Supporting Information

Sequence Diversification by Divergent C-Terminal Elongation of Peptides

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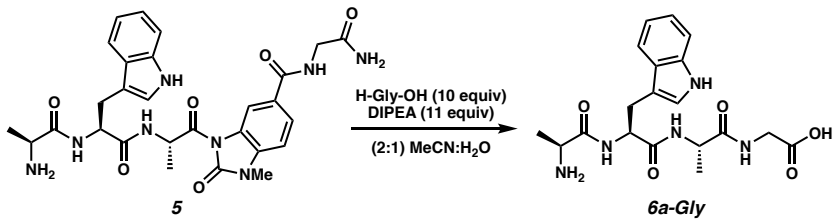
[†] These authors contributed equally to this work.

Table 1. Solution phase C-terminal peptide elongation.



5
Chemical Formula: $C_{28}H_{32}N_8O_6$
Exact Mass: 576.2445

6a-Gly
Chemical Formula: $C_{19}H_{25}N_5O_5$
Exact Mass: 403.1856

Table 1, entry 1: 10 equiv H-Gly-OH was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**5**, 35 μmol) was dissolved in 300 μL of (2:1) MeCN: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.

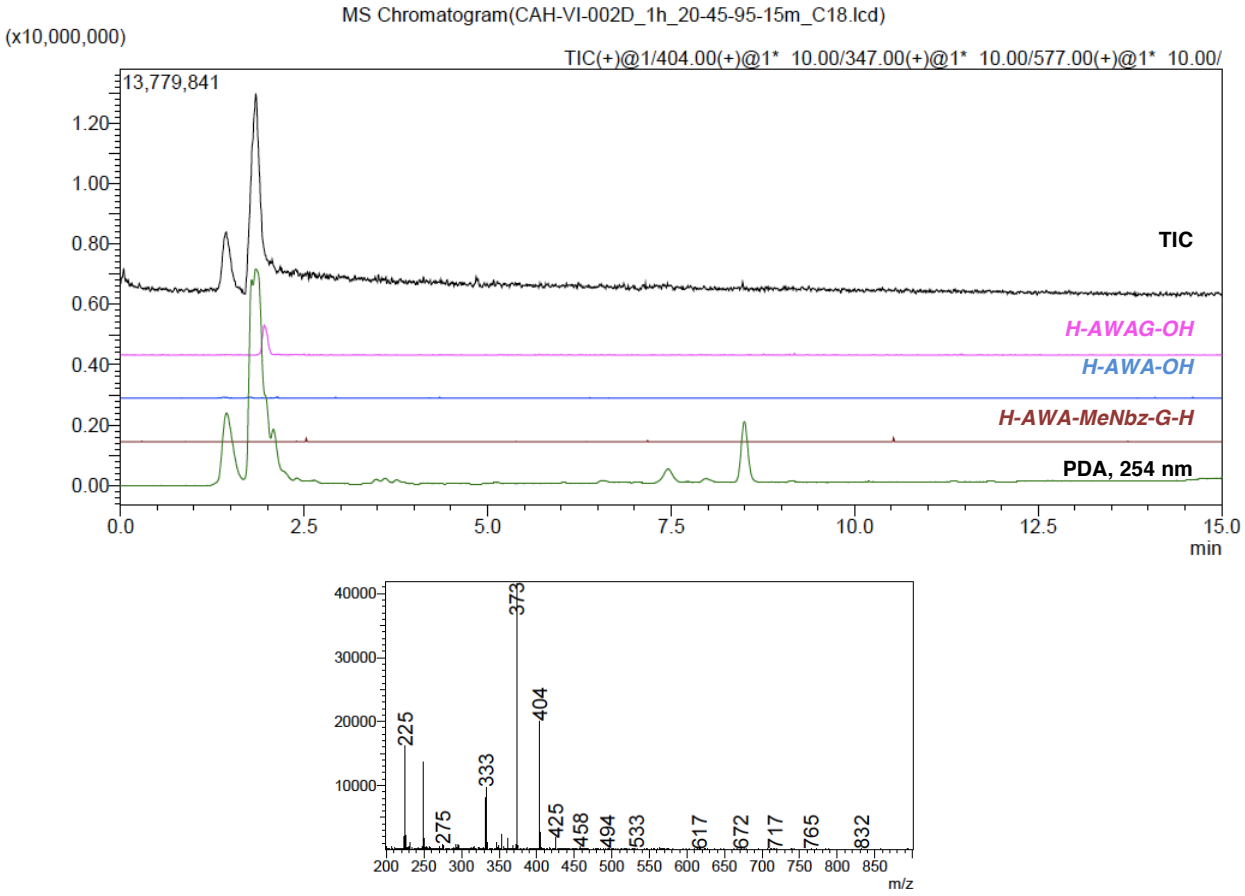


Figure SI-01. Reaction progress after 30 min of H-Gly-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

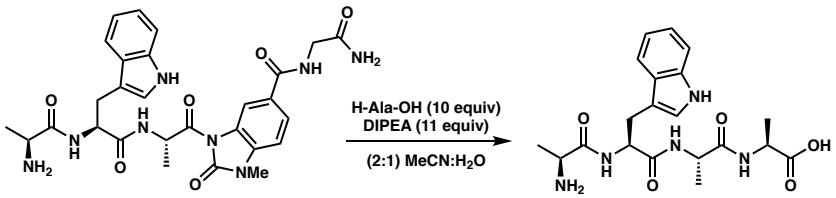


Table 1, entry 2: 10 equiv H-Ala-OH was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**5**, 35 μmol) was dissolved in 300 μL of (2:1) MeCN:H_2O and added to the amino acid containing vial. 11 equiv of freshly distilled DIEA was added to the vial and allowed to stir at ambient temperature for 30 min. After 30 min, the reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

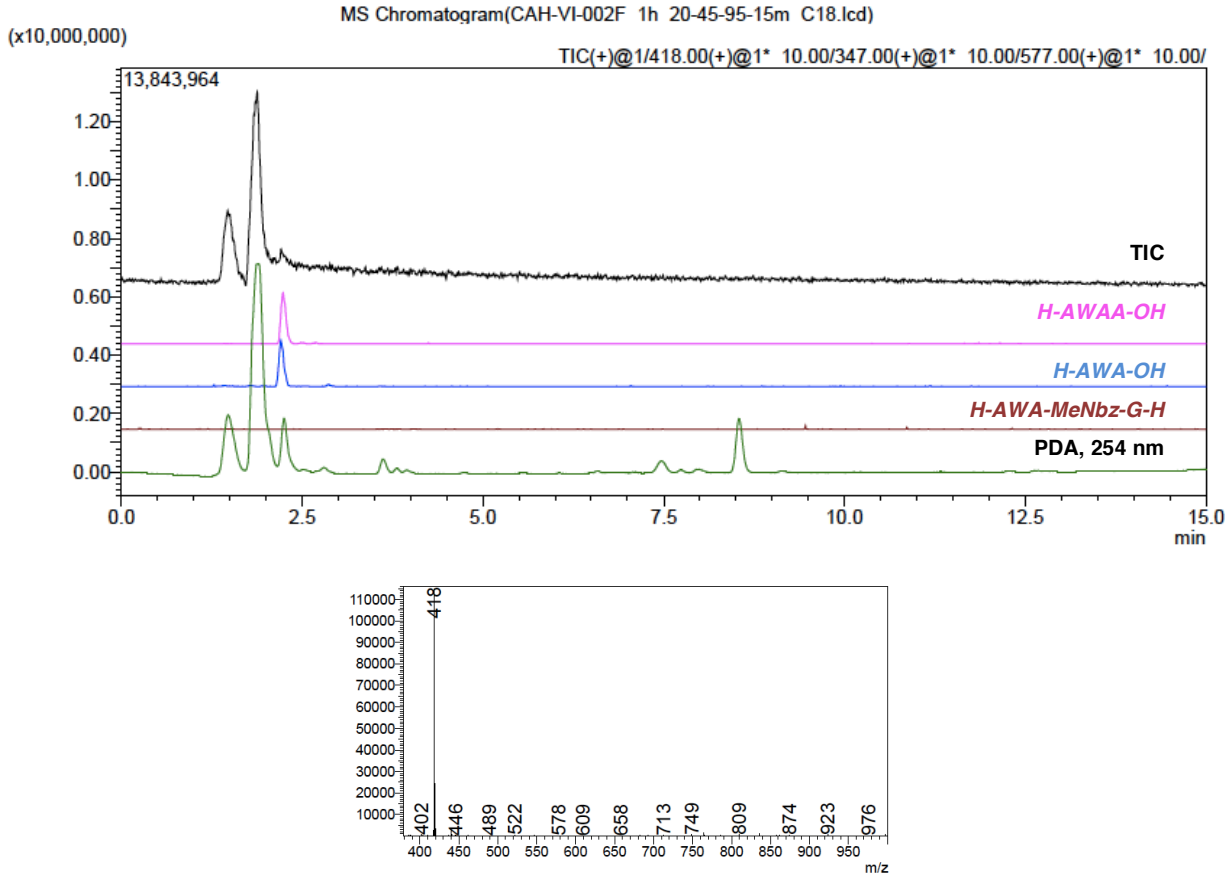


Figure SI-02. Reaction progress after 30 min of H-Ala-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

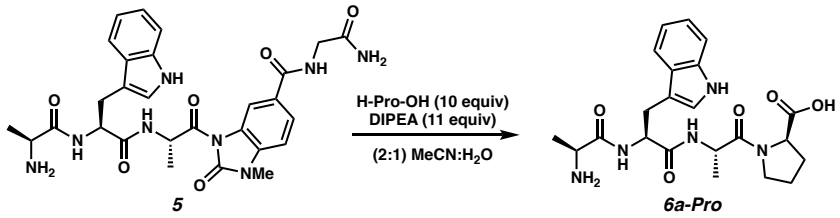


Table 1, entry 3: 10 equiv H-Pro-OH was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**5**, 35 μmol) was dissolved in 300 μL of (2:1) MeCN:H_2O and added to the amino acid containing vial. 11 equiv of freshly distilled DIEA was added to the vial and allowed to stir at ambient temperature for 6 h, after 6 h the reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

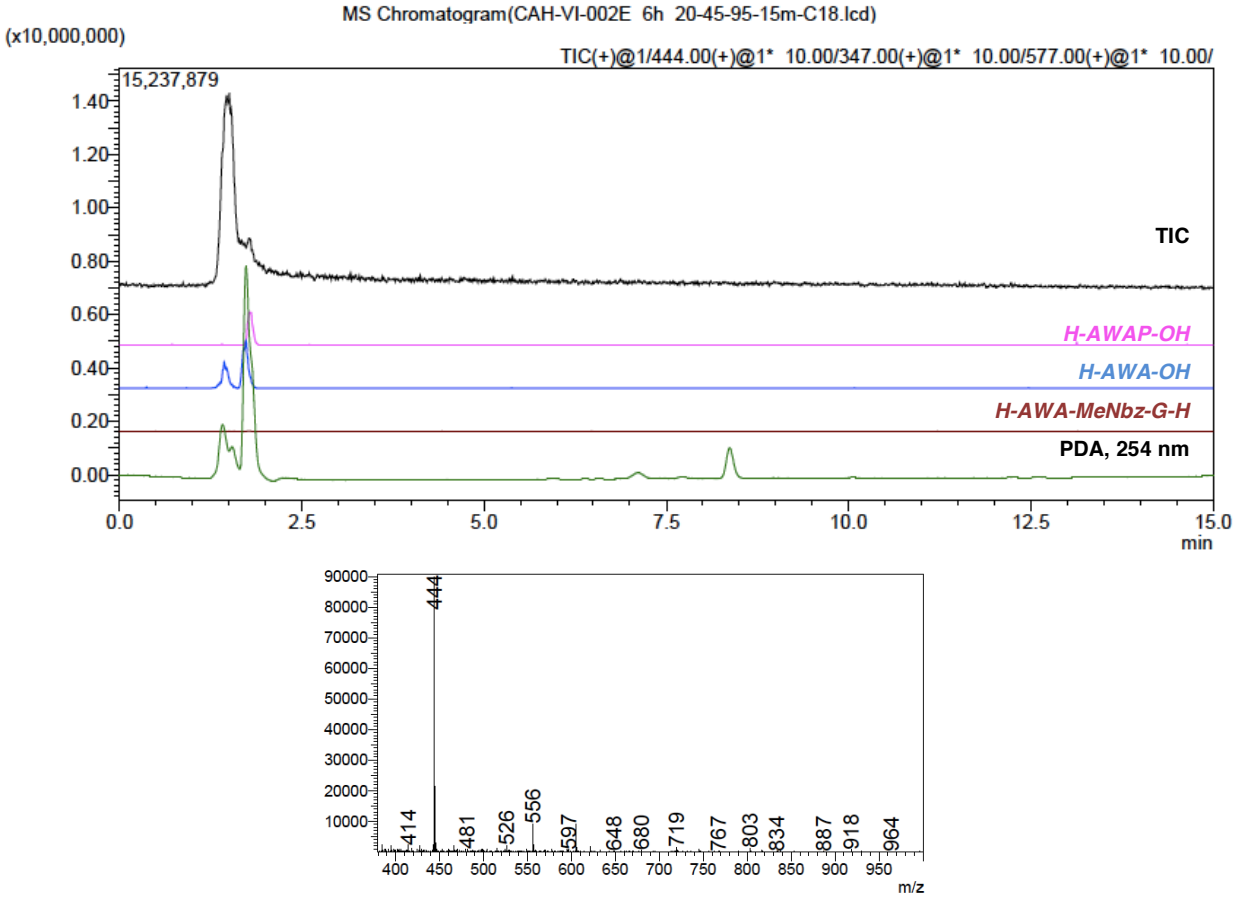


Figure SI-03. Reaction progress after 6 h of H-Pro-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

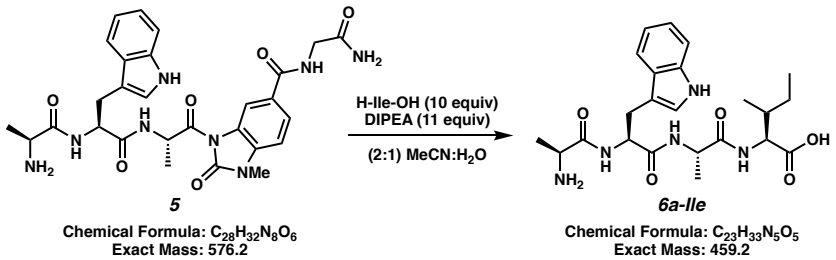


Table 1, entry 4: 10 equiv H-Ile-OH was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**5**, 35 μmol) was dissolved in 300 μL of (2:1) MeCN:H_2O and added to the amino acid containing vial. 11 equiv of freshly distilled DIEA was added to the vial and allowed to stir at ambient temperature for 30 min. After 30 min, the reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

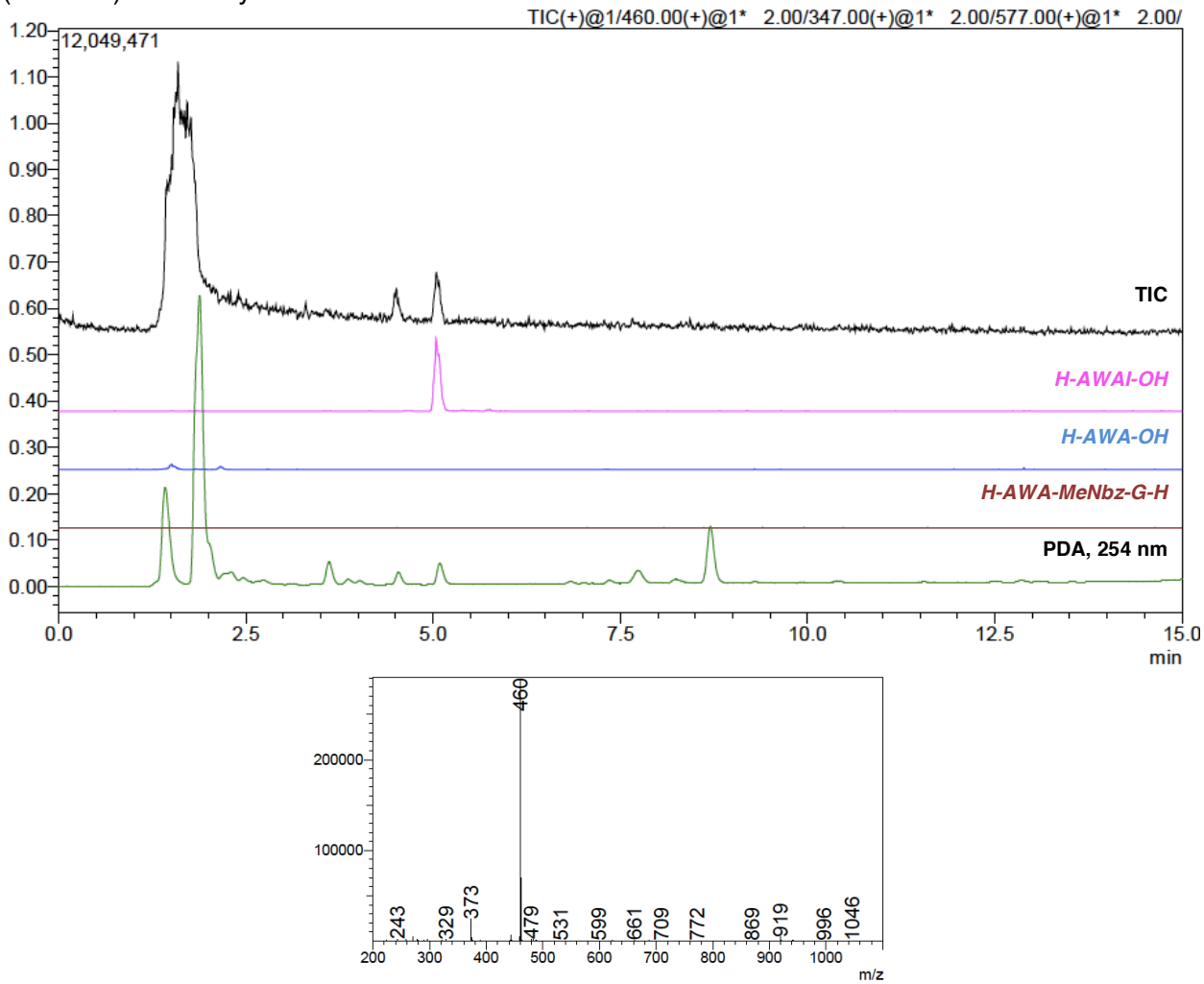
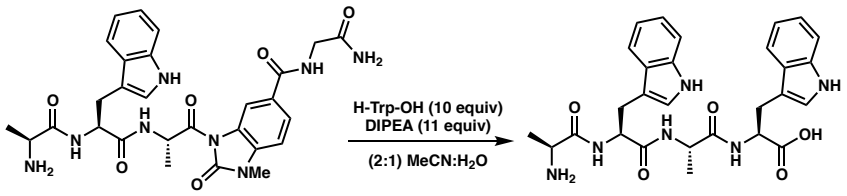


Figure SI-04. Reaction progress after 1 h of H-Ile-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.



5

Chemical Formula: $C_{28}H_{32}N_8O_6$
Exact Mass: 576.2445

6a-Ile

Chemical Formula: $C_{28}H_{32}N_6O_5$
Exact Mass: 532.2434

Table 1, entry 5: 10 equiv H-Trp-OH was weighed out into a vial containing a stir bar. 20 mg of crude peptide (**5**, 35 μmol) was dissolved in 300 μL of (2:1) MeCN: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.

