Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Epimerization-Free Access to C-Terminal Cysteine Peptide Acids, Carboxamides, Amides, and Esters via Complimentary Strategies

Christine A. Arbour, Thilini D. Kondasinghe, Hasina Y. Saraha, Teanna L. Vorlicek and Jennifer L. Stockdill*

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, United States

Table of Contents

General Information. Solid-Phase Peptide Synthesis was performed on a Biotage SP *Wave* Initiator$^+$ and all peptides were synthesized on either pre-loaded glycine Wang Resin (Fmoc-Gly-Wang Resin loading: 0.62 mmol/g from Chem-Impex Int'l. Inc.) or Rink Amide Resin (Novabiochem, 100-200 mesh, loading: 0.77 mmol/g). ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Varian MR-400, Agilent MR-400, or a Varian V-500 MHz instrument with a multinuclear broadband probe at ambient temperature unless otherwise stated. Chemical shifts are reported in parts per million relative to residual solvent peaks (as established by Stoltz, et. Al. in *Organometallics* **2010**, *29*, 2176). All ^{13}C spectra are recorded with complete proton decoupling. All HPLC analyses and purifications were performed on a Custom Reverse Phase Shimadzu Liquid Chromatograph Mass Spectrometer (LCMS-2020), which can toggle between analytical and semi-preparative columns. This instrument has a photodiode array (PDA) detector (D2 & W lamp), which collects a range of wavelengths, in place of a traditional single channel UV detector. RP-HPLC-MS mobile phases (MeCN and H_2O) contained 0.1% Formic Acid. Analytical HPLC was performed on a Phenomenex Kinetex C18 column (5 μm, 250 x 4.6 mm) and a Thermo Scientific Hypersil Gold C8 column (5 μm, 250 x 4.6 mm). Semi-Preparative HPLC was conducted using a Thermo Scientific Hypersil Gold C8 column (5 μm, 150 x 10 mm). Thin layer chromatography was performed using glass-backed SiliaPlate™ TLC Plates (cat. # TLG-R10011B-323) cut to the desired size then visualized with short-wave UV lamps and $KMnO_4$, CAM, PMA, or Anisaldehyde stains prepared according to standard recipes.[1] All yields refer to chromatographically and spectroscopically pure products. All peptide yields are calculated based on the final loading. Optical rotation data was collected on a Perkin-Elmer 341 automated Polarimeter at the concentration noted.

Reagents and Materials.

Unless otherwise specified, all commercially available reagents were used without further purification. Anhydrous Et_2O, PhMe, *n*-hexane, MeCN, DMF, DMSO, CH_2Cl_2 were purchased from Fisher, THF was purchased from EMD, PhH was purchased from Sigma-Aldrich and HPLC grade MeCN and H_2O were purchased from Fisher. These were passed through a commercial solvent purification system (2 columns of alumina) and used without further drying. Triethylamine, diisopropylamine, pyridine, and Hünig's base were distilled over CaH_2 immediately prior to use. All L- and D- amino acids were purchased from Chem-Impex Int'l. Inc. unless otherwise noted. HATU were purchased from Chem-Impex Int'l. Inc. 4-Nitrophenyl chloroformate was purchased from Alfa Aesar. 4-fluoro-3-nitrobenzoic acid, and *N*-(3-bromopropyl)phthalimide were purchased from Oakwood Chemical. 33 wt.% methylamine in absolute ethanol was purchased from Sigma-Aldrich. The authentic sample of α-Conotoxin ImI was obtained free of charge from Alomone Labs.

[1] Leonard, J.; Lygo, B.; Procter, G. In *Advanced Practical Organic Chemistry*; CRC Press: Boca Raton, FL, 2013; pp 158. (ISBN: 978-1-4398-6097-7)

SPPS.

All substrates were made on either pre-loaded Gly-Wang Resin or Rink Amide Resin on a 500 mg scale (loadings varied).

Semi-Automated Microwave Synthesis of Peptides:

- *Reactor Vials [Vial size (Volume range allowed)]:* 2 mL reactor vial (0.8-1.1 mL), 5 mL reactor vial (1.6-3.2 mL), and 10 mL reactor vial (3.2-6.4 mL)
- *Swell + Heat:* DMF was added and vortexed at 1200 RPM for 20 min at 70 ºC. The solvent was then removed over 1 m followed by two DMF washes (DMF was added and the suspension was vortexed at 600 RPM for 45 s, followed by the removal of solvent (over 2 min)).
- *Coupling:* A solution of Fmoc-aa-OH (5 equiv), HATU (4.9 equiv), and DIEA (10 equiv) in DMF was made immediately prior to addition to the reaction vial containing the resin. Once the solution was added, the suspension was heated to 75 ºC for 5 min with a vortex rate of 1200 RPM. After the reaction, the solution was removed (over 2 min) and the resin was rinsed with DMF 4 times (after addition of DMF, the suspension was agitated at a vortex rate of 1200 RPM for 1 min, solvent removal was at a rate of 2 m). Fmoc-Cys(Trt)-OH was coupled using a *modified* method established by Dawson, P. E. *JACS* **2015**, to minimize epimerization, using Fmoc-Cys-OH (1.55 mmol), HATU (1.55 mmol), DIEA (1.705 mmol) in 4 mL DMF at 75 ºC for 5 min.[2]
- *Fmoc Removal (Deprotection):* The reactor vial was filled with 20% piperidine in DMF. The suspension was vortexed at 1200 RPM for 3 min at RT. The solvent is removed followed by addition of 20% piperidine in DMF. The suspension is vortexed again at 1200 RPM for 10 min at RT. The solvent was removed over 2 min, followed by 4 DMF washes (after addition of DMF, the suspension was agitated at a vortex rate of 1200 RPM for 1 min, solvent removal was at a rate of 2 min).
- *Wash*: DMF was added to the reaction vial and agitated at a vortex rate of 1200 RPM for 1 min. The solvent was removed over 1 min and repeated for a total of 4 times.
- *Final Wash:* Resin was rinsed with CH_2Cl_2 (3 x 1 mL) and MeOH (3 x 1 mL).
- *Drying for Storage/Weighing:* After the final wash, the resin was placed on the lyophilizer overnight for drying.

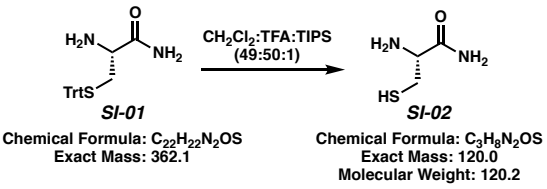
Native Chemical Ligation Buffer:
The buffer was made using a previously established protocol.[3]

MeDbz activation:
Activation conditions were conducted using a previously established protocol.[2]

[2] Blanco-Canosa, J. B.; Nardone, B.; Albericio, F.; Dawson, P. E. *J. Am. Chem. Soc.* **2015**, *137*, 7197–7209.
[3] Aussedat, B.; Fasching, B.; Johnston, E.; Sane, N.; Nagorny, P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 3532–3541.

Calculating *Conversion* of On Resin Reactions.

After the nucleophilic cleavage was conducted, the resin was subjected to 95:2.5:2.5 TFA:TIPS:H_2O to cleave the remaining *MeNbz-Gly* and unreacted peptide-*MeNbz-Gly*. The solvent was concentrated using a constant stream of air until a small amount of residue was left. Then cold diethyl ether was added to the vial to crash out the crude peptide. After centrifugation, the ether was decanted off and the solid was dissolved in 1:1 MeCN:H_2O and lyophilized. The lyophilized solid was then dissolved in 20% MeCN/H_2O and analyzed via RP-HPLC-MS using a gradient of 20-80% MeCN/H_2O over 15 min. Conversion was calculated based on the ratio of H_2N-peptide-*MeNbz-Gly*-OH[4] to H_2N-*MeNbz-Gly*-OH.



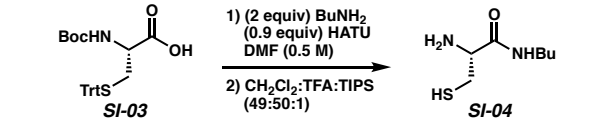
SI-01
Chemical Formula: $C_{22}H_{22}N_2OS$
Exact Mass: 362.1

SI-02
Chemical Formula: $C_3H_8N_2OS$
Exact Mass: 120.0
Molecular Weight: 120.2

Synthesis of cysteine amide.

1 g (2.76 mmol) of H-Cys(Trt)-NH_2 was weighed out into a 100 mL round bottom flask containing a stir bar. The amino acid was then dissolved in 10 mL of (49:50:1) CH_2Cl_2:TFA:TIPS and stirred at ambient temperature. After 15 min, TIPS was added dropwise until the solution turned from bright red-orange to a pale yellow solution. The volatile solvent was then removed under reduced pressure after 30 min, followed by addition of 40 mL water. A white solid was filtered off and the filtrate was frozen (LN_2), and lyophilized to yield the crude cysteine amide in >99% yield (745 mg).^{1}H NMR (499 MHz, Acetonitrile-d_3) δ 7.16 (s, 1H), 6.68 (s, 1H), 4.20 (t, J = 5.3 Hz, 1H), 3.06 (qd, J = 14.9, 5.1 Hz, 2H). ^{13}C NMR (126 MHz, CD_3CN) δ 169.8, 55.3, 26.0.

[4] In some cases, Fmoc was still intact and incorporated into the % conversion calculation.



Chemical Formula: $C_{27}H_{29}NO_4S$
Exact Mass: 463.2

Chemical Formula: $C_7H_{16}N_2OS$
Exact Mass: 176.1
Molecular Weight: 176.3

Synthesis of cysteine butylamide.

Step 1 (Synthesis Boc-Cys(Trt)-NHBu): To a microwave vial was added Boc-Cys(Trt)-OH and HATU (plus stir bar) and the vial was sealed with a cap. Thereafter, DMF was added and the reagents were fully dissolved. Upon completion of the reagents dissolving, butylamine was added (vial felt warm). Then, the vial was placed in the microwave for 5.5 mins at 75 ºC. This was done two times, and the reaction was monitored by direct MS. Unfortunately, the reaction did not go to completion after these two rounds of microwaving. The reaction was allowed to stir vigorously for 2 days at ambient temperature. Again, the starting material was observed. The reaction was then quenched/diluted with water (white precipitate). Then, ethyl acetate was added. A clear line was not achievable between the aqueous and organic layer. Therefore, brine was added and the extraction solution (aqueous) became clear, and it was obvious where the layers sectioned for extraction. The aqueous layer was extracted 5 times, and the organic layer was dried over sodium sulfate. After filtration, the organic layer was concentrated and purified using column chromatography (DCM to 5% MeOH/DCM). 243.2 mg (43% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 (d, *J* = 7.4 Hz, 6H), 7.28 (t, *J* = 7.5 Hz, 6H), 7.21 (t, *J* = 7.2 Hz, 3H), 6.05 (s, 1H), 4.94-4.84 (bs, 1H), 3.95-3.74 (bs, 1H), 3.17 (q, *J* = 6.7 Hz, 2H), 2.69 (dd, *J* = 12.5, 7.0 Hz, 1H), 2.52 (dd, *J* = 12.8, 5.4 Hz, 1H), 1.41 (s, 10H), 1.29 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.29, 155.37, 144.44, 129.57, 128.02, 126.83, 80.14, 67.13, 53.62, 39.18, 33.91, 31.43, 28.27, 19.94, 13.71.

Step 2 (Synthesis SI-04): To a round bottom flask containing Boc-Cys(Trt)-NHBu, 40 mL of deprotection cocktail (49:50:1 of DCM:TFA:TIS) was added to dissolve the residue, and the deprotection was carried out for 30 minutes at room temperature. The solvent was removed under vacuum, and the residue was suspended in 20 mL of water, and then filtered. The filtrate was collected. Thereafter, the aqueous layer was rinsed once with hexanes. The hexanes was discarded, and the aqueous layer was frozen using liquid nitrogen and lyophilized. 142.3 mg (>99% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ 3.98 – 3.91 (m, 1H), 3.32 – 3.17 (m, 3H), 3.05 – 2.89 (m, 2H), 1.57 – 1.47 (m, 2H), 1.38 (dd, *J* = 15.1, 7.3 Hz, 3H), 0.94 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 166.75, 54.74, 39.02, 30.92, 24.89, 19.62, 12.58. $[\alpha]_D^{25}$ +5.05 (c 1.01, MeOH).

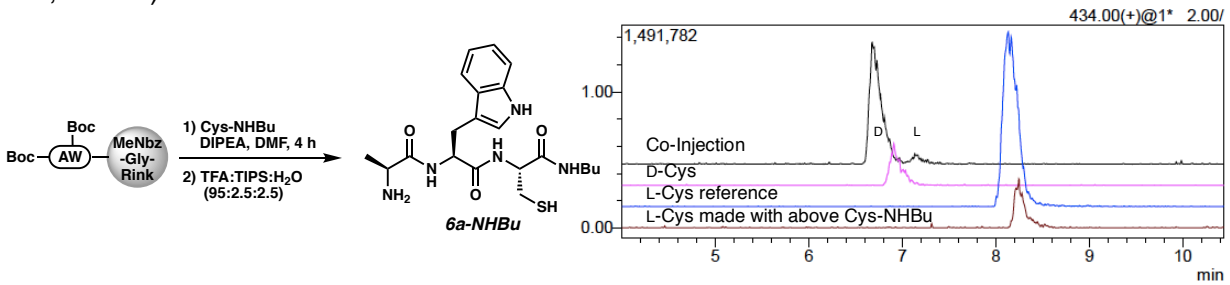
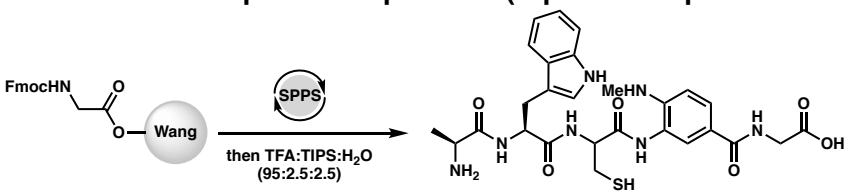


Figure SI-1. A) Co-injection, B) D-Cys, C) L-Cys made from AWC-MeNbz-Gly-Rink, and D) L-Cys from AW-MeNbz-Gly-Rink, gradient: 20-40% MeCN/H_2O + 1% HCOOH over 15 min.[5]

[5] The cysteine butylamide was used to displace AW-MeNbz-Gly-Rink to generate AWC-NHBu (**6a-NHBu**). This allowed us to confirm its enantiopurity.

Experimental Procedures and Spectroscopic Data (Piperidine Epimerization)



SI-05

Peptide **SI-05** was synthesized using a standard Fmoc-SPPS protocol described on S3. Both D- and L-Cys(Trt) substrates were made.[2]

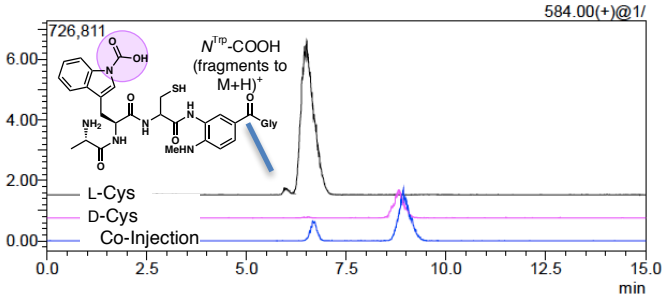
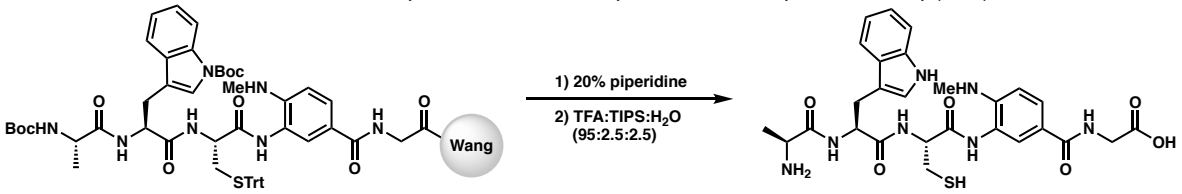


Figure SI-2. A) L-Cys, B) D-Cys, and C) Co-injection after SPPS, gradient: 40-95% MeCN/H_2O + 1% HCOOH over 15 min. **Note*: The indicated peak in the above spectra is a side product of Trp(Boc) in TFA.[6]



SI-05-Wang ***SI-05***

10 mg of resin was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 30 min. After this time the solvent was removed and the resin was subjected to 20% piperidine in DMF for 2,4, & 24 h. Then the solvent was removed and the resin was rinsed with DMF (3 x 1 mL) and CH_2Cl_2 (3 x 1 mL). The resin was then subjected to 95:2.5:2.5 TFA:TIPS:H_2O for 0.5 h. The solvent was collected after this time and concentrated under a constant stream of air. The sample was then crashed out with cold diethyl ether, centrifuged, and decanted to yield the crude peptide. The peptide was dissolved in 50% MeCN:H_2O and analyzed via RP-HPLC-MS using a gradient of 20% MeCN:H_2O over 15 min.

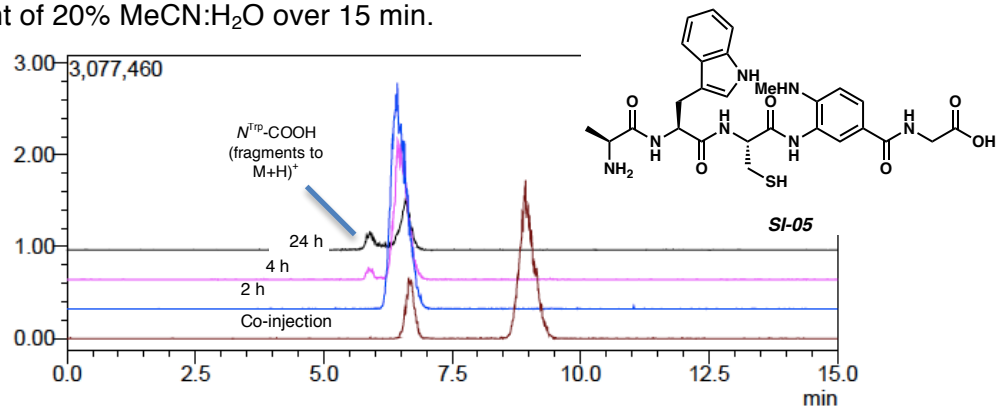


Figure SI-3. A) L-Cys after 24 h, B) 4 h, C) 2 h, and D) co-injection, gradient: 40-95% MeCN/H_2O + 1% HCOOH over 15 min.

[6] Franzén, H.; Grehn, L.; Ragnarsson, U. *J. Chem. Soc., Chem. Commun.* **1984**, *0*, 1699–1700.

Table 1. Evaluation of cysteine epimerization.

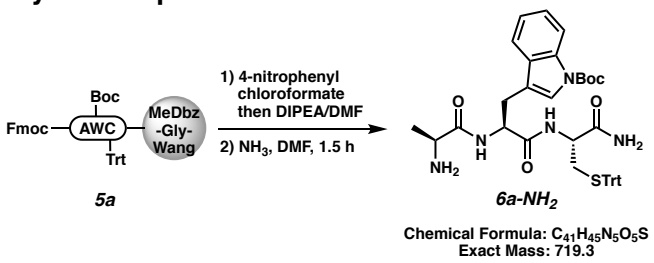


Table 1, Entry 1: H_2N-AWC-NH_2 (6a-NH_2). 100 mg of peptide on resin (dry) was weighed out into a 5 mL fritted reaction vial with an attached needle and rubber stopper. The resin was swelled in CH_2Cl_2 (500 μL – 4 mL) for 30 min. The MeDbz linker was activated by a previously established protocol.[2,7] Next, 500 μL of DMF was added and the reaction vial was closed off with a septum (needle and stopper still attached at the bottom). A double balloon was made and filled with ammonia. A hypodermic needle was attached to the balloon and inserted into the septum and submerged into the DMF solution. The smallest (blue) gauge needle was placed into the septum to allow for the slow exiting of gas. The ammonia gas was bubbled into DMF for 1.5 h. Then the solvent was removed and collected. Additional rinsing was required to completely remove the desired peptide from resin. CH_2Cl_2 (3 x 1 mL) was used to rinse the resin, followed by MeCN (3 x 1 mL), and lastly CH_2Cl_2 (3 x 1 mL). The washes were combined with the initial DMF solution, blown down with air, subjected to 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude product. Purification was conducted after 3 cycles of lyophilization (until crude product composition was a fluffy white solid). A silica column was used with a mobile phase eluent of 50-100% MeCN/Ethyl Acetate followed by 0-10% MeOH/CH_2Cl_2. The product was isolated, concentrated under reduced pressure, and lyophilized to yield a fluffy white solid (11.56 mg, 54%). ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.37 (d, J = 8.2 Hz, 1H), 8.16 (s, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.45 (s, 1H), 7.39 – 7.18 (m, 19H), 7.16 (s, 1H), 4.64 (s, 1H), 4.32 (q, J = 6.9 Hz, 1H), 3.26 (q, J = 6.9 Hz, 2H), 3.12 (dd, J = 14.7, 4.1 Hz, 1H), 2.98 – 2.88 (m, 1H), 2.37 (d, J = 6.7 Hz, 2H), 1.60 (s, 9H), 1.02 (d, J = 6.9 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 171.8, 171.1, 168.6, 144.7, 144.2, 129.5, 128.5, 127.2, 124.7, 124.5, 122.9, 122.8, 119.9, 116.7, 108.5, 83.9, 66.2, 52.2, 51.9, 50.6, 34.5, 31.2, 28.2, 27.8, 21.4.

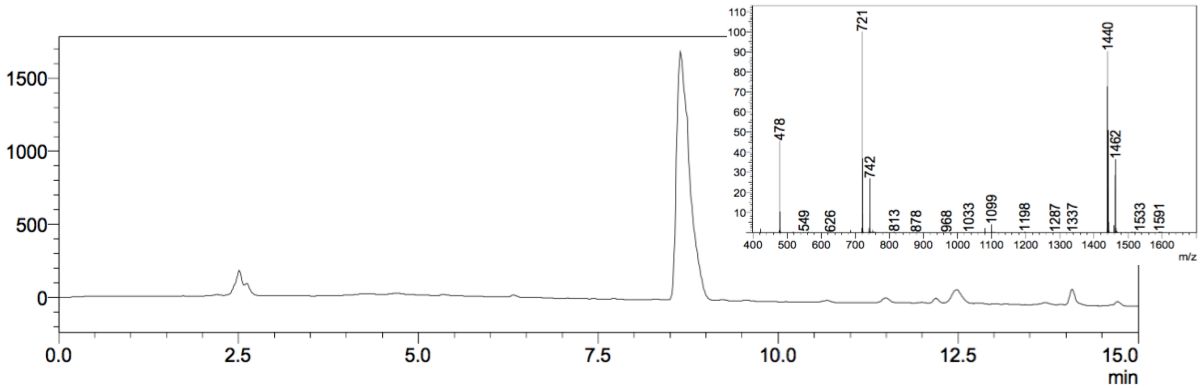


Figure SI-4. Analytical Column PDA of purified peptide at 190 nm. Gradient: 40-95% MeCN/H_2O + 1% HCOOH over 15 min. Retention time: 8.77 min.

[7] Blanco-Canosa, J. B.; Dawson, P. E. *Angew. Chem. Int. Ed.* **2008**, *47*, 6851–6855.

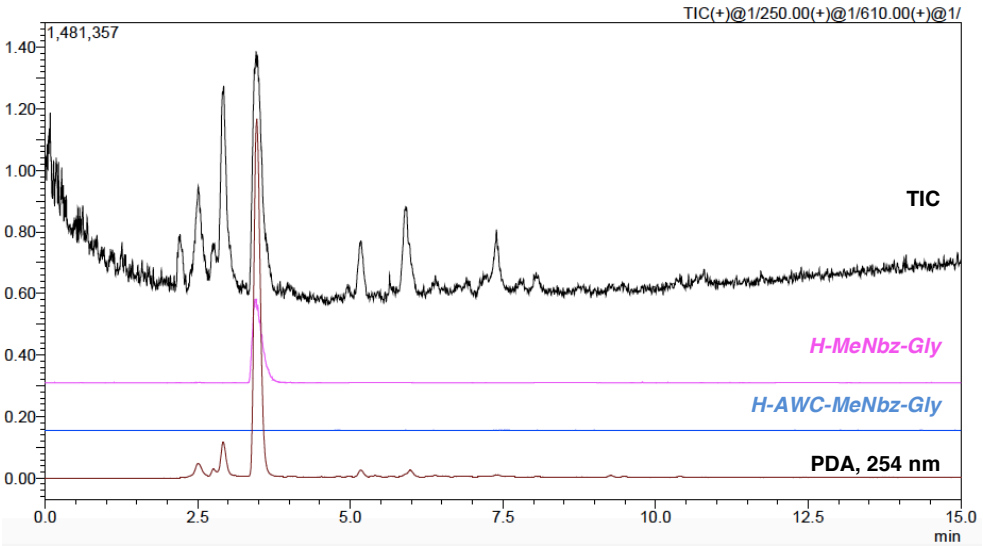


Figure SI-5. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

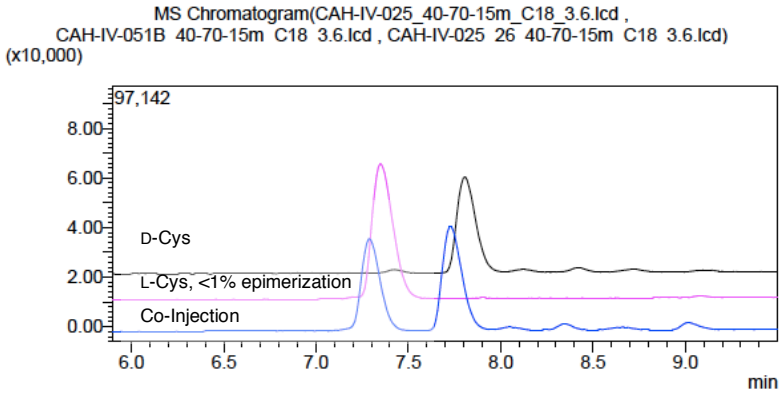


Figure SI-6. A) D-Cys, B) L-Cys, and C) Co-injection, gradient: 40-70% MeCN/H_2O + 1% HCOOH over 15 min.

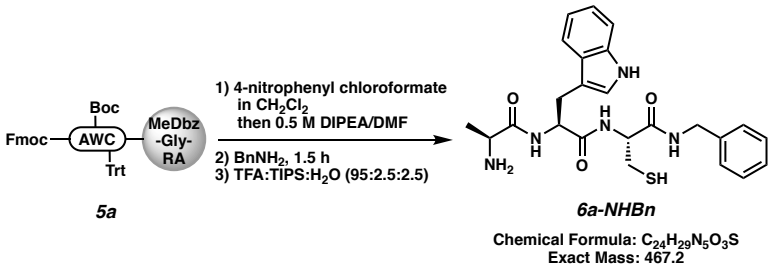


Table 1, Entry 2: H_2N-AWC-NHBn (6a-NHBn). 20 mg of resin (**5a**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of $BnNH_2$ was added to the resin and agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 3 mL of TFA:TIPS:H_2O (95:2.5:2.5) and agitated for 30 min. After this time, the solvent was blown down with a constant stream of air, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

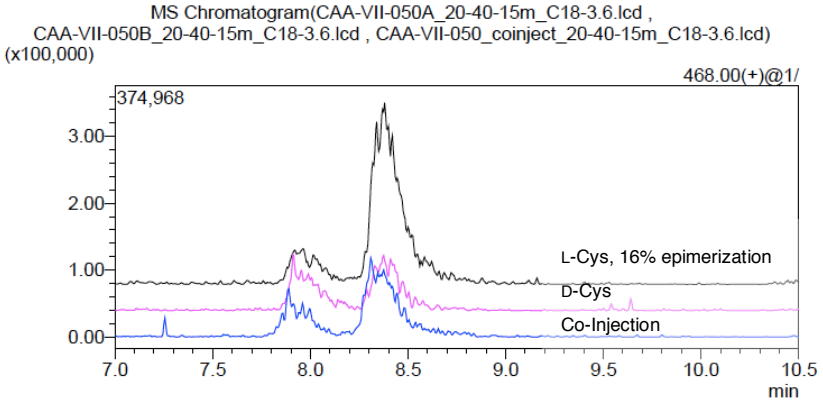


Figure SI-7. A) L-Cys, B) D-Cys, and C) Co-injection, gradient: 20-40% MeCN/H_2O + 1% HCOOH over 15 min.

