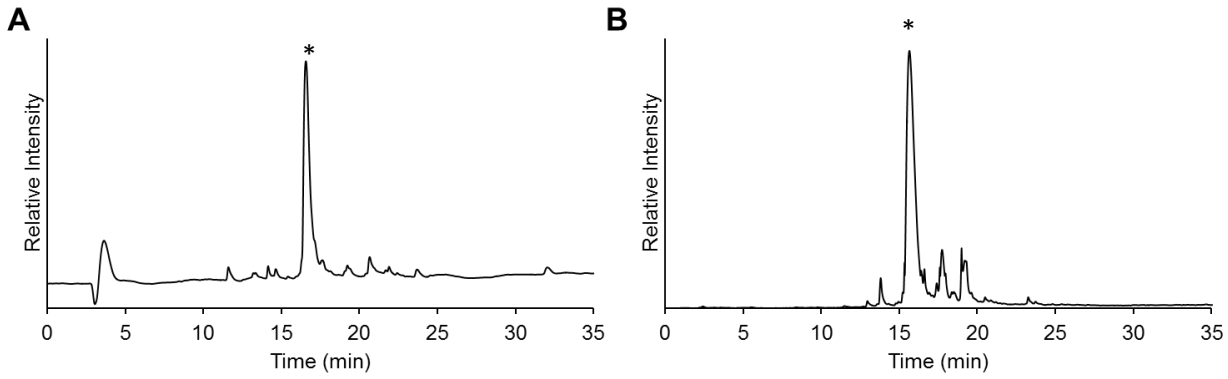


Figure S13. RP-HPLC spectra of crude N-terminal Cys-containing peptides after TFA-cleavage from resin. (A) Crude CRGD(D-F)-MEGA peptide (B) Crude CASHEW-MEGA peptide. RP-HPLC performed on C18 analytical column, 0-73% B, 30 min gradient. Asterisks indicate the desired product.