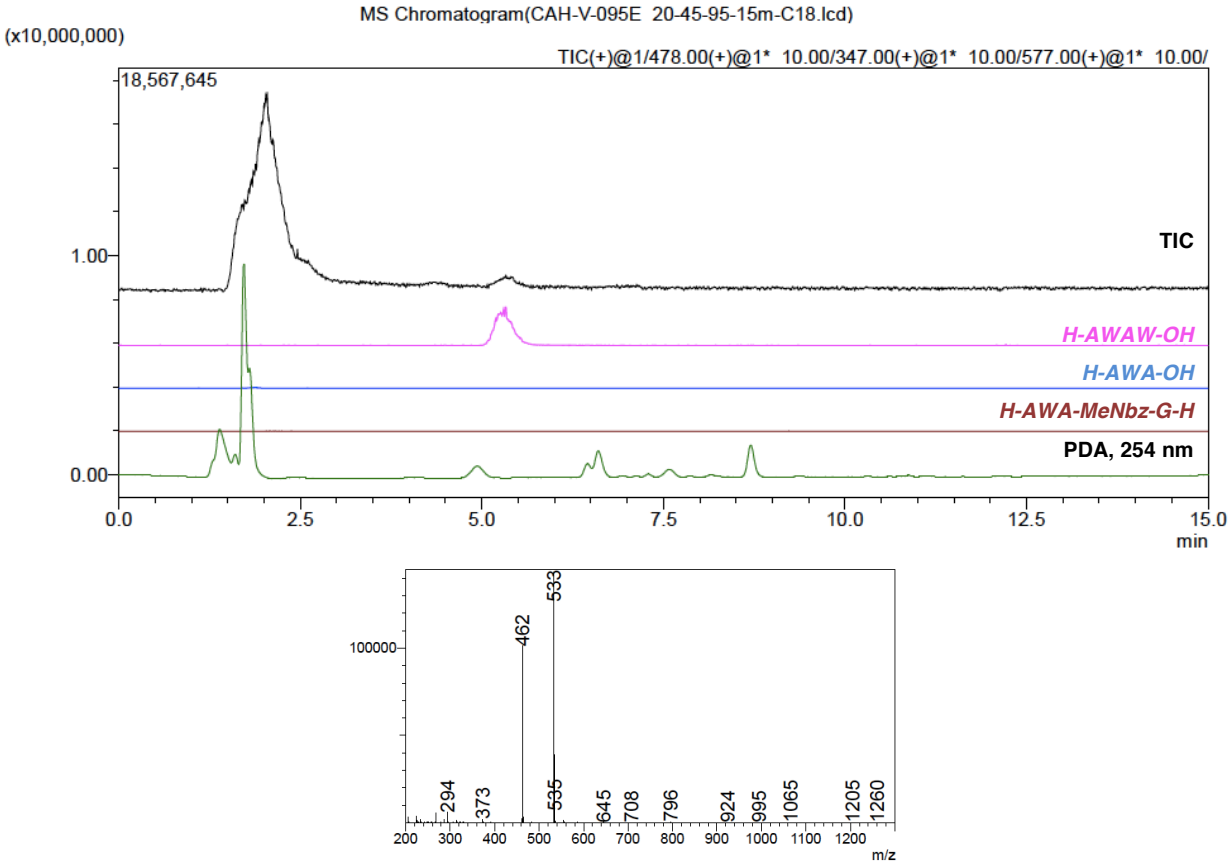


Figure SI-05. Reaction progress after 30 min of H-Trp-OH addition, gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

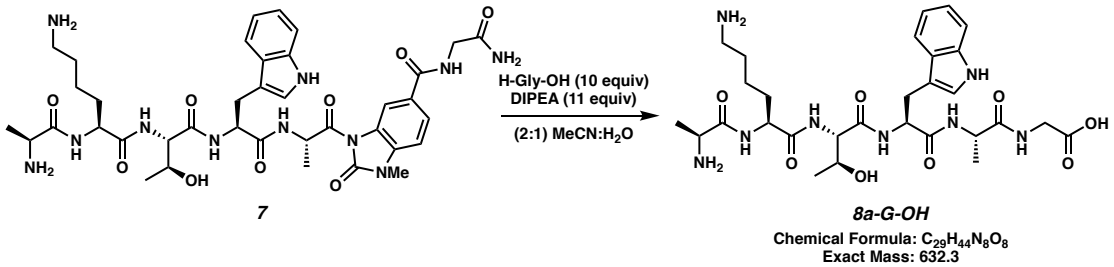


Table 1, entry 6: 10 equiv of H-Gly-OH was weighed out into a vial containing a stir bar. 20 mg of **7** (25 μmol) was dissolved in 300 μL of (2:1) MeCN:H_2O and added to the amino acid. Then 11 equiv of freshly distilled DIEA was added and allowed to stir at ambient temperature for 30 min. The reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

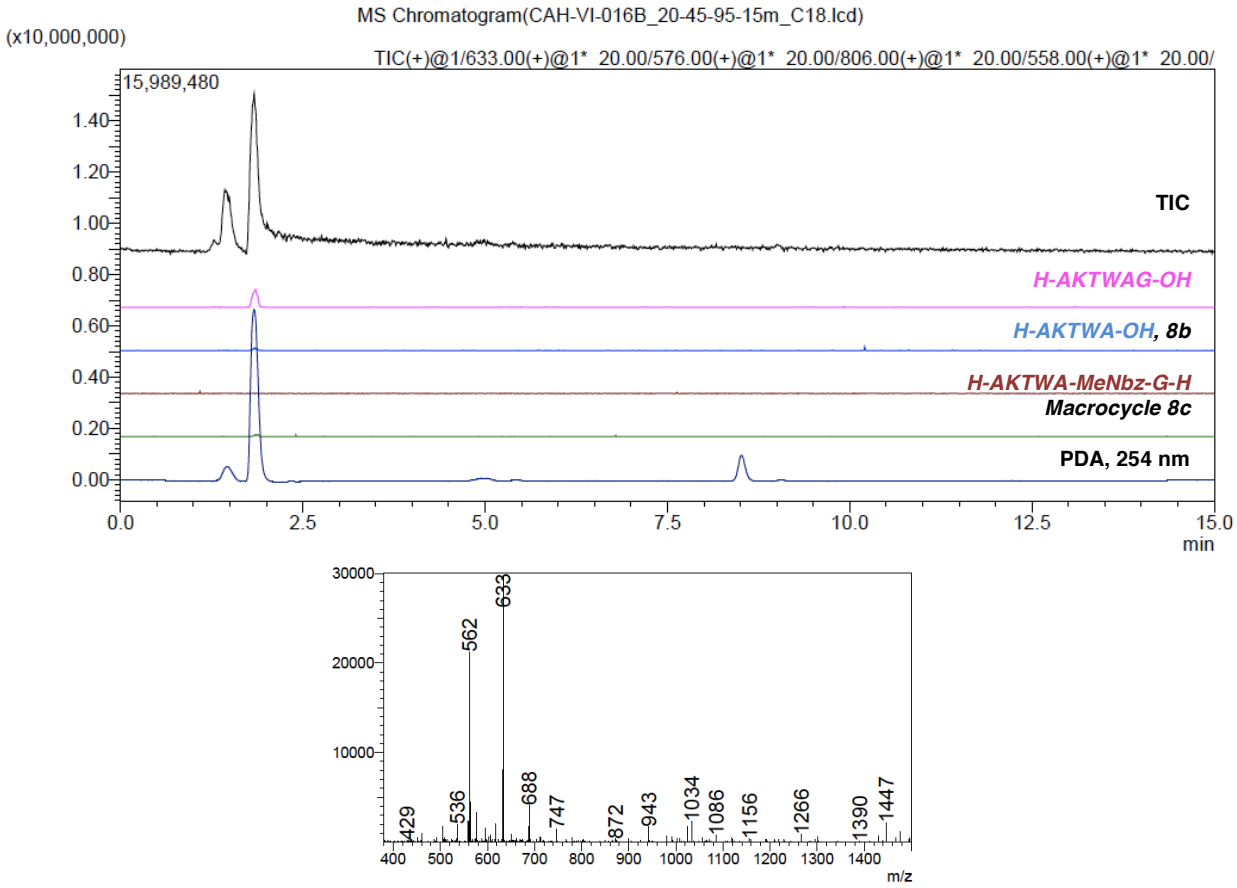


Figure SI-06. Reaction progress after 30 m of H-Gly-OH addition, gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

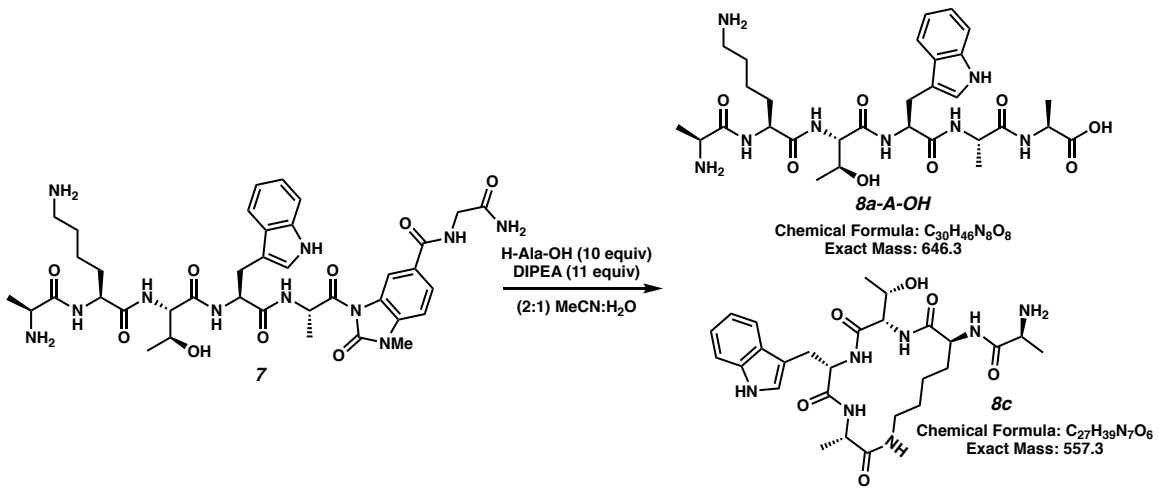


Table 1, entry 7: 10 equiv of H-Ala-OH was weighed out into a vial containing a stir bar. 20 mg of **7** (25 μmol) was dissolved in 300 μL of (2:1) MeCN:H_2O and added to the amino acid. Then 11 equiv of freshly distilled DIEA was added and allowed to stir at ambient temperature for 30 min. The reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

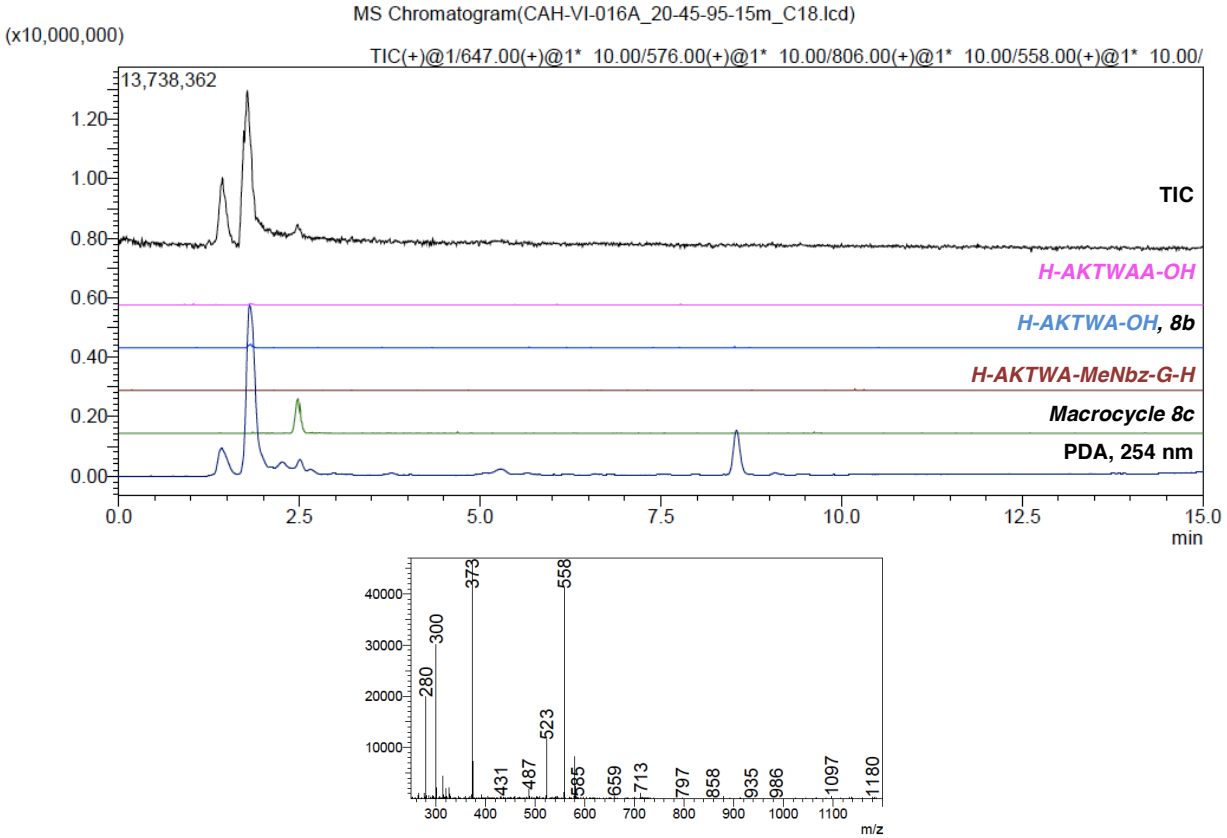


Figure SI-07. Reaction progress after 30 m of H-Ala-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

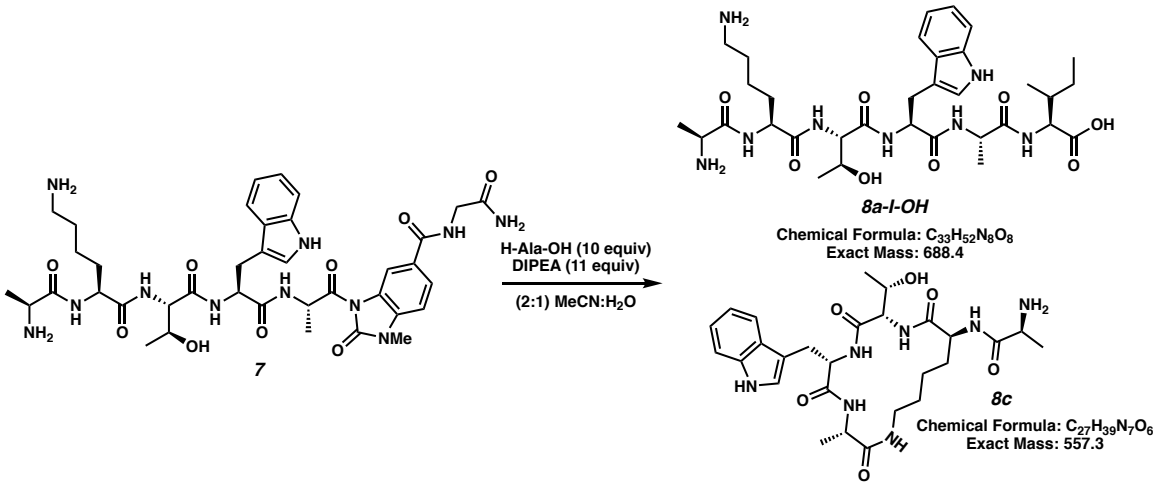


Table 1, entry 8: 10 equiv of H-Ile-OH was weighed out into a vial containing a stir bar. 20 mg of **7** (25 μmol) was dissolved in 300 μL of (2:1) MeCN:H_2O and added to the amino acid. Then 11 equiv of freshly distilled DIEA was added and allowed to stir at ambient temperature for 30 min. The reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS.

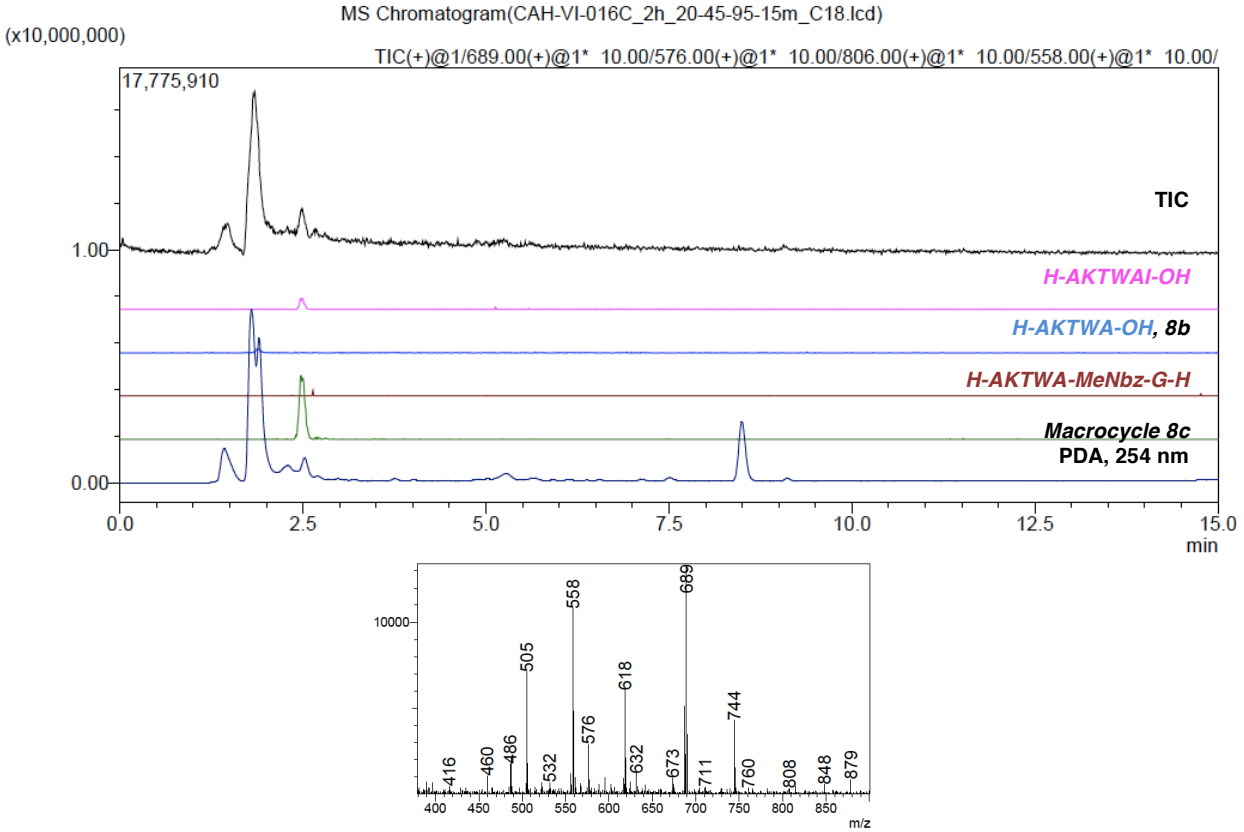


Figure SI-08. Reaction progress after 2 h of H-Ile-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

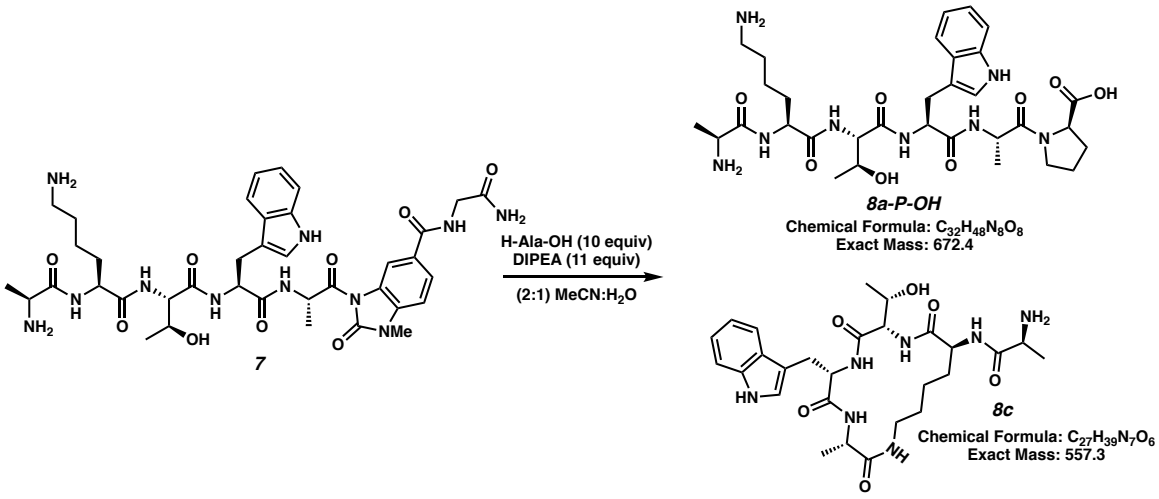


Table 1, entry 9: 10 equiv of H-Pro-OH was weighed out into a vial containing a stir bar. 20 mg of **7** (25 μmol) was dissolved in 300 μL of (2:1) MeCN:H_2O and added to the amino acid. Then 11 equiv of freshly distilled DIEA was added and allowed to stir at ambient temperature for 30 min. The reactions were quenched in 20% MeCN/H_2O (1% TFA) and analyzed via RP-HPLC-MS. Only macrocycle was observed.