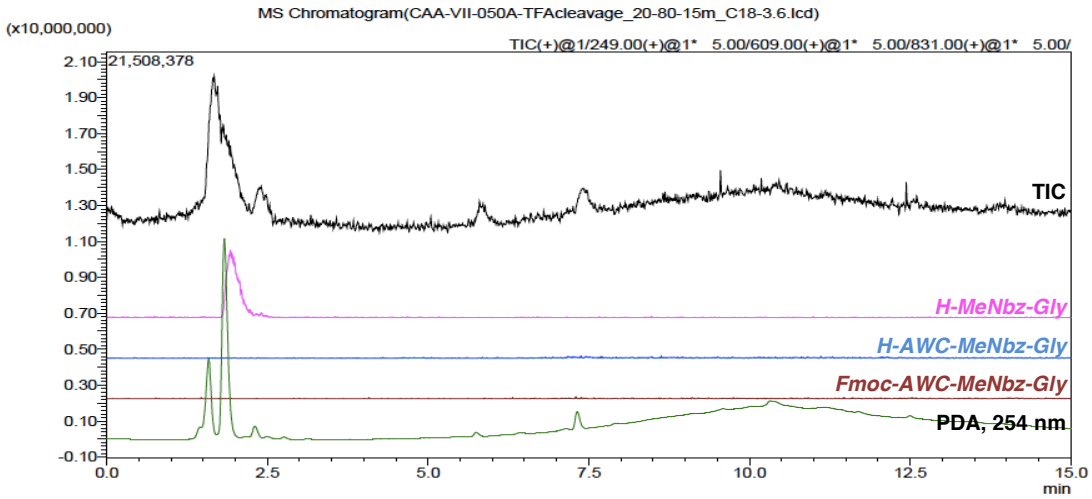


Figure SI-8. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

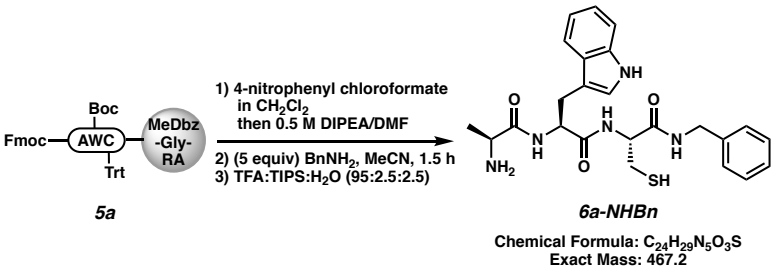


Table 1, Entry 3: H_2N-AWC-NHBn (6a-NHBn). 20 mg of resin (**5a**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL MeCN was added to the resin followed by 5 equiv $BnNH_2$ and agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

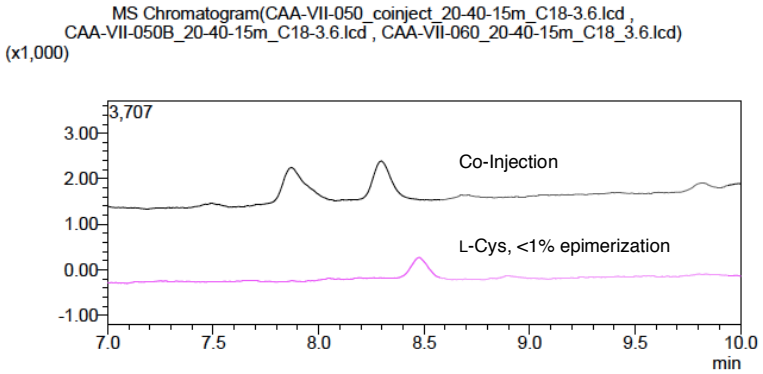


Figure SI-9. A) Co-injection and B) L-Cys, gradient: 20-40% MeCN/H_2O + 1% HCOOH over 15 min.

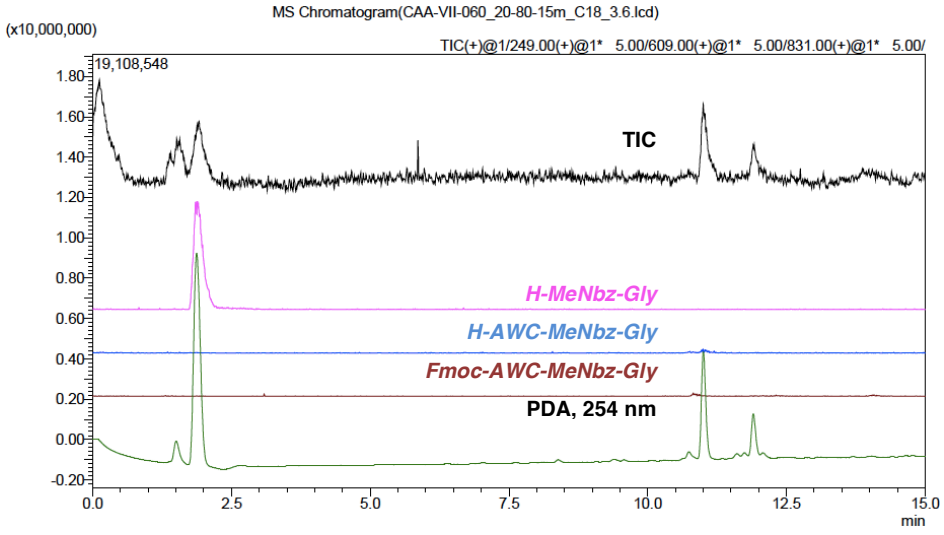
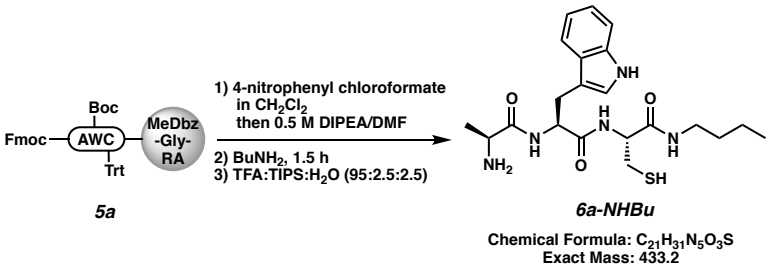


Figure SI-10. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.



5a *6a-NHBu*

Chemical Formula: $C_{21}H_{31}N_5O_3S$
Exact Mass: 433.2

Table 1, Entry 4: H_2N-AWC-NHBu (6a-NHBu). 20 mg of resin (**5a**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of $BuNH_2$ was added to the resin and agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 3 mL of TFA:TIPS:H_2O (95:2.5:2.5) and agitated for 30 min. After this time, the solvent was blown down with a constant stream of air, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

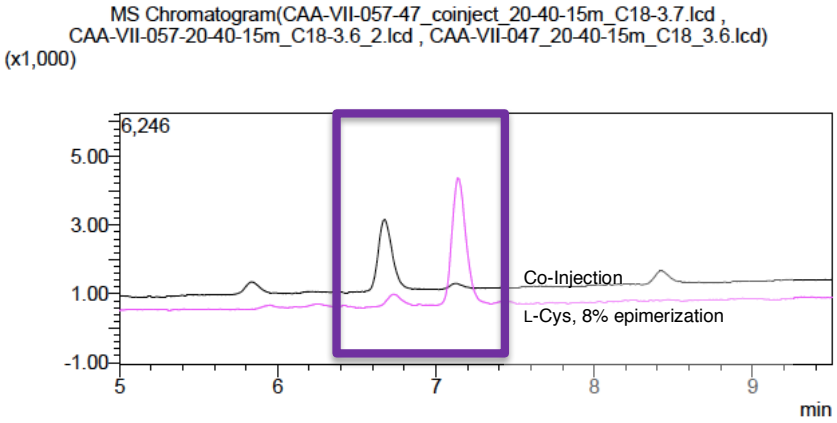


Figure SI-11. A) Co-injection and B) L-Cys, gradient: 20-40% MeCN/H_2O + 1% HCOOH over 15 min.

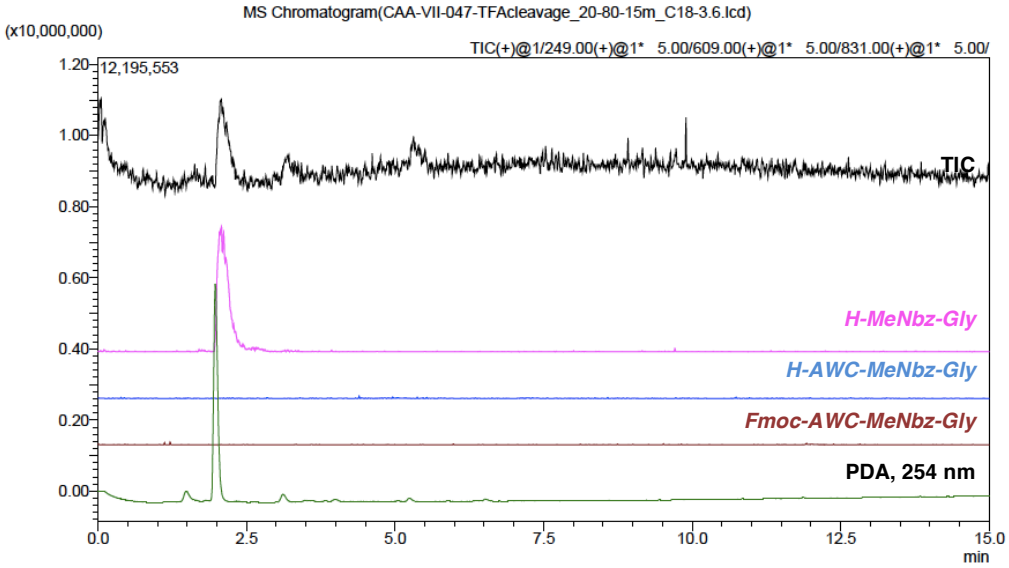


Figure SI-12. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

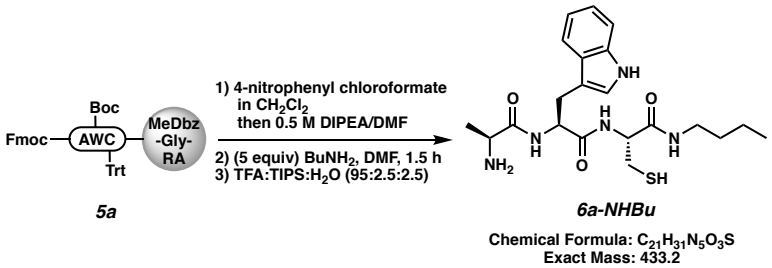


Table 1, Entry 5: AWC-NHBu (6a-NHBu). 20 mg of resin (**5a**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 10 equiv butylamine in 200 μL DMF was added to the resin and agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

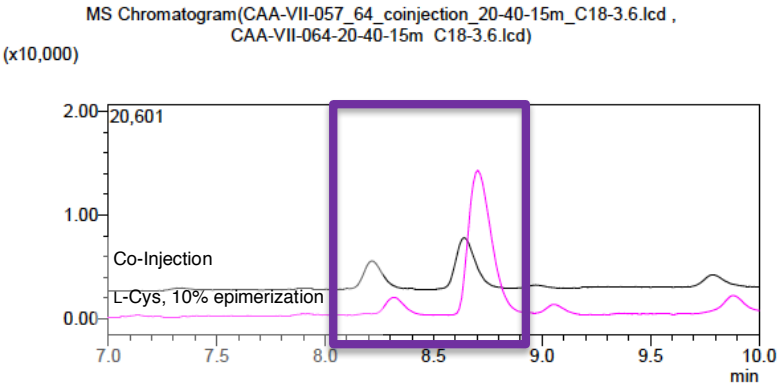


Figure SI-13. A) Co-injection and B) L-Cys, using a gradient of 20-40% MeCN/H_2O + 1% HCOOH over 15 min.

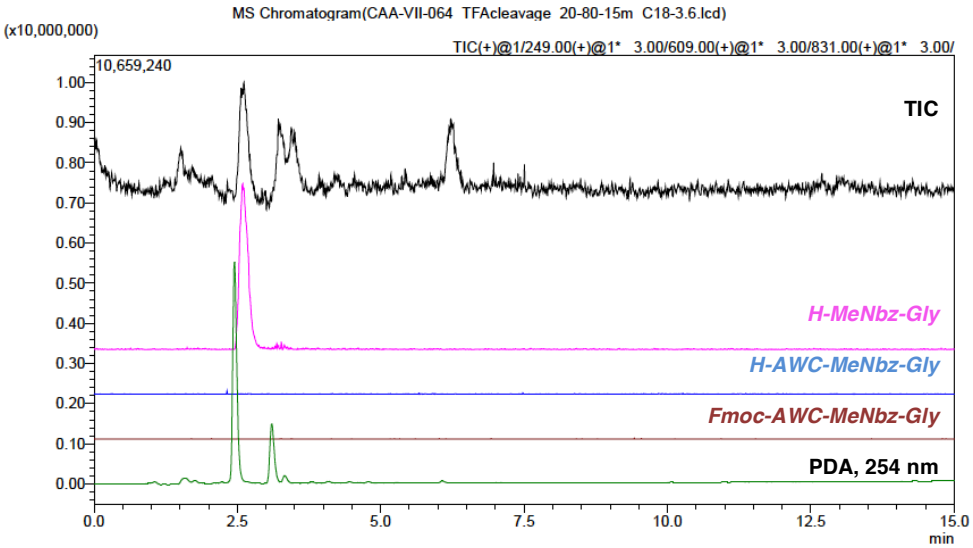
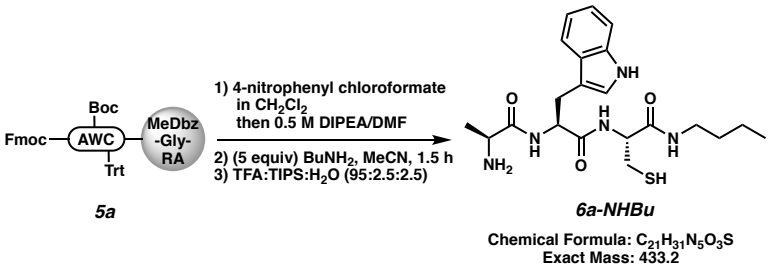


Figure SI-14. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.



5a *6a-NHBu*

Chemical Formula: $C_{21}H_{31}N_5O_3S$
Exact Mass: 433.2

Table 1, Entry 6: AWC-NHBu (6a-NHBu). 20 mg of resin (**5a**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeCN and added to the resin followed by 5 equiv butylamine. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

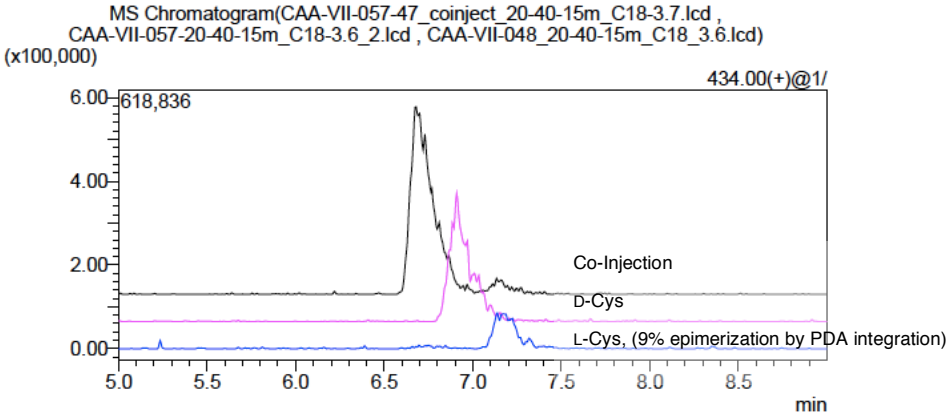


Figure SI-15. A) Co-injection, B) D-Cys, and C) L-Cys, using a gradient of 20-40% MeCN/H_2O + 1% HCOOH over 15 min.

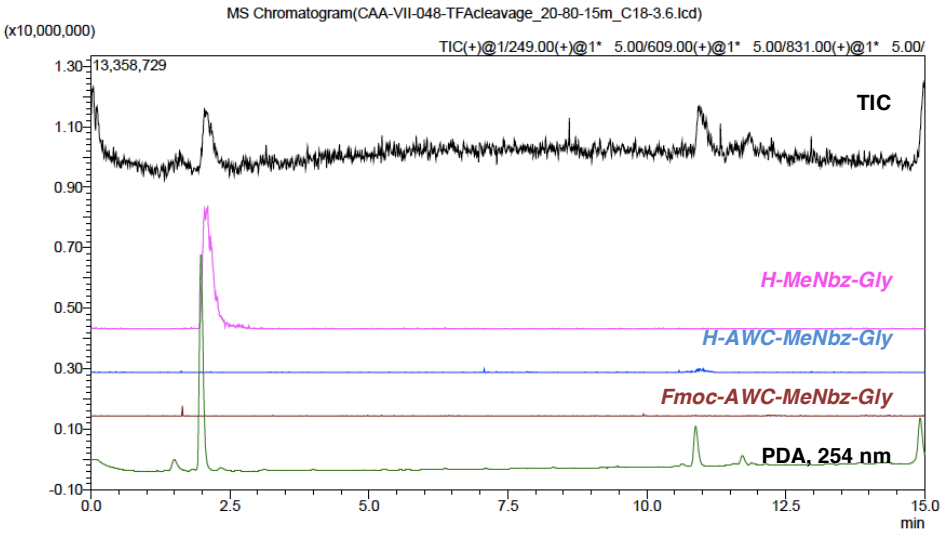


Figure SI-16. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

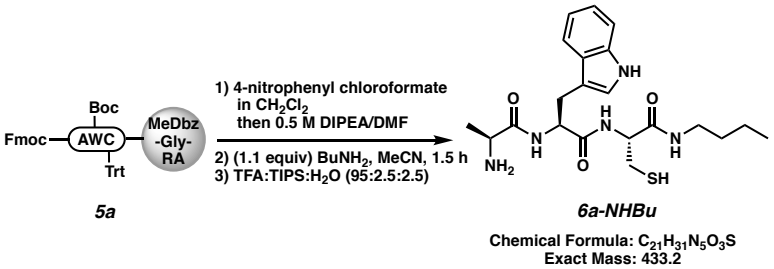


Table 1, Entry 7: AWC-NHBu (6a-NHBu). 20 mg of resin (**5a**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeCN and added to the resin followed by 1.1 equiv butylamine. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

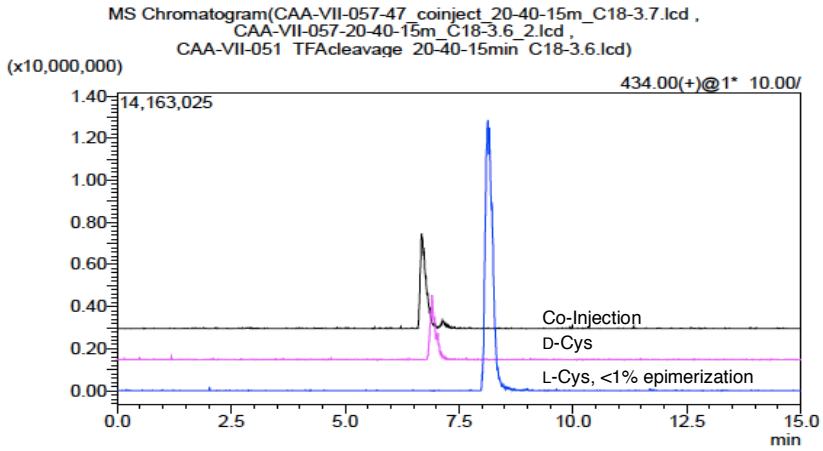


Figure SI-17. A) Co-injection, B) D-Cys, and C) L-Cys, gradient of 20-40% MeCN/H_2O + 1% HCOOH over 15 min.

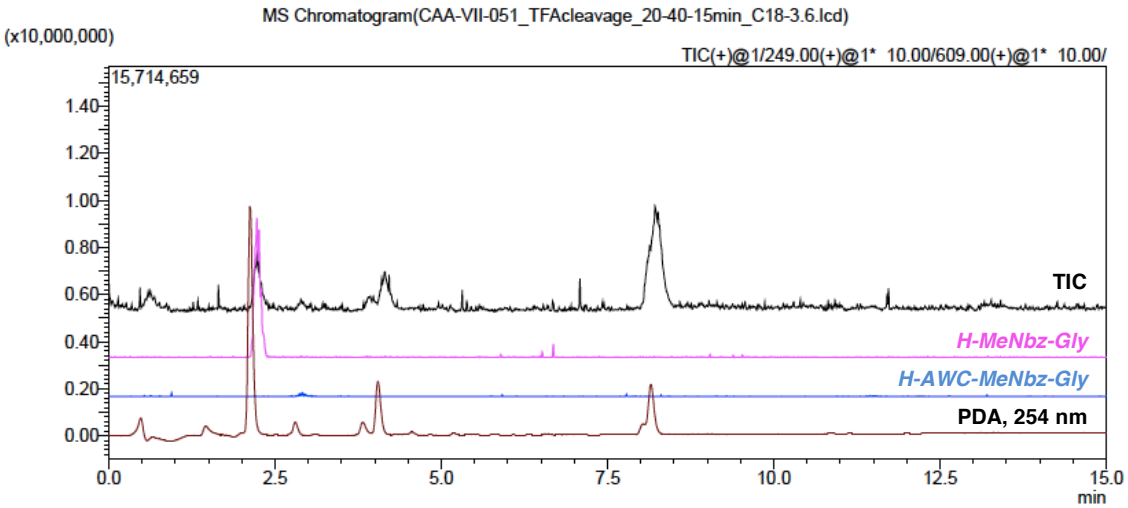
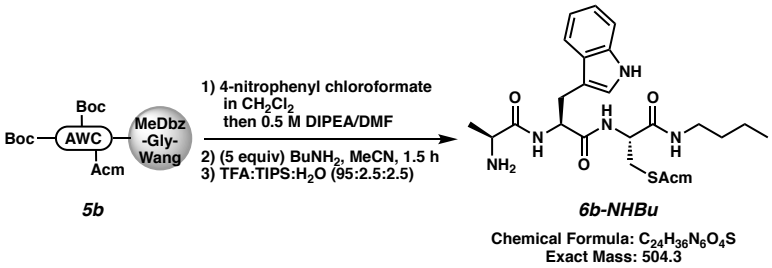


Figure SI-18. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.



5b *6b-NHBu*

Chemical Formula: $C_{24}H_{36}N_6O_4S$
Exact Mass: 504.3

Table 1, Entry 8: AWC(Acm)-NHBu (6b-NHBu). 20 mg of resin (**5b**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeCN and added to the resin followed by 5 equiv butylamine. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

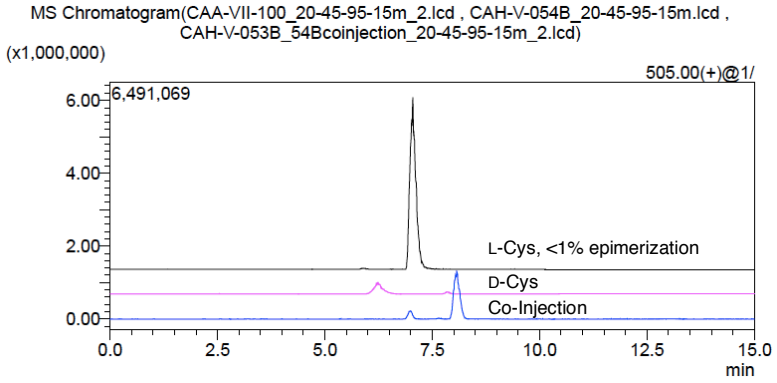


Figure SI-19. A) L-Cys, B) D-Cys, and C) co-injection gradient of 20-45-95% MeCN/H_2O + 1% HCOOH over 15 min.

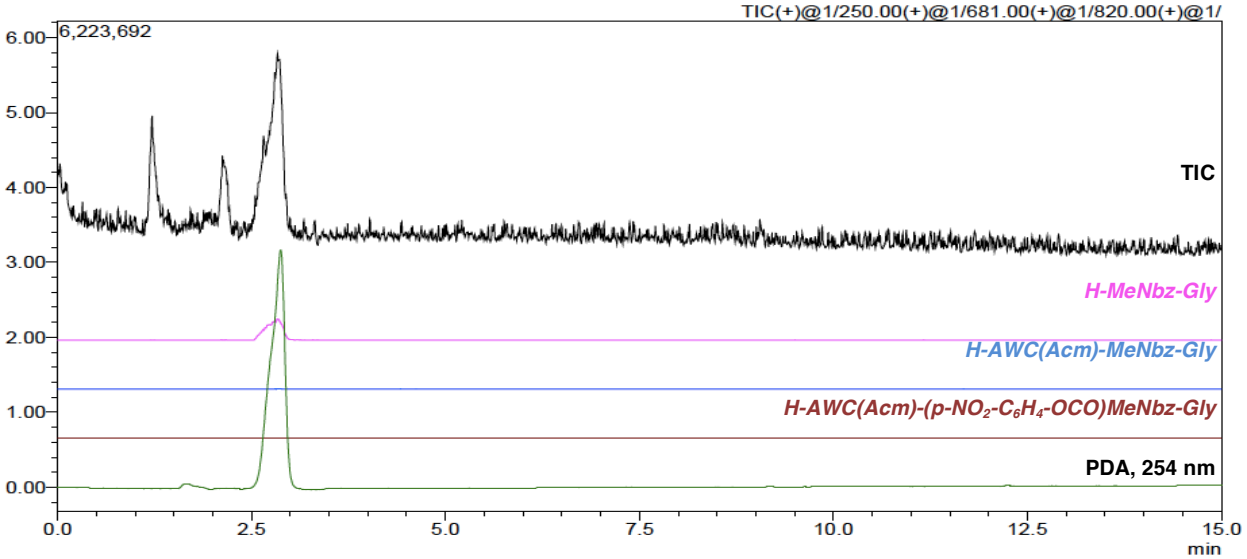


Figure SI-20. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

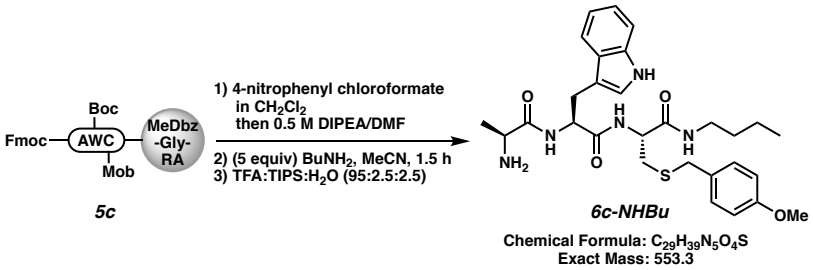


Table 1, Entry 9: AWC(Mob)-NHBu (6c-NHBu). 20 mg of resin (**5c**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeCN and added to the resin followed by 5 equiv butylamine. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

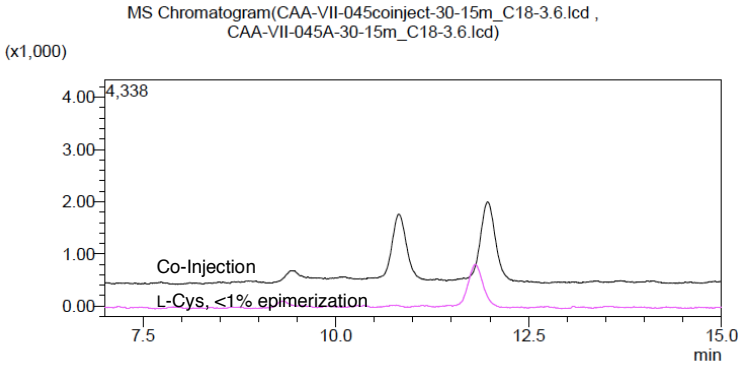


Figure SI-021. A) Co-injection, B) L-Cys, using a gradient of 30% MeCN/H_2O + 1% HCOOH over 15 min, PDA: 254 nm.

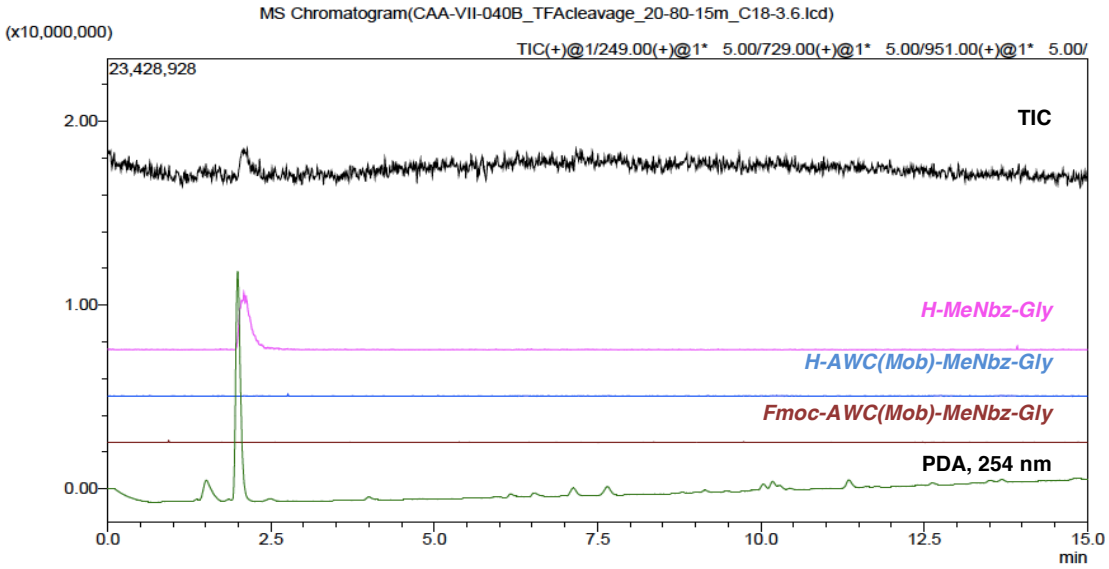


Figure SI-22. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

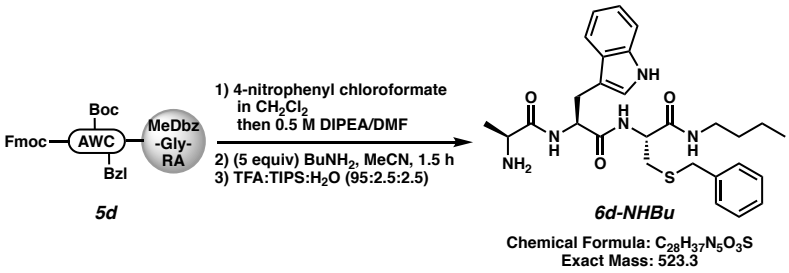


Table 1, Entry 10: AWC(Bzl)-NHBu (6d-NHBu). 20 mg of resin (**5d**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeCN and added to the resin followed by 5 equiv butylamine. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

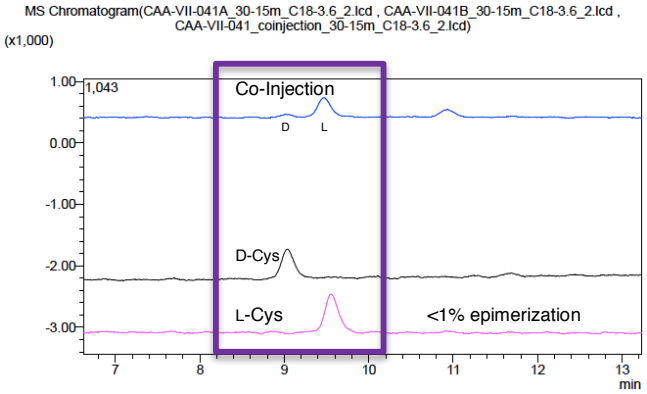


Figure SI-23. A) Co-injection, B) D-Cys, and C) L-Cys, gradient of 30% MeCN/H_2O + 1% HCOOH over 15 min, PDA: 254 nm.