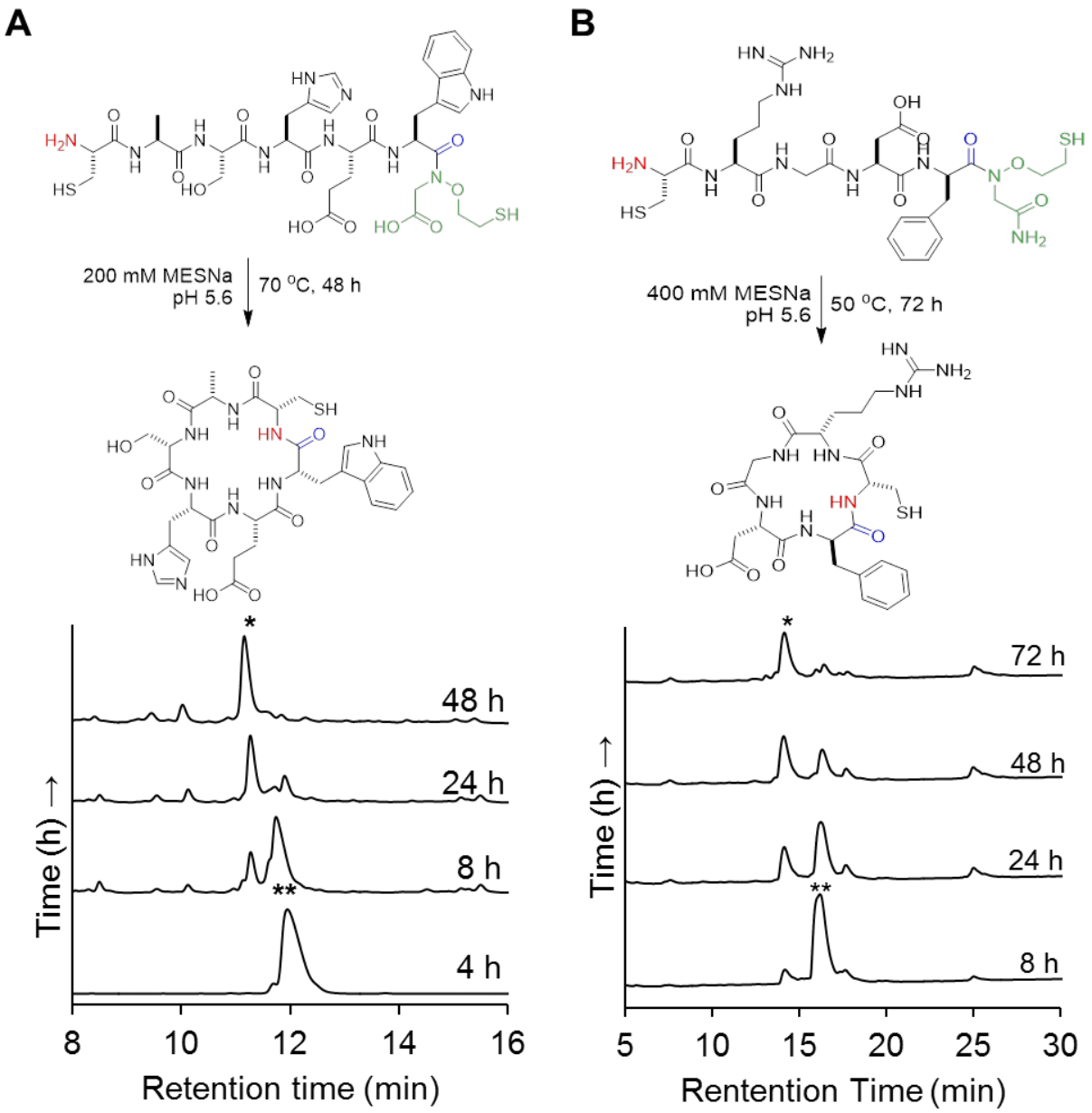


Figure S14. Cyclization of CASHEW-MEGA and CRGD(D-F)-MEGA peptides. (A) Scheme depicting the cyclization reaction of CASHEW-MEGA (top) and RP-HPLC time-course of CASHEW-MEGA cyclization (bottom). (B) Scheme depicting the cyclization reaction of CRGD(D-F)-MEGA (top) and RP-HPLC time-course of CRGD(D-F)-MEGA cyclization (bottom). * = Cyclized peptide, ** = Initial MEGA peptide. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.

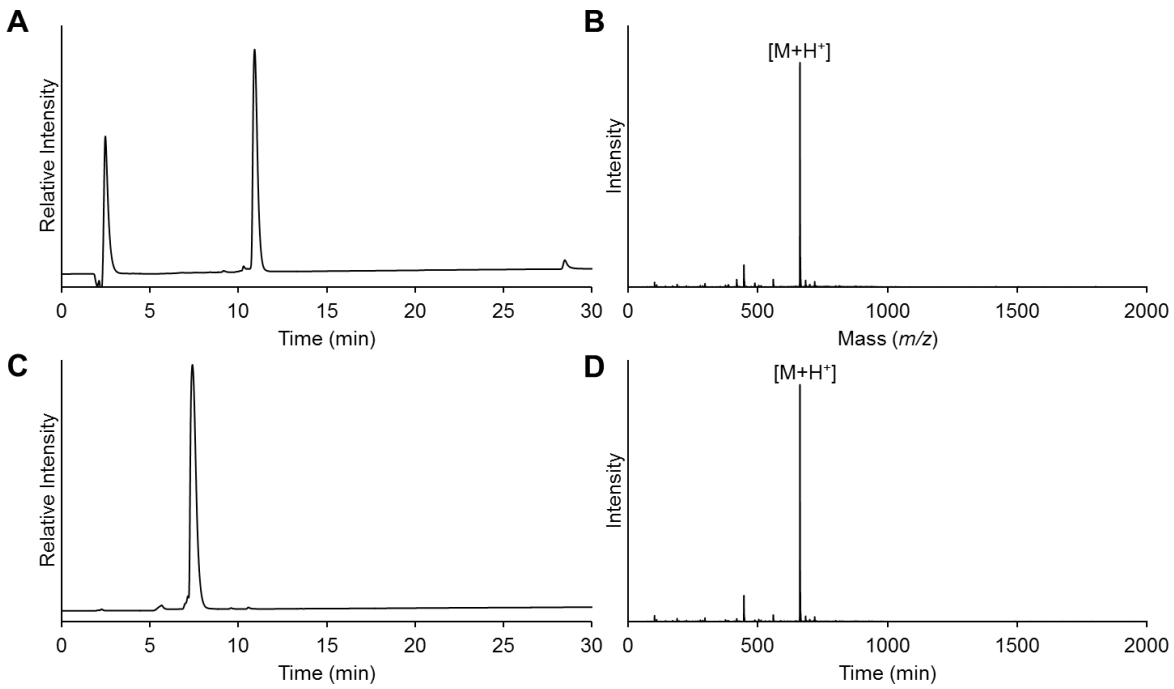


Figure S15. Synthesis of CLAS(D-H)-MEGA and CLAS(L-H)-MEGA peptides (A) C18 analytical RP-HPLC chromatogram of purified CLAS(D-H)-MEGA peptide. (B) ESI-MS of purified CLAS(D-H)-MEGA peptide. Calcd. [M+H$^+$] 662.8 Da, obsd. 662.6 Da. (C) C18 analytical RP-HPLC chromatogram of purified CLAS(L-H)-MEGA peptide. (D) ESI-MS of purified CLAS(D-H)-MEGA peptide. Calcd. [M+H$^+$] 662.8 Da, obsd. 662.5. RP-HPLC performed on C18 analytical column, 0-73% B, 30 min gradient.