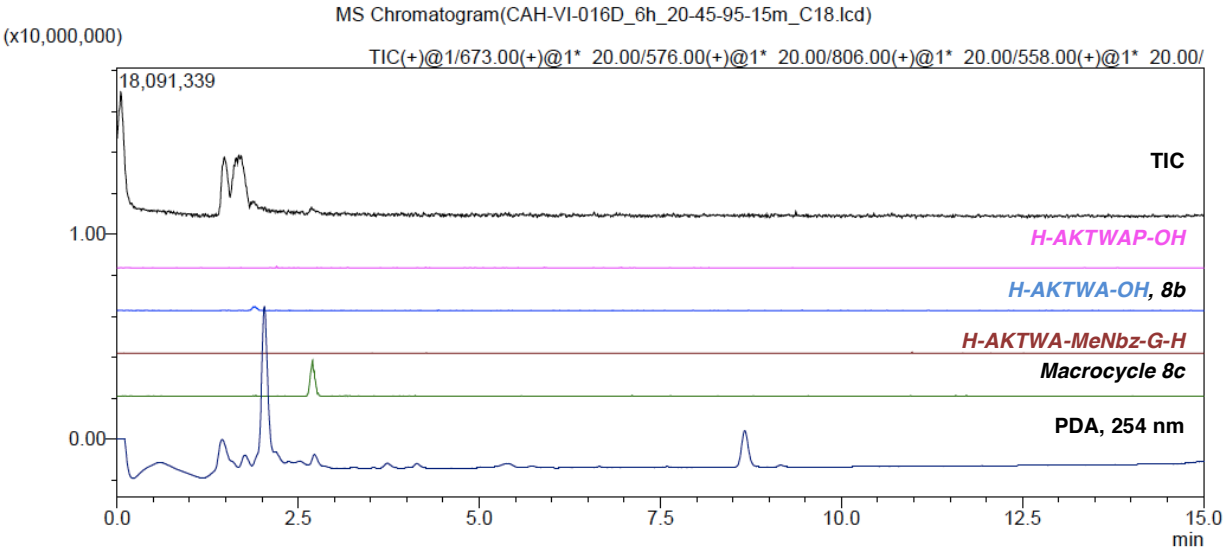


Figure SI-09. Reaction progress after 6 h of H-Pro-OH addition, Gradient: 20-45(12 min)-95(3 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

Table 2. On-resin elongation to access protected peptide acids.

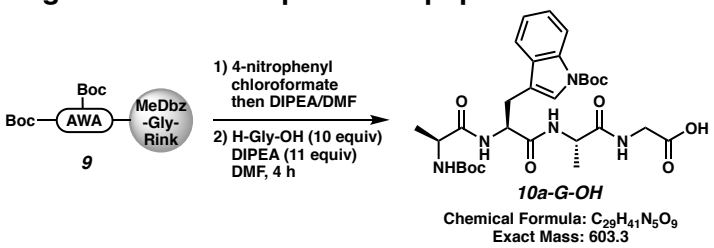


Table 2, entry 1: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Gly-OH was dissolved in 500 μL DMF in a separate vial and added to the 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 (LN_2), and lyophilized to yield the crude peptide.

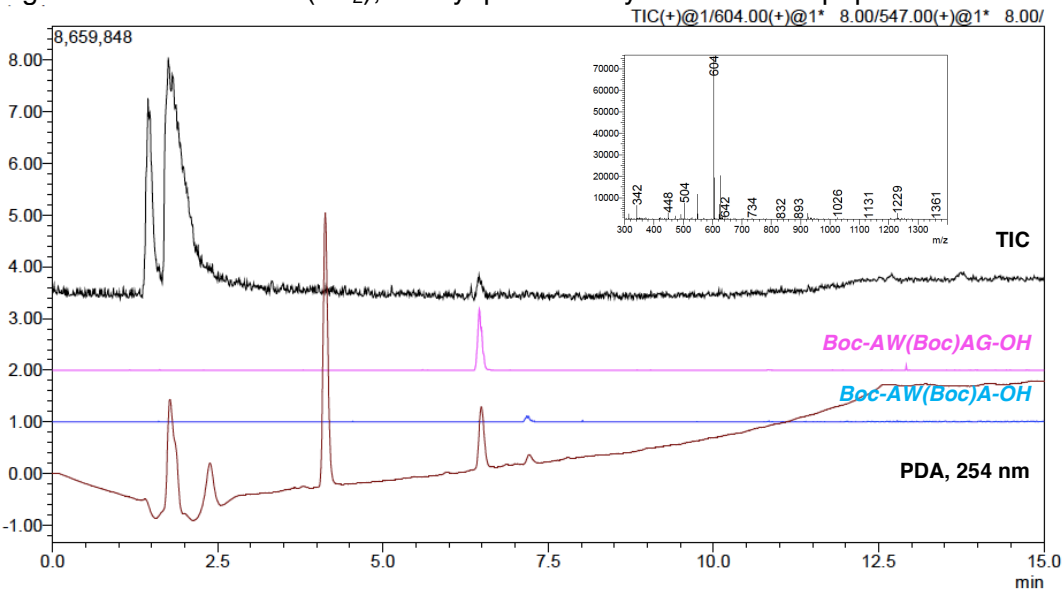


Figure SI-10. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

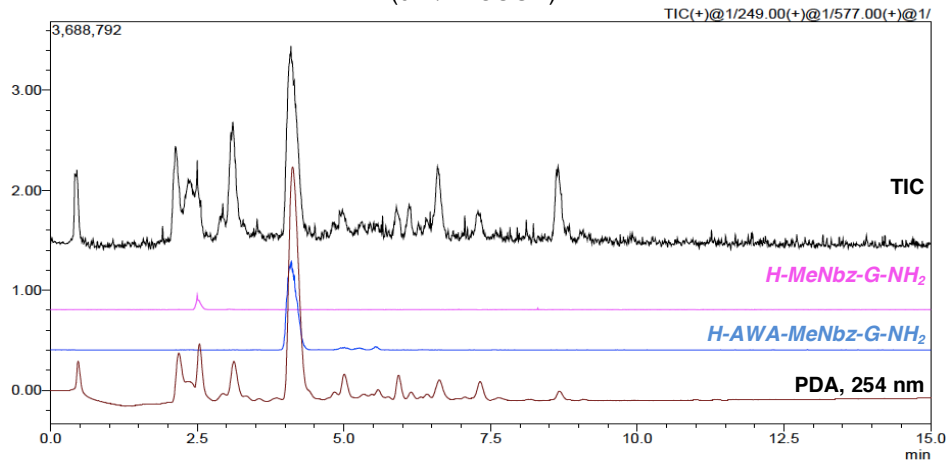


Figure SI-11. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

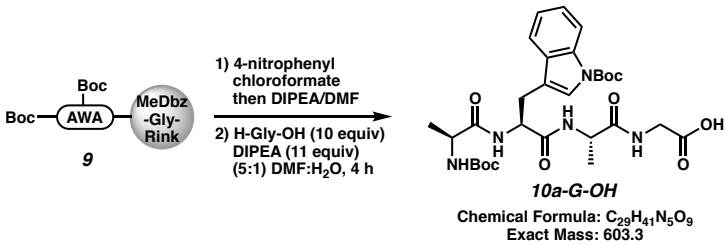


Table 2, entry 2: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Gly-OH was dissolved in 500 μL DMF in a separate vial and added to the 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 (LN_2), and lyophilized to yield the crude peptide.

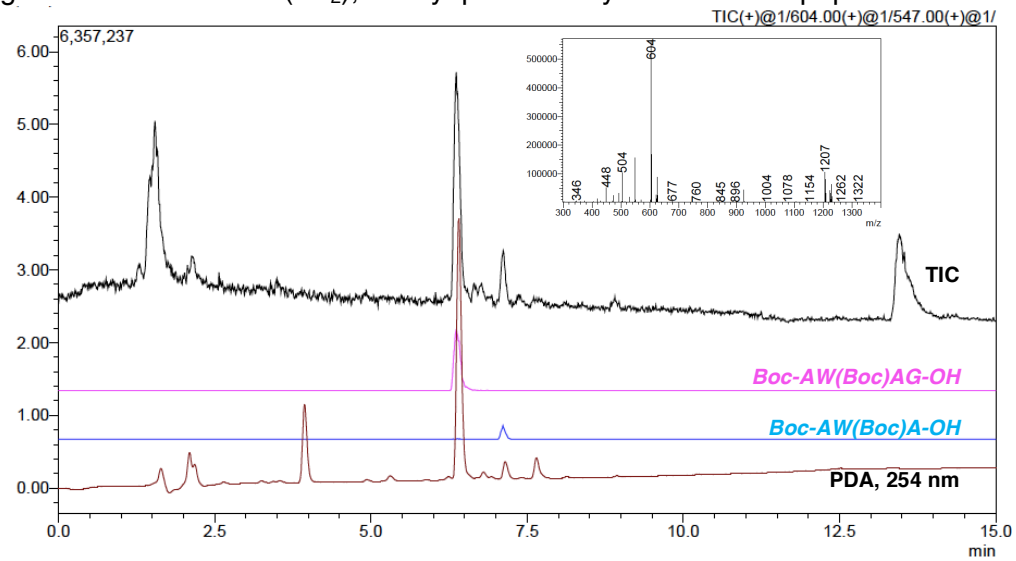


Figure SI-12. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

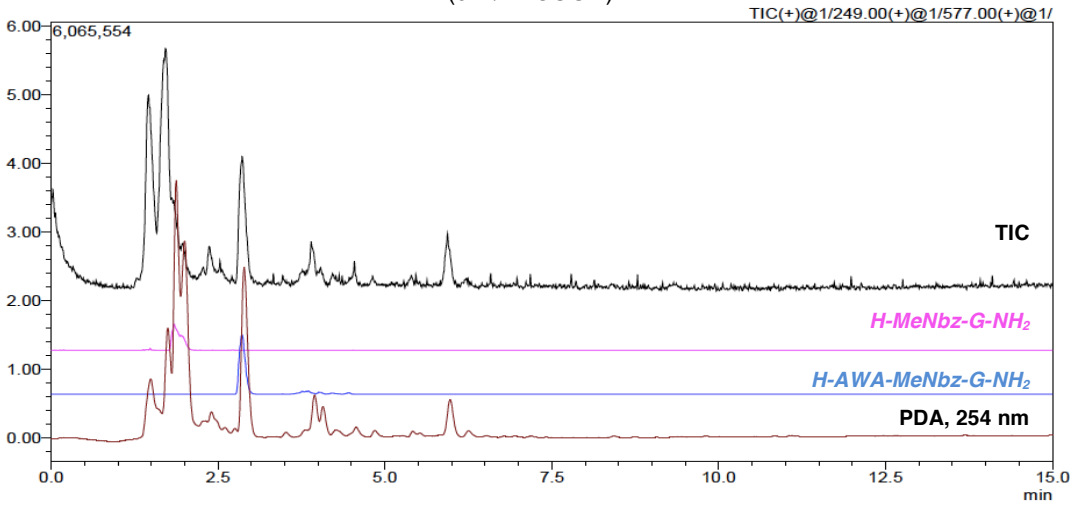


Figure SI-13. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

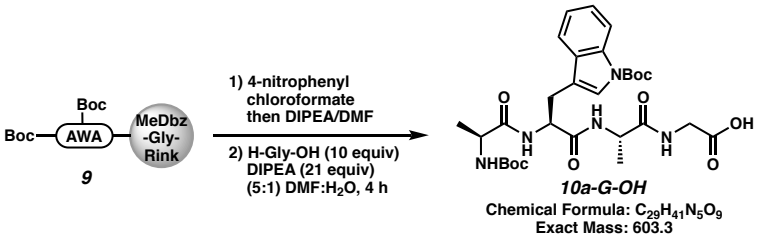


Table 2, entry 3: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Gly-OH was added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide.

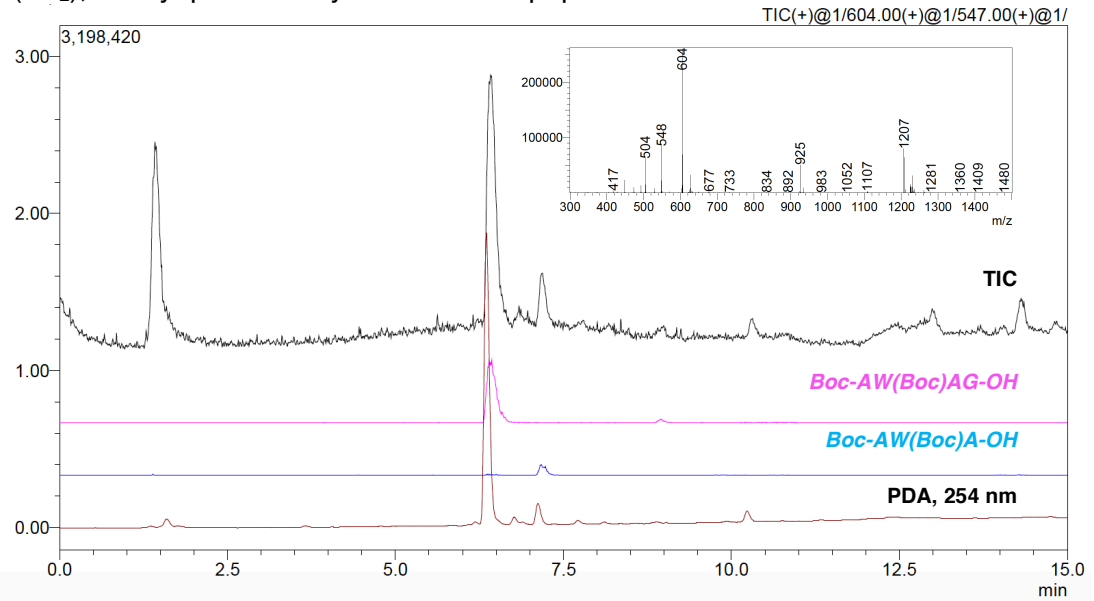


Figure SI-14. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

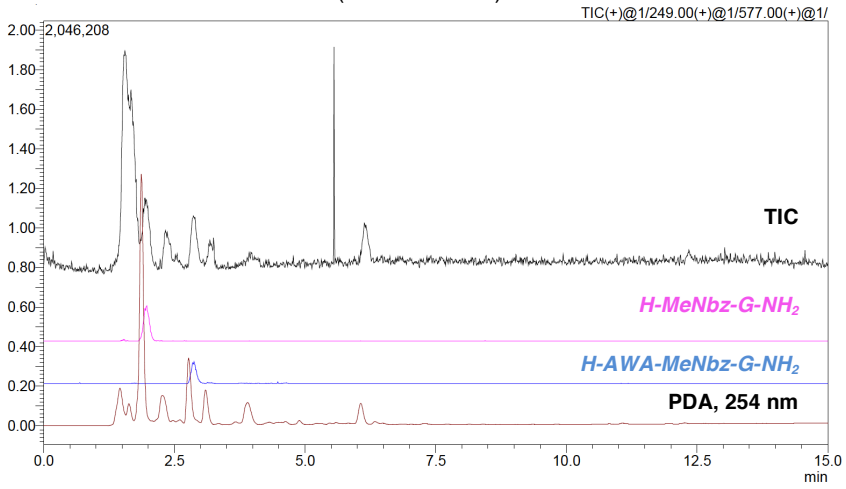


Figure SI-15. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O over 15 min (0.1% HCOOH).

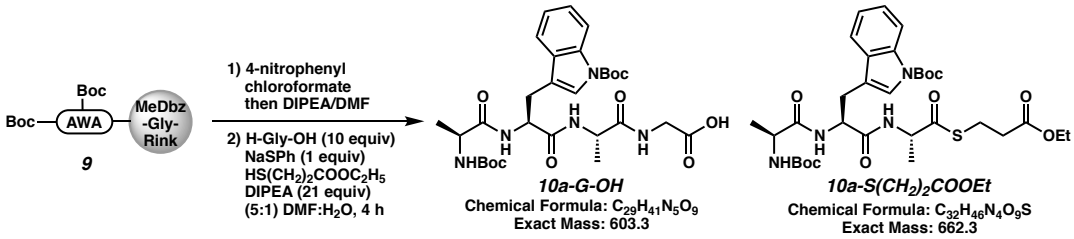


Table 2, entry 4: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure).10 equiv of H-Gly-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:H_2O (5:1) with 100 μL of ethyl-3-mercaptopropionate. Then 21 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 (LN_2), and lyophilized to yield the crude peptide. Exclusively alkyl thioester was observed.

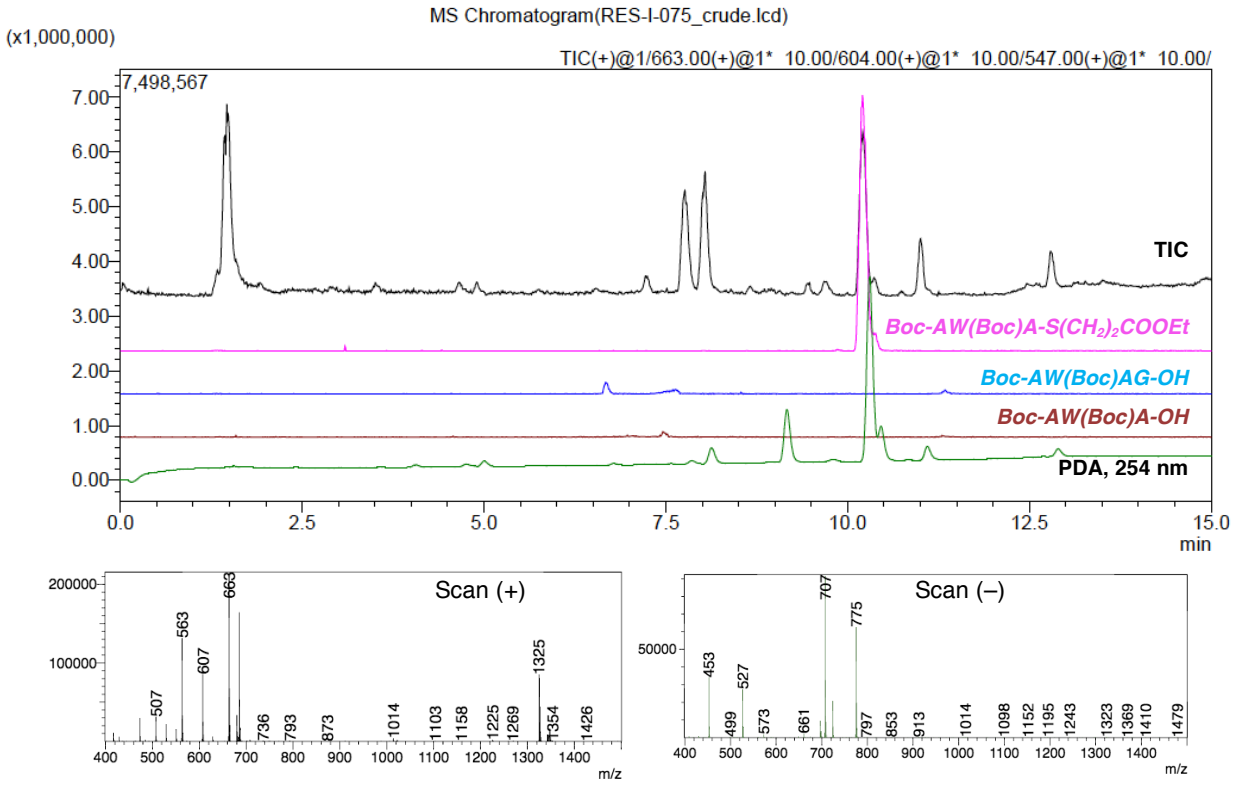


Figure SI-16. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

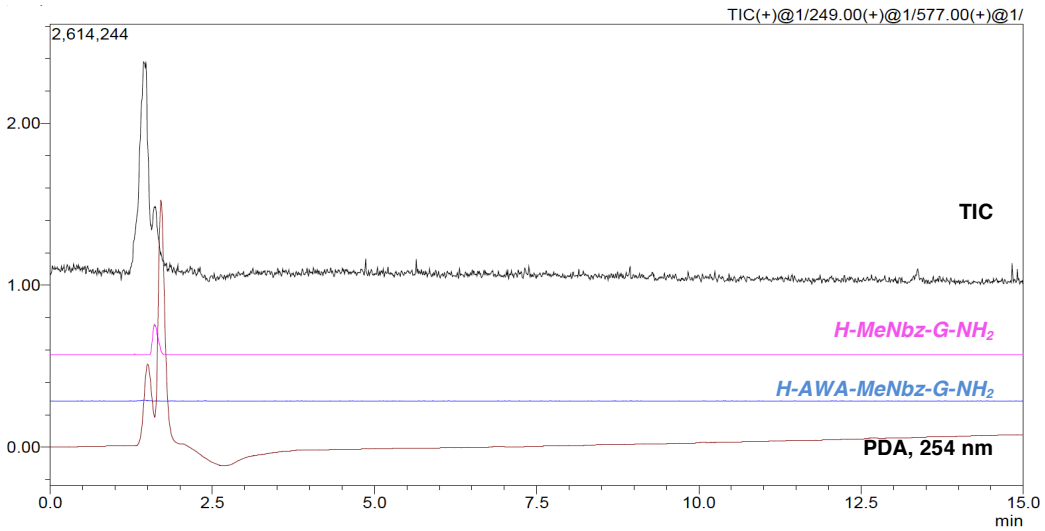


Figure SI-17. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

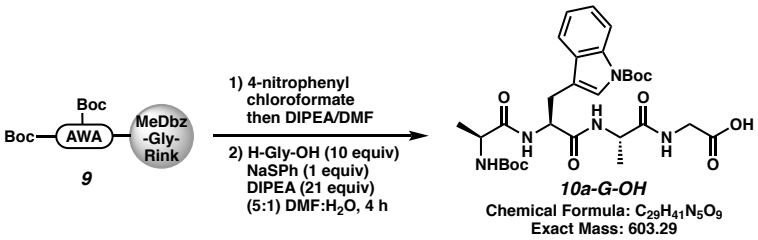


Table 2, entry 5: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Gly-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide.