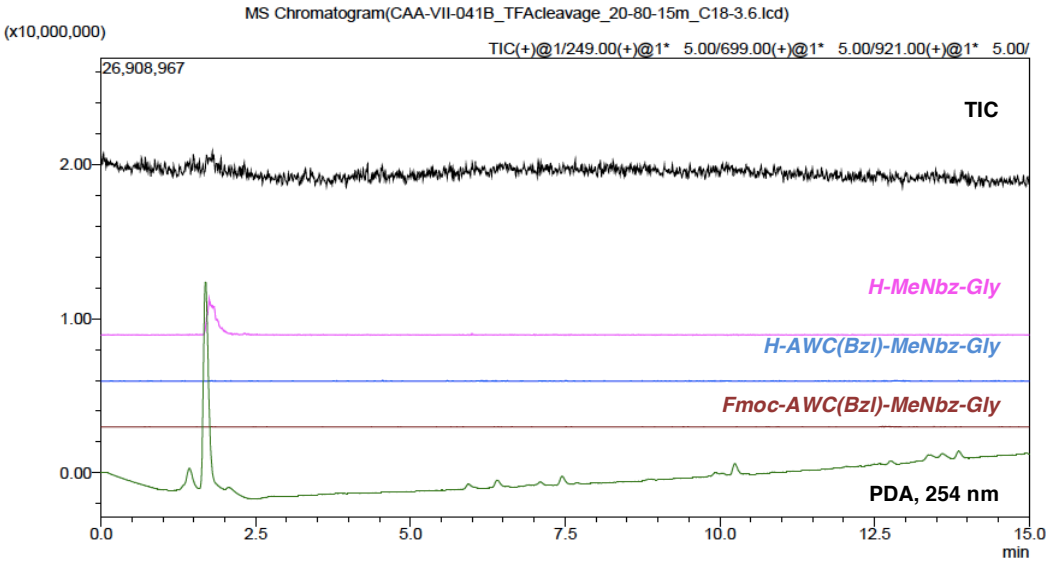


Figure SI-24. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

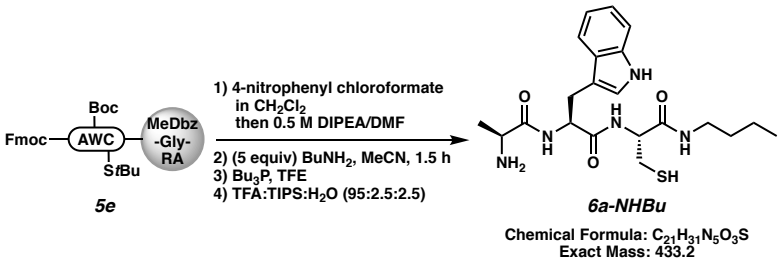


Table 1, Entry 11: AWC-NHBu (6a-NHBu). 20 mg of resin (**5e**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeCN was added to the resin followed by 5 equiv butylamine. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After quenching, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and stirred for 1 h to give the deprotected cystine peptide (**AWC(S*t*Bu)-NHBu**, **6e-NHBu**). The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted, and the solid was dissolved in 500 μL TFE and stirred at ambient temperature with 5 equiv Bu_3P for 2 h. After this time, the solvent was concentrated under a constant stream of air, suspended in H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. The peptide was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

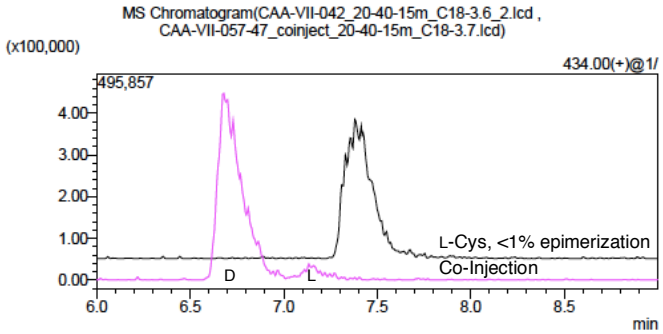


Figure SI-25. A) L-Cys and B) Co-injection, gradient of 20-40% MeCN/H_2O + 1% HCOOH over 15 min.

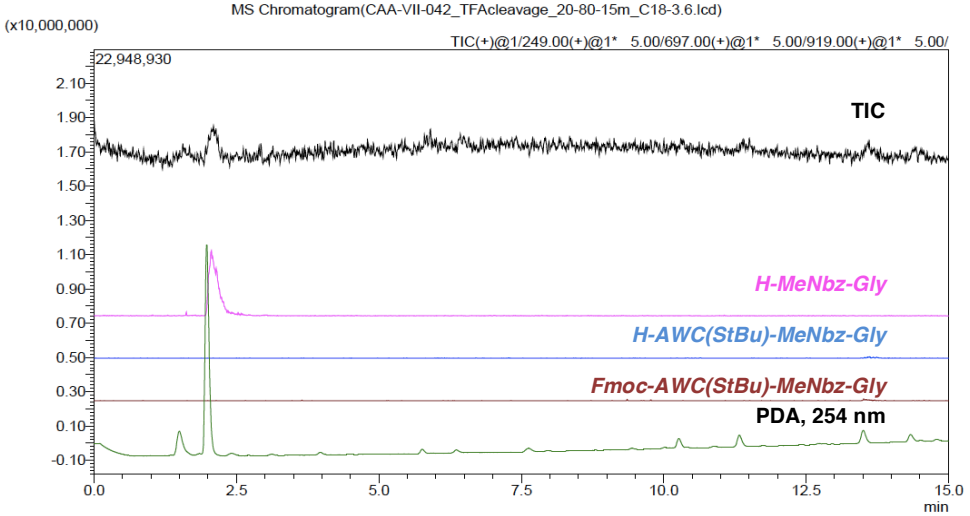


Figure SI-26. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

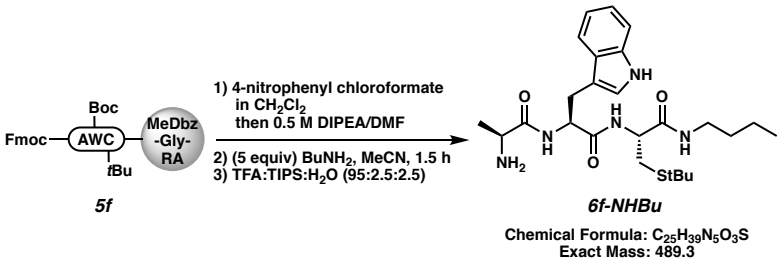


Table 1, Entry 12: AWC(*t*Bu)-NHBu (6f-NHBu). 20 mg of resin (**5f**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeCN and added to the resin followed by 5 equiv butylamine. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

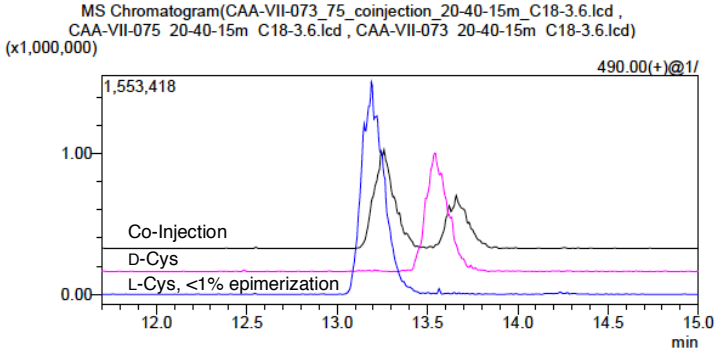


Figure SI-27. A) Co-injection, B) D-Cys, and C) L-Cys, gradient of 20-40% MeCN/H_2O over 15 min.

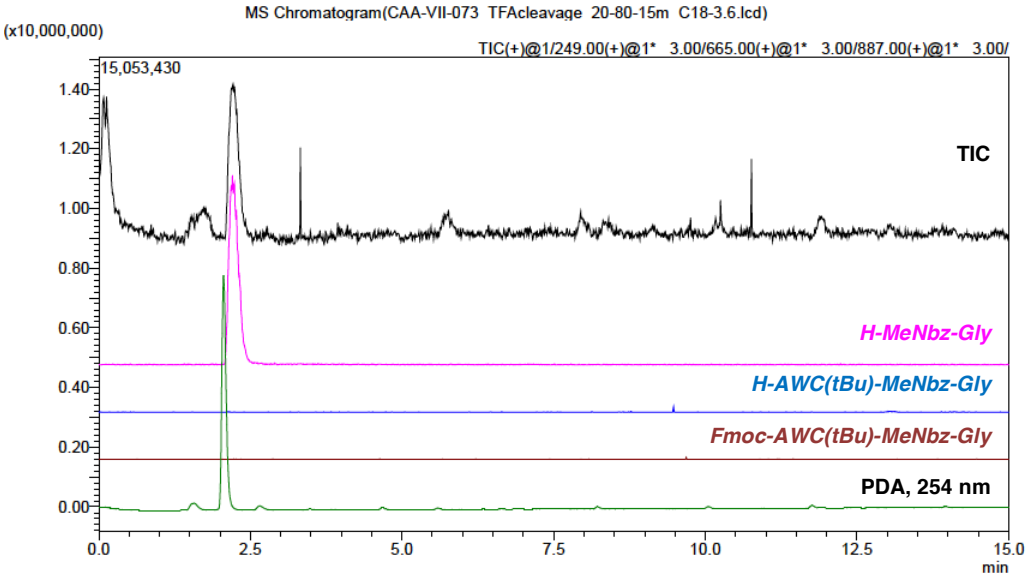
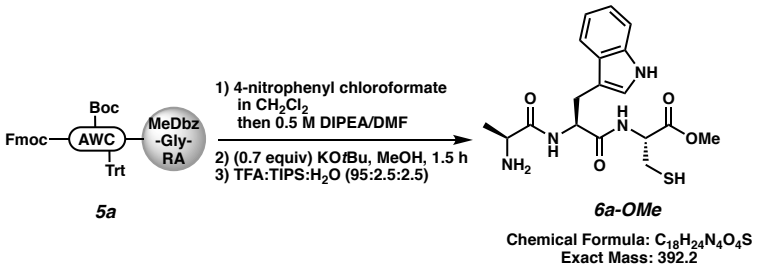


Figure SI-28. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.



5a *6a-OMe*

Chemical Formula: $C_{18}H_{24}N_4O_4S$
Exact Mass: 392.2

Table 1, Entry 13: AWC-OMe (6a-OMe). 20 mg of resin (**5a**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 0.7 equiv KO*t*Bu was dissolved in 200 μL of MeOH and added to the resin. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

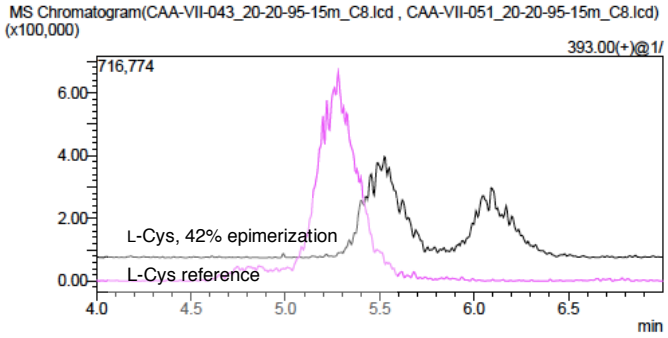


Figure SI-29. A) L-Cys after KO*t*Bu treatment and B) L-Cys reference, using a gradient of 20-20(12 min)-95%(3 min) MeCN/H_2O + 1% HCOOH over 15 min.

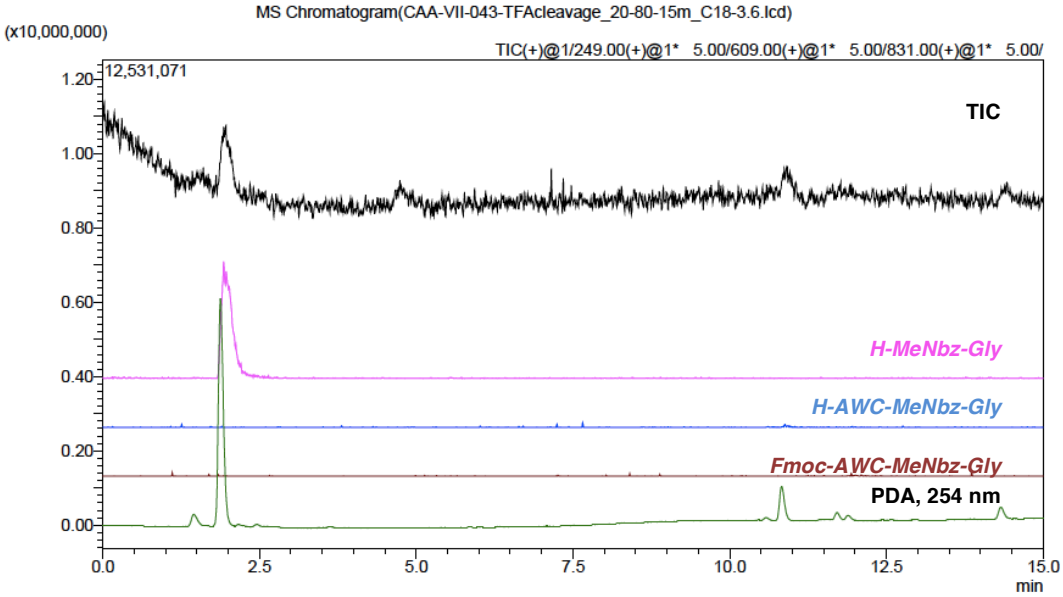
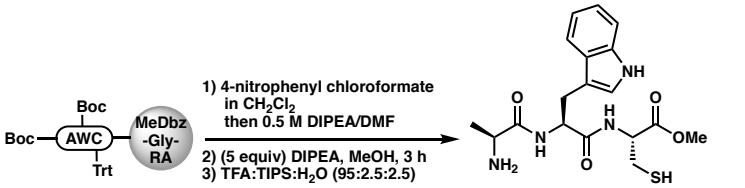


Figure SI-30. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.



5g *6a-OMe*

Chemical Formula: $C_{18}H_{24}N_4O_4S$
Exact Mass: 392.2

Table 1, Entry 14: AWC-OMe (6a-OMe). 20 mg of resin (**5g**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeOH was added to the resin followed by 5 equiv DIEA. The resin was agitated for 3 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was concentrated down, dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

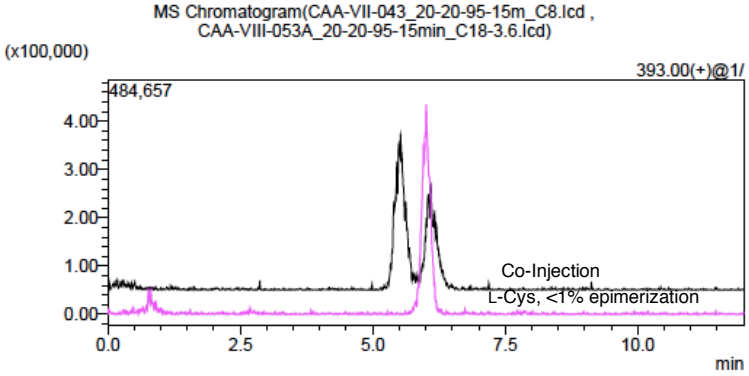


Figure SI-31. Gradient of 20-20(12 min)-95%(3 min) MeCN:H_2O + 1% HCOOH over 15 min.[8]

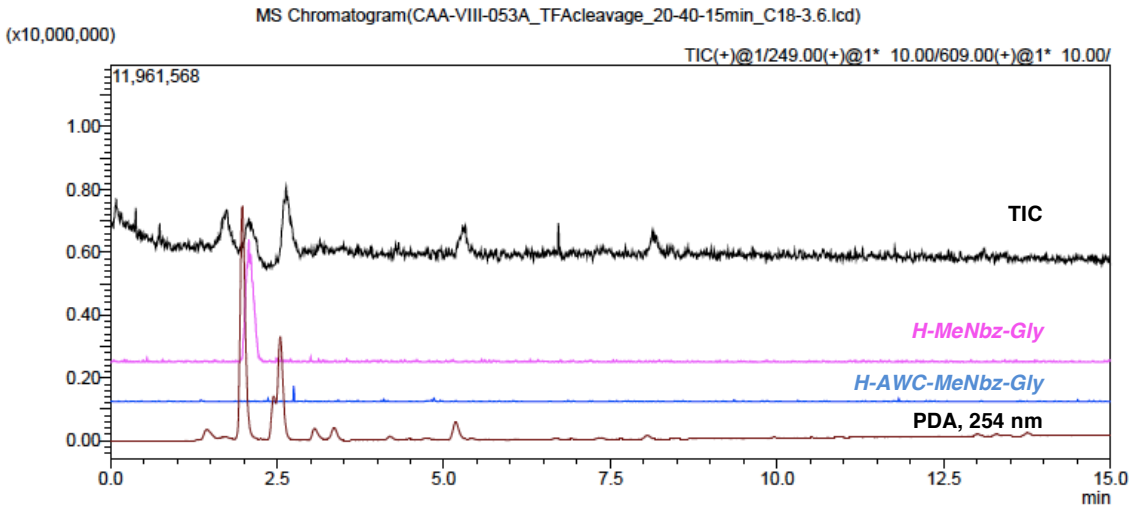
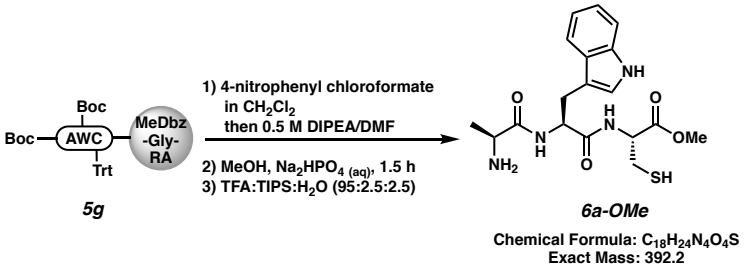


Figure SI-32. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

[8] The L-Cys substrate has a delayed elution in the assay, but since no second peak is observed we reported no detectable epimerization.



5g *6a-OMe*

Chemical Formula: $C_{18}H_{24}N_4O_4S$
Exact Mass: 392.2

Table 1, Entry 15: AWC-OMe (6a-OMe). 20 mg of resin (**5g**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of MeOH was added to the resin along with 200 μL of Na_2HPO_4/NaH_2PO_4 buffer at pH = 8. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

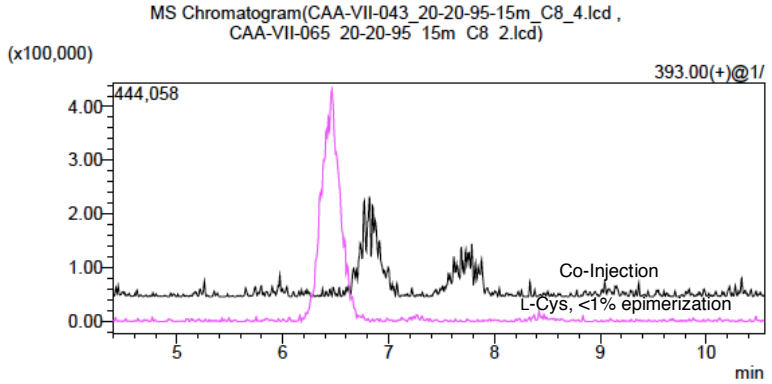


Figure SI-33. Gradient of 20-20(12 min)-95%(3 min) MeCN:H_2O + 1% HCOOH over 15 min.

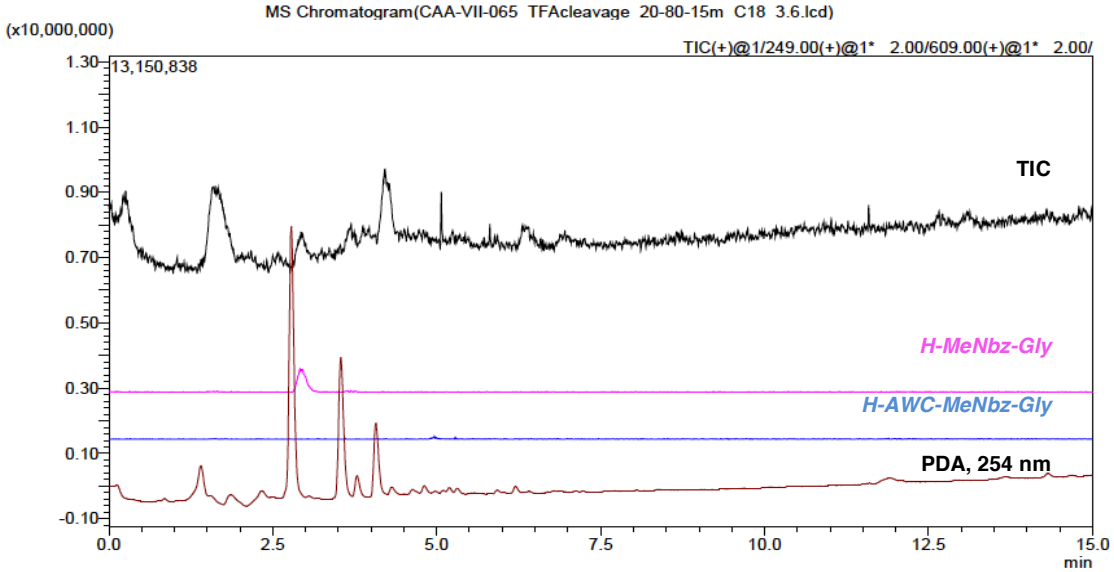


Figure SI-34. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

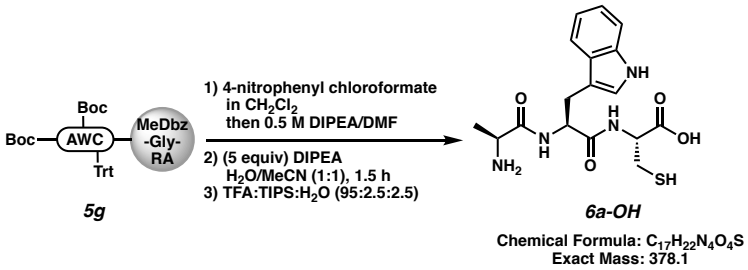


Table 1, Entry 16: AWC-OH (6a-OH). 20 mg of resin (**5g**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of (1:1) H_2O/MeCN was added to the resin followed by 5 equiv DIEA. The resin was agitated for 1.5 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

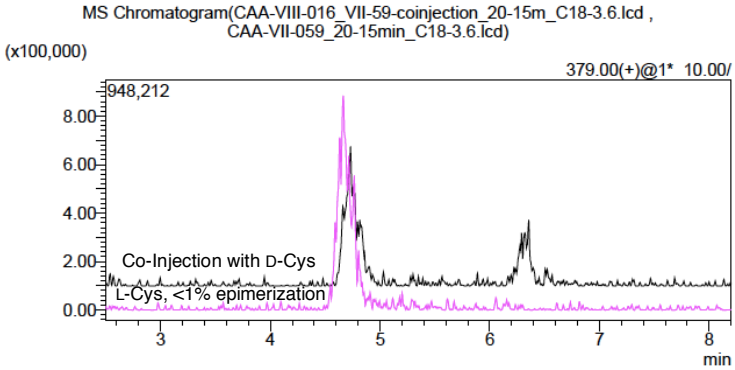


Figure SI-35. A) Co-injection and B) L-Cys, Gradient of 20% MeCN:H_2O + 1% HCOOH over 15 min.

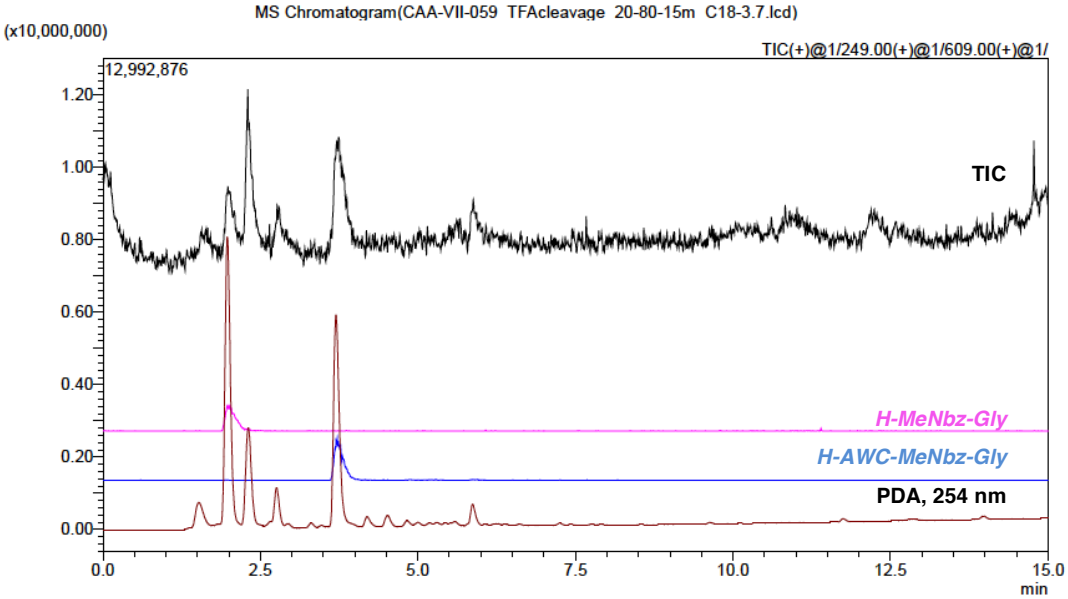


Figure SI-36. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

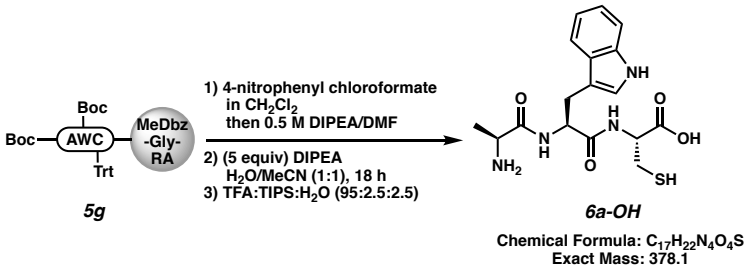


Table 1, Entry SI-01: AWC-OH (6a-OH). 20 mg of resin (**5g**) was weighed out into a 2 mL reactor vial and swelled in CH_2Cl_2 for 15 min. After this time the solvent was removed and the resin was subjected to activation conditions. Then 200 μL of (1:1) H_2O/MeCN was added to the resin followed by 5 equiv DIEA. The resin was agitated for 18 h at ambient temperature. The solvent was then removed and immediately subjected to 0.5 mL of TFA:TIPS:H_2O (95:2.5:2.5). After this time, the solvent was frozen (LN_2), and lyophilized to yield the crude peptide. Then 2 mL of TFA:TIPS:H_2O (95:2.5:2.5) was added to the crude peptide solid and agitated for 1 h. The solvent was blown down with a constant stream of air, crashed out with cold diethyl ether, centrifuged, decanted and the solid was dissolved in 20% MeCN:H_2O (1% TFA) and analyzed via RP-HPLC-MS.

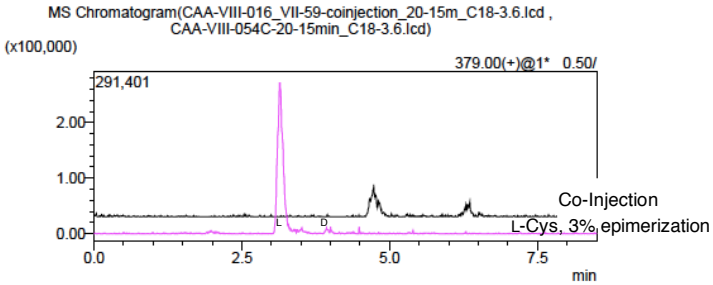


Figure SI-37. A) Co-injection and B) L-Cys, Gradient of 20% MeCN:H_2O + 1% HCOOH over 15 min.