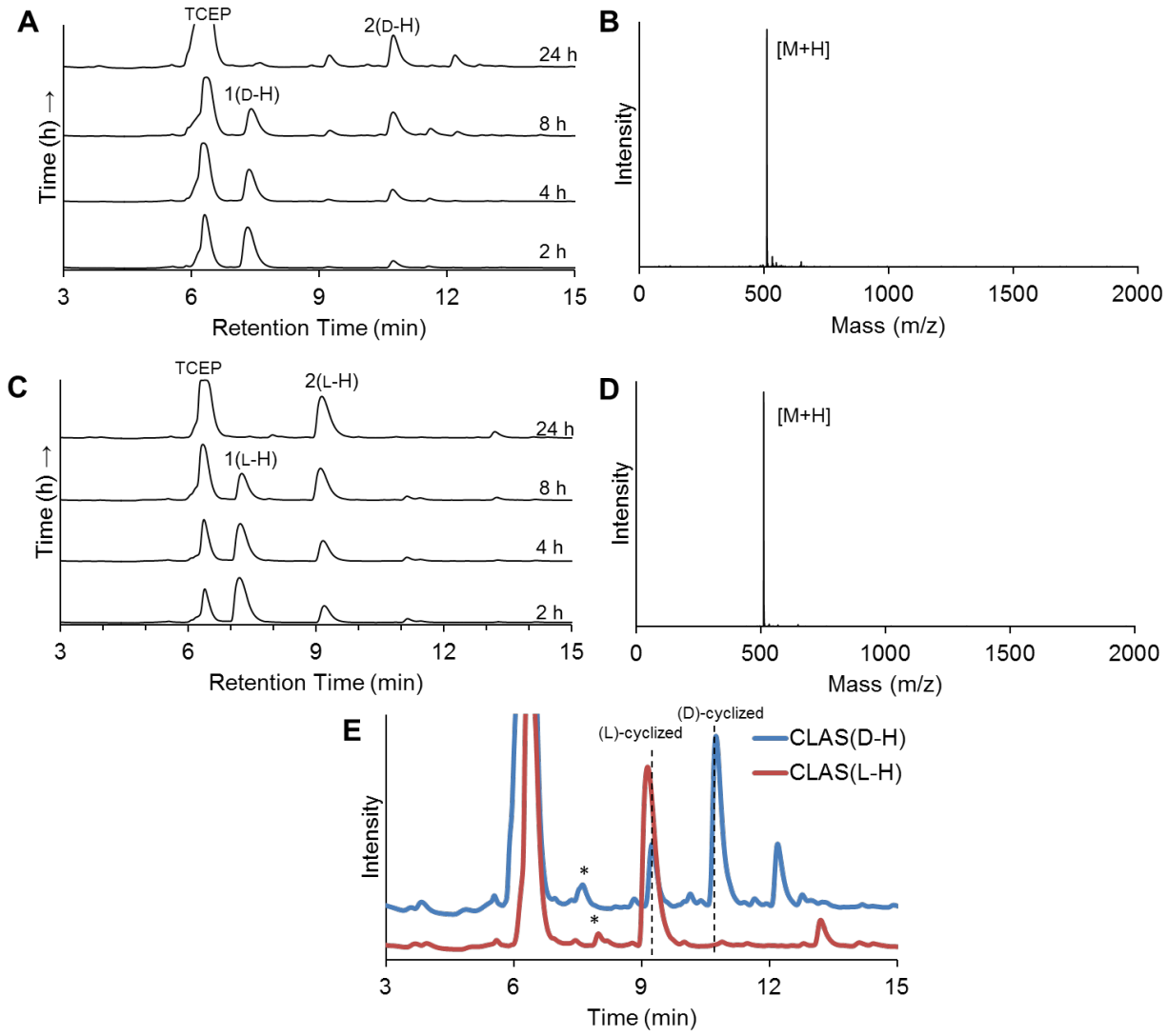


Figure S16. Cyclization of CLAS(D-H)-MEGA and CLAS(L-H)-MEGA peptides (A) CLAS(D-H) cyclization time-course. (B) ESI-MS of purified cyclic CLAS(D-H) peptide. Calcd. [M+H$^+$] 512.2 Da, obsd. 512.4 Da. (C) CLAS(L-H) cyclization time-course. (D) ESI-MS of purified cyclic CLAS(L-H) peptide. Calcd. [M+H$^+$] 512.2 Da, obsd. 512.4 Da. (E) Overlaid C18 analytical RP-HPLC of CLAS(D-H)-MEGA and CLAS(L-H)-MEGA cyclization reactions at 24 h; 24.8% and 1.8% epimerization were observed for CLAS(D-H)-MEGA and CLAS(L-H)-MEGA, respectively. **1** = CLASH-MEGA, **2** = Cyclized product, * = Unreacted CLASH-MEGA. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.