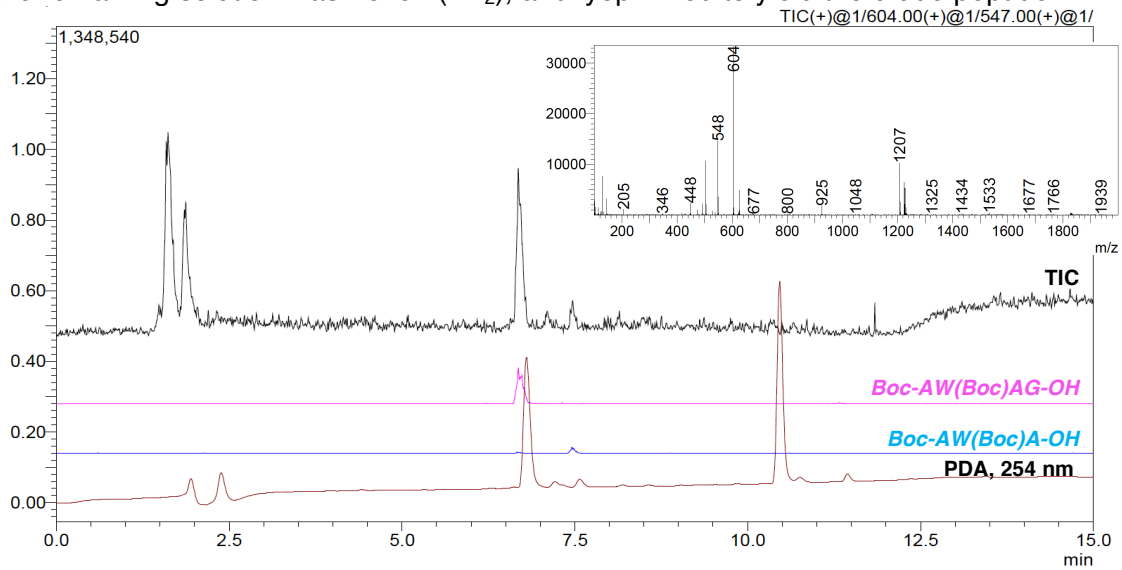


Figure SI-18. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

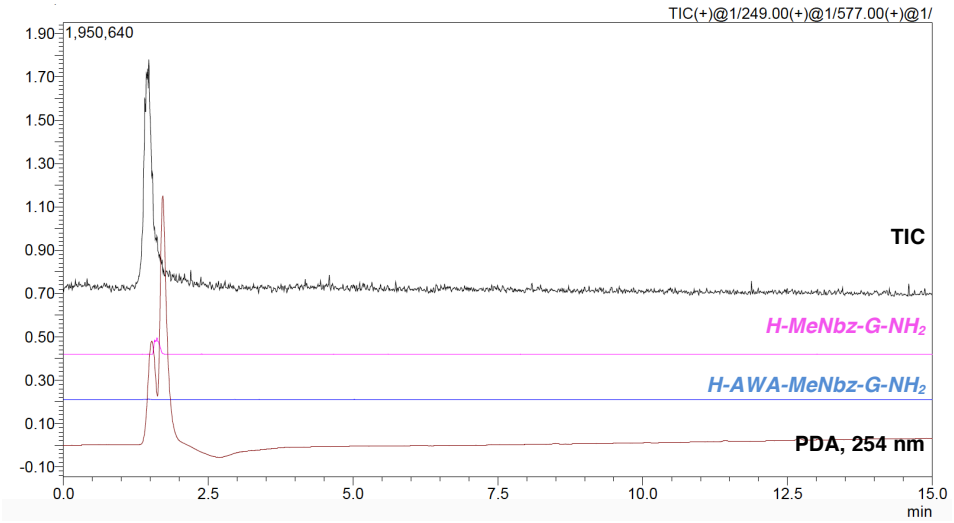


Figure SI-19. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

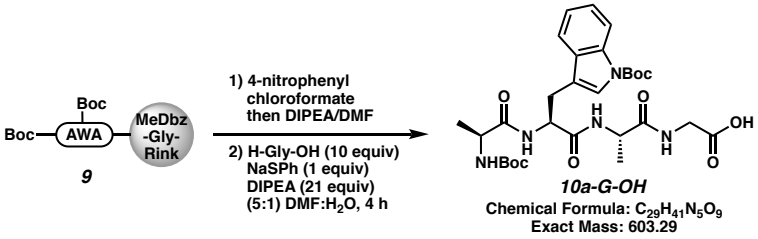


Table 2, entry 5: 100 mg (16 μmol) of **9** was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Gly-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was purified using RP-HPLC-MS to yield the pure peptide **10a-G-OH** in 39% yield (4.7 mg). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.98 (dd, J = 33.3, 7.4 Hz, 1H), 7.81 (d, J = 7.9 Hz, 1H), 7.67 (d, J = 8.2 Hz, 1H), 7.47 (s, 1H), 7.30 (q, J = 7.2, 6.7 Hz, 1H), 7.23 (q, J = 6.8 Hz, 1H), 6.96 (dd, J = 27.6, 7.2 Hz, 1H), 4.58 (dd, J = 14.0, 5.8 Hz, 1H), 4.36 – 4.25 (m, 1H), 3.91 – 3.84 (m, 1H), 3.79 – 3.68 (m, 3H), 3.11 (d, J = 11.0 Hz, 1H), 2.93 (dd, J = 14.9, 8.3 Hz, 1H), 1.60 (s, 9H), 1.34 (s, 8H), 1.21 (d, J = 7.0 Hz, 2H), 1.11 (dq, J = 16.0, 9.0, 8.1 Hz, 4H). ^{13}C NMR (151 MHz, DMSO) δ 172.6, 172.3, 171.1, 170.5, 155.0, 149.1, 134.7, 130.4, 124.2, 122.4, 119.5, 116.1, 114.5, 83.4, 78.2, 52.4, 51.9, 50.1, 48.1, 40.8, 28.2, 27.7, 18.3, 18.1. HRMS (ESI+) *m/z* calc'd for $C_{29}H_{41}N_5O_9Na$ $[M+Na]^+$ 626.2802, found 626.2786.

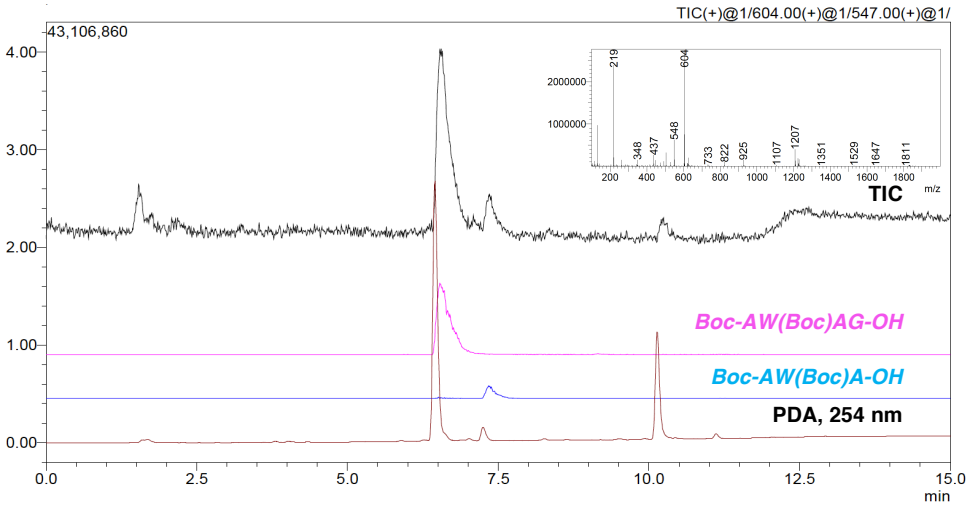


Figure SI-20. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

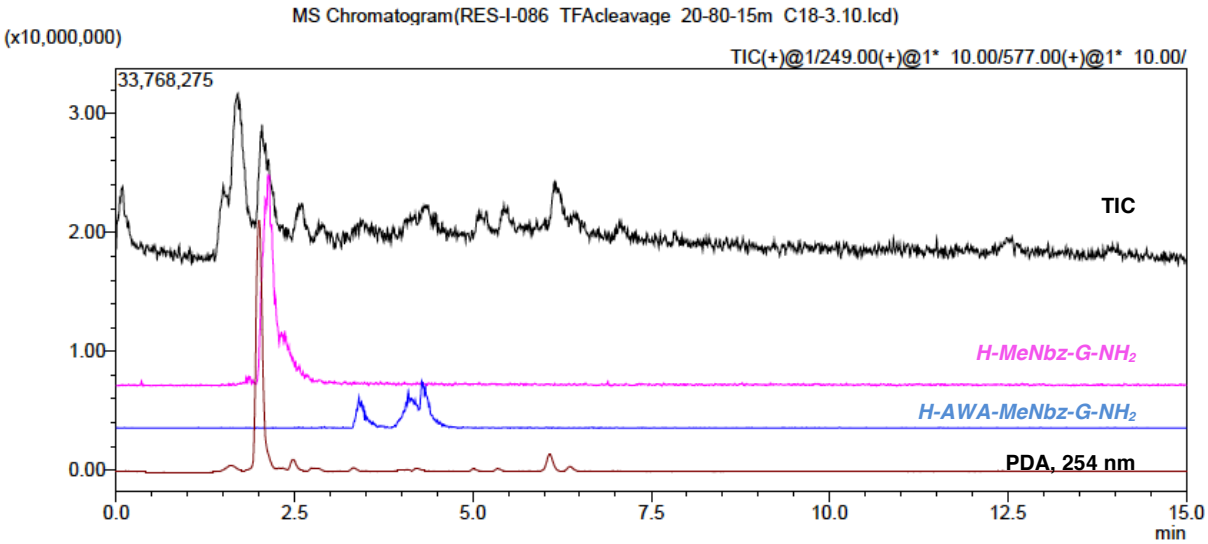


Figure SI-21. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

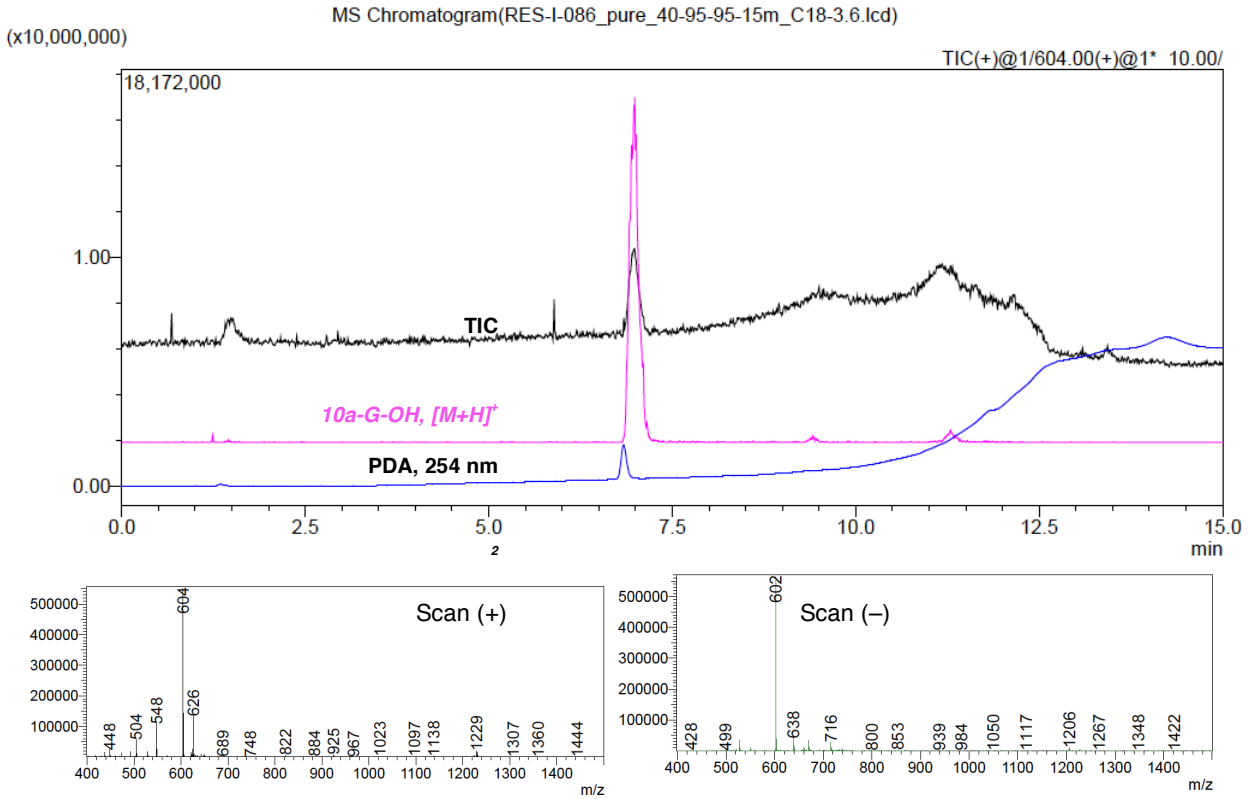


Figure SI-22. Analytical Column PDA of pure peptide, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

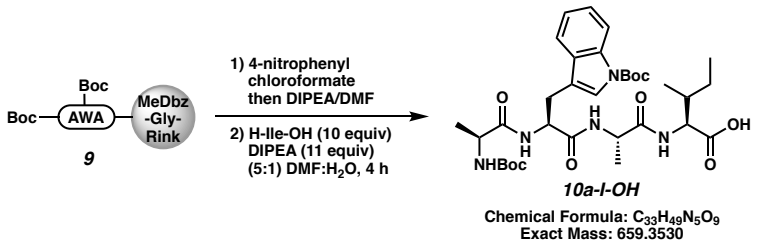


Table 2, entry 6: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH was dissolved in 500 μL DMF and 100 μL H_2O in a separate vial 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 (LN_2), and lyophilized to yield the crude peptide.

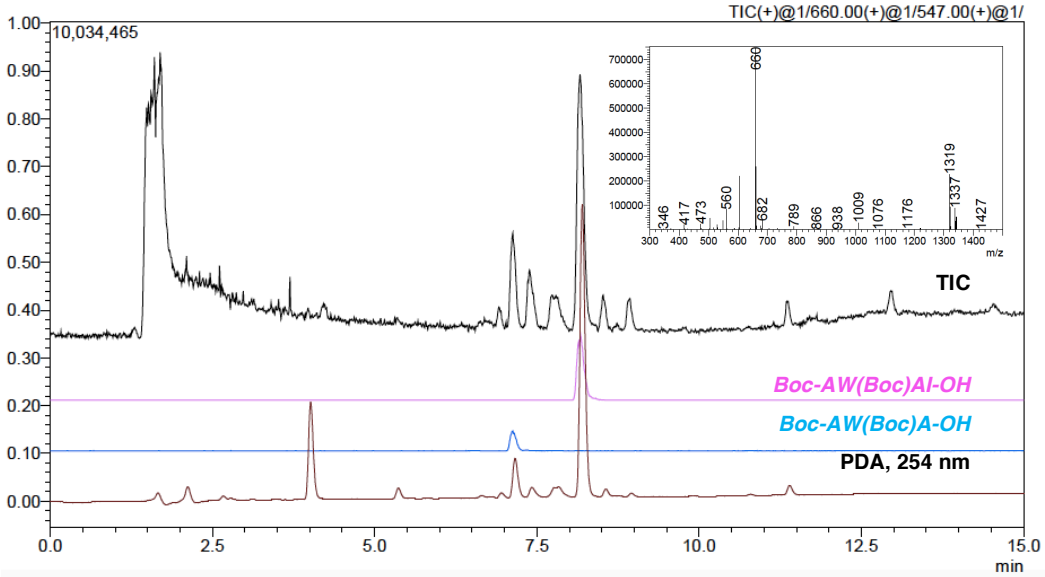


Figure SI-23. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

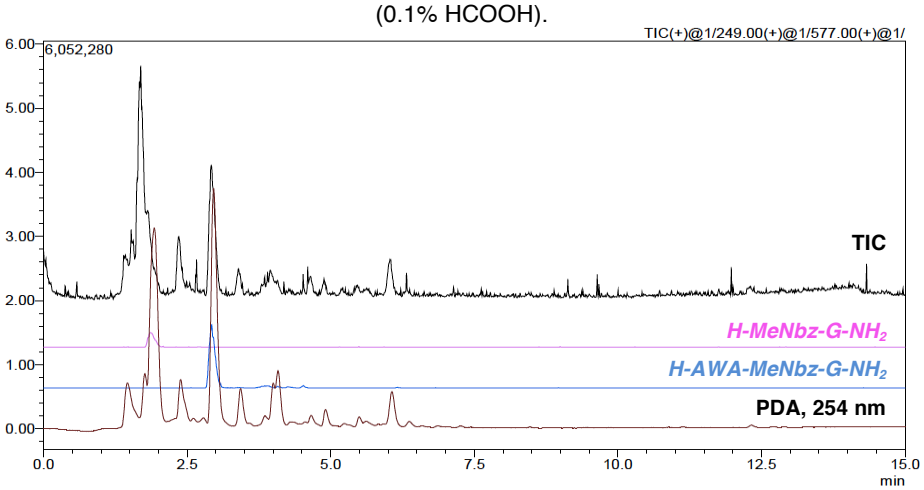


Figure SI-24. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

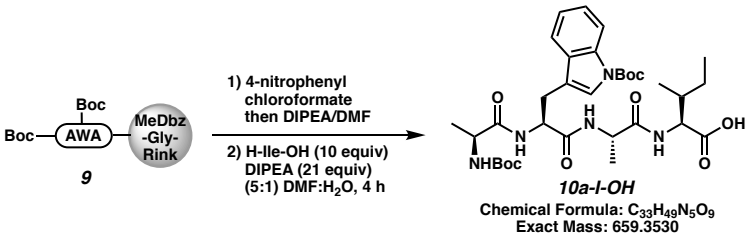


Table 2, entry 7: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH was added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide.