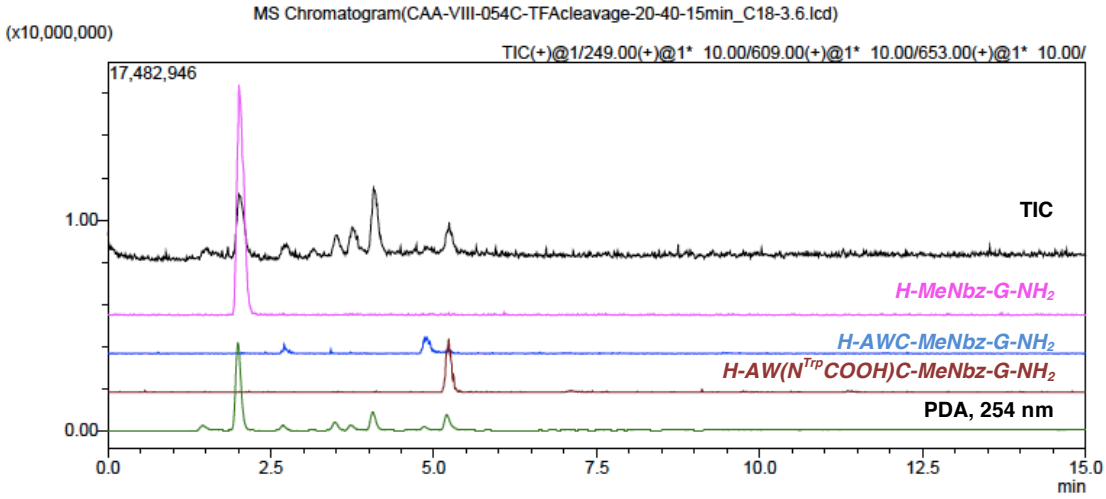
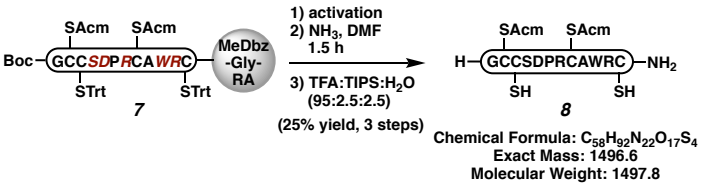


Figure SI-38. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

Scheme 2. Synthesis of α-Conotoxin Iml.



100 mg (24 μmol) of peptide **7** was weighed out into a 5 mL fritted peptide vial and swelled in CH_2Cl_2 for 15 min. The solvent was removed and the peptide was activated (See MeDbz activation). Next, 500 μL of DMF was added and the reaction vial was closed off with a septum (needle and stopper still attached at the bottom). A double balloon was made and filled with ammonia. A hypodermic needle was attached to the balloon and inserted into the septum and submerged into the DMF solution. The smallest (blue) gauge needle was placed into the septum to allow for the slow exiting of gas. The ammonia gas was bubbled into DMF for 1.5 h. Then the solvent was removed and collected. Additional rinsing was required to completely remove the desired peptide from resin. CH_2Cl_2 (3 x 1 mL) was used to rinse the resin, followed by MeCN (3 x 1 mL), and lastly CH_2Cl_2 (3 x 1 mL). The washes were combined with the initial DMF solution, blown down with air, subjected to 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized to yield the crude product (70% crude yield, 86% HPLC purity). The crude peptide was then fully deprotected using TFA:TIPS:H_2O (95:2.5:2.5) for 1.5 h. The solvent was blown down, crashed out with cold diethyl ether, centrifuged, and the ether was decanted off to yield the crude fully deprotected peptide. The solid was dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized. The peptide was purified by RP-HPLC-MS to yield the reduced peptide in 25% yield (8.9 mg).

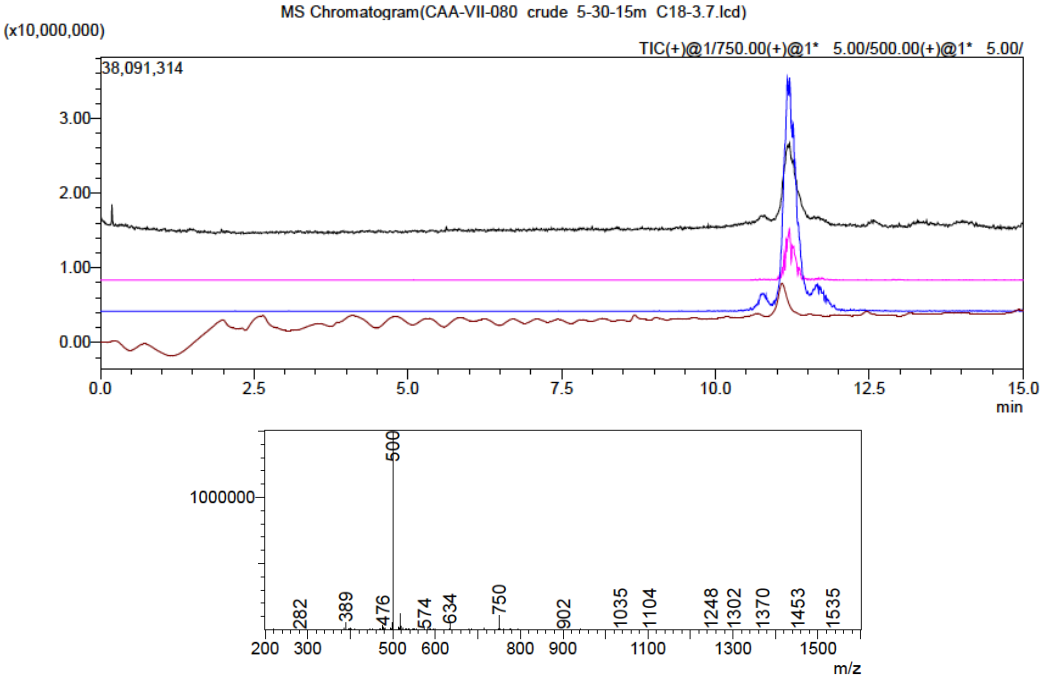


Figure SI-39. Analytical Column PDA of crude peptide **8**, gradient of 5-30% MeCN/H_2O + 1% HCOOH over 15 min.

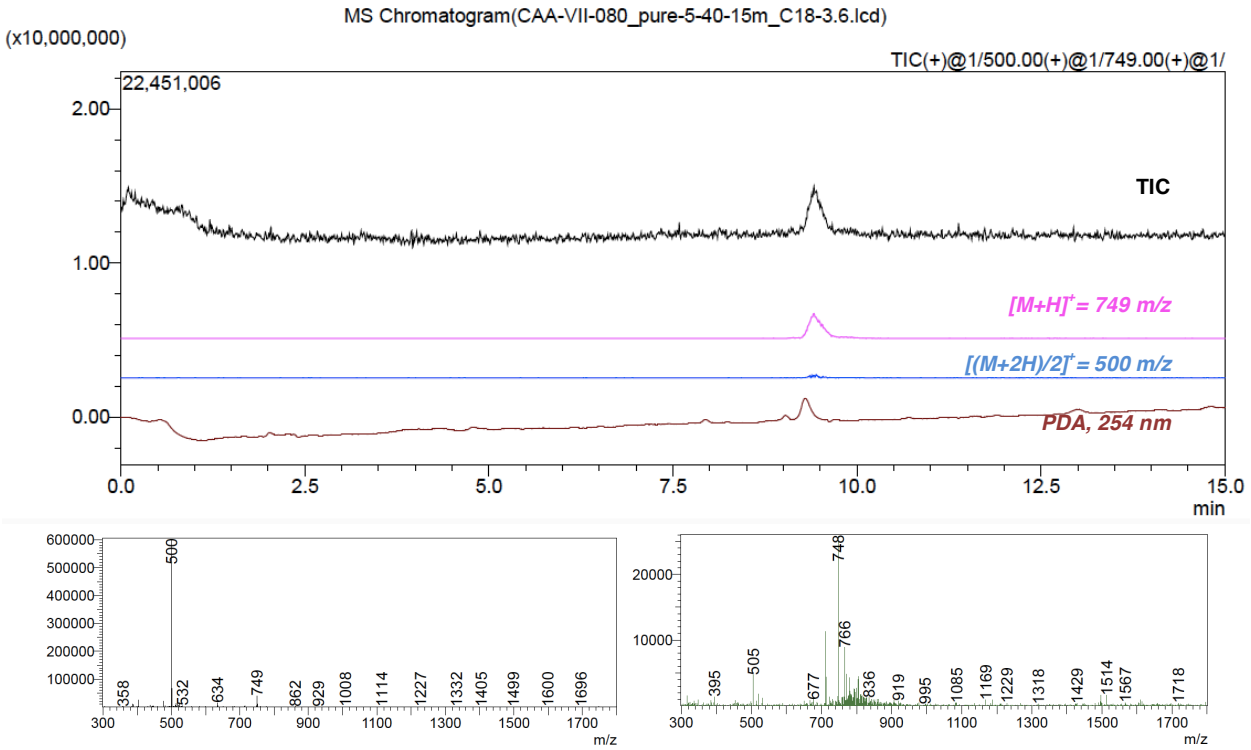
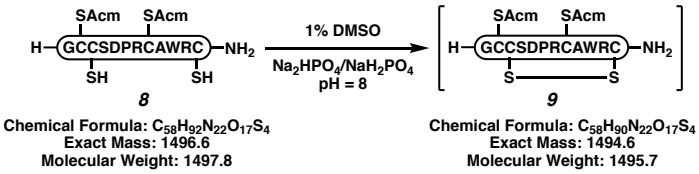


Figure SI-40. Analytical Column PDA of pure peptide **8**, gradient of 5-40% MeCN/H_2O + 1% HCOOH over 15 min.



8
Chemical Formula: $C_{58}H_{92}N_{22}O_{17}S_4$
Exact Mass: 1496.6
Molecular Weight: 1497.8

9
Chemical Formula: $C_{58}H_{90}N_{22}O_{17}S_4$
Exact Mass: 1494.6
Molecular Weight: 1495.7

8.6 mg (5.7 μmol) of **8** was dissolved in 6 mL Na_2HPO_4/NaH_2PO_4 buffer (that was previously adjusted to pH 8 with 5 M NaOH). Then 60 μL of DMSO was added to the mixture and allowed to stir overnight at ambient temperature. After complete consumption of starting material, which was monitored by RP-HPLC-MS, peptide **9** was further oxidized (see next scheme) without removal from this reaction mixture.

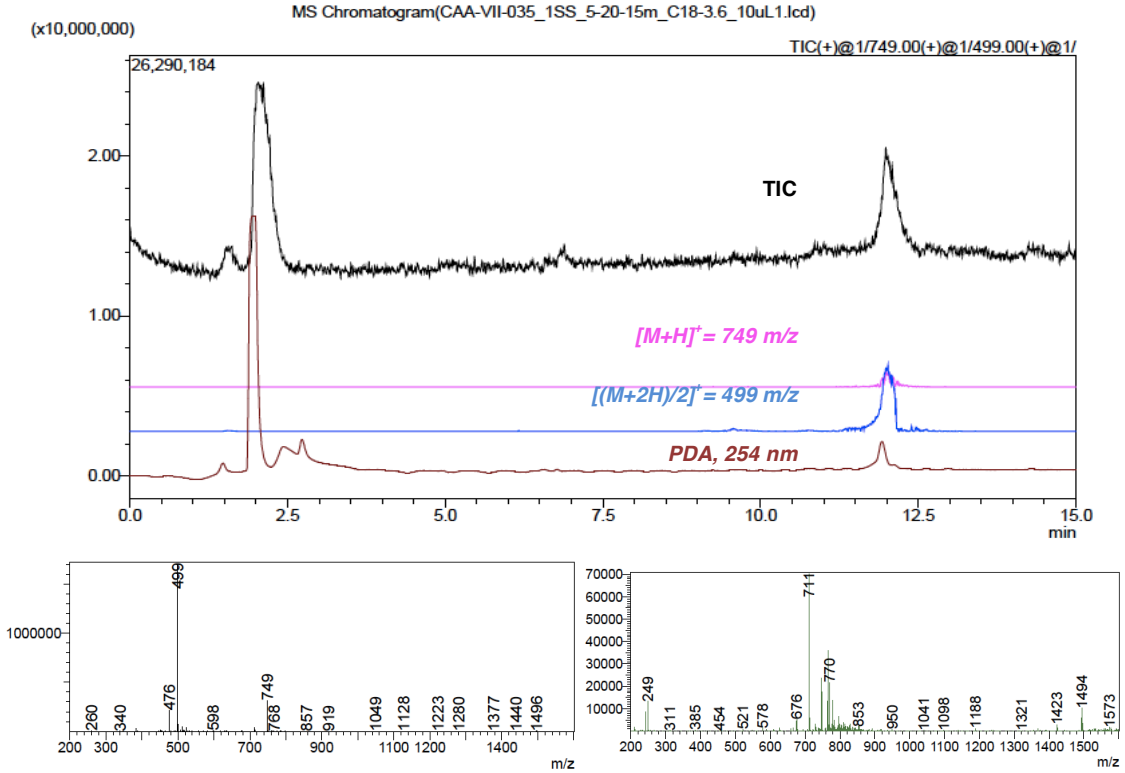
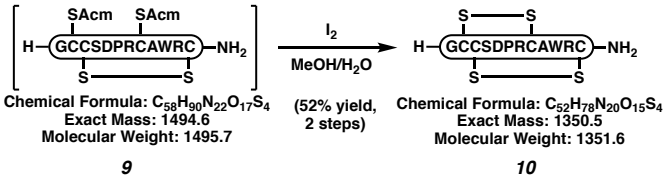


Figure SI-41. Analytical Column PDA of crude peptide **9** at 254 nm, gradient of 5-20% MeCN/H_2O + 1% HCOOH over 15 min.



Chemical Formula: $C_{58}H_{90}N_{22}O_{17}S_4$
Exact Mass: 1494.6
Molecular Weight: 1495.7

9

Chemical Formula: $C_{52}H_{78}N_{20}O_{15}S_4$
Exact Mass: 1350.5
Molecular Weight: 1351.6

10

The solution of peptide **9**, in the previously mentioned buffer, was subjected to 8 equiv iodine in a solution of (4:1) MeOH:H_2O (the MeOH/H_2O mixture was added until the iodine was completely dissolved). The removal of Acm and final folding was monitored by RP-HPLC-MS. After complete consumption of starting material, the mixture was quenched with ascorbic acid. The solid ascorbic acid was added portion-wise until the red solution turned clear. The mixture was then frozen (LN_2), and lyophilized to yield the crude peptide **10**. The peptide was purified using RP-HPLC-MS to yield the pure peptide in 52% yield (4.2 mg).

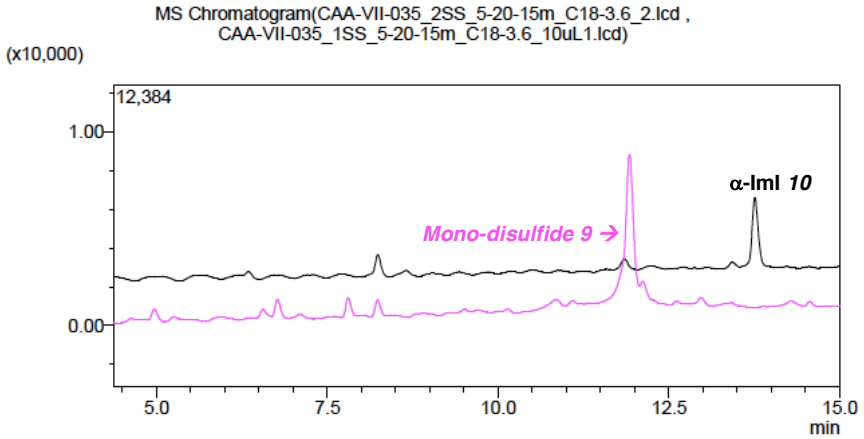


Figure SI-42. Analytical Column PDA illustrating the final folding step of α-conotoxin lml, gradient of 5-20% MeCN/H_2O + 1% HCOOH over 15 min.

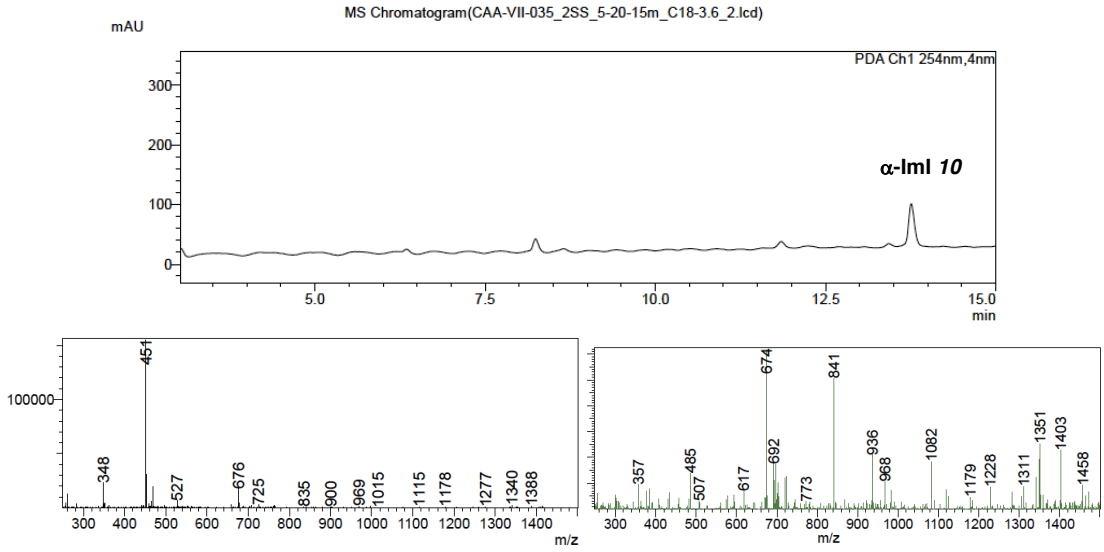


Figure SI-43. Analytical Column PDA of pure peptide **10**, gradient of 5-20% MeCN/H_2O + 1% HCOOH over 15 min. Retention time: 13.84 min.

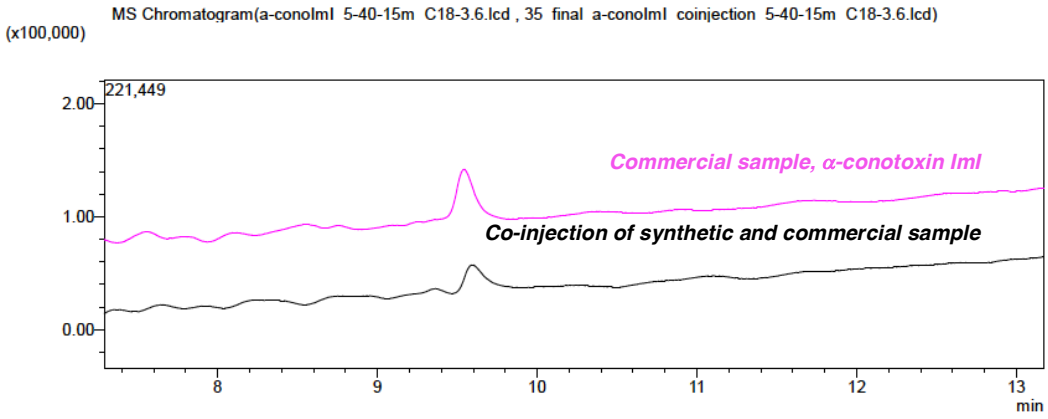
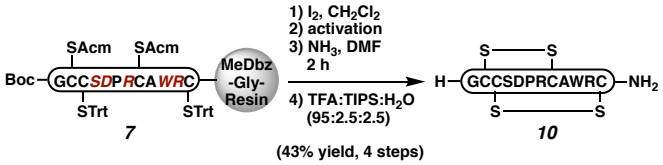


Figure SI-44. A) Analytical Column PDA of commercial α-conotoxin lml at 254 nm, B) co-injection of synthetic (solution-phase folding) and commercial sample, gradient of 5-40% MeCN/H_2O + 1% HCOOH over 15 min.

On-resin folding then activation/cleavage of α-conotoxin lmi



80.0 mg (19.2 μmol) of **7** was swelled in DMF for 30 min. Then 2.0 mL solution of I_2 (5 equiv, 40.6 mg) in CH_2Cl_2 was added to the resin and allowed to agitate for 90 min at ambient temperature. Next, the reaction mixture was filtered and the resin was washed thoroughly with CH_2Cl_2 (10 x 1 mL) and DMF (10 x 1 mL). The peptide was activated (See MeDbz activation). Then, 500 μL of DMF was added and the reaction vial was closed off with a septum (needle and stopper still attached at the bottom). A double balloon was made and filled with ammonia. A hypodermic needle was attached to the balloon and inserted into the septum and submerged into the DMF solution. The smallest (blue) gauge needle was placed into the septum to allow for the slow exiting of gas. The ammonia gas was bubbled into DMF for 2.0 h. Then the solvent was removed and collected. Additional rinsing was required to completely remove the desired peptide from resin. CH_2Cl_2 (3 x 1 mL) was used to rinse the resin, followed by MeCN (3 x 1 mL), and lastly CH_2Cl_2 (3 x 1 mL). The washes were combined with the initial DMF solution, blown down with air, subjected to 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized. The resulting crude peptide was then fully deprotected using TFA:TIPS:H_2O (95:2.5:2.5) for 2.0 h. The solvent was blown down, crashed out with cold diethyl ether, centrifuged, and the ether was decanted off to yield the crude fully deprotected peptide **10**. The solid was dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized. The peptide was purified by RP-HPLC-MS to afford a mixture of conotoxins foldamers in 43% yield (11.2 mg).

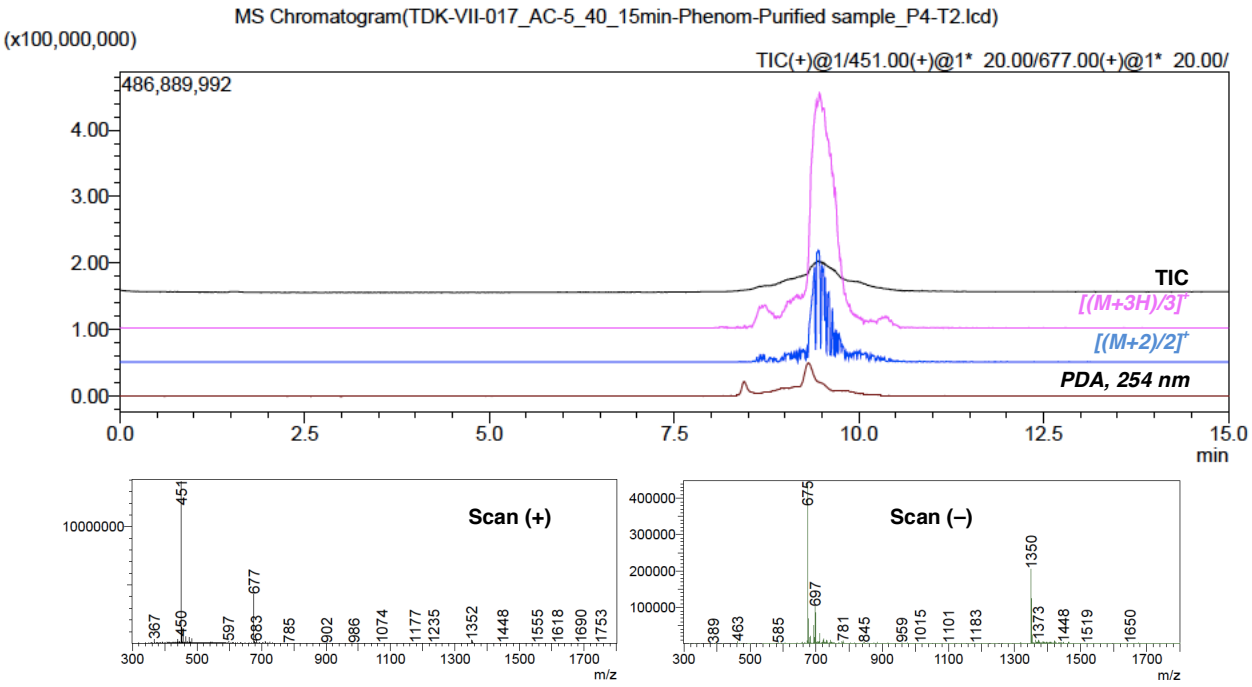


Figure SI-45. Analytical Column PDA of pure peptide **10** at 254 nm, gradient of 5-40% MeCN/H_2O + 1% HCOOH over 15 min. Retention time: 9.08 min.

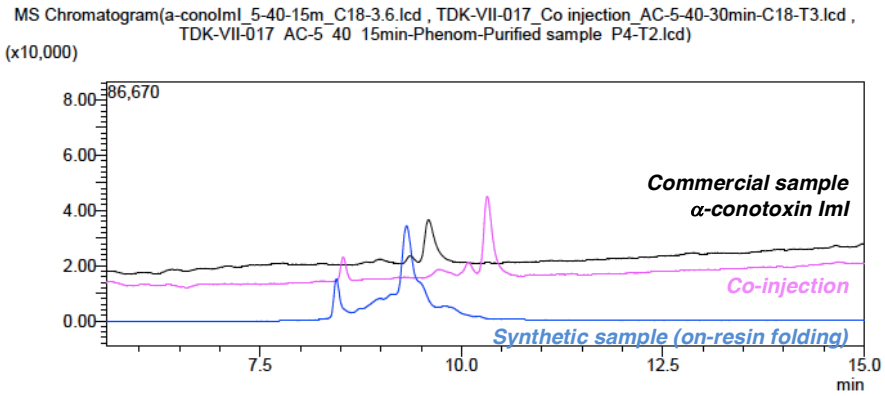


Figure SI-46. A) Analytical Column PDA of commercial α-conotoxin Iml at 254 nm, B) co-injection, C) synthetic sample, gradient of 5-40% MeCN/H_2O + 1% HCOOH over 15 min.

Table 2. On-Resin C-Terminal Ligation.

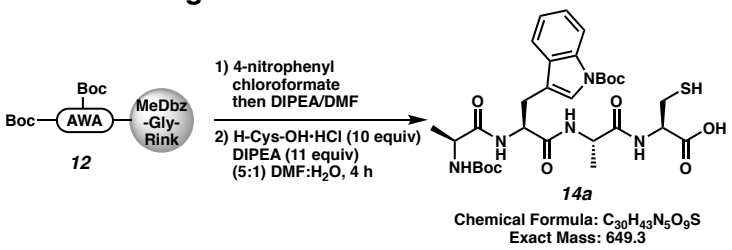


Table 2, Entry 1: 20 mg of **12** was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the linker was activated. 10 equiv of H-Cys-OH•HCl was dissolved in 500 μL DMF and 100 μL H_2O in a separate vial and added to the swelled resin. Then 11 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. The solvent was removed from the vial and quenched in (1% TFA) 20% MeCN/H_2O. The resin was further rinsed with CH_2Cl_2 (3 x 1 mL) and combined with the initial solvent. The volatile solvent was blown down and the remaining solution was frozen in liquid nitrogen, and lyophilized to yield the crude peptide **14a**.

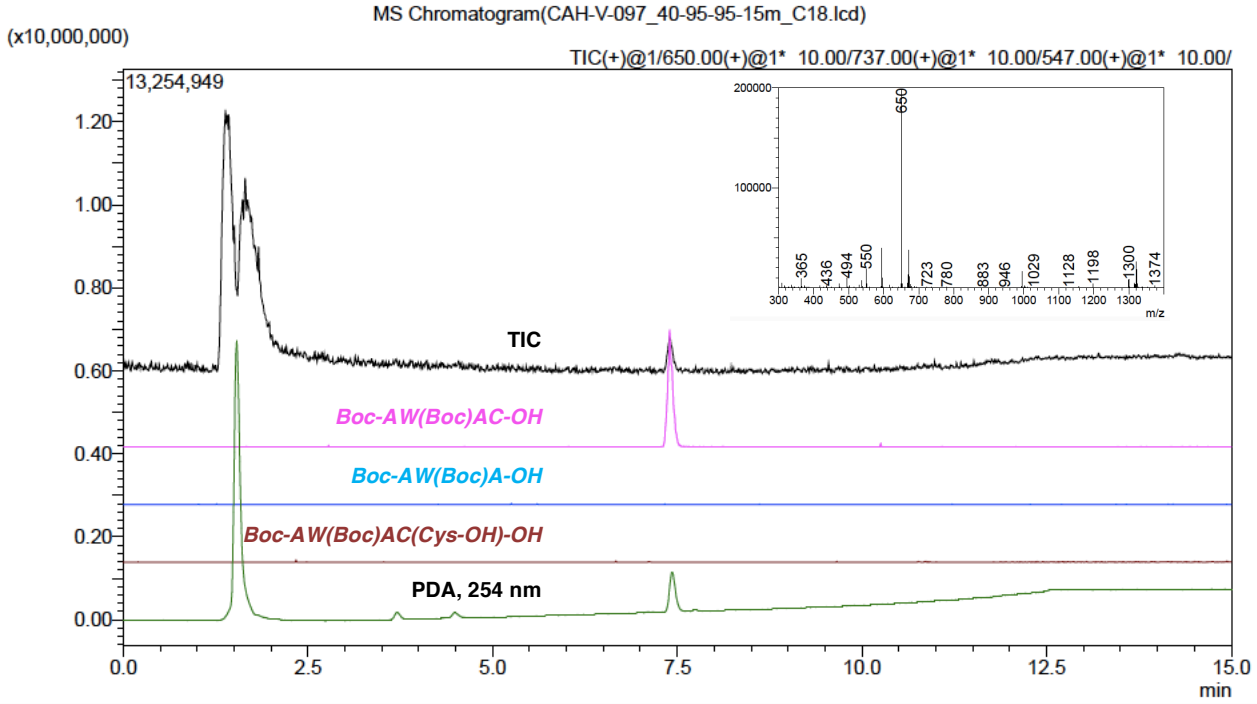


Figure SI-47. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95-95% MeCN/H_2O + 1% HCOOH.