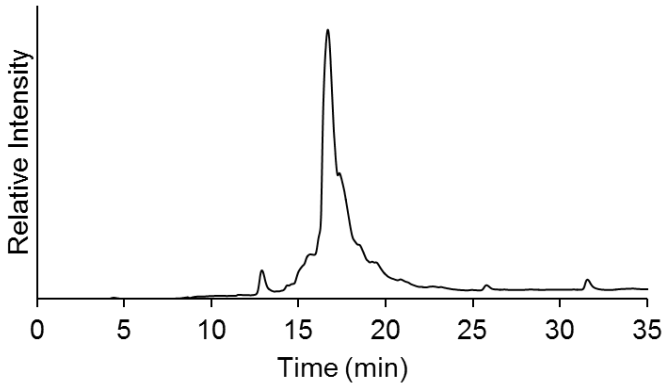


Figure S17. RP-HPLC spectrum of crude SFT-1 (I10G)-MEGA peptide after TFA-cleavage from resin. RP-HPLC performed on C18 analytical column, 0-73% B, 30 min gradient.

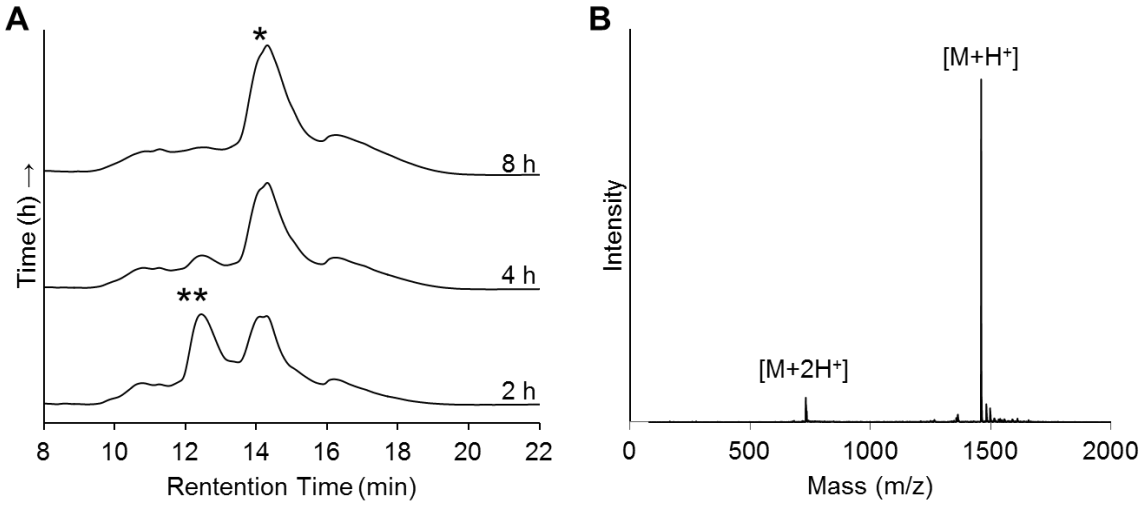


Figure S18. SFT-1 (I10G)-MEGA cyclization. (A) RP-HPLC time-course of SFT-1 (I10G)-MEGA thioesterification. Buffer: 400 mM MESNa, 100 mM NaH_2PO_4, 25 mM TCEP, pH 5.6, 70 °C. * = Cyclized product, ** = SFT-1 (I10G)-MEGA peptide. (B) ESI-MS of SFT-1 (I10G) cyclized product. Calcd. [M+H$^+$] 1,459.7 Da, obsd. 1,459.4 Da. RP-HPLC performed on C18 analytical column, 10-60% B, 30 min gradient.

References

1. Coin, I.; Beyermann, M.; Bienert, M. *Nat. Protoc.* **2007,** *2,* 3247.
2. Weller, C. E.; Huang, W.; Chatterjee, C. *ChemBioChem* **2014,** *15,* 1263.
3. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972,** *37,* 3404.
4. Bacsa, B.; Kappe, C. O. *Nat. Protoc.* **2007,** *2,* 2222.
5. Lavens, S. E.; Proud, D.; Warner, J. A. *J. Immunol. Methods* **1993,** *166,* 93.
6. Quimbar, P.; Malik, U.; Sommerhoff, C. P.; Kaas, Q.; Chan, L. Y.; Huang, Y. H.; Grundhuber, M.; Dunse, K.; Craik, D. J.; Anderson, M. A.; Daly, N. L. *J. Biol. Chem.* **2013,** *288,* 13885.