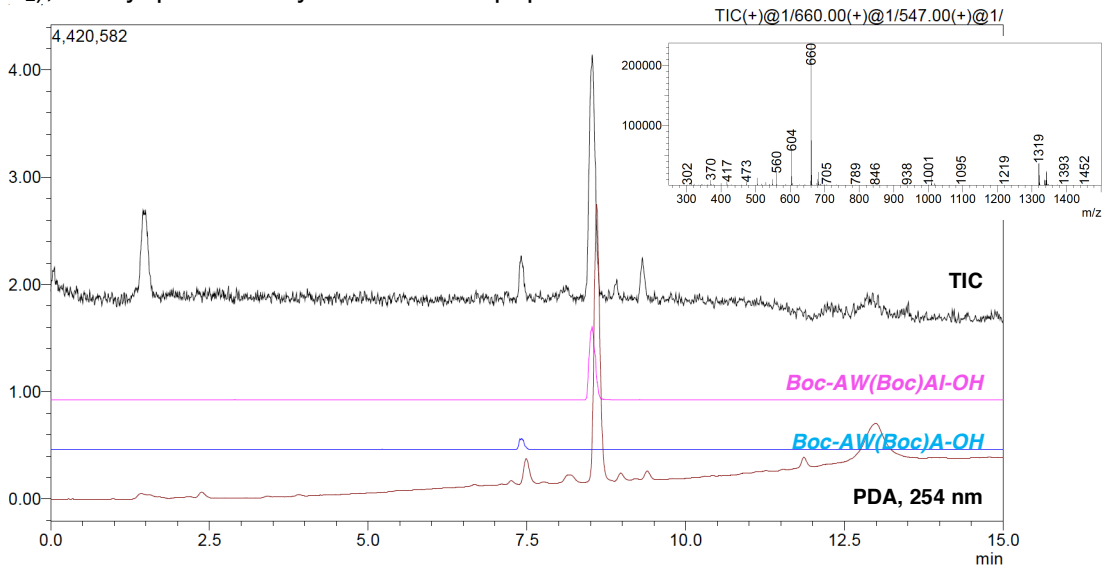


Figure SI-25. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

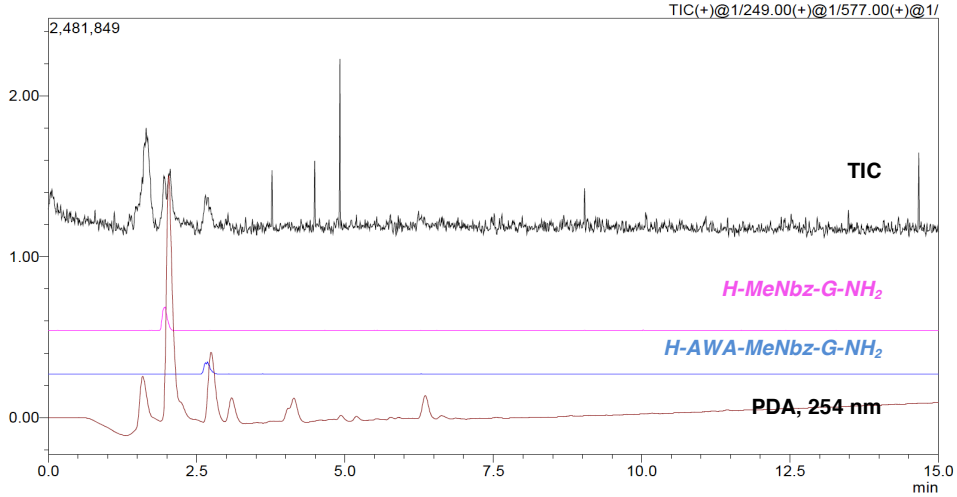


Figure SI-26. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

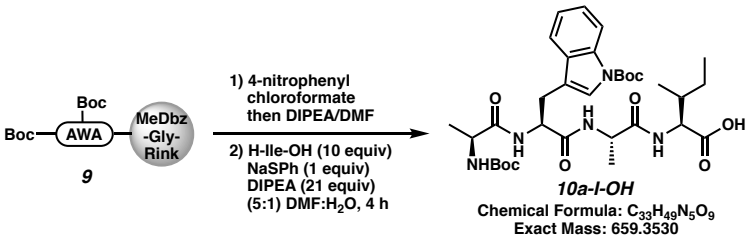


Table 2, Entry 8: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH and 1 equiv of NaSPh was added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide.

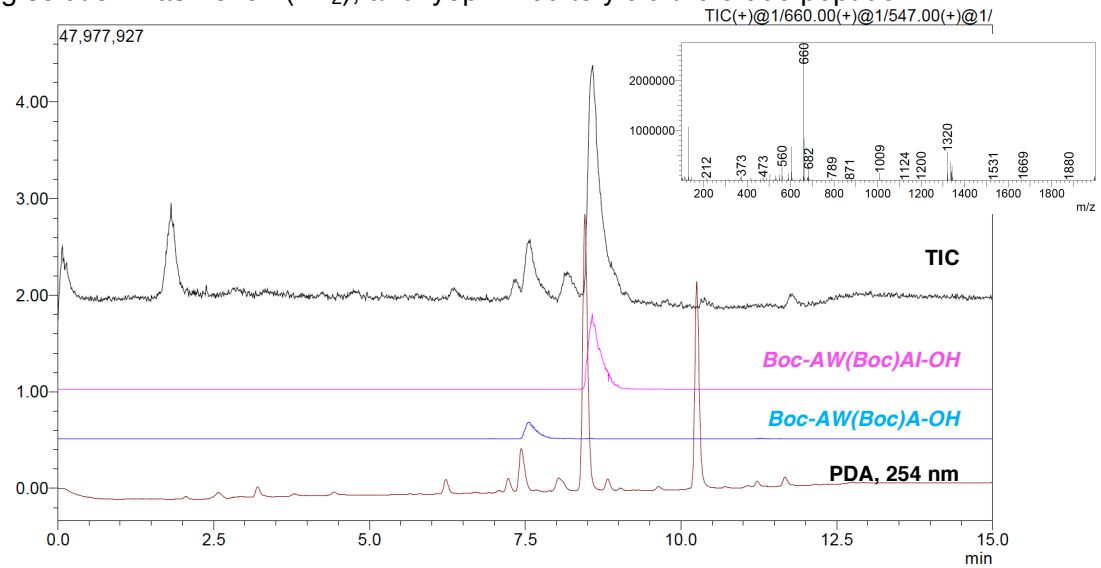


Figure SI-27. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

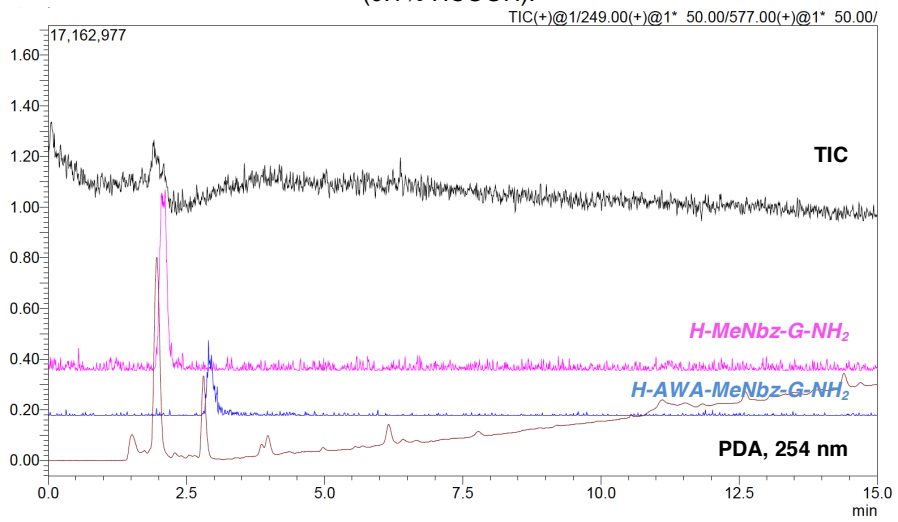


Figure SI-28. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

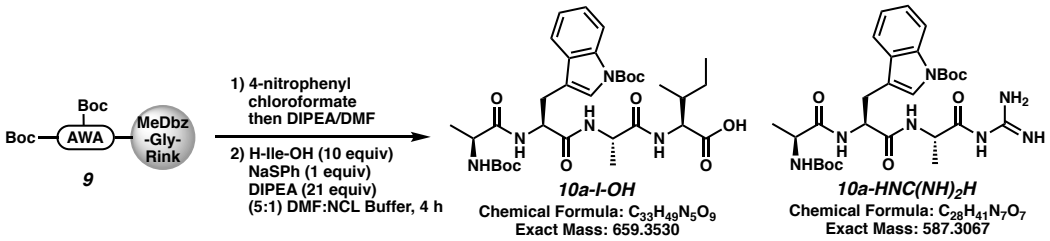


Table 2, entry SI-01: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. A fresh sodium phosphate buffer was prepared (see sodium phosphate buffer with guanidine procedure). After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF: modified NCL buffer (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. Mostly guanidine addition was observed.

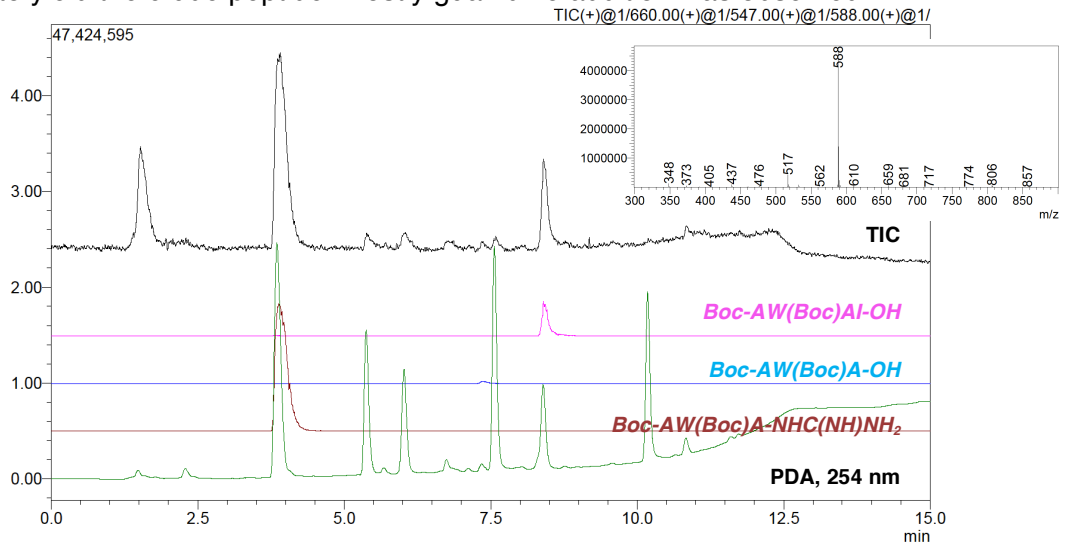


Figure SI-29. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

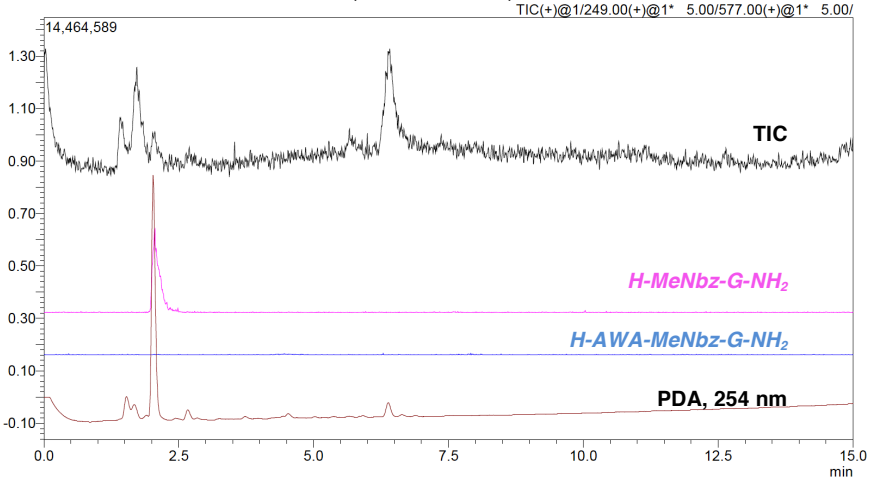


Figure SI-30. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

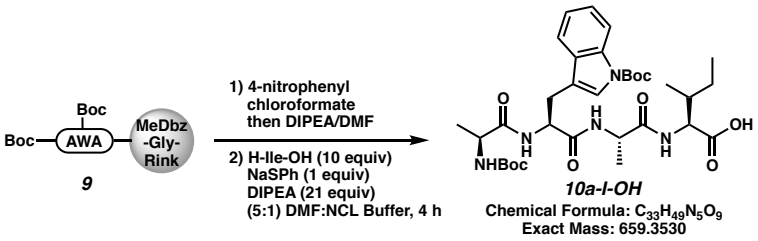


Table 2, entry 9: 100 mg of **9** (16 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. A fresh sodium phosphate buffer was prepared (see Sodium phosphate buffer, guanidine-free). After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:NCL Buffer (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was purified using RP-HPLC-MS to give the pure peptide in 22% yield (4.4 mg). ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.21 (d, J = 7.1 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.49 (s, 1H), 7.29 (t, J = 7.7 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 6.92 (d, J = 7.3 Hz, 1H), 4.66 – 4.53 (m, 1H), 4.37 (q, J = 6.9 Hz, 1H), 4.17 (dd, J = 8.2, 5.8 Hz, 1H), 3.92 – 3.83 (m, 1H), 3.13 – 3.02 (m, 1H), 2.97 – 2.87 (m, 1H), 1.76 (dq, J = 13.7, 7.7, 7.2 Hz, 1H), 1.60 (s, 9H), 1.33 (s, 8H), 1.20 (t, J = 6.9 Hz, 3H), 1.10 (d, J = 7.0 Hz, 4H), 0.84 (t, J = 7.8 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.9, 172.6, 172.1, 170.6, 155.0, 149.1, 134.7, 130.4, 124.2, 122.4, 119.4, 116.2, 114.6, 83.4, 78.2, 56.3, 51.8, 50.2, 48.0, 36.5, 28.2, 27.7, 27.4, 24.7, 18.2, 15.6, 11.4; HRMS (ESI+) *m/z* calc'd for $C_{33}H_{49}N_5O_9Na$ $[M+Na]^+$ 682.3428, found 682.3428.

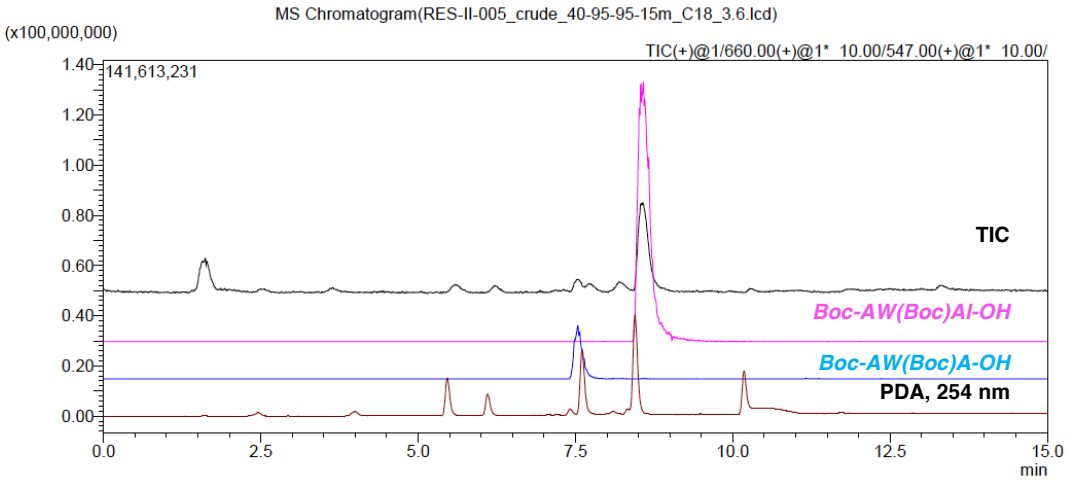


Figure SI-31. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

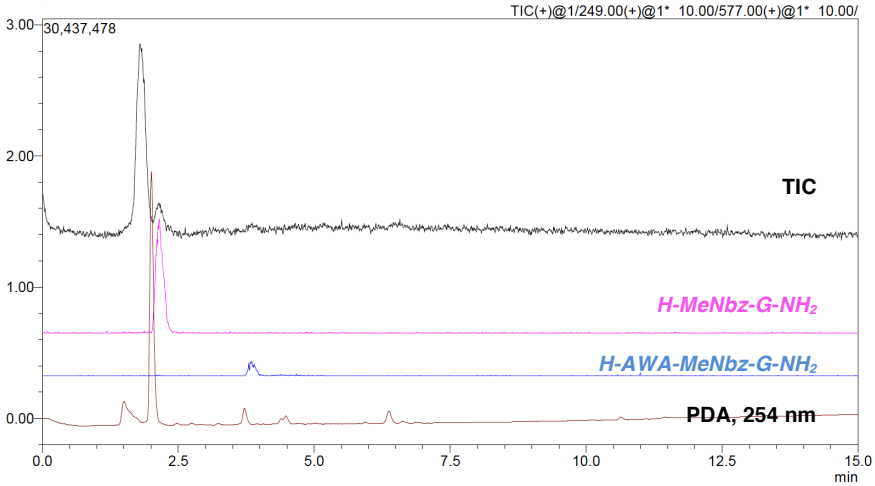


Figure SI-32. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

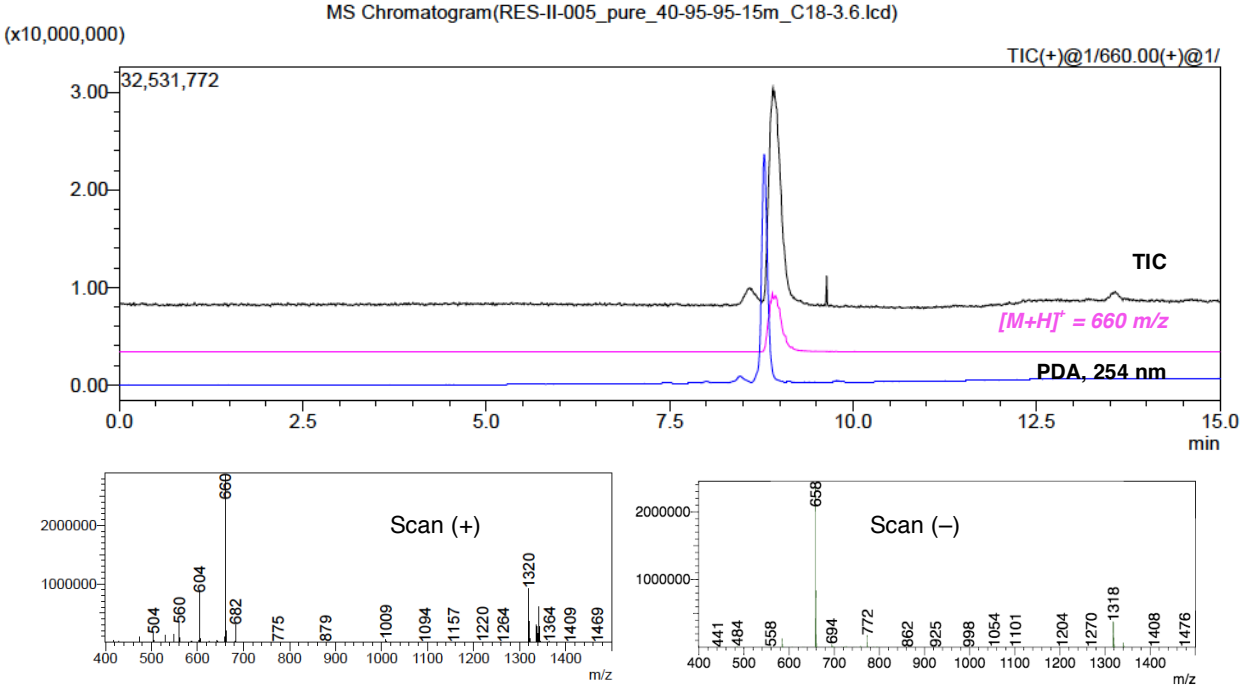


Figure SI-33. Analytical Column PDA of pure peptide, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

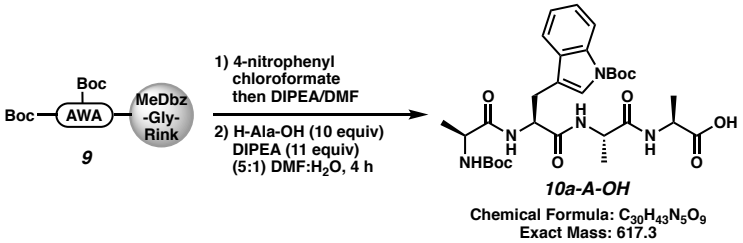


Table 2, entry 10: 20 mg of **9** was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ala-OH was dissolved in 500 μL DMF and 100 μL H_2O in a separate vial and added 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 (LN_2), and lyophilized to yield the crude peptide.