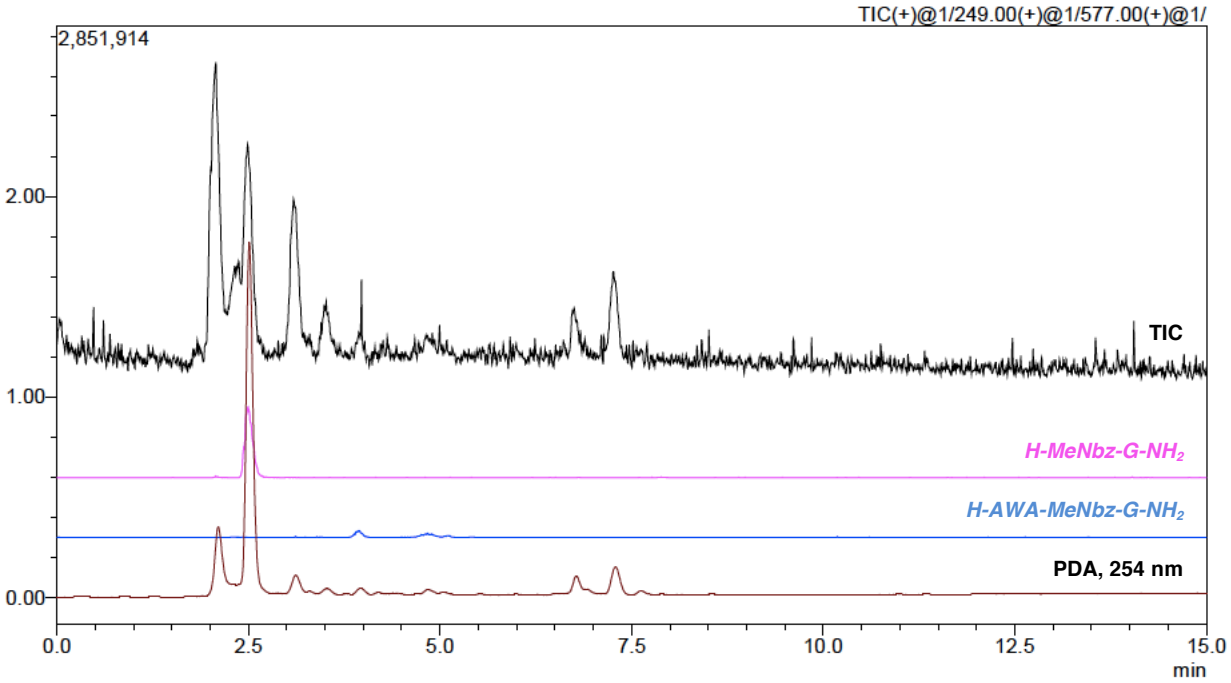


Figure SI-48. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

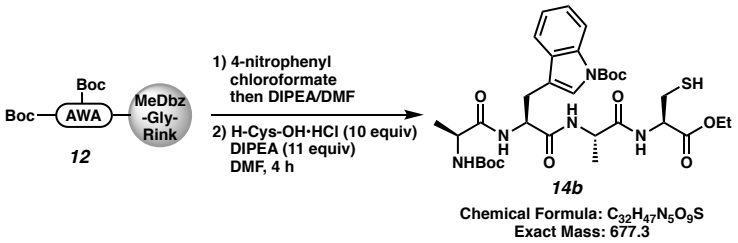


Table 2, Entry 2: 20 mg of **12** was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the linker was activated. 10 equiv of H-Cys-OEt•HCl was dissolved in 500 μL DMF and added to the swelled resin. Then 11 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. The solvent was removed from the vial and quenched in (1% TFA) 20% MeCN/H_2O. The resin was further rinsed with CH_2Cl_2 (3 x 1 mL) and combined with the initial solvent. The volatile solvent was blown down and the remaining solution was frozen in liquid nitrogen, and lyophilized to yield the crude peptide **14b**.

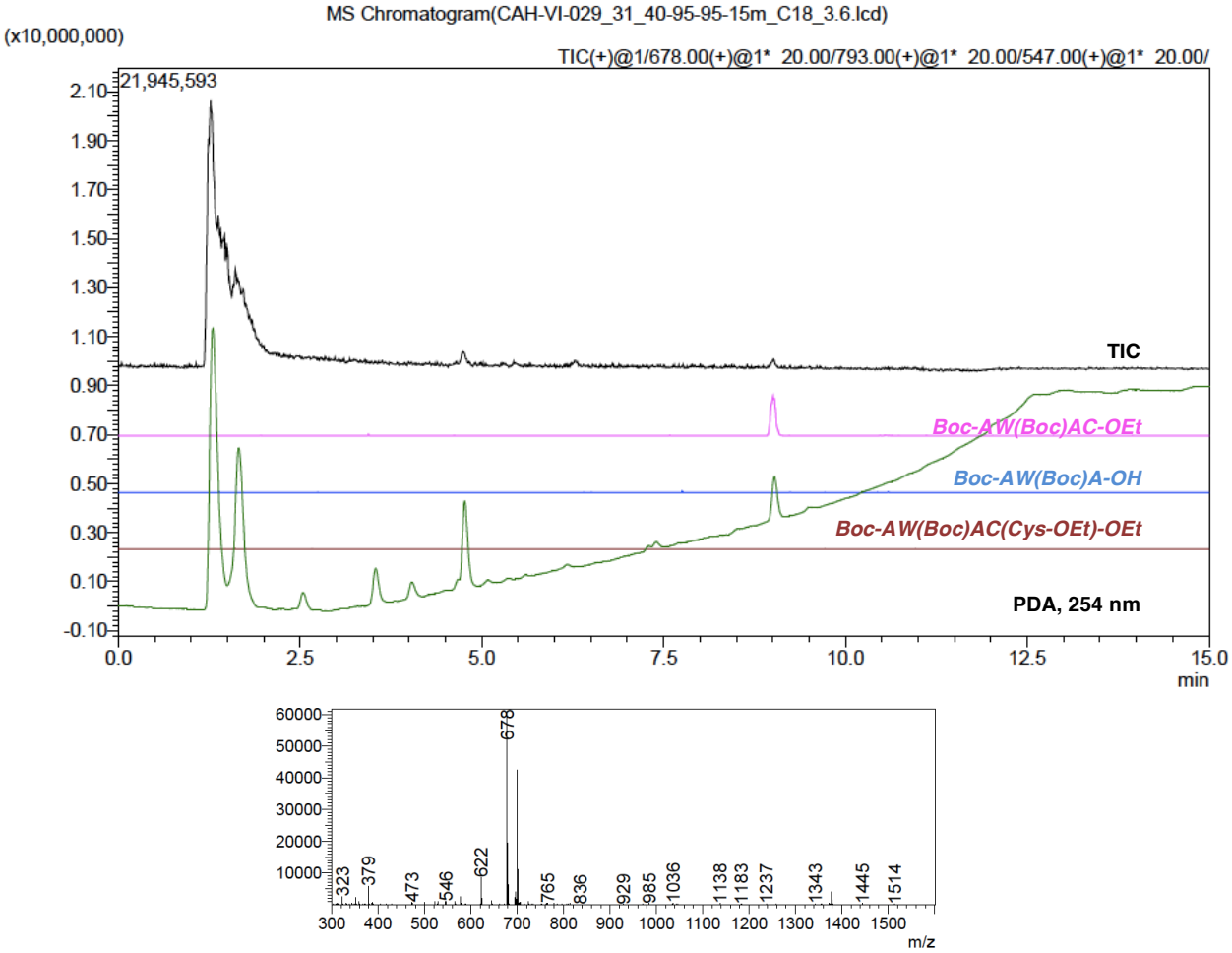


Figure SI-49. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95-95% MeCN/H_2O + 1% HCOOH. Retention time: 9.00 min.

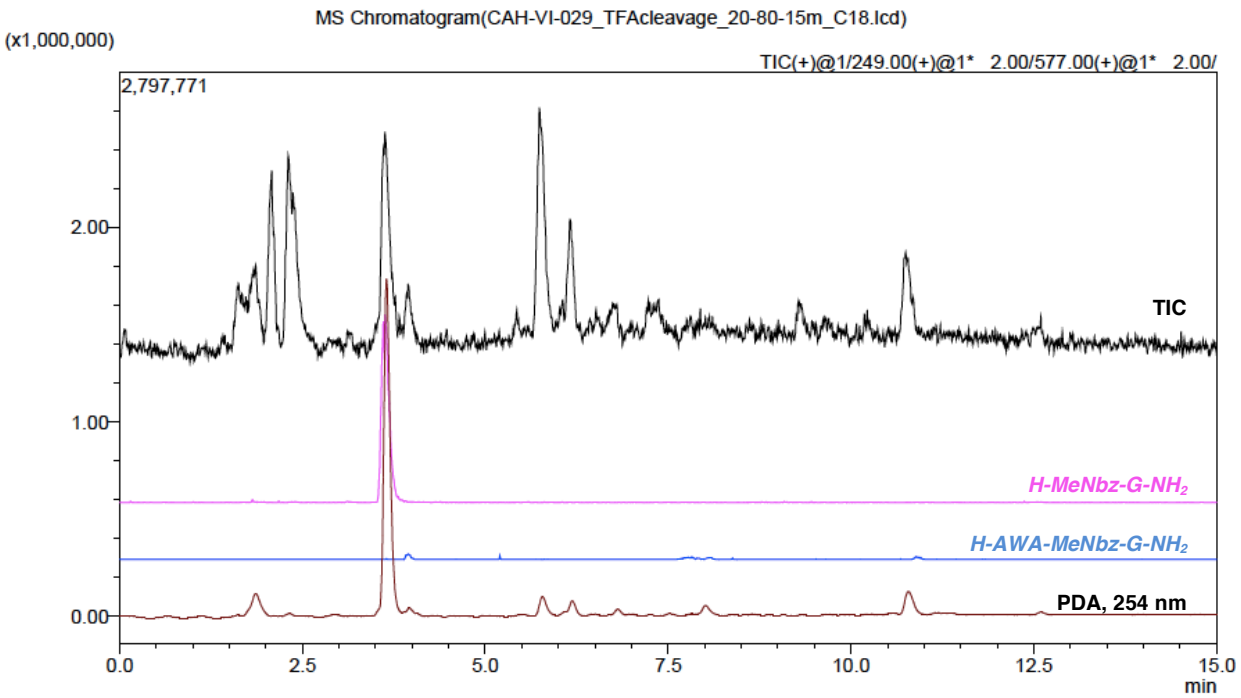


Figure SI-50. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

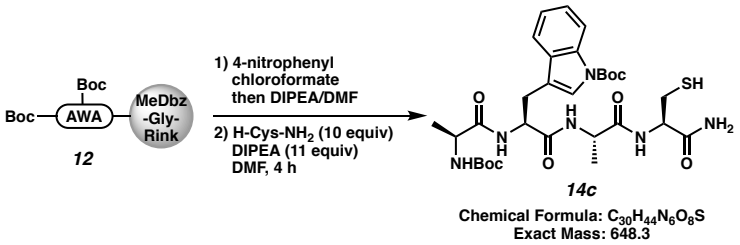


Table 2, Entry 3: 20 mg of **12** was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the linker was activated. 10 equiv of H-Cys-NH_2•TFA was dissolved in 500 μL DMF and added to the swelled resin. Then 11 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. The solvent was removed from the vial and quenched in (1% TFA) 20% MeCN/H_2O. The resin was further rinsed with CH_2Cl_2 (3 x 1 mL) and combined with the initial solvent. The volatile solvent was blown down and the remaining solution was frozen in liquid nitrogen, and lyophilized to yield the crude peptide **14c**.

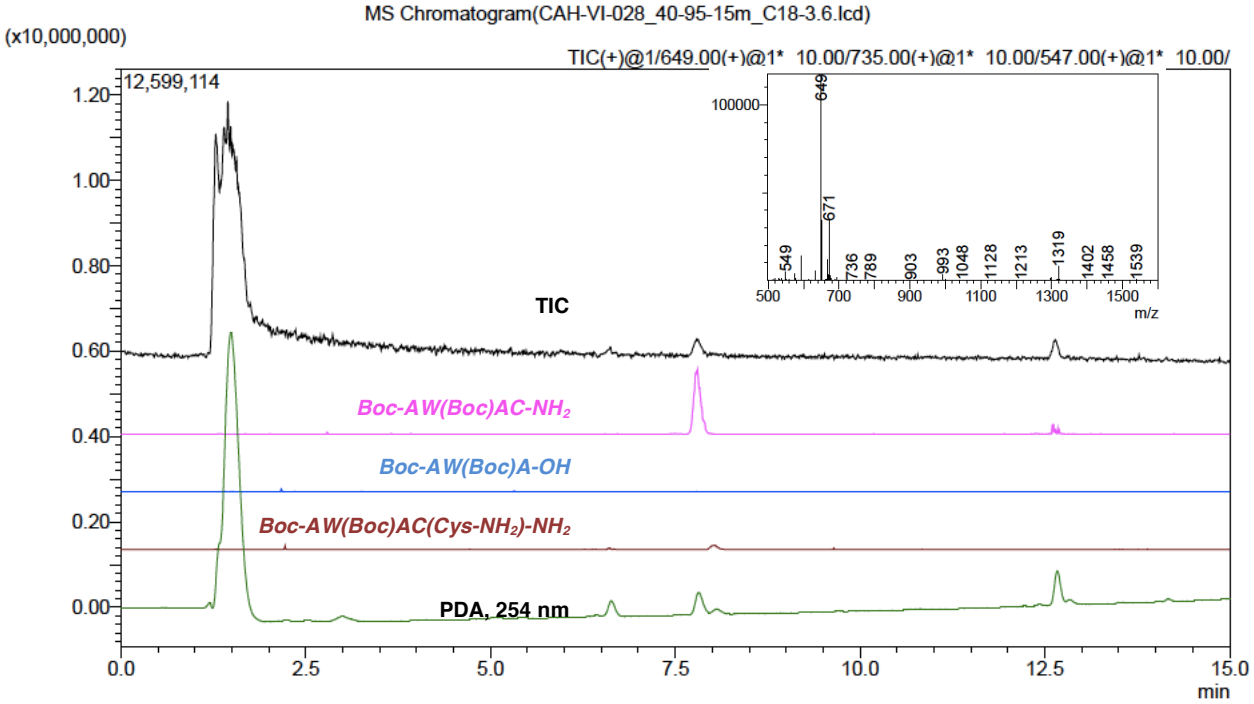


Figure SI-51. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95-95% MeCN/H_2O + 1% HCOOH. Retention time: 7.80 min.

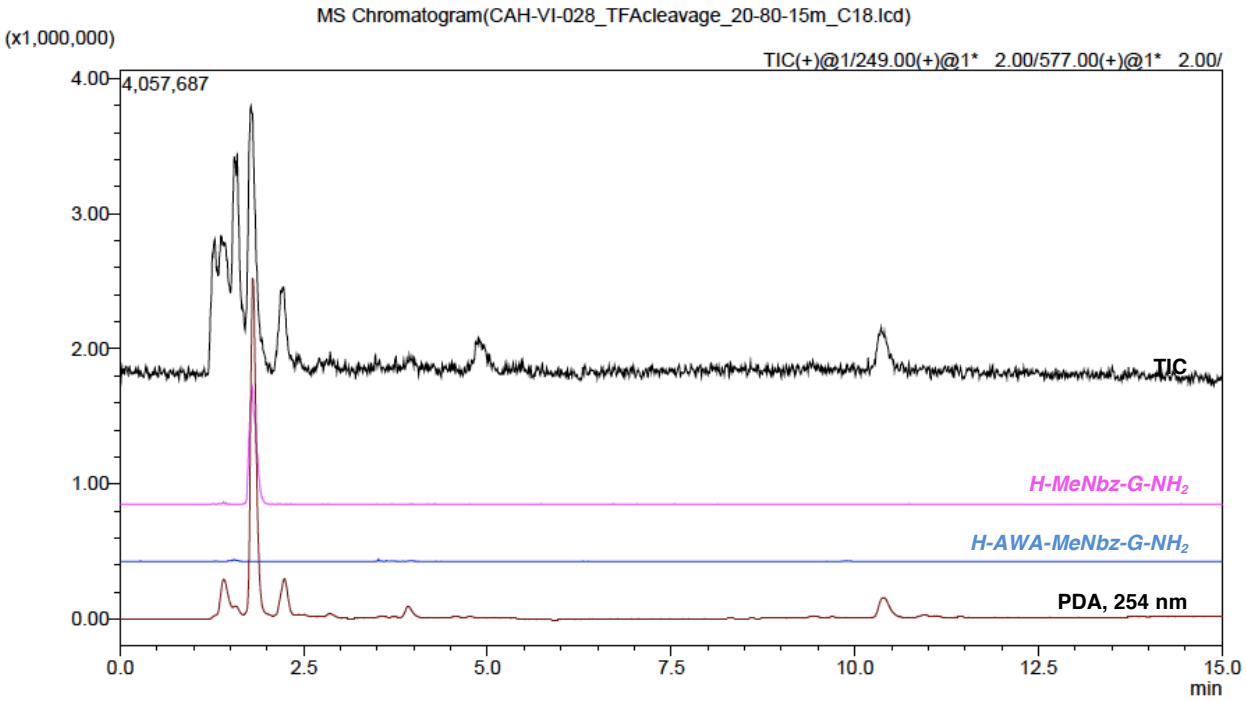


Figure SI-52. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

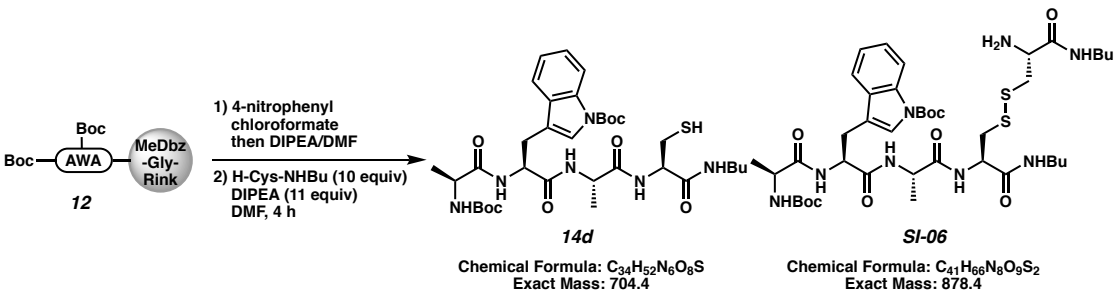


Table 2, Entry 4: 20 mg of **12** was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the linker was activated. 10 equiv of H-Cys-NHBu was dissolved in 500 μL DMF and added to the swelled resin. Then 11 equiv of DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. The solvent was removed from the vial and quenched in 20% MeCN/H_2O (1% TFA). The resin was further rinsed with CH_2Cl_2 (3 x 1 mL) and combined with the initial solvent. The volatile solvent was blown down and the remaining solution was frozen in liquid nitrogen, and lyophilized to yield the crude peptide **SI-06**.

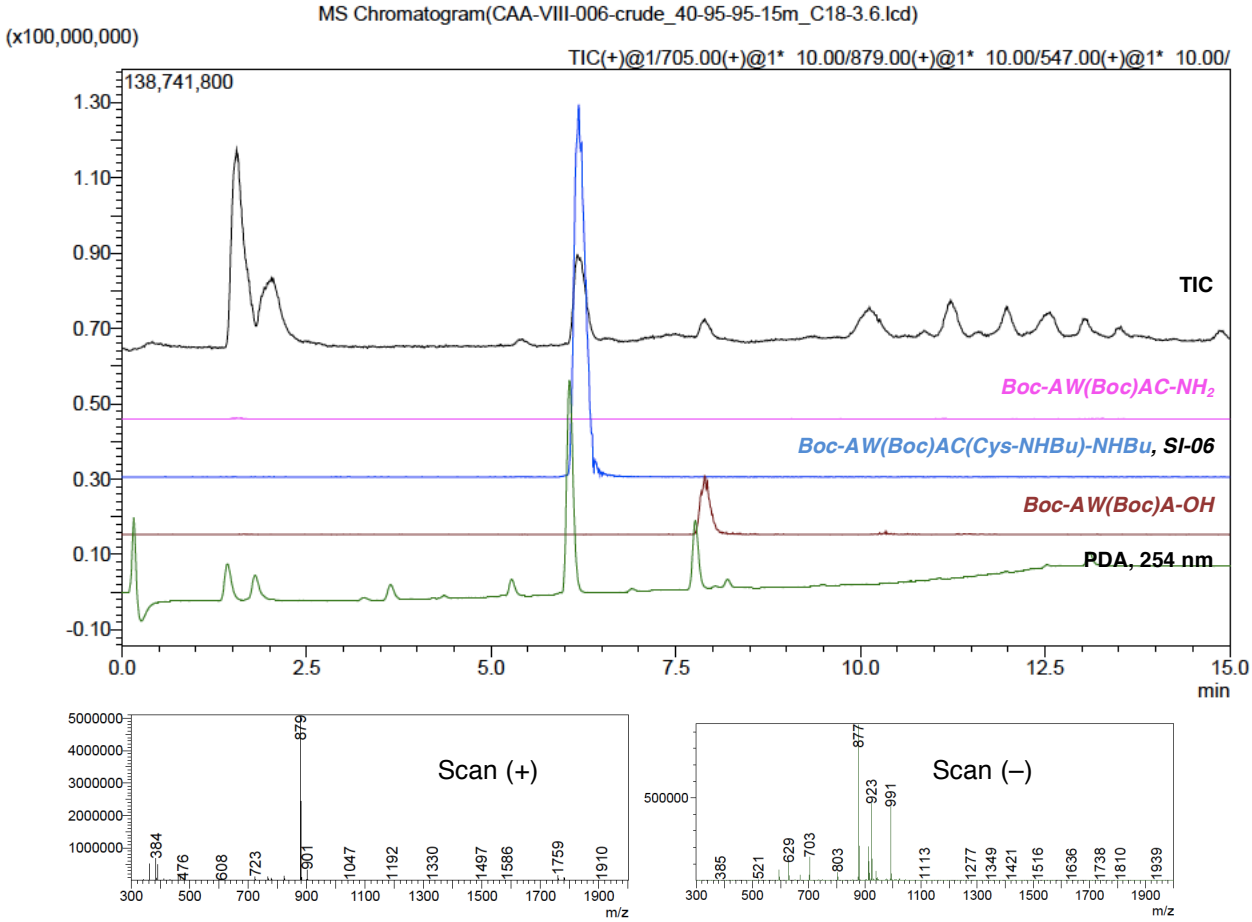


Figure SI-53. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95-95% MeCN/H_2O + 1% HCOOH. Retention time: 6.24 min.

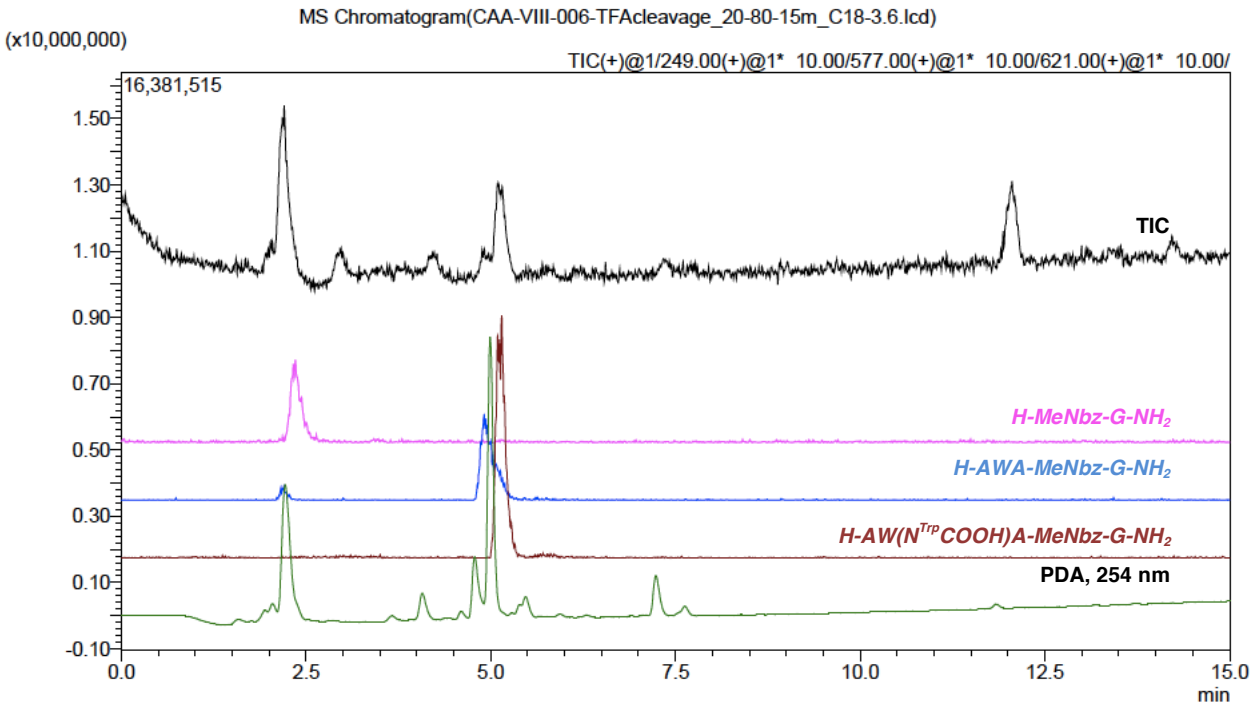


Figure SI-054. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O over 15 min.[6]

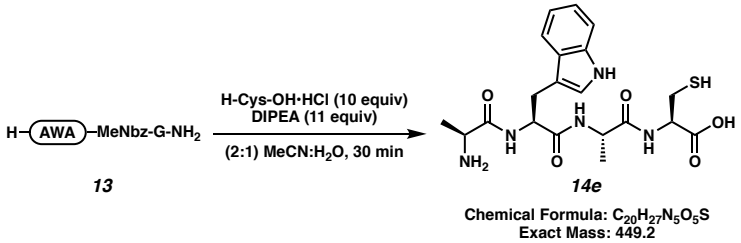


Table 2, Entry 5: 10 equiv H-Cys-OH•HCl was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**13**) was dissolved in 200 μL of HPLC grade MeCN and 100 μL H_2O and added to the amino acid containing vial. 11 equiv of freshly distilled DIEA was added to the vial and allowed to stir at ambient temperature for 30 min. After 30 min, the reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

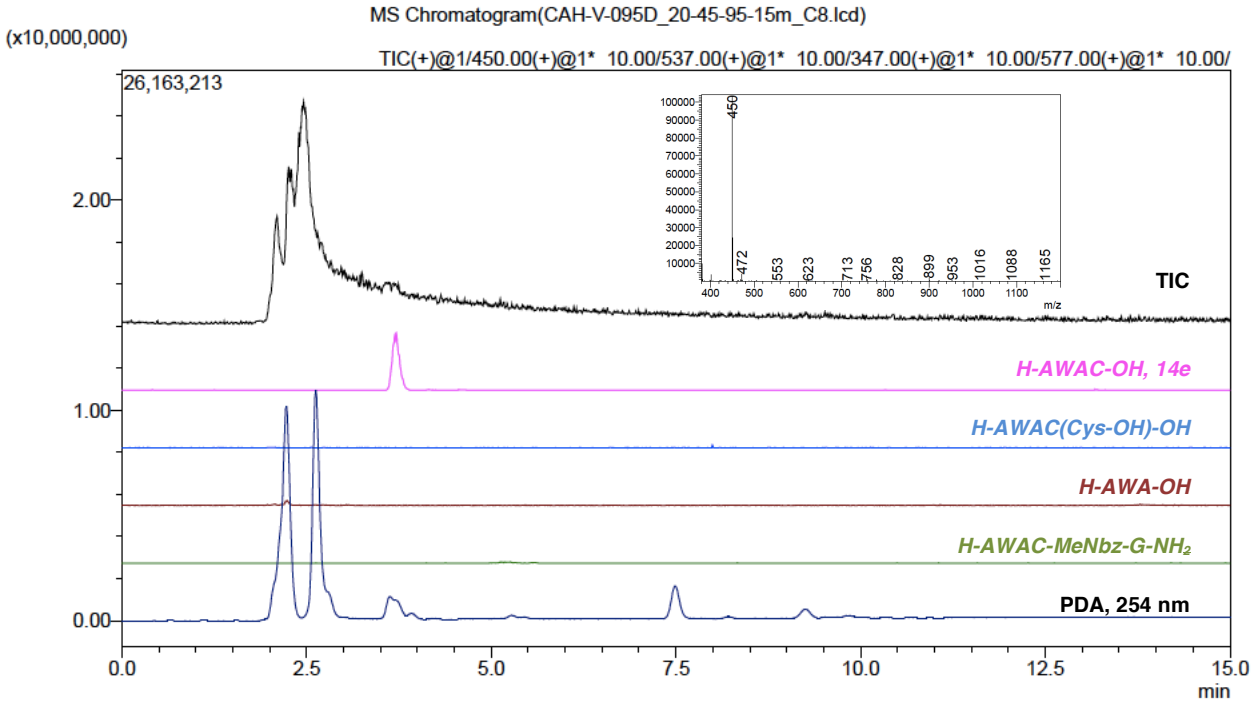


Figure SI-55. Reaction progress after 30 min of H-Cys-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O + 1% HCOOH over 15 min.

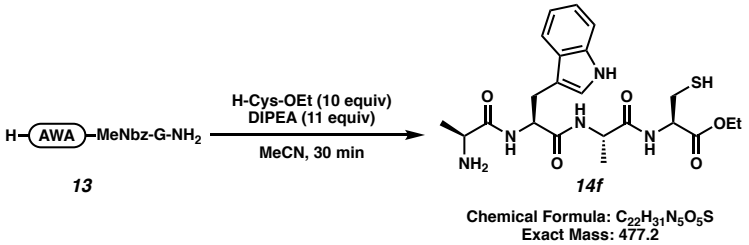


Table 2, Entry 6: 10 equiv H-Cys-OEt was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**13**) was dissolved in 200 μL of HPLC grade MeCN and added to the amino acid containing vial. 11 equiv of freshly distilled DIEA was added to the vial and allowed to stir at ambient temperature for 30 min. After 30 min, the reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

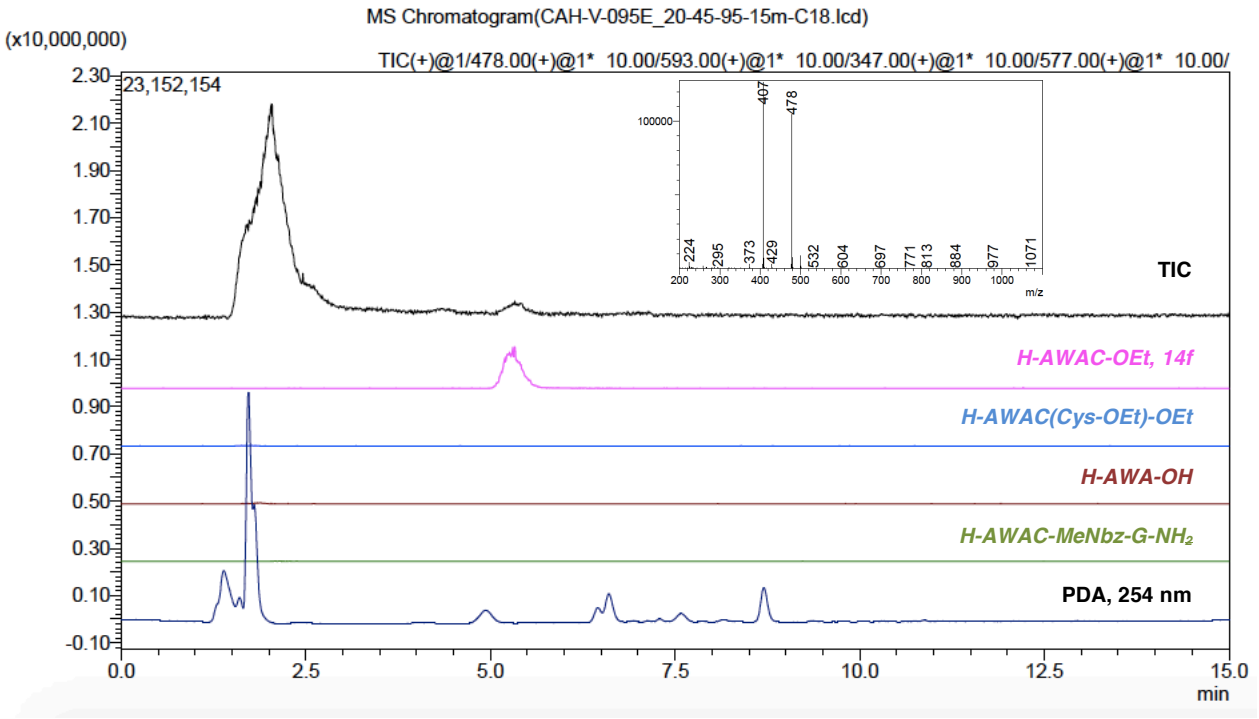


Figure SI-56. Reaction progress after 30 min of H-Cys-OEt addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O + 1% HCOOH over 15 min.

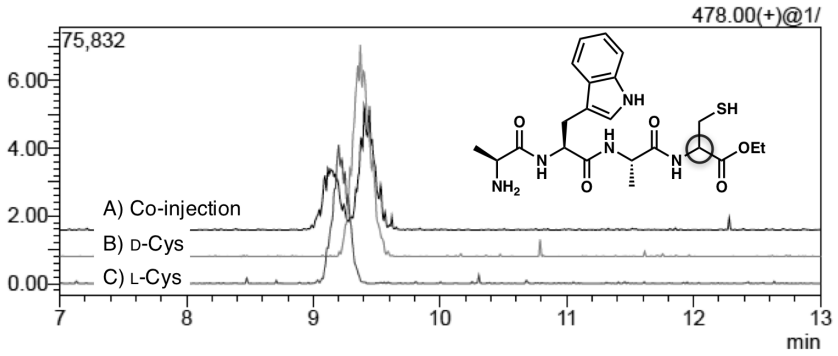


Figure SI-57. A) Co-injection, B) D-Cys, and C) L-Cys of in solution elongation of Cys-OEt, Gradient: 20-20(10 min)-45(5 min)% MeCN/H_2O + 1% HCOOH over 15 min.

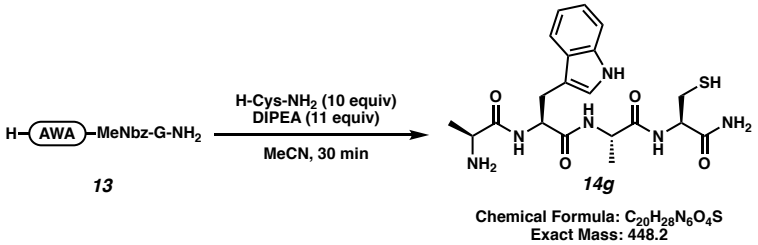


Table 2, Entry 7: 10 equiv H-Cys-NH_2 was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**13**) was dissolved in 200 μL of HPLC grade MeCN and added to the amino acid containing vial. 11 equiv of freshly distilled DIEA was added to the vial and allowed to stir at ambient temperature for 30 min. After 30 min, the reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

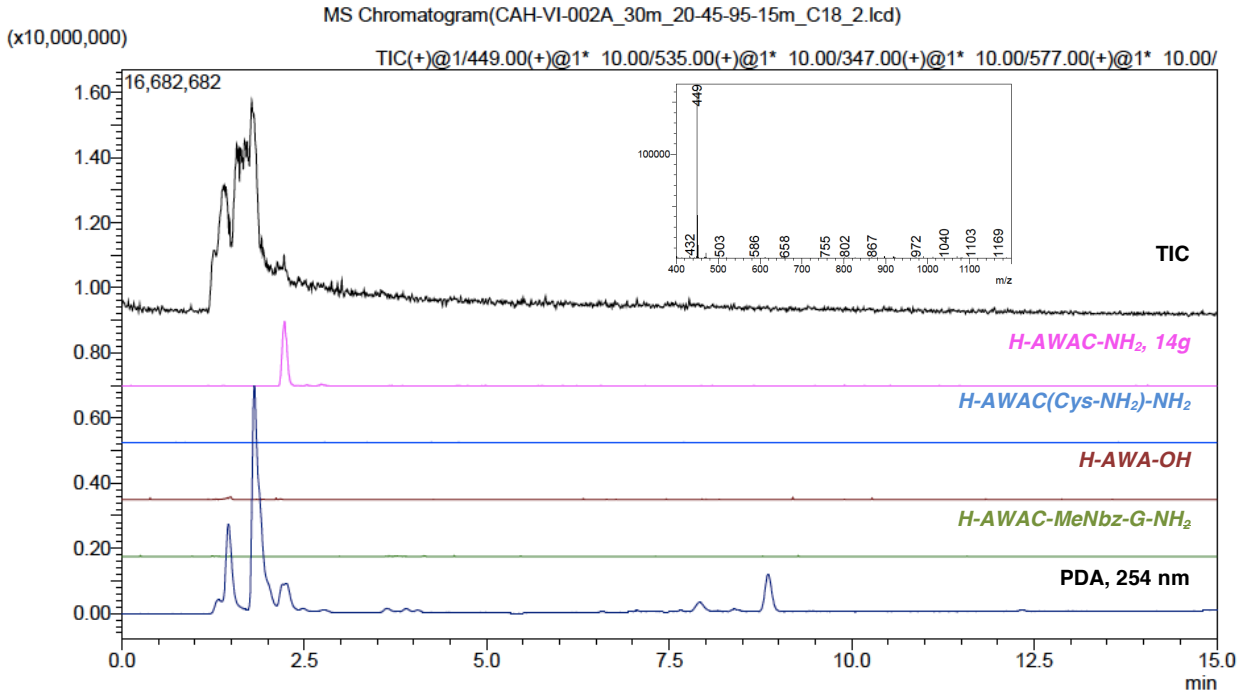


Figure SI-58. Reaction progress after 30 min of H-Cys-NH_2 addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O + 1% HCOOH over 15 min.

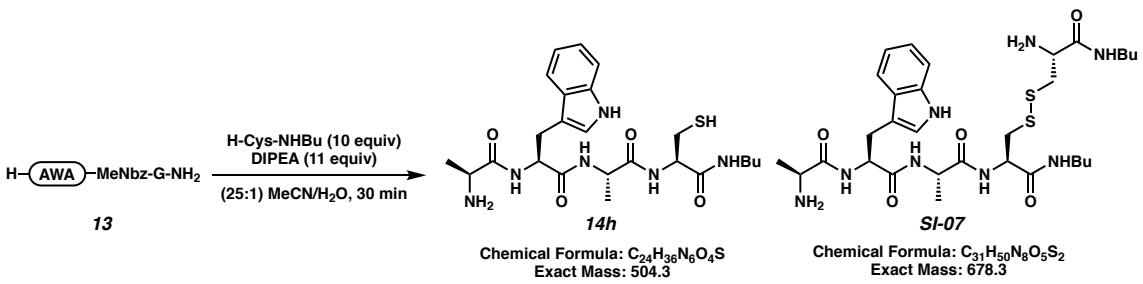


Table 2, Entry 8: 10 equiv H-Cys-NHBu was weighed out into a vial containing a stir bar. 3.8 mg of crude peptide (**13**) was dissolved in 500 μL of HPLC grade MeCN and 20 μL H_2O and added to the amino acid containing vial. 11 equiv of freshly distilled DIEA was added to the vial and allowed to stir at ambient temperature for 30 min. After 30 min, the reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

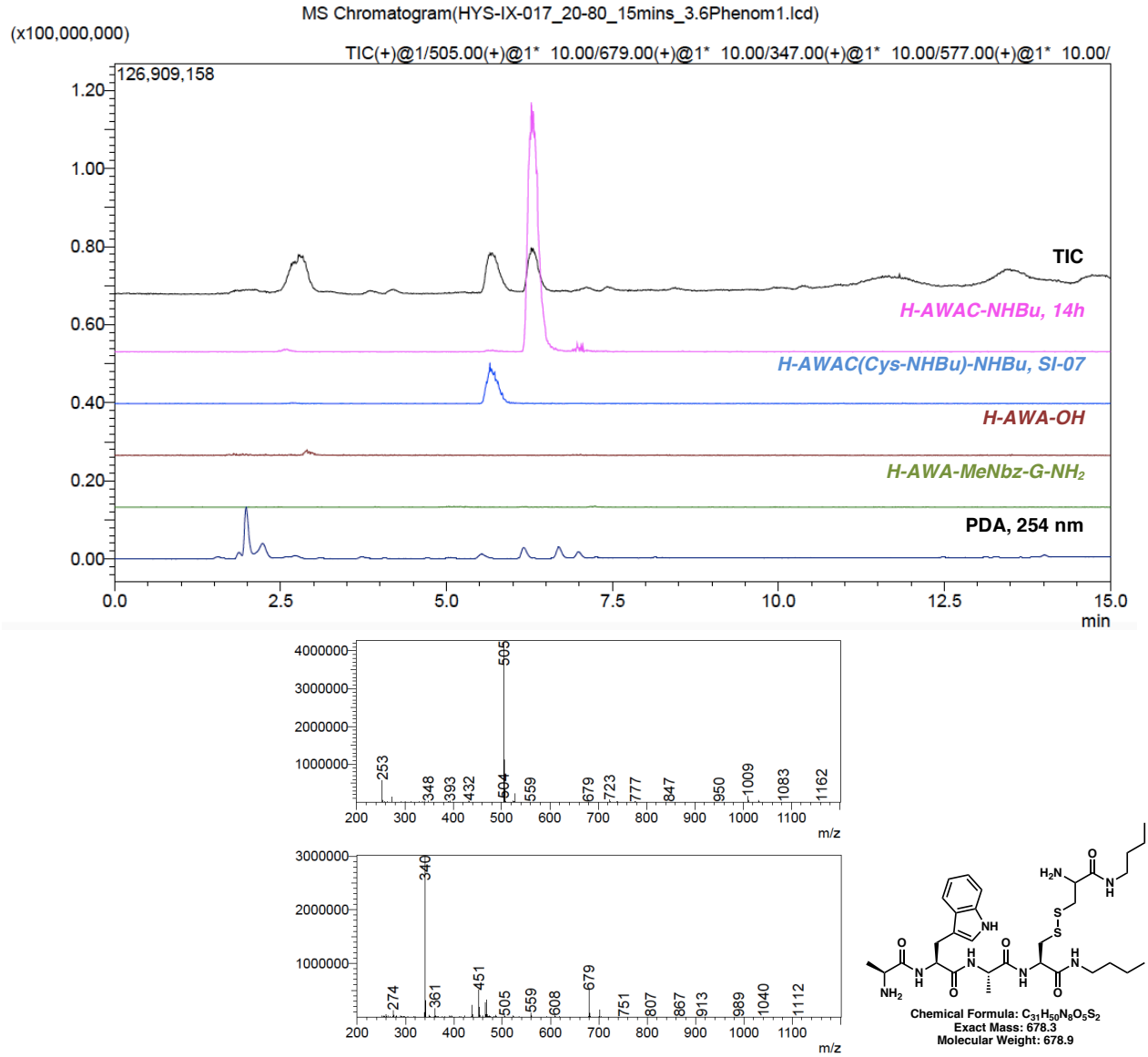


Figure SI-59. Reaction progress after 30 min of H-Cys-NH_2 addition, Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

Co-injection of C-terminal Cys-OH elongation and authentic sample.

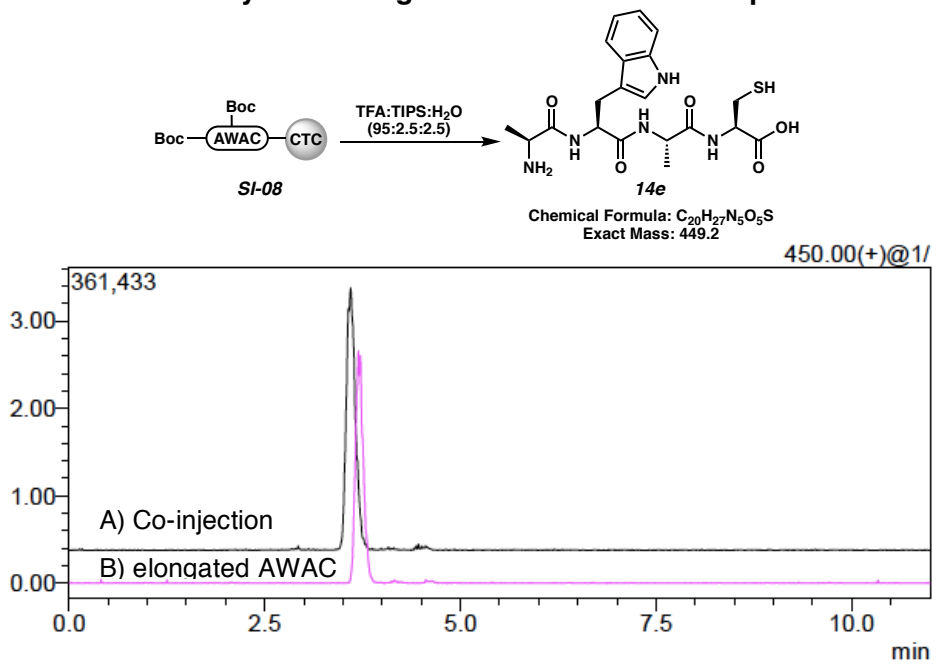
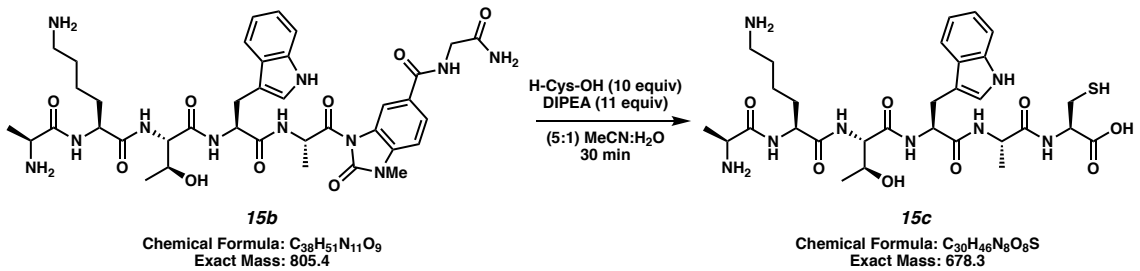


Figure SI-60. A) Co-injection of authentic sample and elongated product, **B)** C-terminal Cys-OH elongated product, AWAC-OH, gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O + 1% HCOOH over 15 min.

Table 3. Cysteine elongation to generate C-terminal acids, carboxamides, and esters.



15b
Chemical Formula: $C_{38}H_{51}N_{11}O_9$
Exact Mass: 805.4

15c
Chemical Formula: $C_{30}H_{46}N_8O_8S$
Exact Mass: 678.3

Table 3, entry 1. 20 mg of peptide **15b** (24.8 μmol) was dissolved in 600 μL of (5:1) MeCN:H_2O. 39 mg of H-Cys-OH•HCl (10 equiv, 0.25 mmol) was weighed out into a vial containing a stir bar. The solution of peptide was transferred to the vial containing the amino acid, followed by addition of 48 μL DIEA (11 equiv, 0.27 mmol). The mixture was stirred at ambient temperature for 30 min. The reaction was quenched with 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

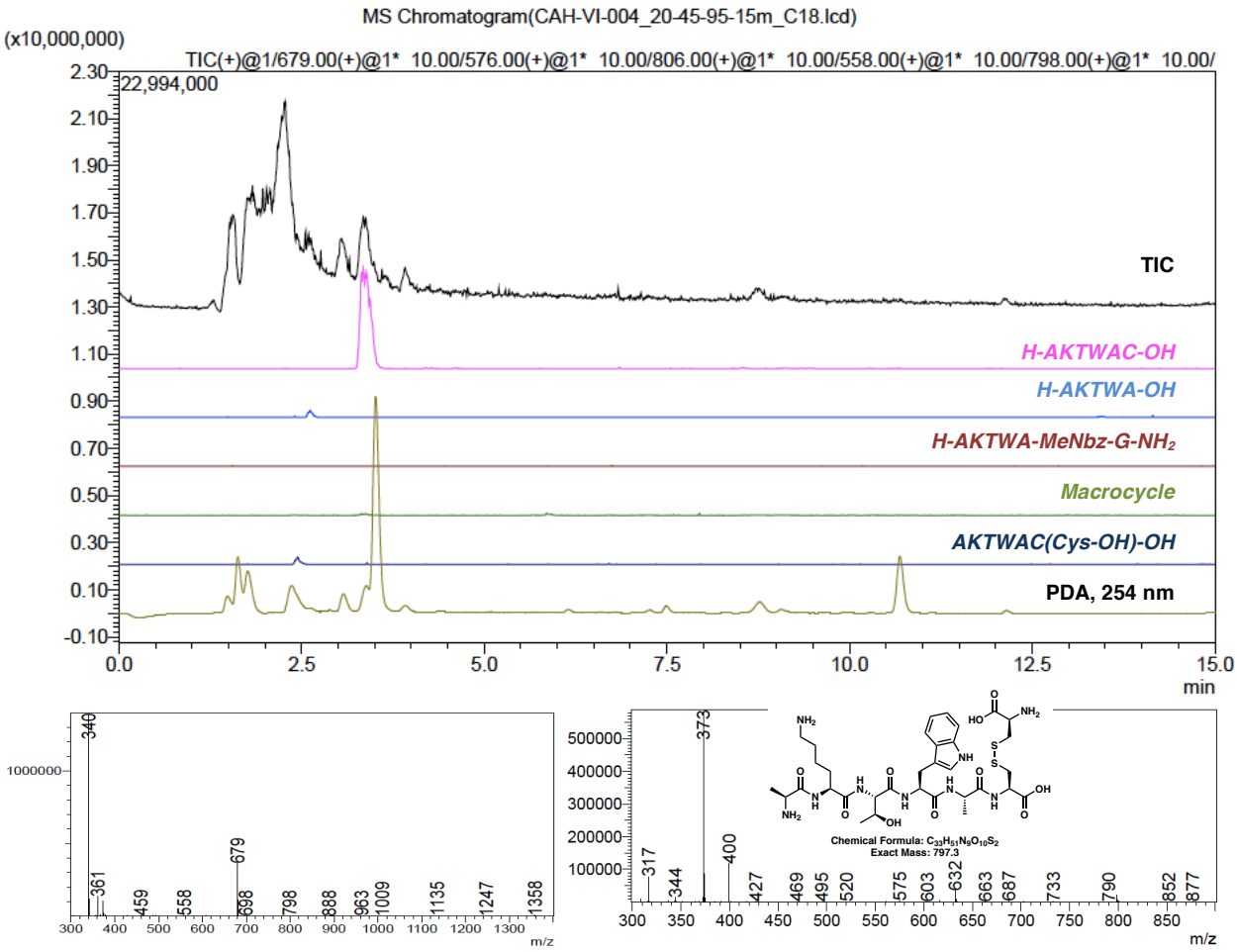


Figure SI-61. Reaction progress after 30 min of H-Cys-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O + 1% HCOOH over 15 min.

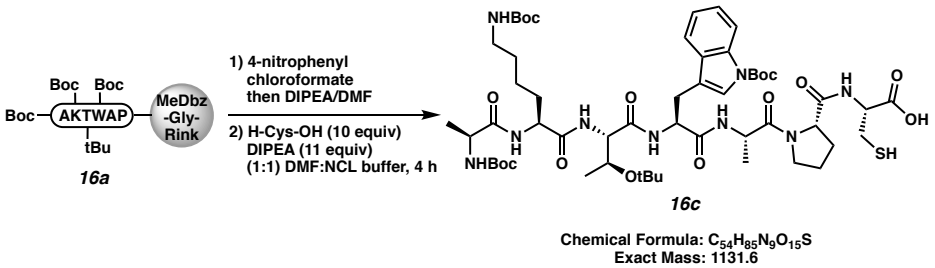


Table 3, entry 2. 20 mg of peptide **16a** (7 μmol) was swelled in CH_2Cl_2 for 30 min. After swelling the solvent was removed and the peptide was activated. In a separate vial, 8.5 mg of H-Cys-OH•HCl (10 equiv, 70 μmol) was dissolved in 500 μL (1:1) DMF:NCL buffer followed by the addition of 13 μL DIEA (11 equiv, 77 μmol). The solution of the amino acid was transferred to the resin and agitated at ambient temperature for 4 h. The reaction was quenched with 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

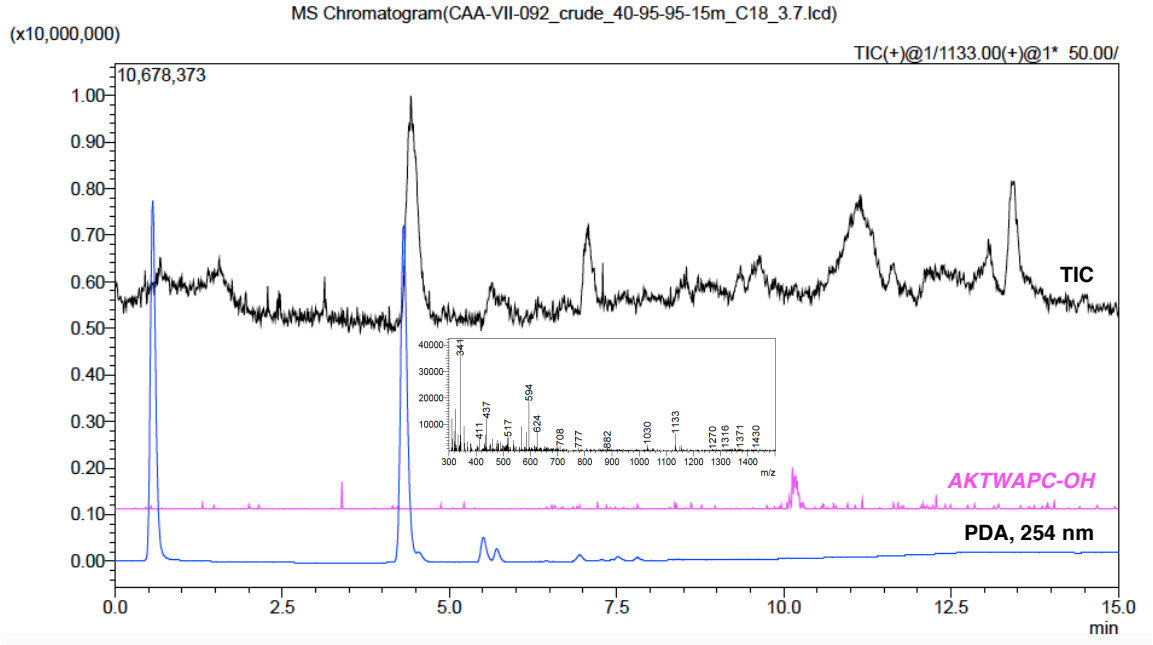


Figure SI-62. Analytical Column PDA of crude peptide **16c**, gradient of 40-95-95% MeCN/H_2O + 1% HCOOH over 15 min.

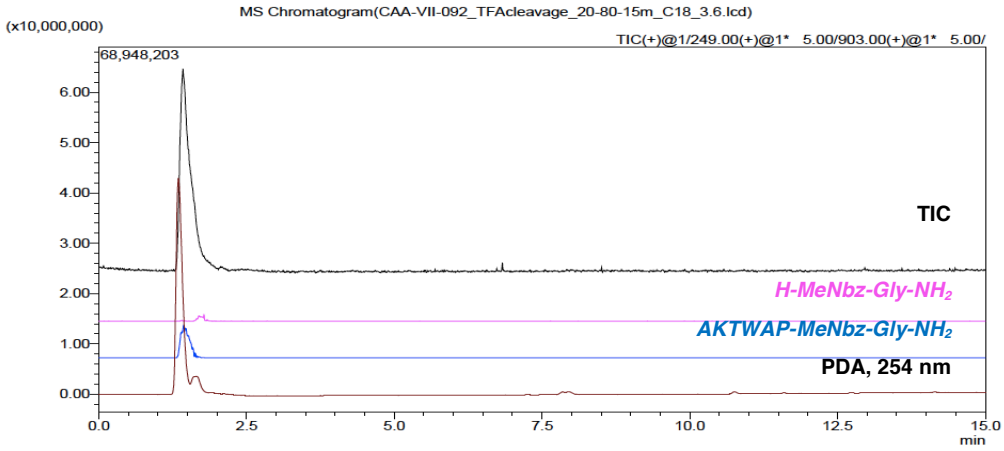


Figure SI-63. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

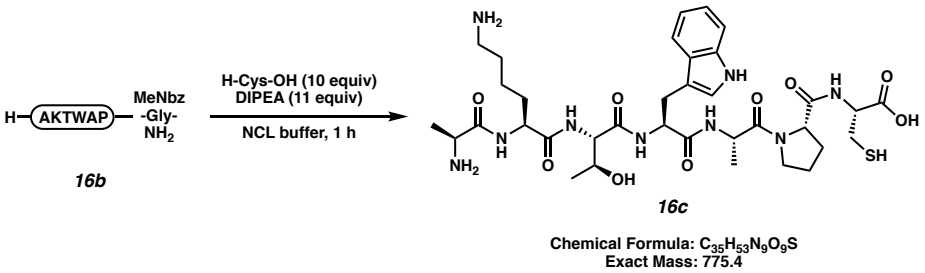


Table 3, entry 3. 10 mg of peptide **16b** (11 μmol) was dissolved in 250 μL of NCL buffer. 17.5 mg of H-Cys-OH•HCl (10 equiv, 0.11 mmol) was weighed out into a vial containing a stir bar. The solution of peptide was transferred to the vial containing the amino acid, followed by addition of 21 μL DIEA (11 equiv, 0.12 mmol). The mixture was stirred at ambient temperature for 1 h. The reaction was quenched with 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

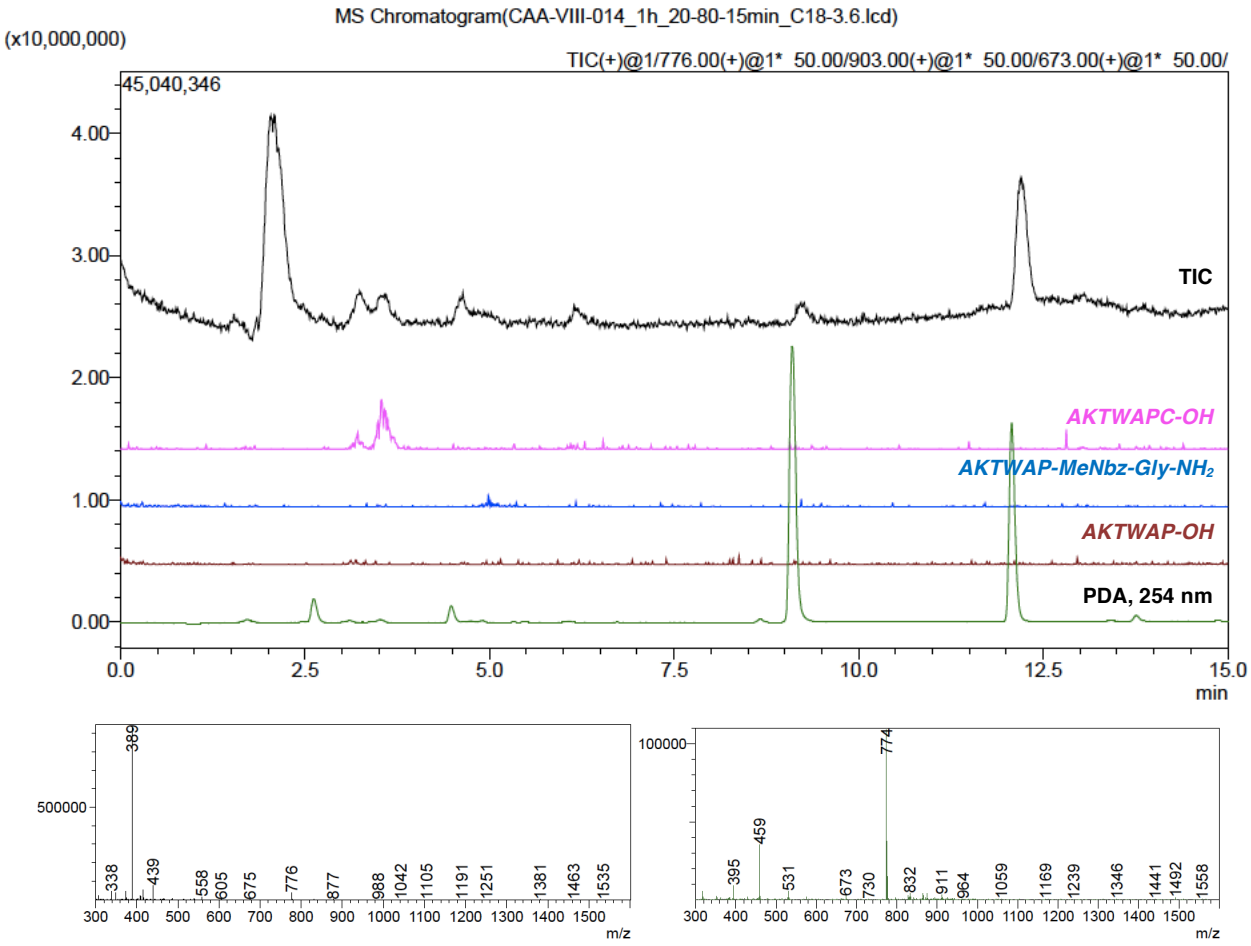


Figure SI-64. Analytical Column PDA of crude peptide **16c** after 1 h, gradient of 40-95-95% MeCN/H_2O + 1% HCOOH over 15 min.