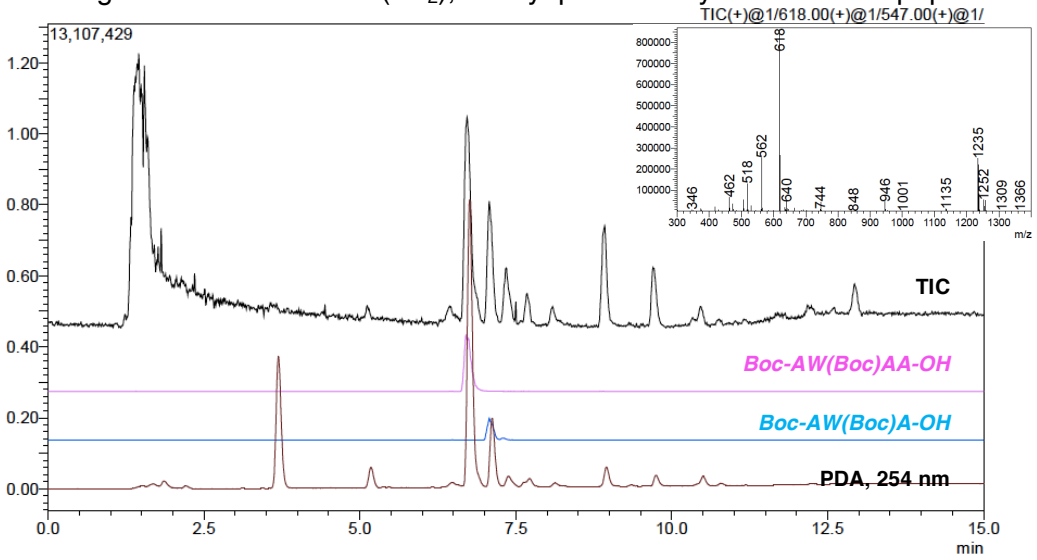


Figure SI-34. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

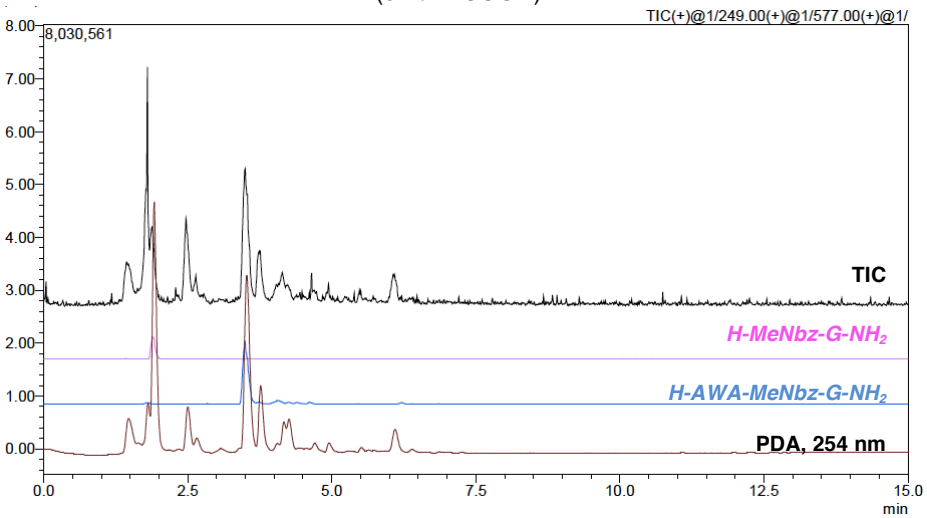


Figure SI-35. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

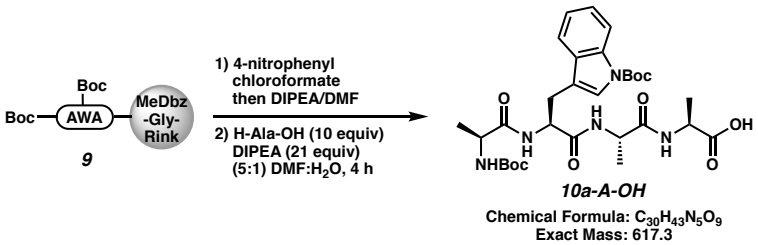


Table 2, entry 11: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ala-OH was added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide.

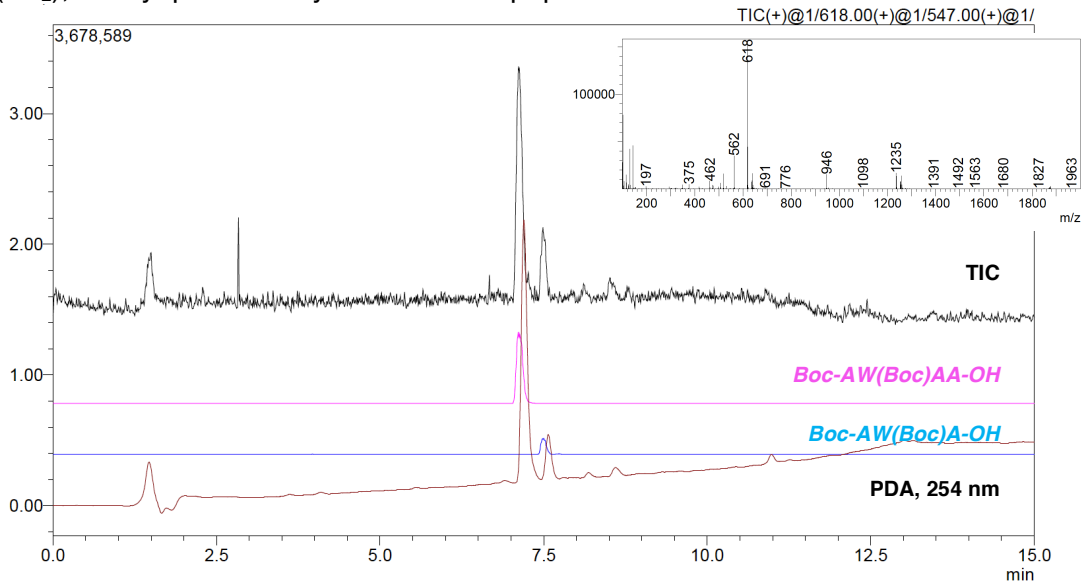


Figure SI-36. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

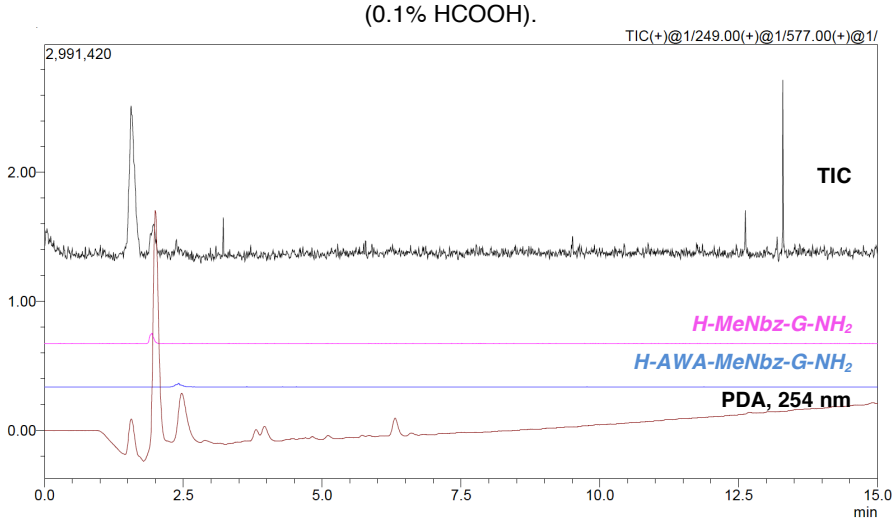


Figure SI-37. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

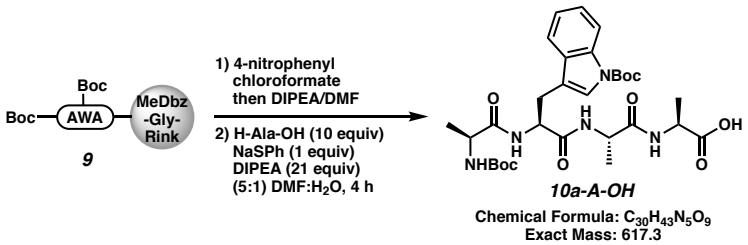


Table 2, entry 12: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ala-OH and 1 equiv of NaSPh was added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide.

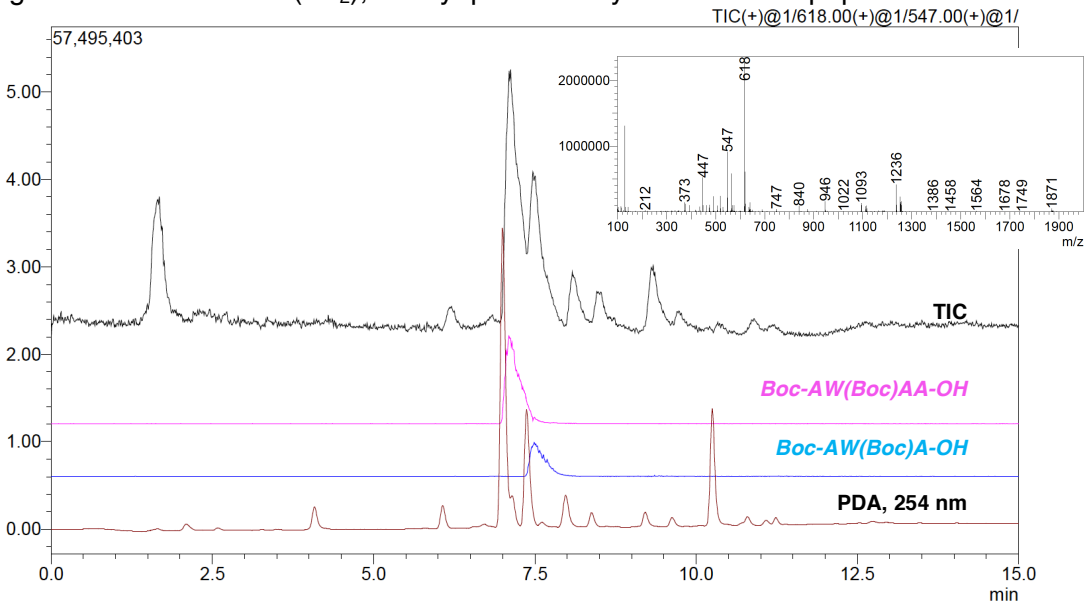


Figure SI-38. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

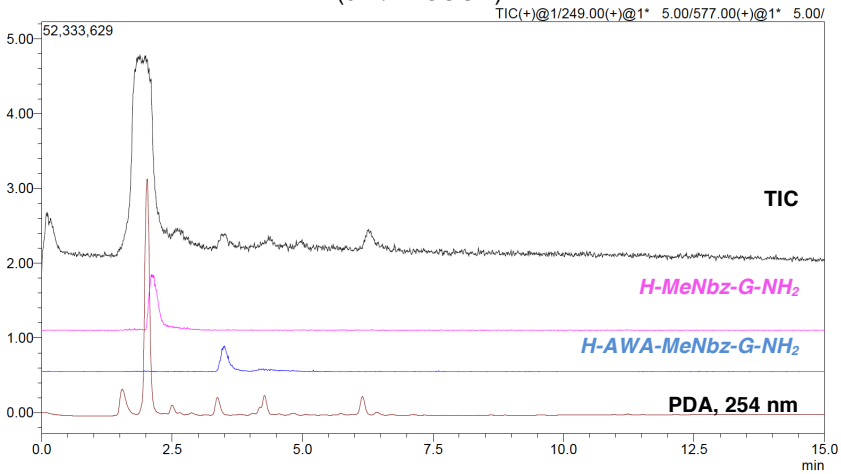


Figure SI-39. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

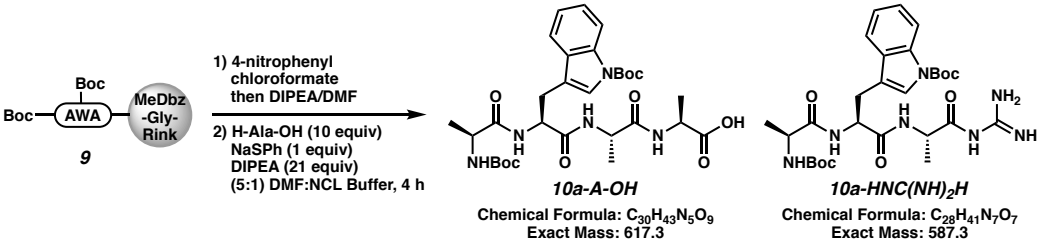


Table 2, entry SI-02: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. A fresh sodium phosphate buffer was prepared. (See Sodium phosphate buffer with guanidine procedure). After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ala-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:modified NCL Buffer (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. Mostly guanidine addition was observed.

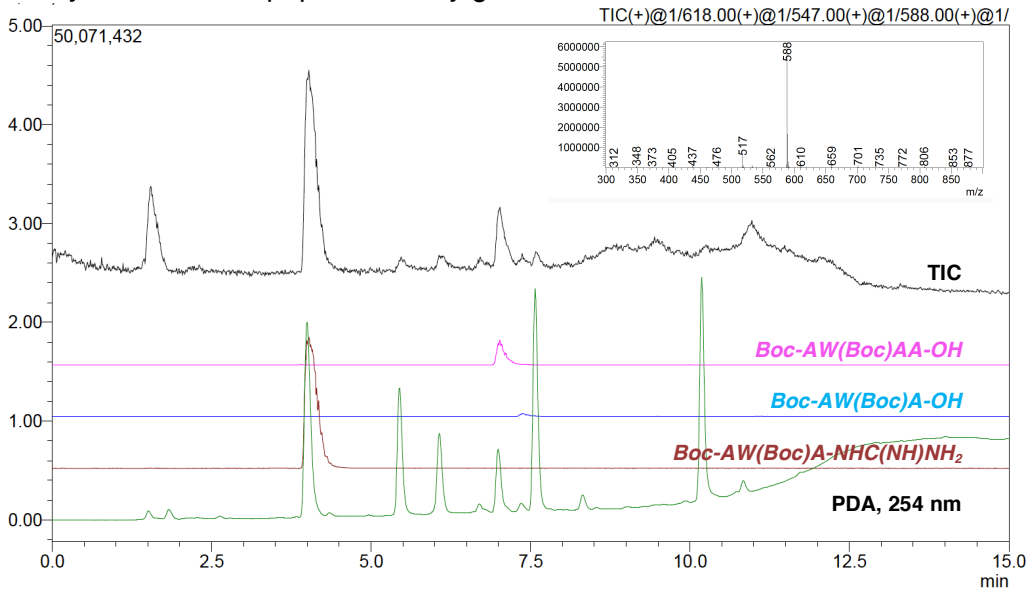


Figure SI-40. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H2O (0.1% HCOOH).

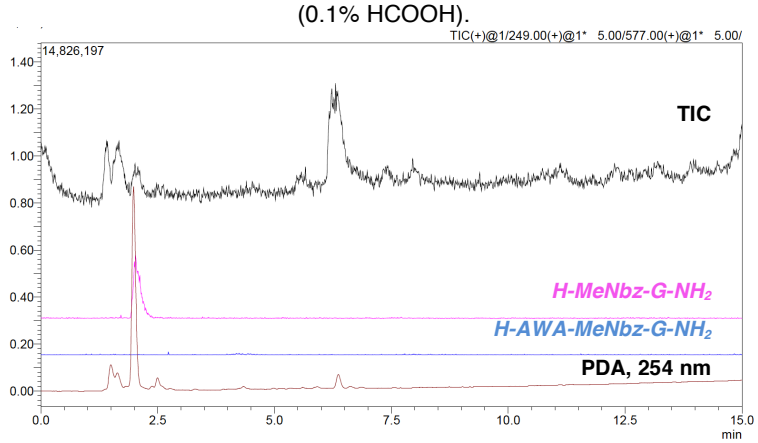


Figure SI-41. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

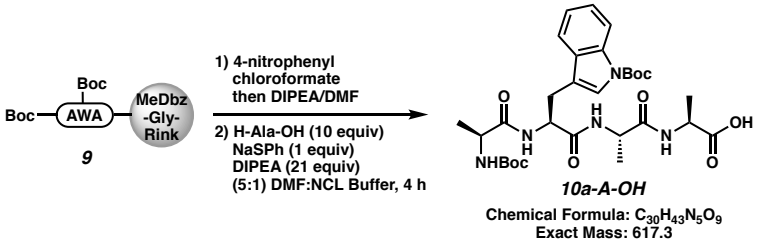


Table 2, entry 13: 100 mg of **9** (16 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. A fresh sodium phosphate buffer was prepared. (See Sodium phosphate buffer (Guanidine-free) procedure). After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ala-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:modified NCL Buffer (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was purified using RP-HPLC-MS to isolate 2.3 mg of pure peptide and 5.2 mg of mixed peptide (61% HPLC purity) to give 29% yield. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.67 (s, 1H), 7.48 (s, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.26 – 7.18 (m, 1H), 6.94 (d, J = 6.4 Hz, 1H), 4.59 (s, 1H), 4.28 (d, J = 6.6 Hz, 1H), 4.16 (s, 1H), 3.90 – 3.85 (m, 1H), 3.08 (s, 1H), 2.92 (dd, J = 14.8, 8.3 Hz, 1H), 1.60 (s, 9H), 1.33 (s, 7H), 1.28 – 1.22 (m, 3H), 1.19 (d, J = 6.9 Hz, 3H), 1.10 (d, J = 7.3 Hz, 4H). ^{13}C NMR (151 MHz, DMSO) δ 174.0, 172.6, 171.6, 170.4, 155.0, 149.1, 134.7, 130.4, 124.2, 122.4, 119.5, 116.2, 114.6, 83.4, 78.2, 51.9, 50.1, 47.9, 47.6, 28.2, 27.7, 18.2, 18.08, 17.3. HRMS (ESI+) *m/z* calc'd for $C_{30}H_{43}N_5O_9Na$ $[M+Na]^+$ 640.2958, found 640.2940.

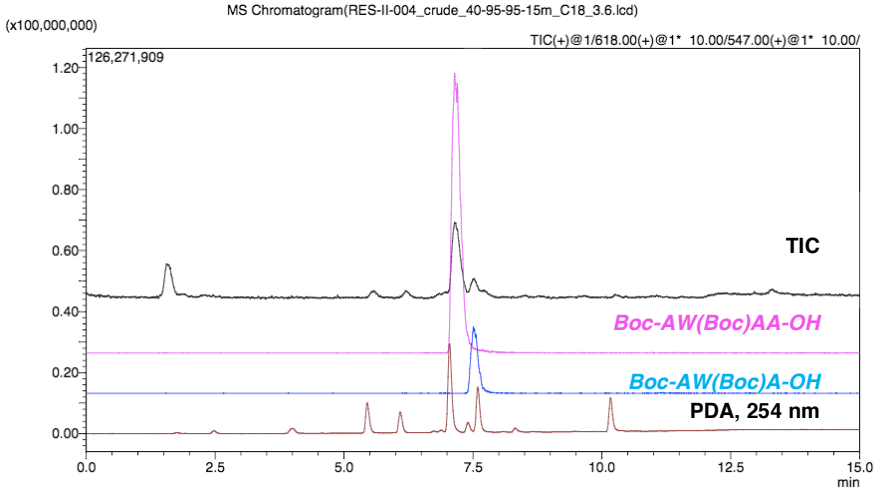


Figure SI-42. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

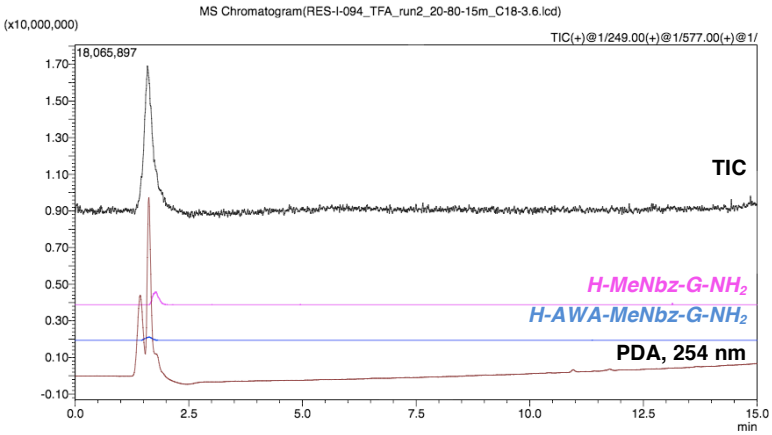


Figure SI-43. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

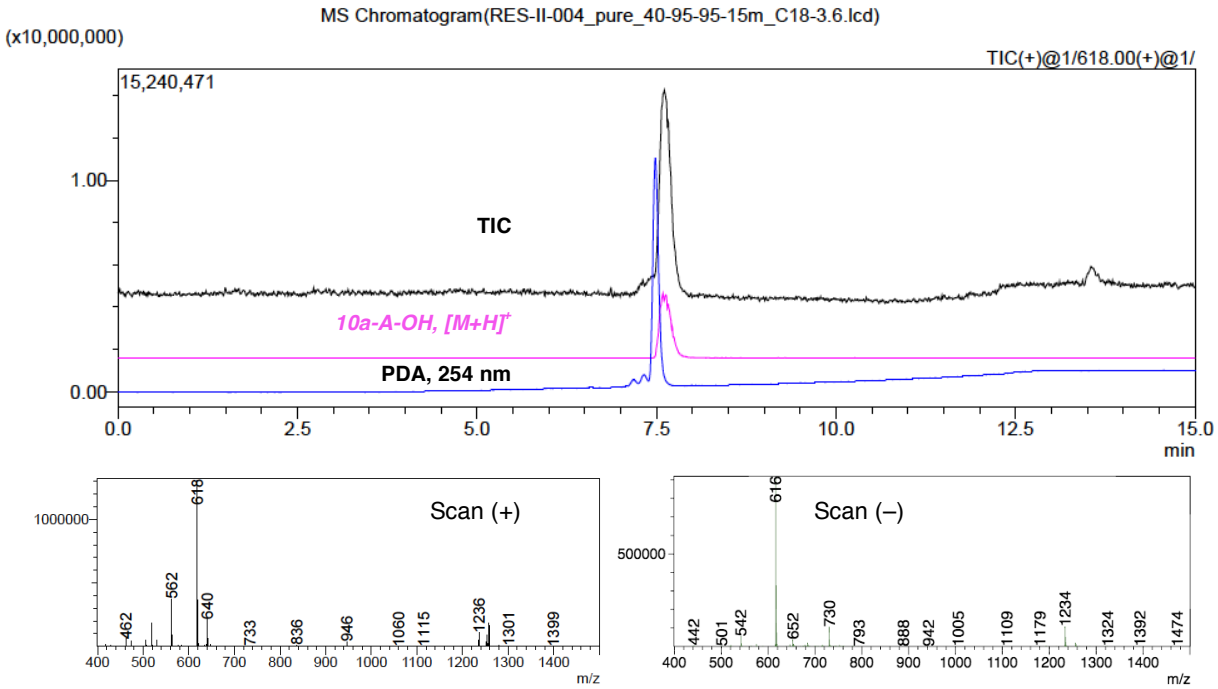


Figure SI-44. Analytical Column PDA of pure peptide, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

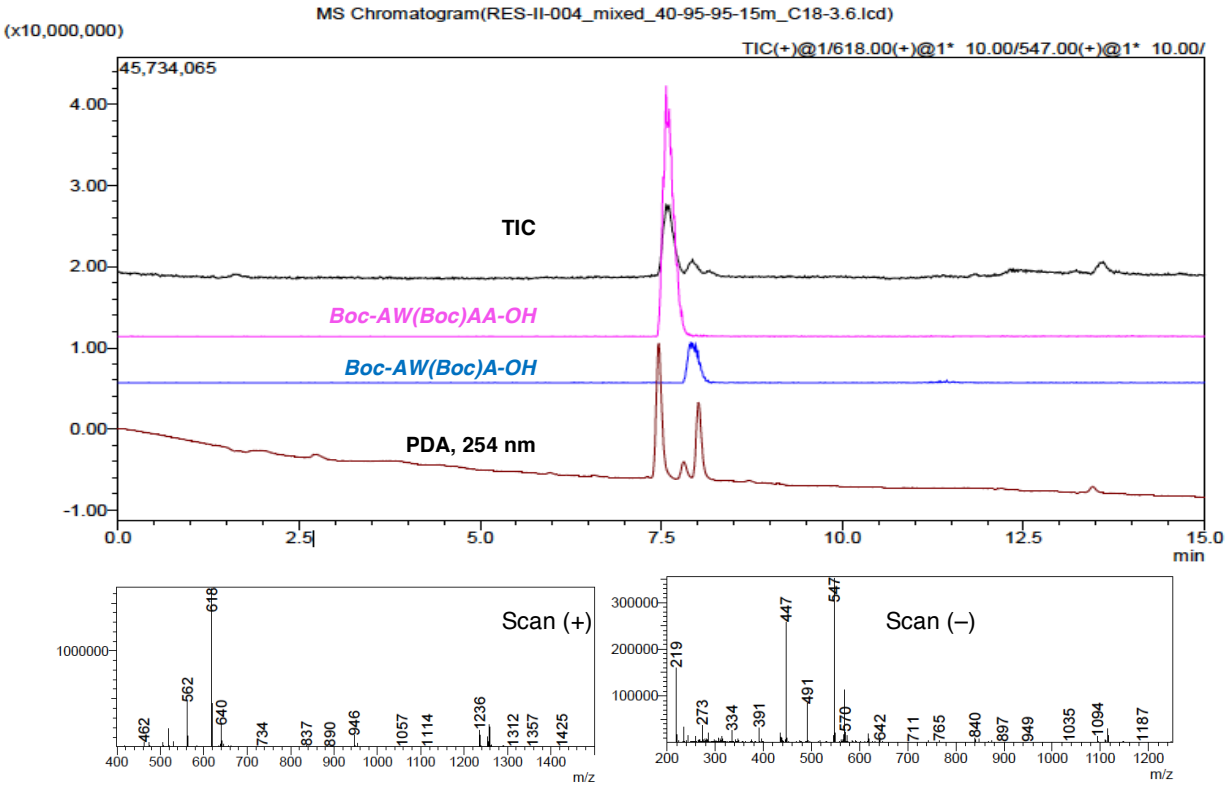


Figure SI-45. Analytical Column PDA of mixed peptides, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

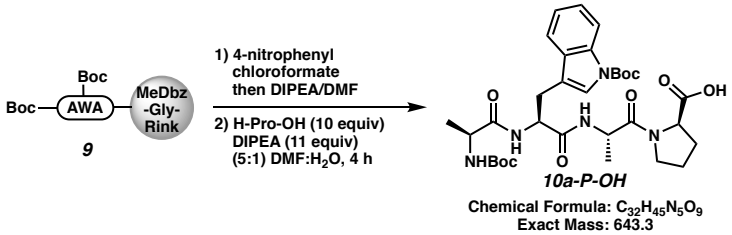


Table 2, entry 14: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was dissolved in 500 μL DMF and 100 μL H_2O in a separate vial 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 (LN_2), and lyophilized to yield the crude peptide.

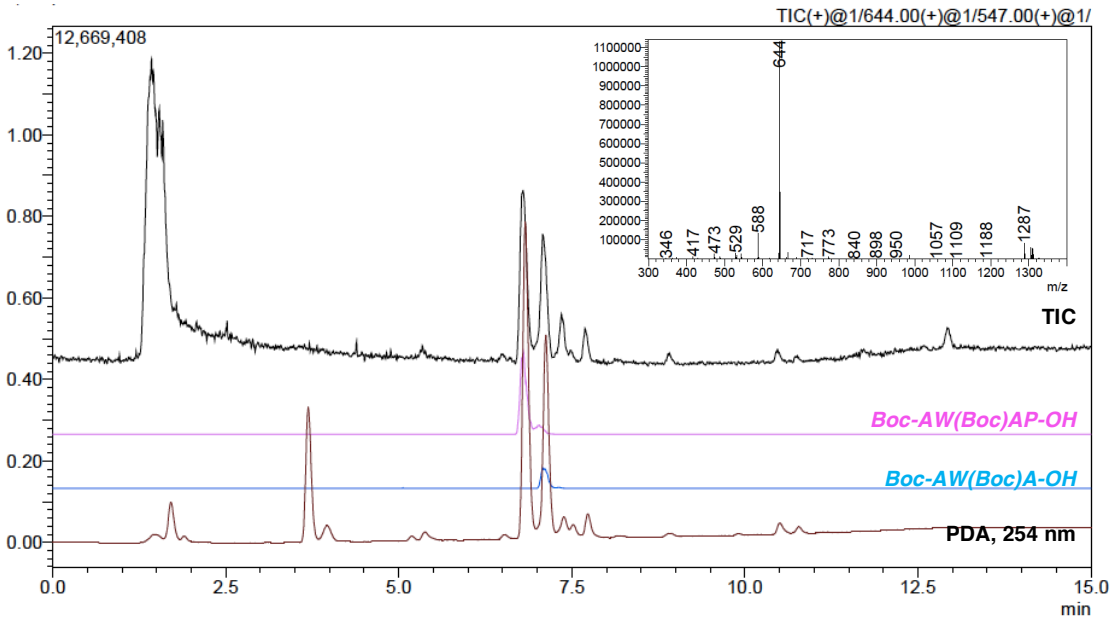


Figure SI-46. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

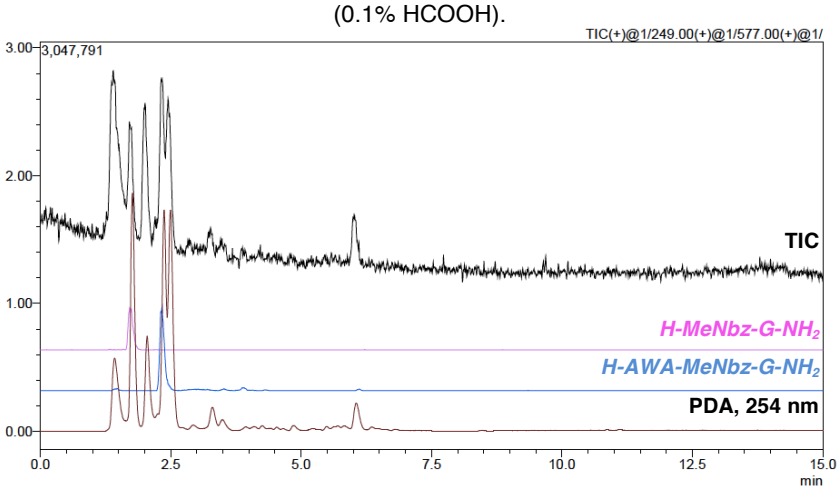


Figure SI-47. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

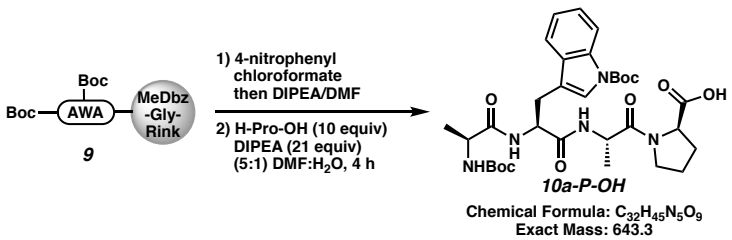


Table 2, entry 15a: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide.

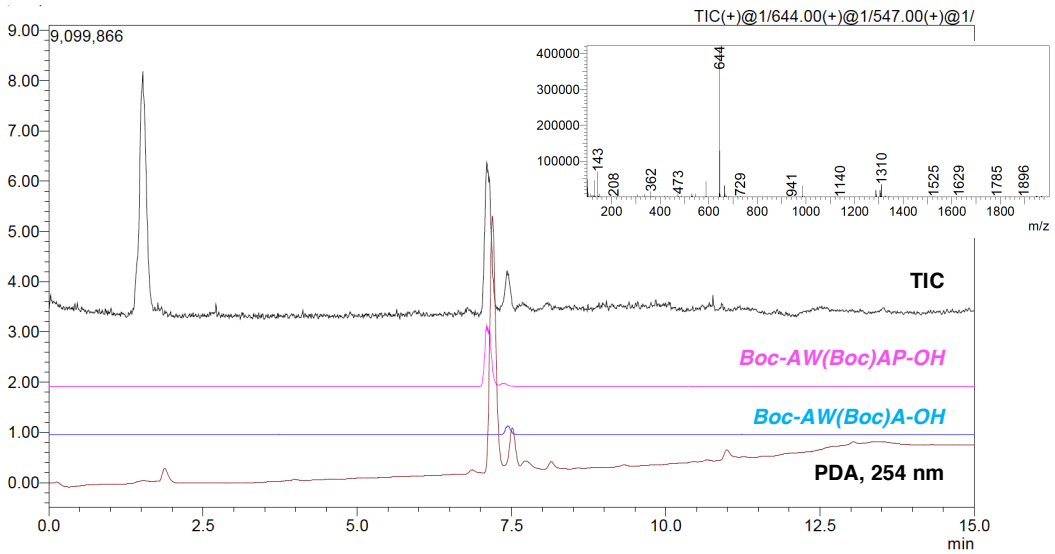


Figure SI-48. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

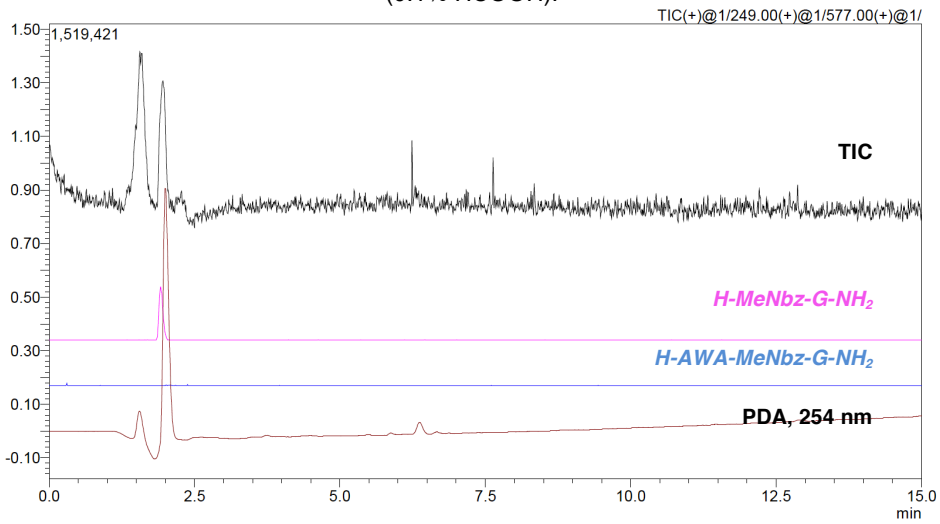


Figure SI-49. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

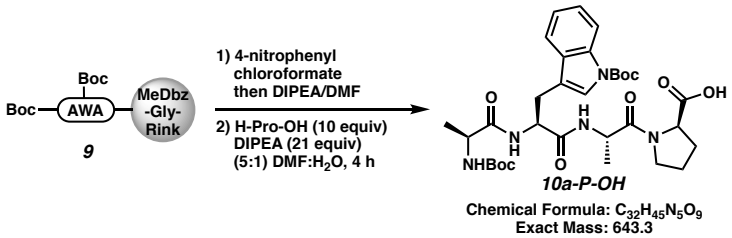


Table 2, entry 15b: 100 mg of **9** (16 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was purified using RP-HPLC-MS yielding 1 mg of pure peptide (**10a-P-OH**) and 5.9 mg of mixed peptide (79% HPLC purity) giving 44% yield. ^{1}H NMR (499 MHz, DMSO-d_6) δ 12.43 (s, 1H), 8.23 (d, J = 7.1 Hz, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.48 (s, 1H), 7.30 (t, J = 7.2 Hz, 1H), 7.22 (t, J = 7.5 Hz, 1H), 6.93 (d, J = 7.4 Hz, 1H), 4.60 (d, J = 5.8 Hz, 1H), 4.53 – 4.43 (m, 1H), 4.23 (dd, J = 8.8, 4.1 Hz, 1H), 3.96 – 3.84 (m, 1H), 3.49 (d, J = 6.7 Hz, 2H), 3.11 – 2.98 (m, 1H), 2.91 (dd, J = 13.9, 7.6 Hz, 1H), 2.18 – 2.04 (m, 1H), 1.94 – 1.77 (m, 3H), 1.61 (s, 9H), 1.35 (s, 8H), 1.18 (d, J = 6.7 Hz, 3H), 1.11 (d, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 173.3, 172.5, 170.0, 155.0, 149.1, 134.6, 130.4, 124.2, 122.3, 119.4, 116.1, 114.6, 83.4, 78.2, 59.7, 58.6, 51.9, 50.0, 49.5, 46.3, 28.6, 28.2, 27.7, 24.5, 18.1, 16.9. HRMS (ESI+) *m/z* calc'd for $C_{32}H_{45}N_5O_9Na$ $[M+Na]^+$ 666.3115, found 666.3115.

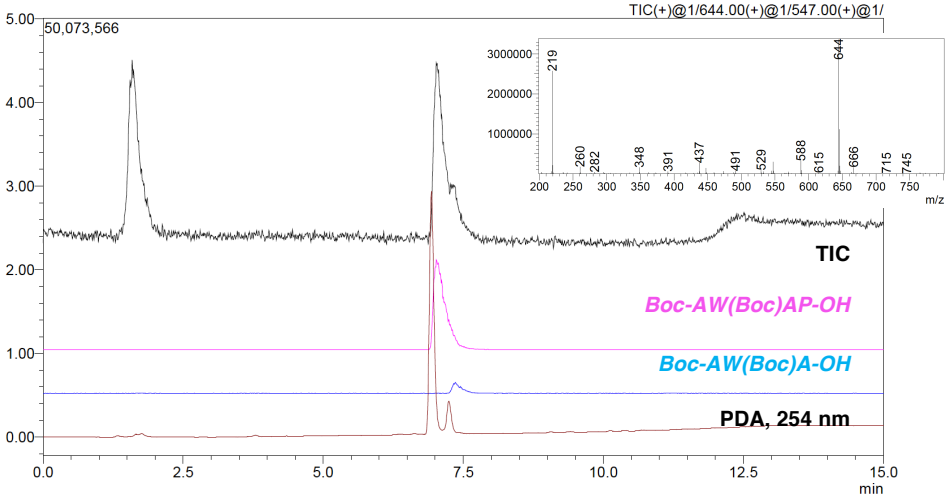


Figure SI-50. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

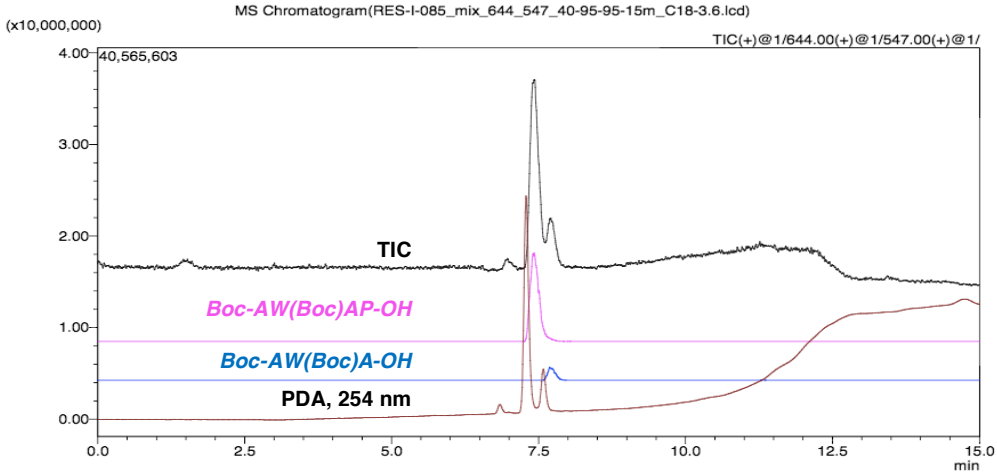


Figure SI-51. Analytical Column PDA of mixed peptide, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

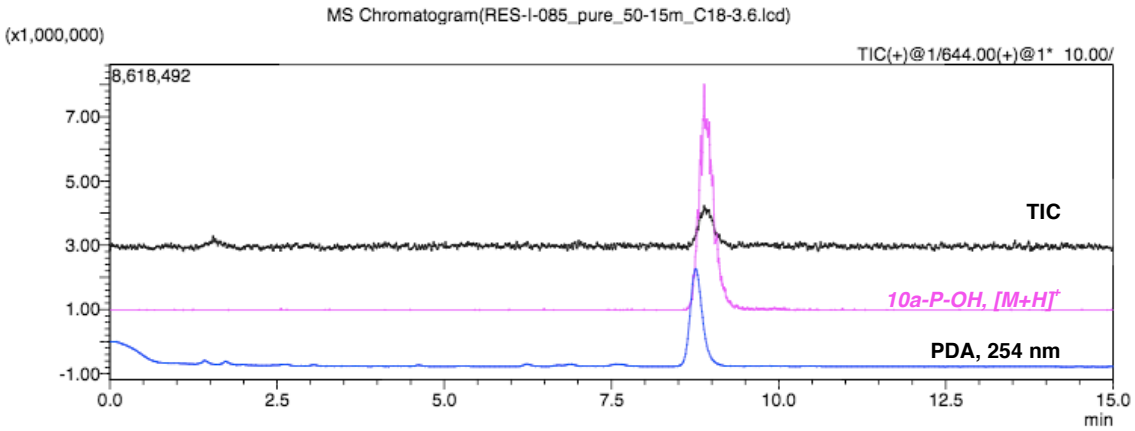


Figure SI-52. Analytical Column PDA of pure peptide, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

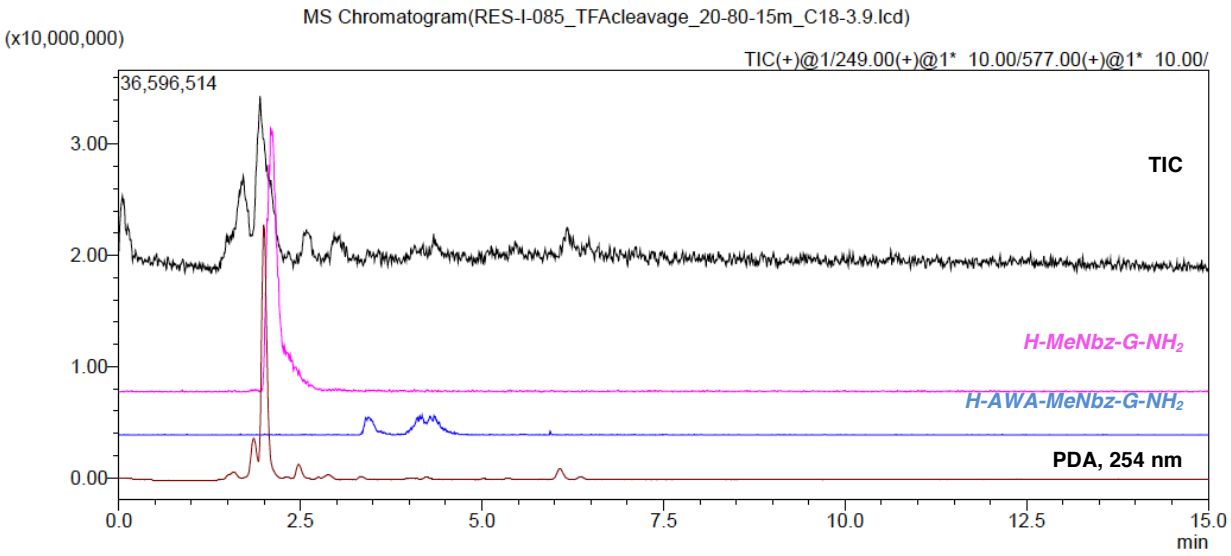


Figure SI-53. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

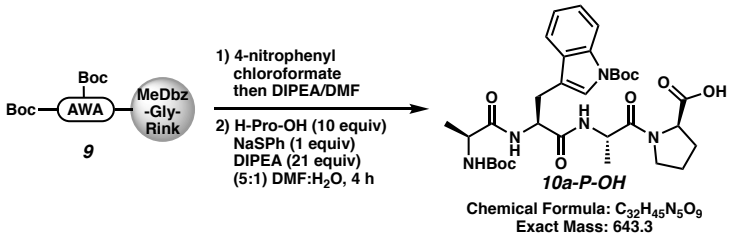


Table 2, entry 16: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**.