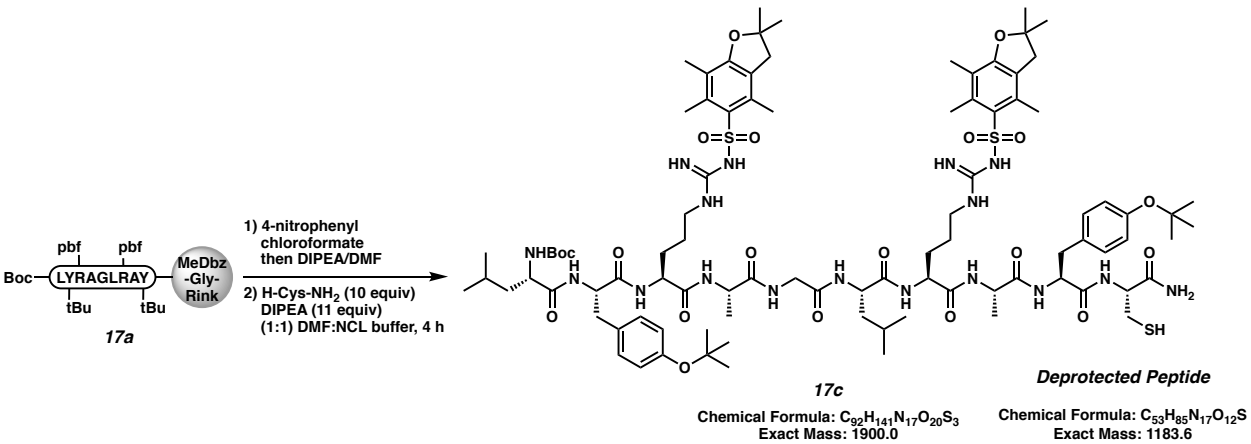


Table 3, entry 4. 20 mg of peptide **17a** (7 μmol) was swelled in CH_2Cl_2 for 30 min. After swelling the solvent was removed and the peptide was activated. In a separate vial, 8.4 mg of H-Cys-NH_2•TFA (10 equiv, 70 μmol) was dissolved in 500 μL (1:1) DMF:NCL buffer followed by the addition of 13 μL DIEA (11 equiv, 77 μmol). The solution of the amino acid was transferred to the resin and agitated at ambient temperature for 4 h. The reaction was quenched with 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS. Due to the protected peptide adhering to the resin, the resin was rinsed with TFA:TIPS:H_2O (95:2.5:2.5), collected, blown down under a constant stream of air, crashed out with cold diethyl ether, centrifuged, and decanted to yield the deprotected peptide which was analyzed via RP-HPLC-MS.

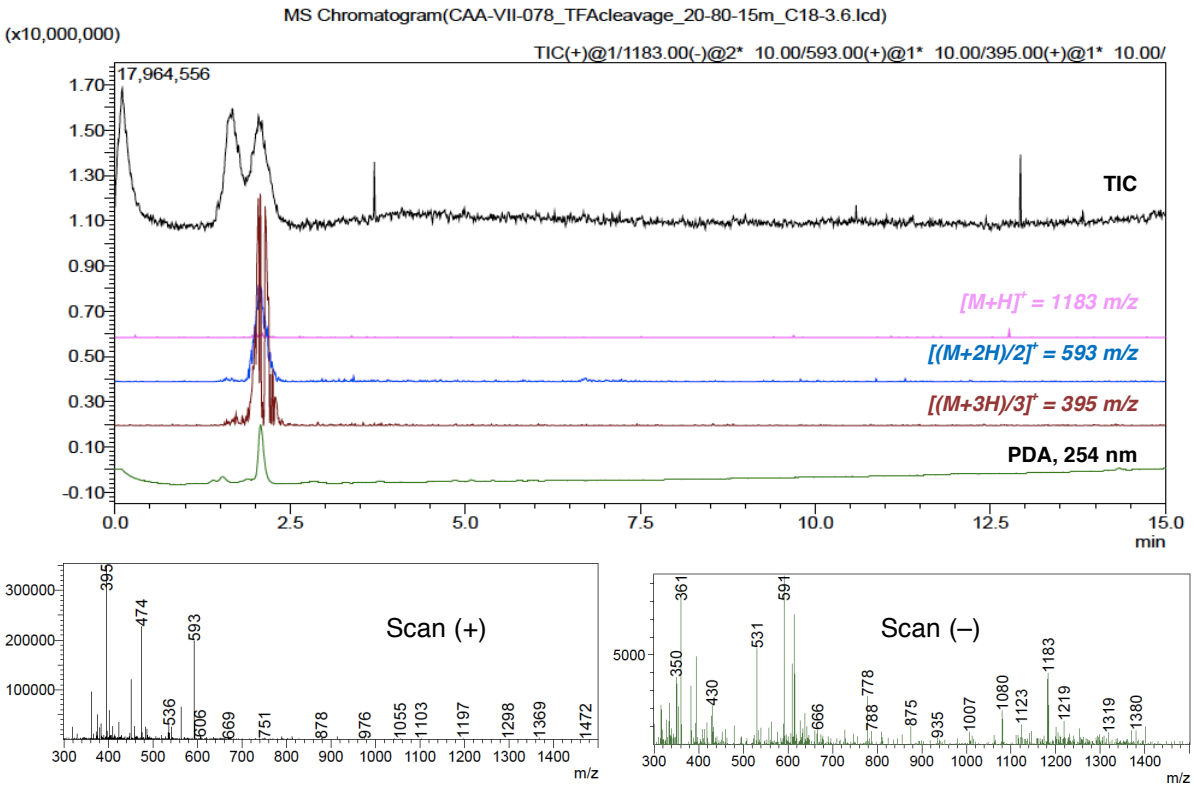


Figure SI-65. Analytical Column PDA of crude peptide **17c**, gradient of 40-95-95% MeCN/H_2O + 1% HCOOH over 15 min.

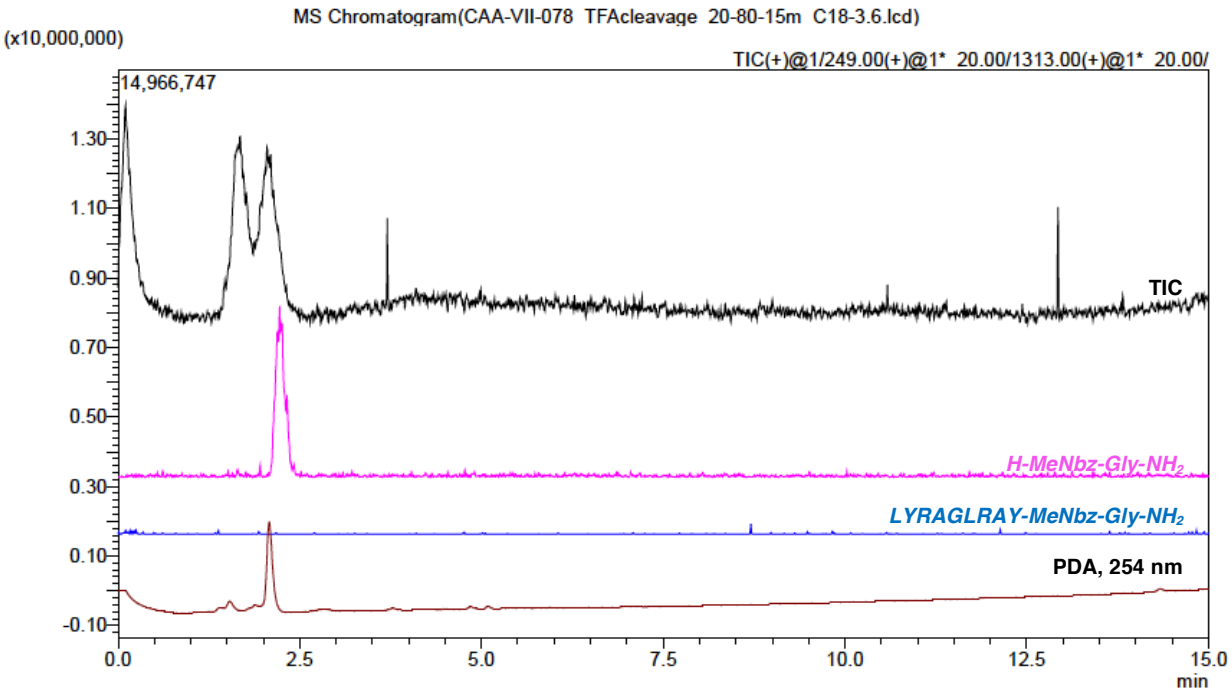


Figure SI-66. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

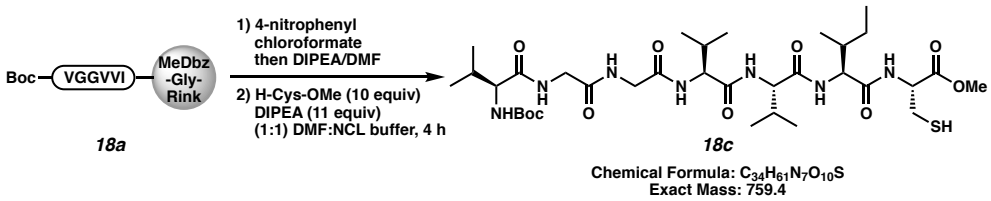


Table 3, entry 5. 20 mg of peptide **18a** (7 μmol) was swelled in CH_2Cl_2 for 30 min. After swelling the solvent was removed and the peptide was activated. In a separate vial, 12 mg of H-Cys-OMe•HCl (10 equiv, 70 μmol) was dissolved in 500 μL (1:1) DMF:NCL buffer followed by the addition of 13 μL DIEA (11 equiv, 77 μmol). The solution of the amino acid was transferred to the resin and agitated at ambient temperature for 4 h. The reaction was quenched with 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS. Due to the protected peptide adhering to the resin, the resin was rinsed with TFA:TIPS:H_2O (95:2.5:2.5), collected, blown down under a constant stream of air, crashed out with cold diethyl ether, centrifuged, and decanted to yield the deprotected peptide which was analyzed via RP-HPLC-MS. *However, product was not observed.*

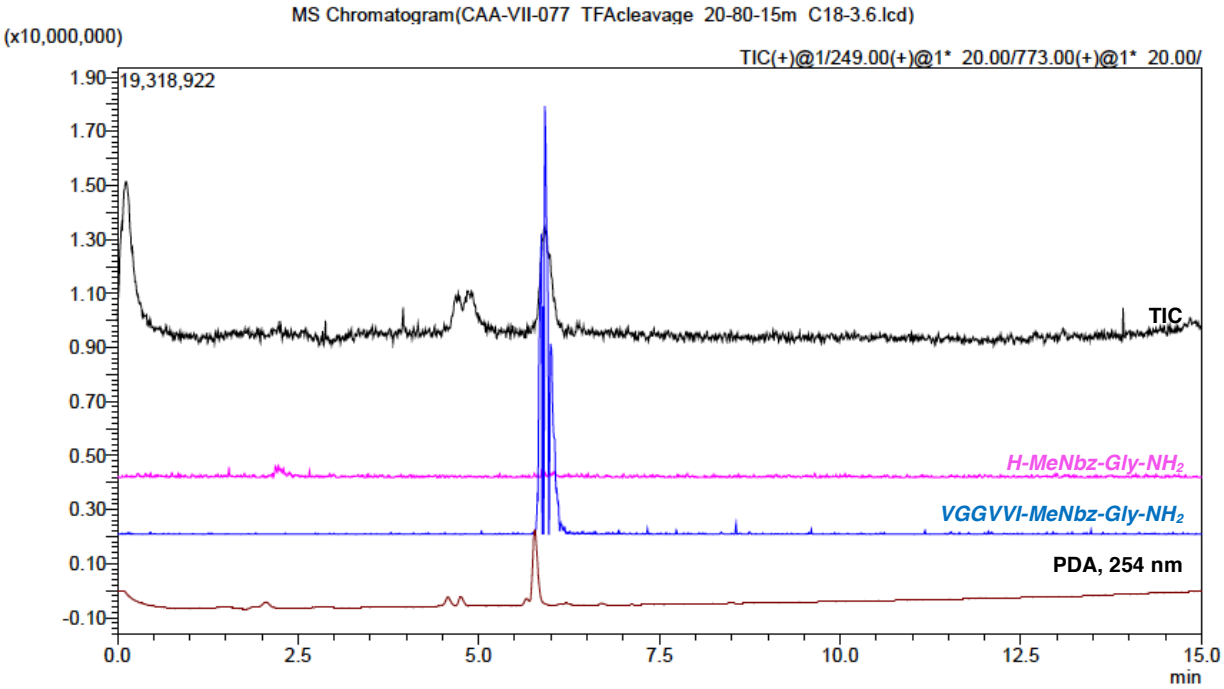


Figure SI-067. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min.

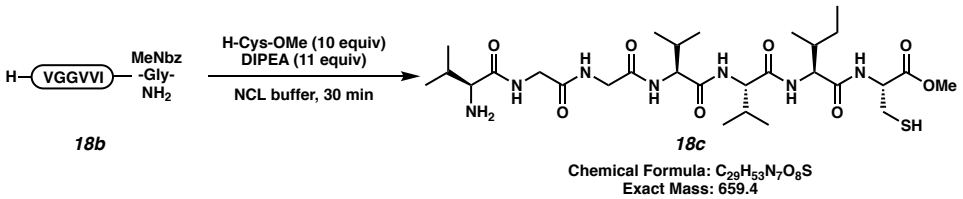


Table 3, entry 6. 67.3 mg of crude peptide **18b** (87 μmol) was dissolved in 250 μL of NCL buffer. 149 mg of H-Cys-OMe•HCl (10 equiv, 0.87 mmol) was weighed out into a vial containing a stir bar. The solution of peptide was transferred to the vial containing the amino acid, followed by addition of 167 μL DIEA (11 equiv, 0.96 mmol). The mixture was stirred at ambient temperature for 30 min. The reaction was quenched with 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

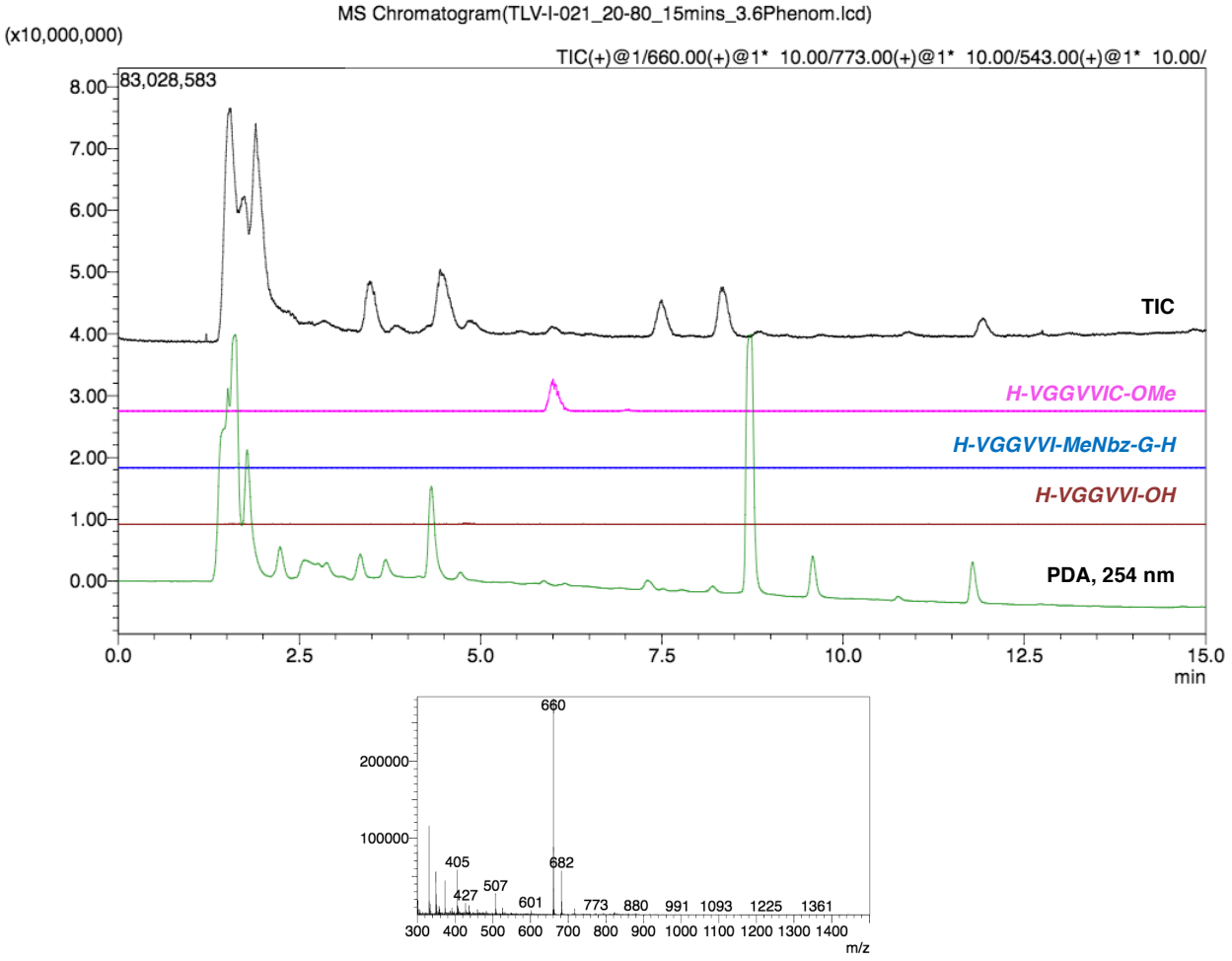
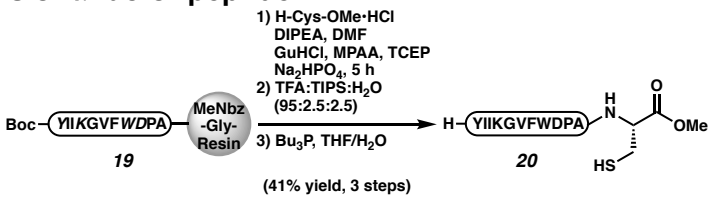


Figure SI-68. Reaction progress after 30 min of H-Cys-OMe addition, Gradient: 20-80% MeCN/H_2O + 1% HCOOH over 15 min, Retention time: 5.98 min.

Scheme 3. Synthesis of α-factor peptide 21.



50 mg (12 μmol) of peptide **19** was activated (See MeDbz activation) and suspended in a pre-mixed solution of (10 equiv) H-Cys-OMe•HCl and (11 equiv) DIEA in (1:1) DMF:NCL buffer (pH 7.2). The mixture was agitated for 4 h at ambient temperature. The solvent was removed and collected. The resin was rinsed with CH_2Cl_2 (2 x), water (2 x), followed by (1:1:3) TFE:AcOH: CH_2Cl_2 (2 x). The rinses were combined with the initial reaction solvent, blown down under a constant stream of air, diluted with water, frozen (LN_2), and lyophilized to yield the fully protected crude peptide. Some remaining peptide was adhered to the resin, which was removed using 95:2.5:2.5 TFA:TIPS:H_2O rinses and combined with the following residue. The fully protected peptide residue was dissolved in (95:2.5:2.5) TFA:TIPS:H_2O and agitated for 1.5 h. The solvent was concentrated down under a constant steam of air, crashed out with cold diethyl ether, centrifuged, decanted off, and the solid was dissolved in 1:1 MeCN:H_2O, frozen (LN_2), and lyophilized. The crude deprotected peptide was then dissolved in 10% THF/H_2O (800 μL) and sparged with argon for 15 min. 5 equiv of Bu_3P was added to the solution and stirred at ambient temperature for 2 h. The volatile solvent was removed under reduced pressure and the aqueous solution was frozen (LN_2) and lyophilized. The solid was purified using RP-HPLC-MS to yield the non-alkylated α-factor precursor **20** in 41% yield (7.0 mg).

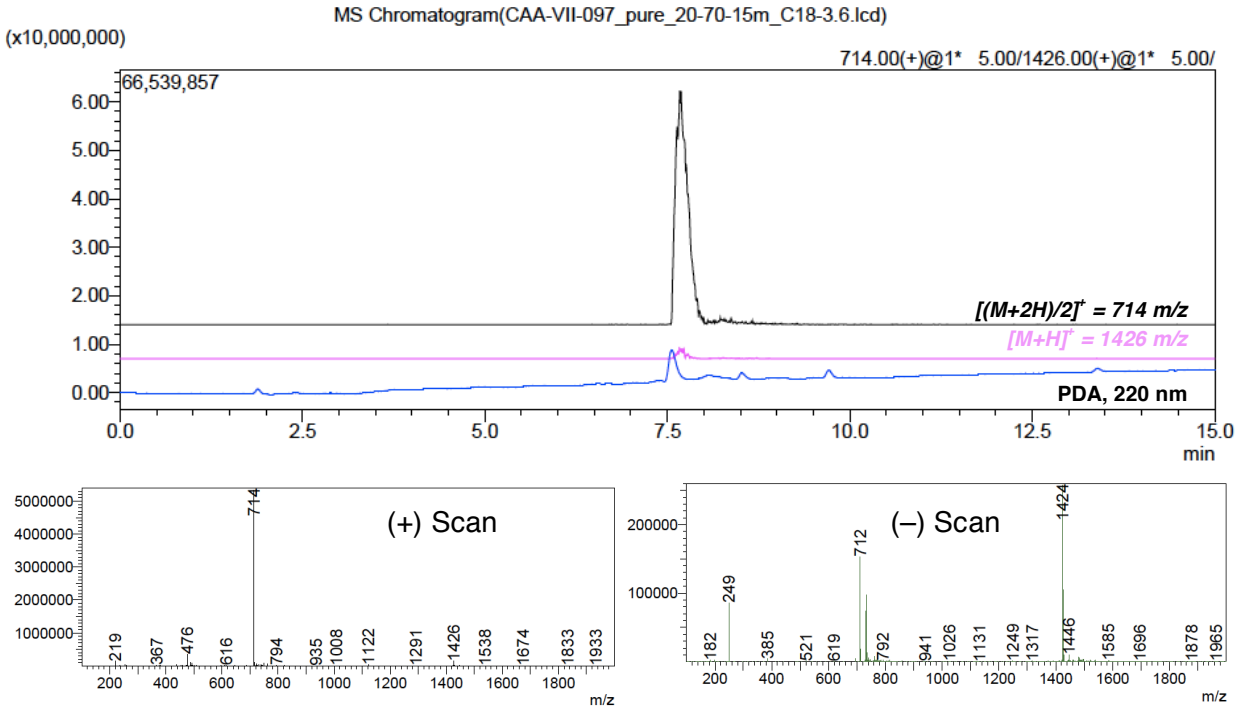


Figure SI-69. Analytical Column PDA of pure peptide **21**, gradient of 20-70% MeCN/H_2O + 1% HCOOH over 15 min. Retention time: 7.75 min.

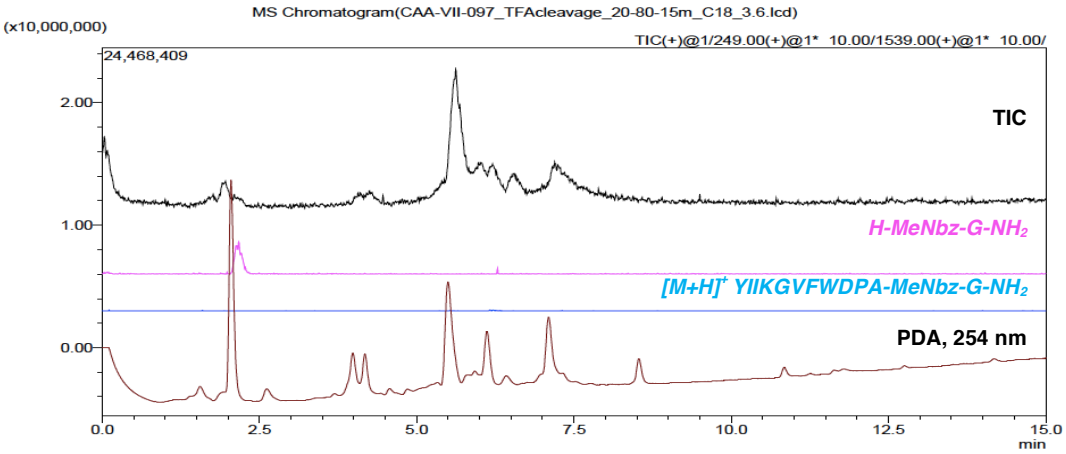
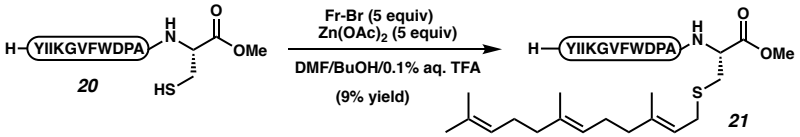


Figure SI-70. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O over 15 min.



2 mg (1.4 μmol) of H-YIIKGVFWDPAC-OMe **20** was weighed out in a 25 mL round bottom flask and dissolved in 0.8 mL DMF/BuOH (2:1). Then 5 equiv of Fr-Br (2.0 mg, 7.0 μmol) was added to the solution followed by a solution of 5 equiv $Zn(OAc)_2$ (1.3 mg, 7.0 μmol) in 0.4 mL 0.1% TFA (aq). The reaction went to completion after 1 h followed by dilution with H_2O, frozen in LN_2, and lyophilized to yield the crude peptide. RP-HPLC-MS purification was conducted resulting in a 9% yield of farnesylated α-factor peptide **21** (0.2 mg).

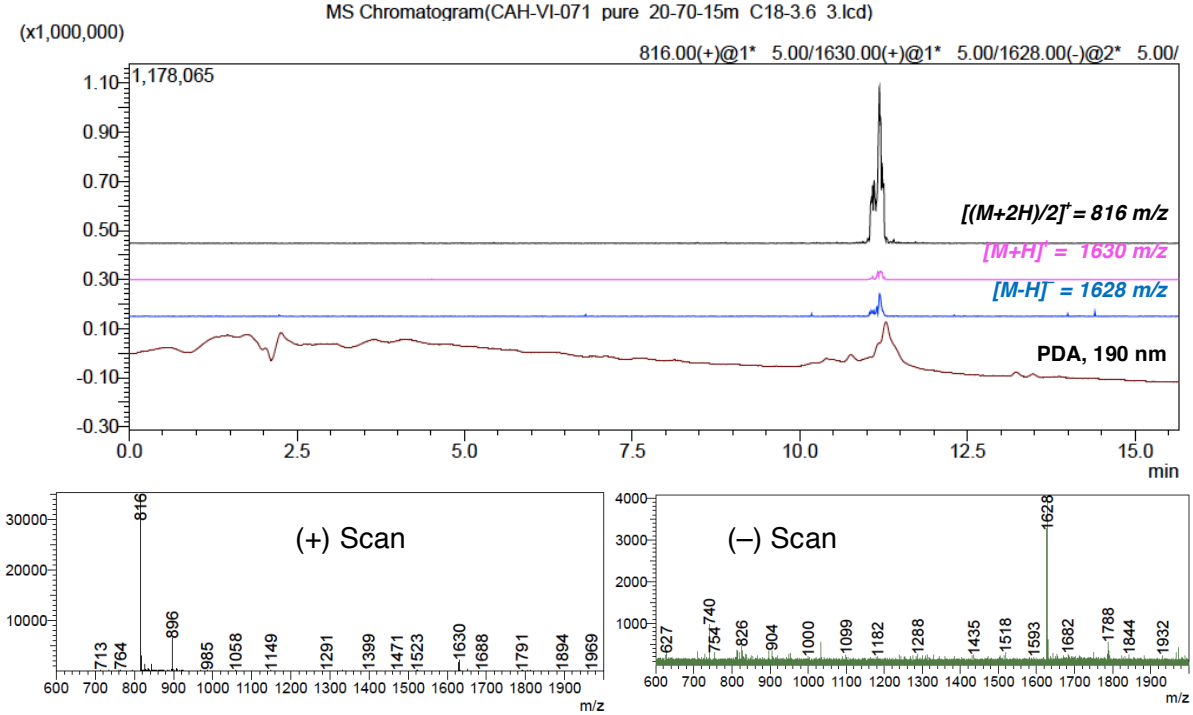
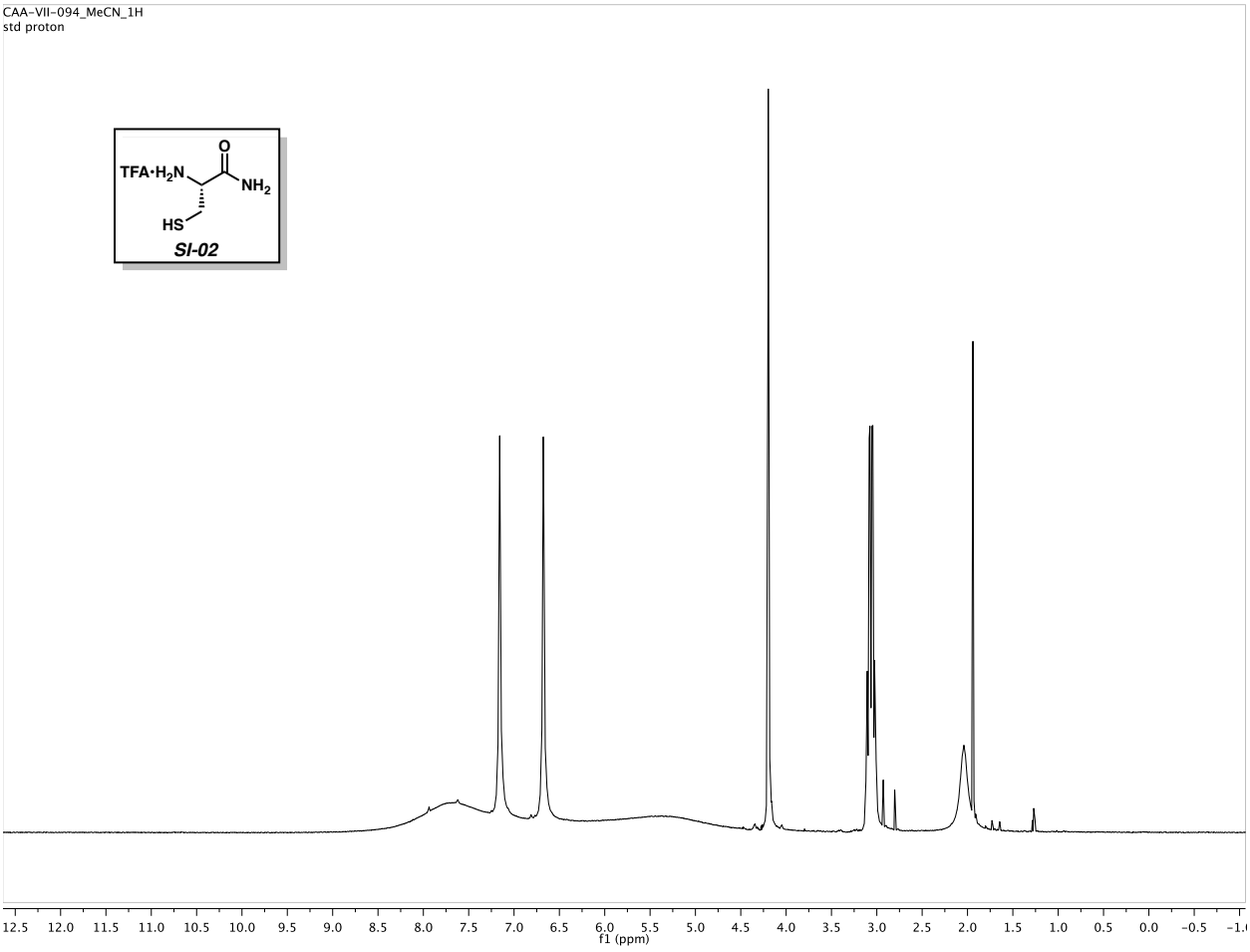
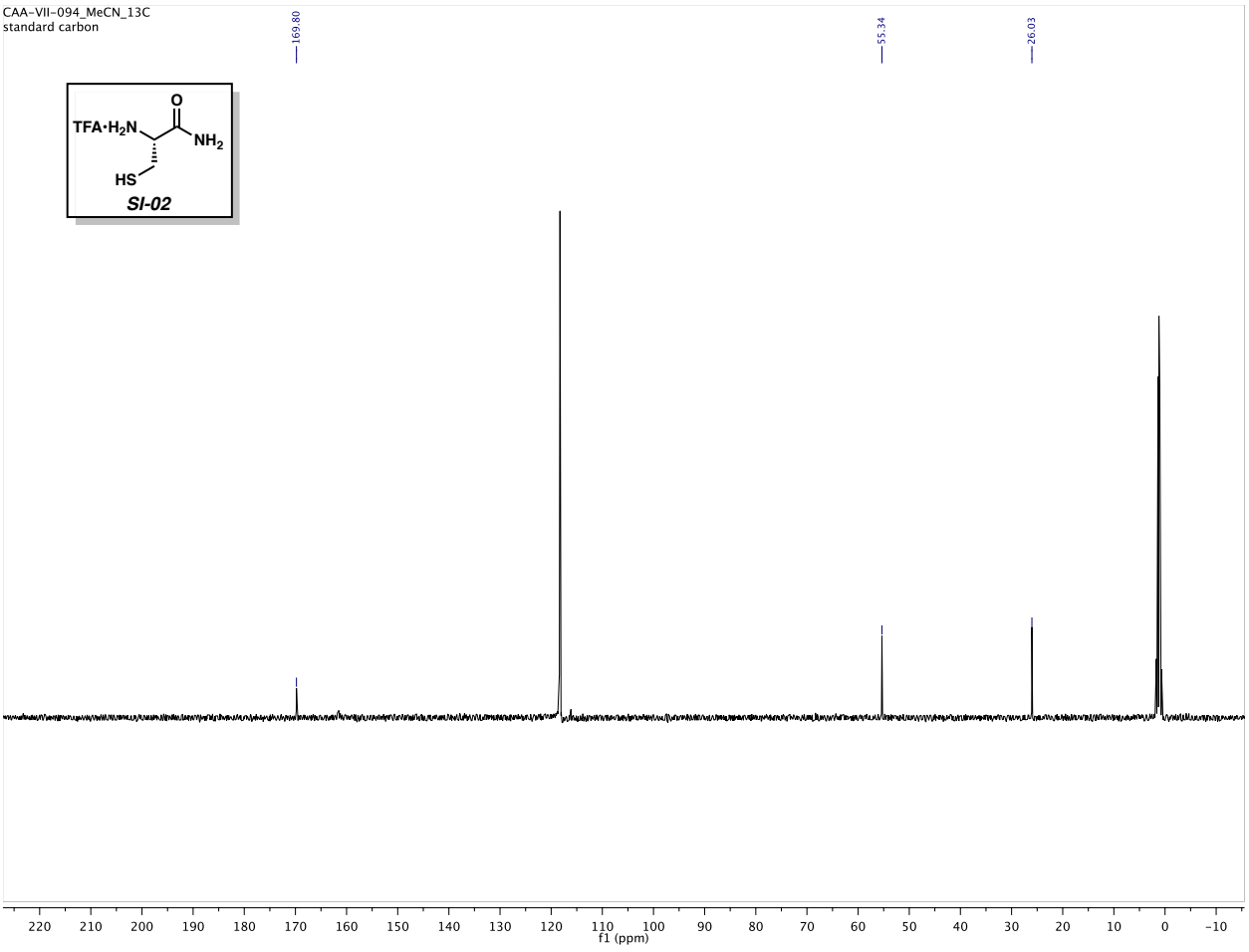
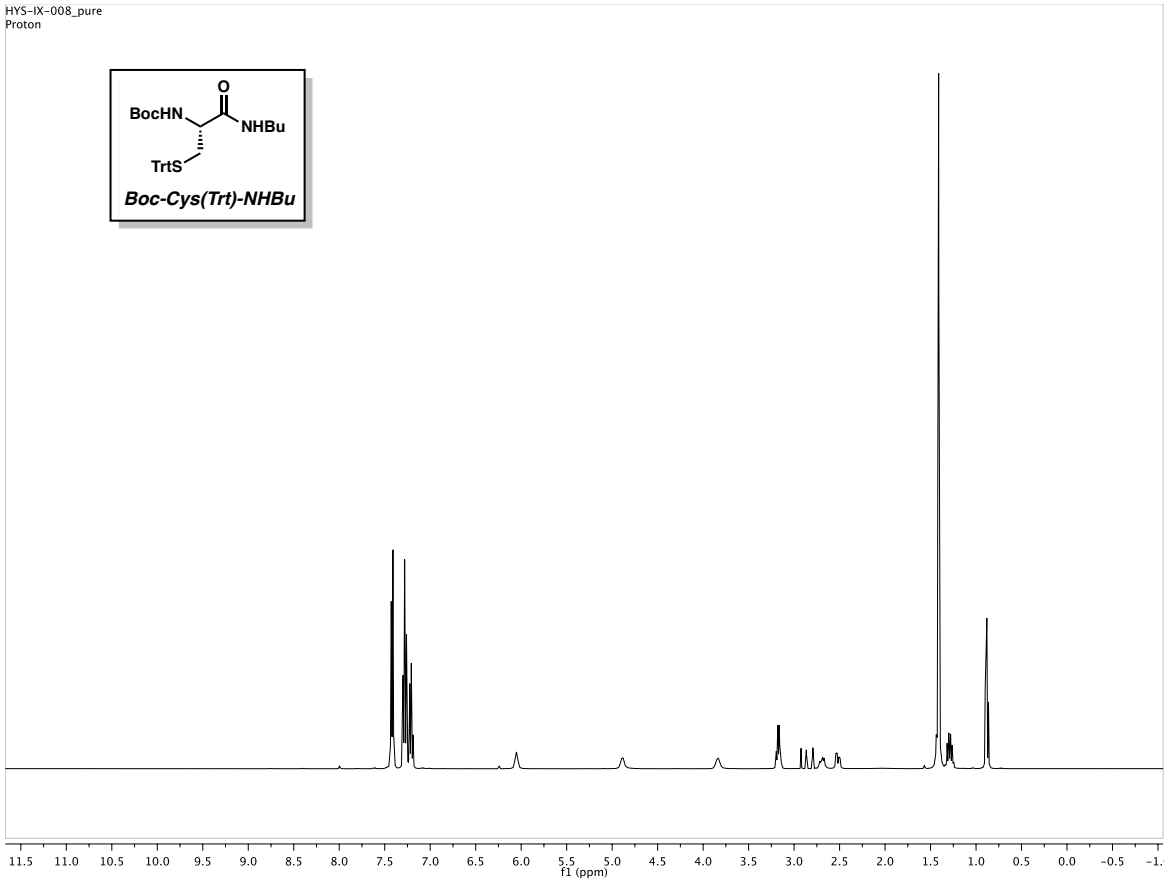


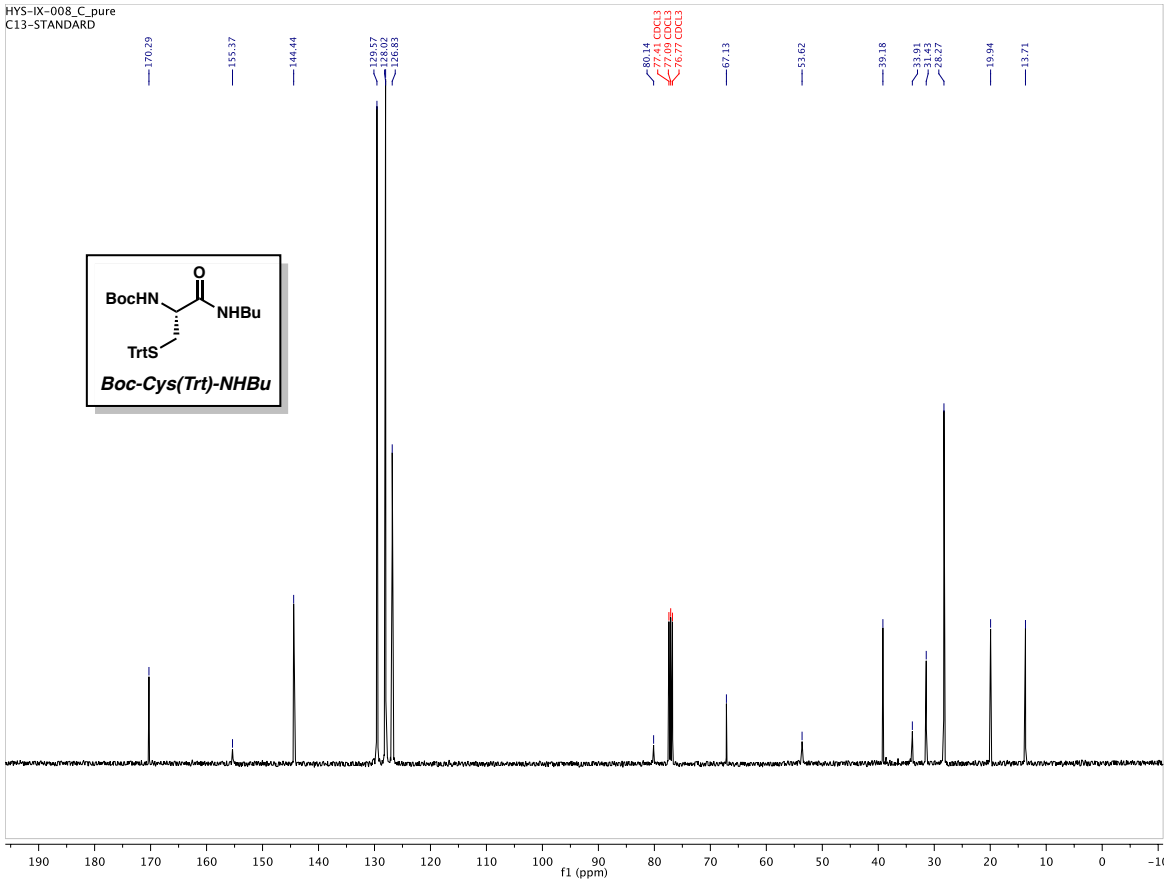
Figure SI-71. Analytical Column PDA of pure peptide at 190 nm. Gradient: 20-70% + 1% HCOOH MeCN/H_2O.

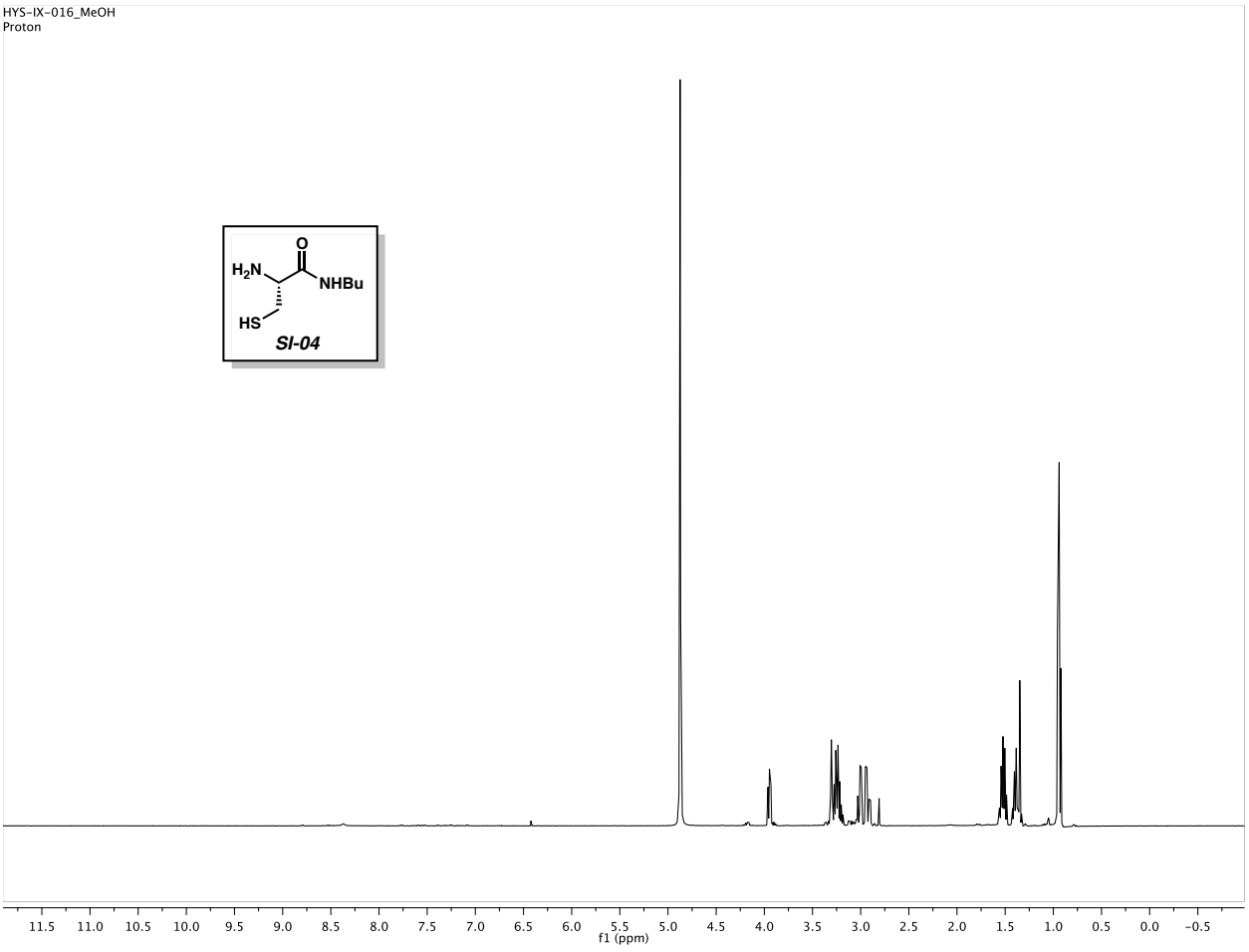
^{1}H and ^{13}C Spectra

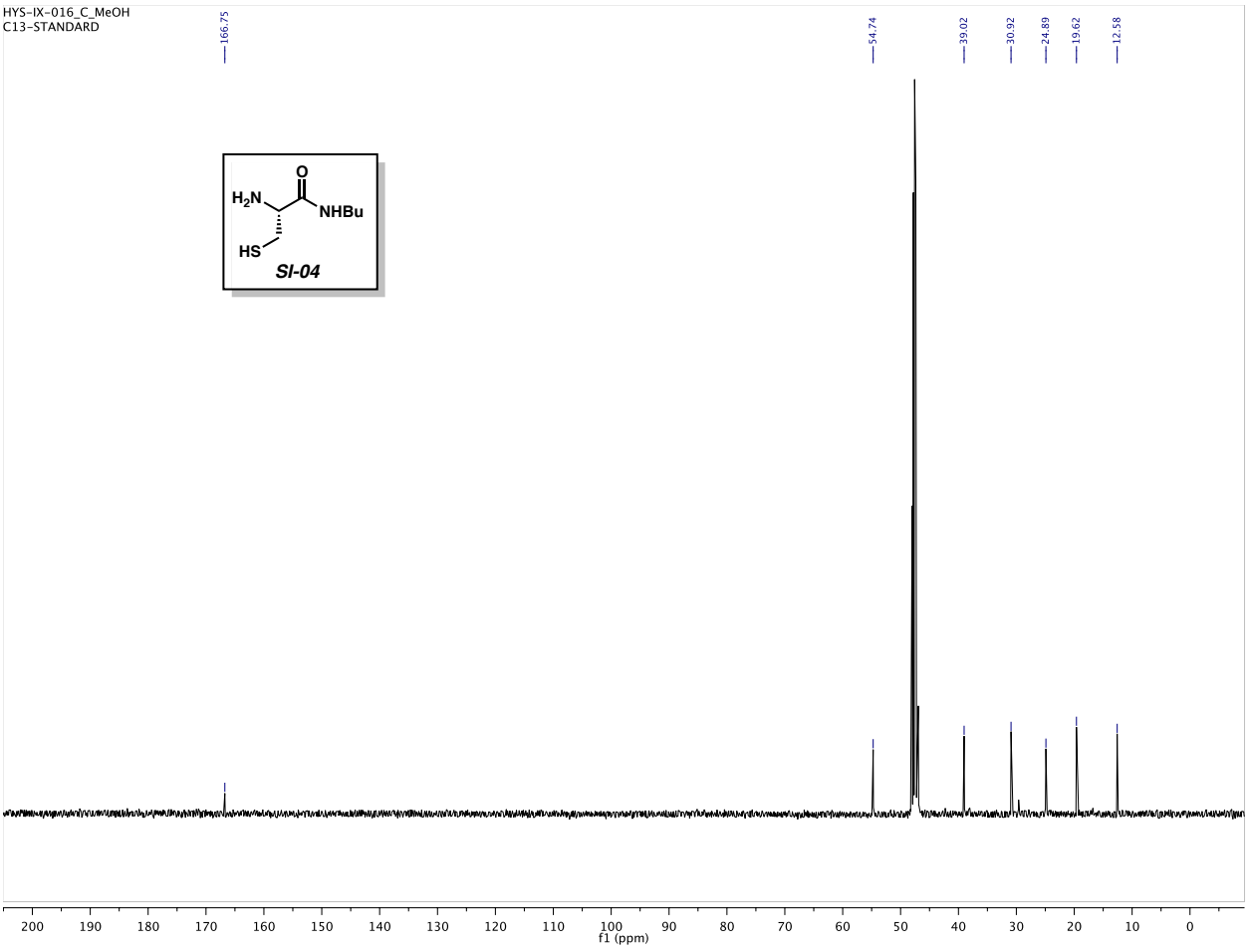


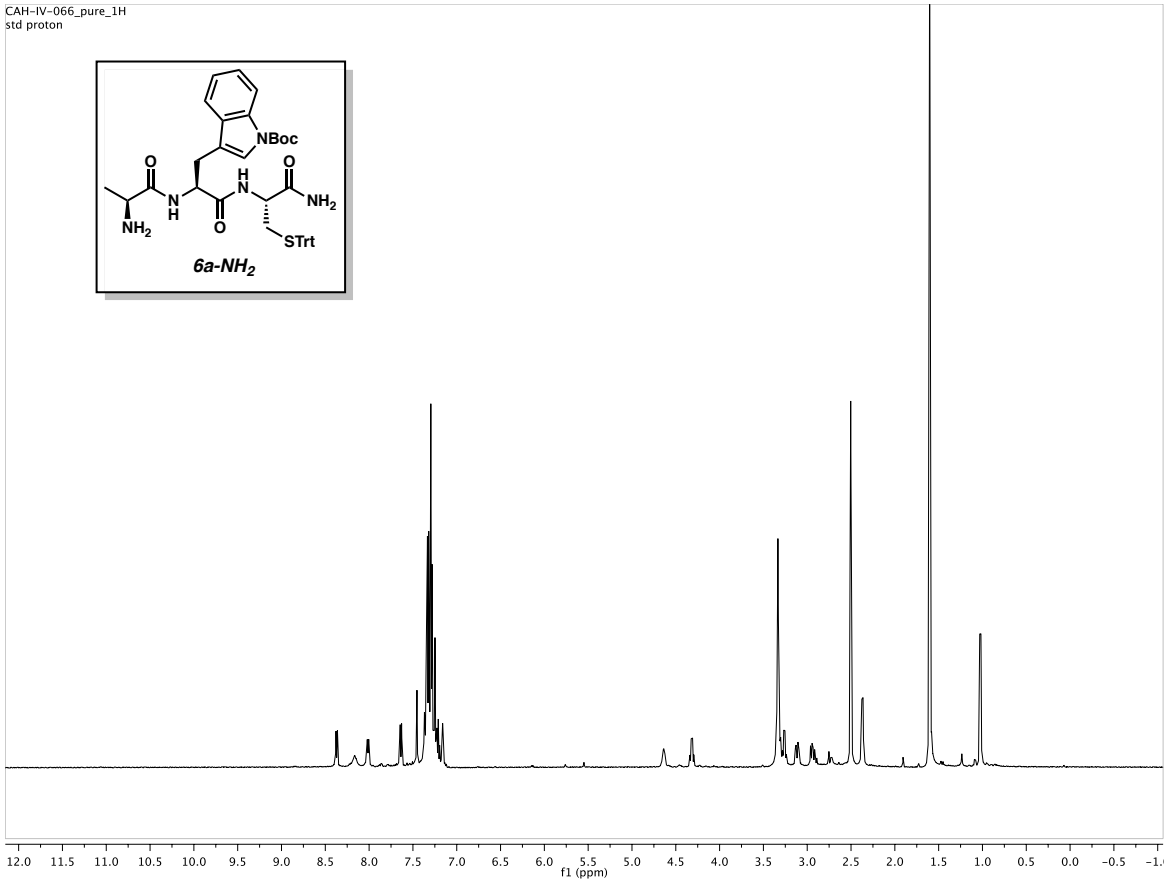

CAA-VII-094_MeCN_13C
standard carbon
169.80
55.34
26.03
TFA·H2N
O
NH2
HS
SI-02
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

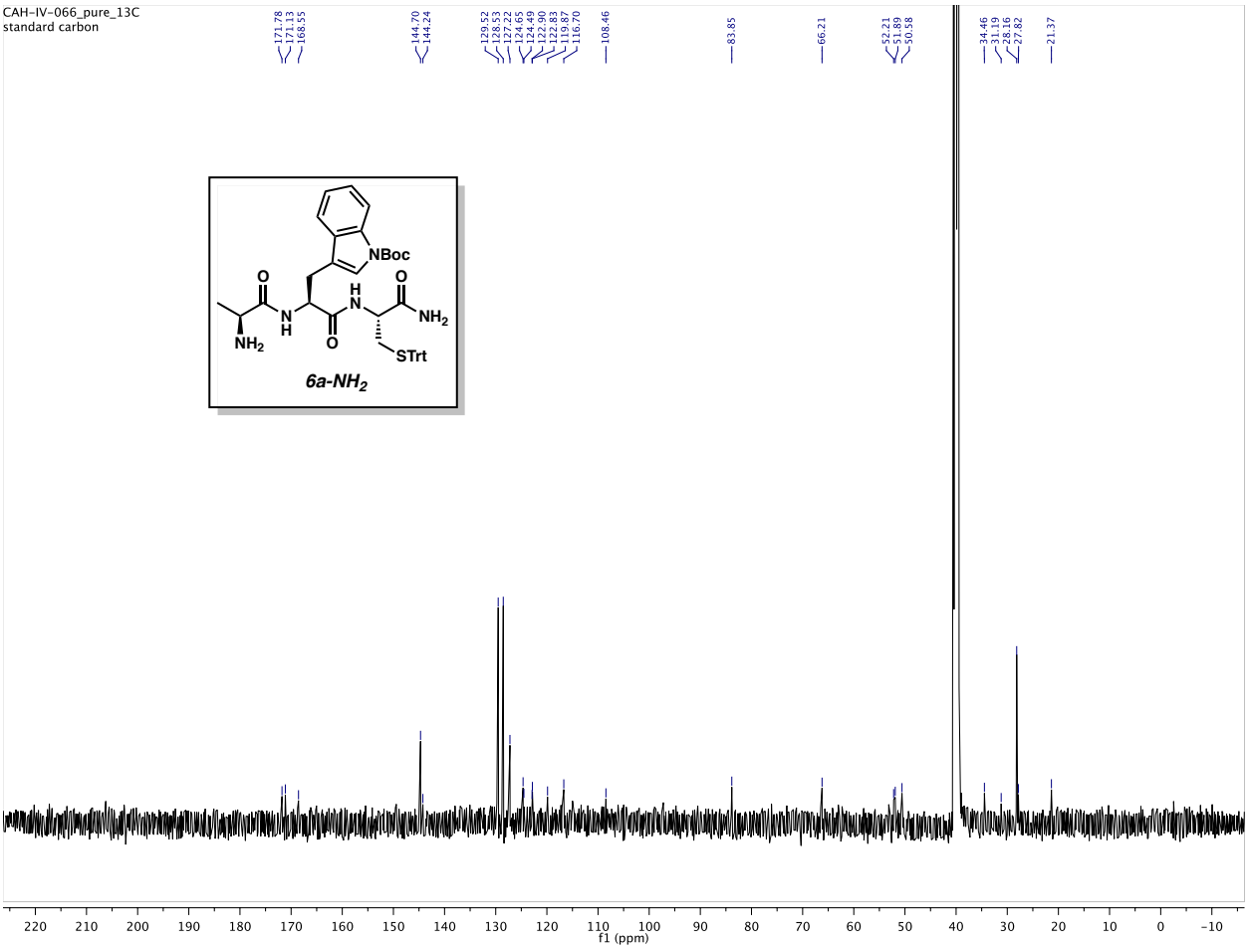

HYS-IX-008_pure
Proton
BocHN
O
NHBu
TrtS
Boc-Cys(Trt)-NHBu
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


HYS-IX-008_C_pure
C13-STANDARD
170.29
155.37
144.44
129.57
128.02
126.83
80.14
77.41 CDCl3
77.09 CDCl3
76.77 CDCl3
67.13
53.62
39.18
33.91
31.43
28.27
19.94
13.71
BocHN
O
NHBu
TrtS
Boc-Cys(Trt)-NHBu
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


HYS-IX-016_MeOH
Proton
O
H2N
NHBu
HS
SI-04
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


HYS-IX-016_C_MeOH
C13-STANDARD
166.75
54.74
39.02
30.92
24.89
19.62
12.58
O
H2N
NHBu
HS
SI-04
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


CAH-IV-066_pure_1H
std proton
NBoc
O
N
H
O
H
N
O
NH2
NH2
STrt
6a-NH2
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


CAH-IV-066_pure_13C
standard carbon
171.78
171.13
168.55
144.70
144.24
129.52
128.53
127.22
124.65
124.49
122.90
122.83
119.27
116.70
108.46
83.85
66.21
52.21
51.89
50.58
34.46
31.19
28.16
27.82
21.37
NBoc
O
O
H
N
N
H
O
NH2
NH2
STrt
6a-NH2
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)