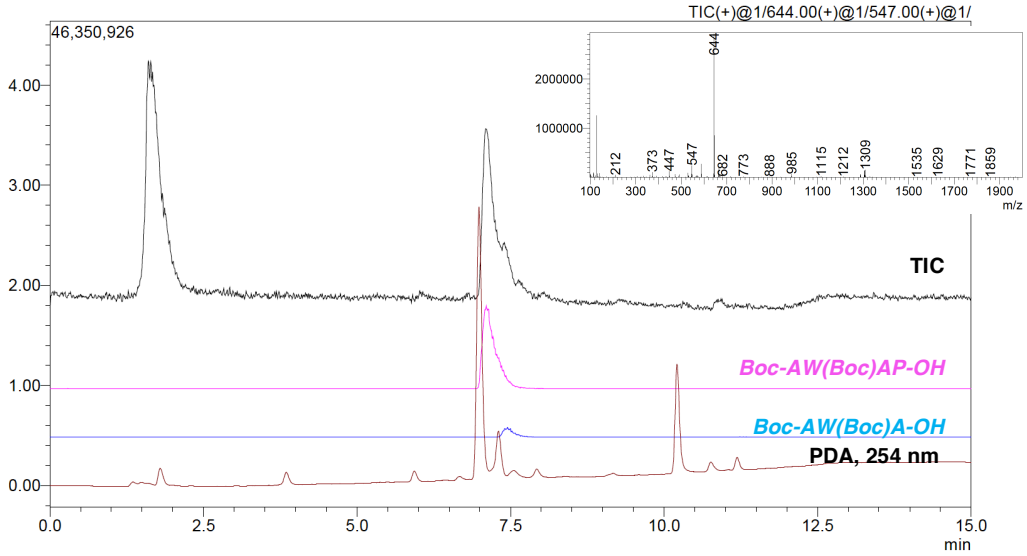


Figure SI-54. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

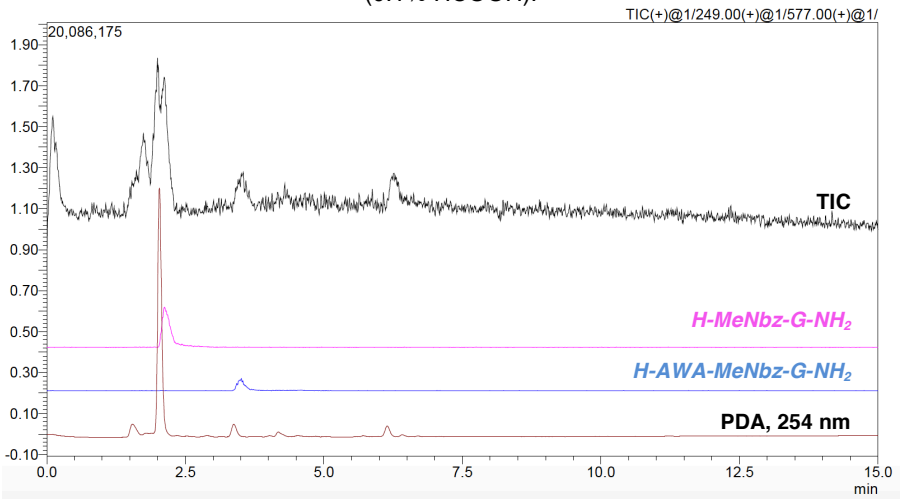


Figure SI-55. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

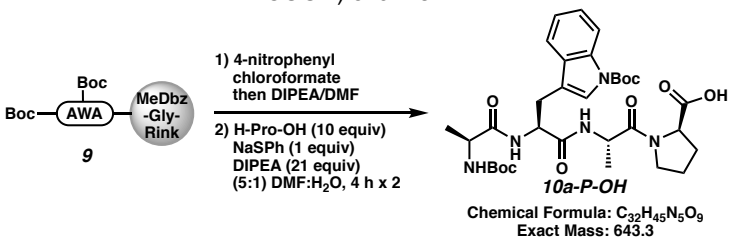


Table 2, entry 17: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. The solvent was removed, collected, and the resin was subjected to the same reactions conditions for an additional 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**.

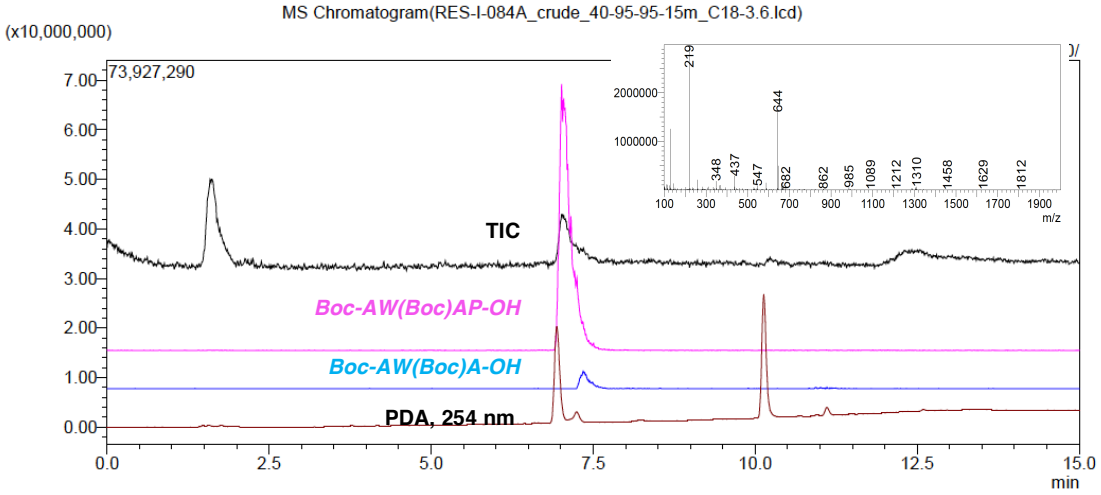


Figure SI-56. Analytical Column PDA of crude peptide (1) at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

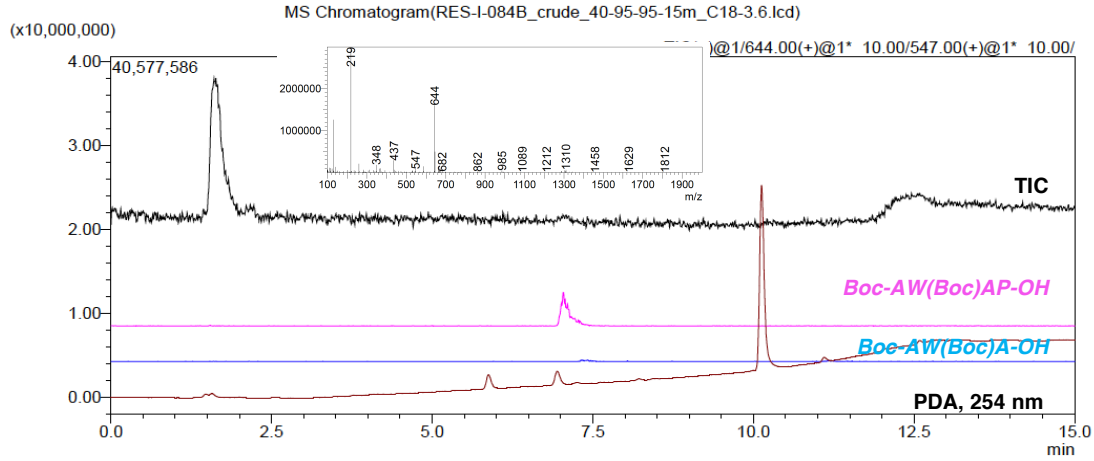


Figure SI-57. Analytical Column PDA of crude peptide (2) at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

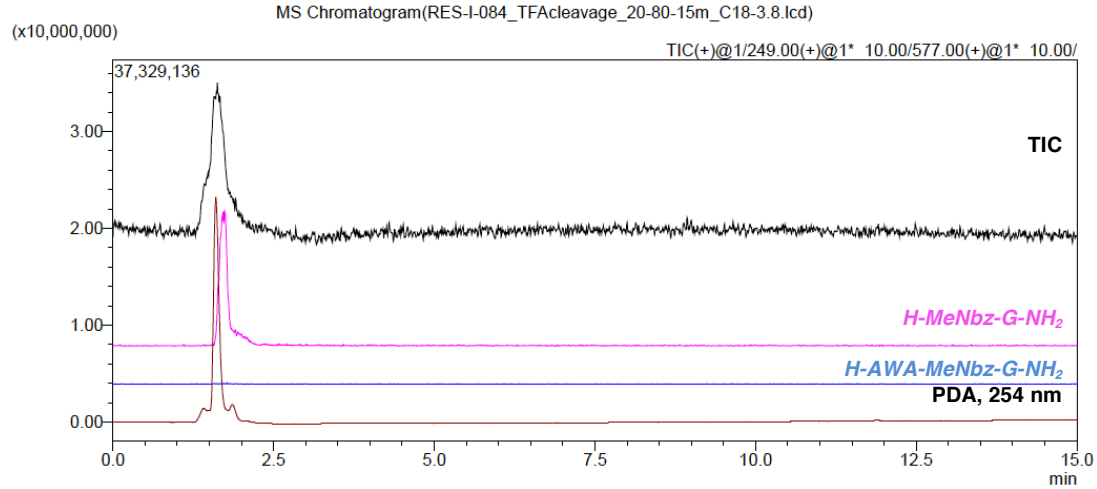


Figure SI-58. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

Table 3. Evaluation of C-terminal alanine epimerization.

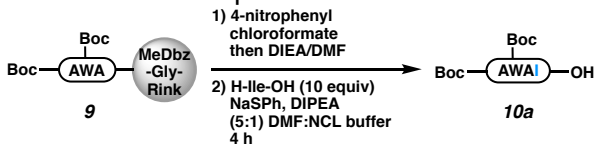


Table 3, entry 1: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH and 1 equiv of NaSPh were added directly to the resin followed by (5:1) 600 μL DMF:modified NCL buffer (without guanidine). Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-I-OH**.

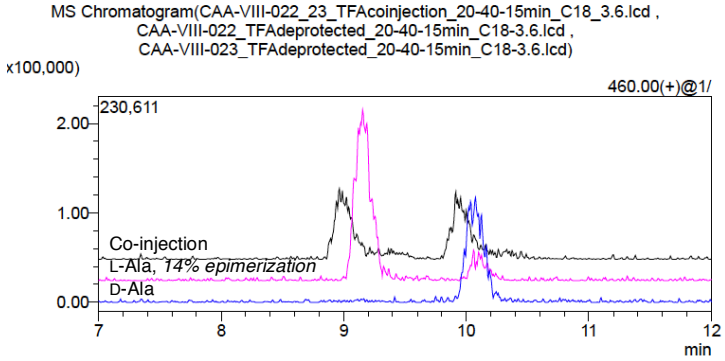


Figure SI-59. Evaluation of C-terminal alanine epimerization with isoleucine. A) L-Ala crude, B) D-Ala crude, and C) co-injection, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min. *For conversion see Figure SI-32.*

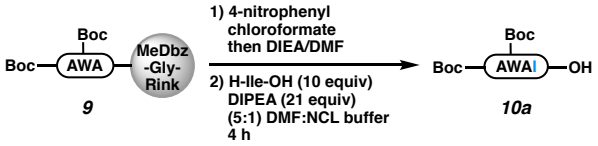


Table 3, entry 2: 10 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH was added directly to the resin followed by (5:1) 300 μL DMF:modified NCL buffer (without guanidine). Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-I-OH**.

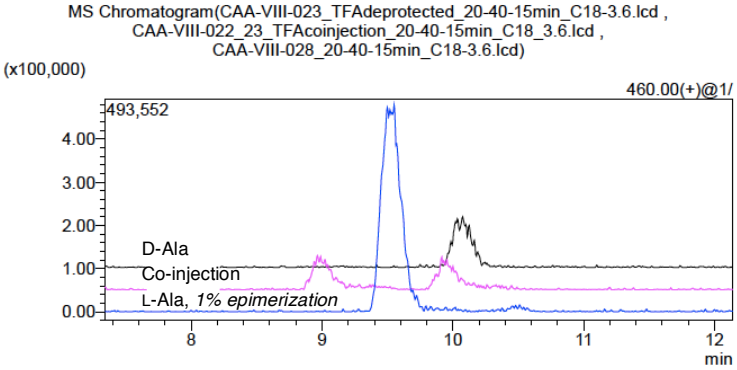


Figure SI-60. Evaluation of C-terminal alanine epimerization with isoleucine. A) D-Ala crude, B) co-injection, and C) L-Ala crude, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

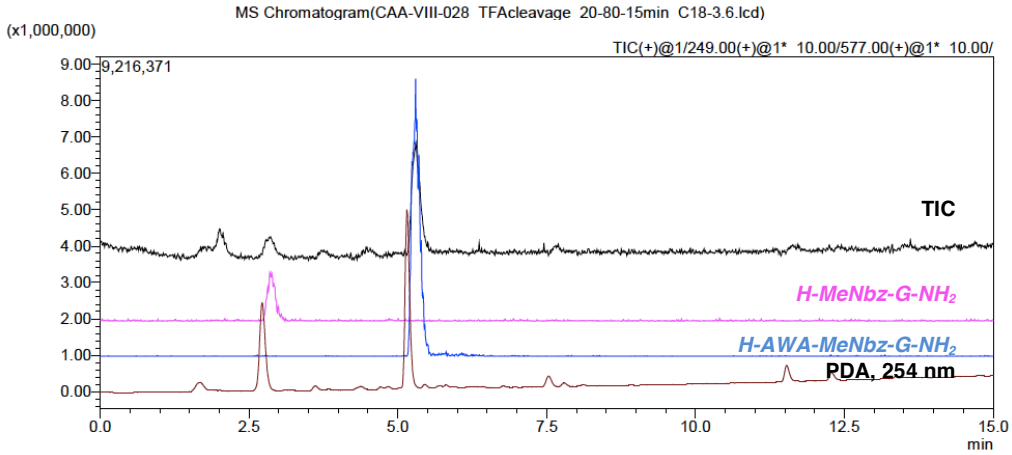


Figure SI-61. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

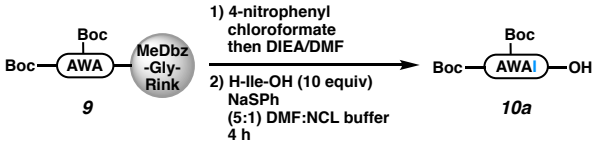


Table 3, entry 3: 10 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH and 1 equiv NaSPh was added directly to the resin followed by (5:1) 300 μL DMF:modified NCL buffer (without guanidine). The reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-I-OH**.

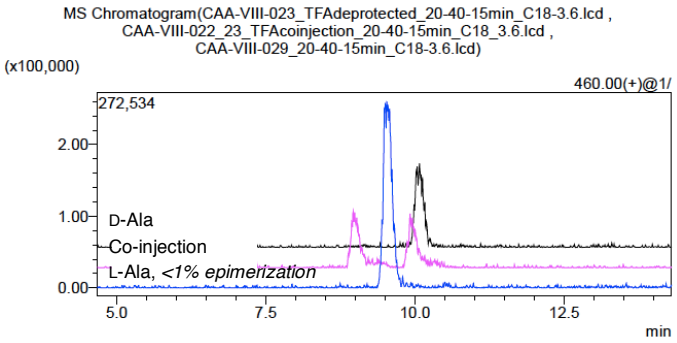


Figure SI-62. Evaluation of C-terminal alanine epimerization with isoleucine. A) D-Ala crude, B) co-injection, and C) D-Ala crude, gradient of 20-40% MeCN/H_2O (0.1% HCOOH) over 15 min.

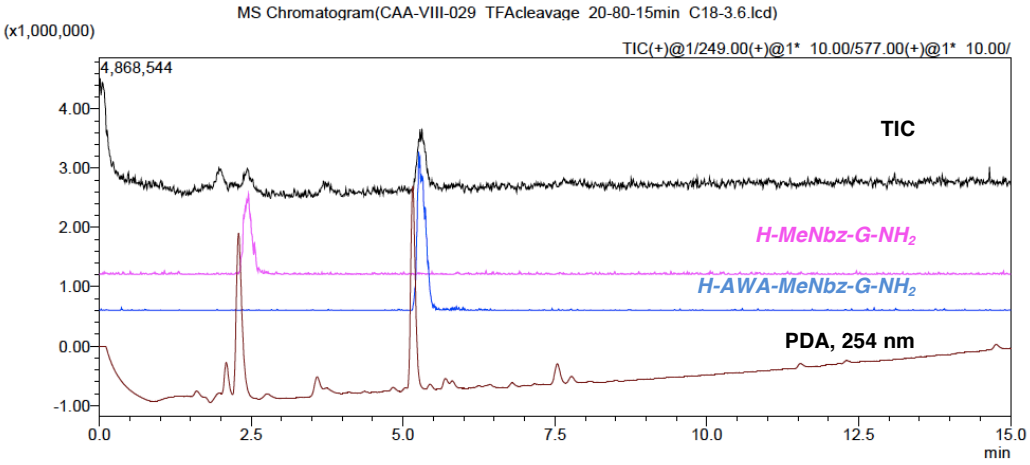


Figure SI-63. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

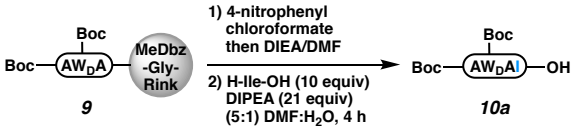


Table 3, entry 4: 24 mg of **9** (5 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH was added directly to the resin followed by (5:1) 600 μL DMF:H_2O. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-I-OH**.

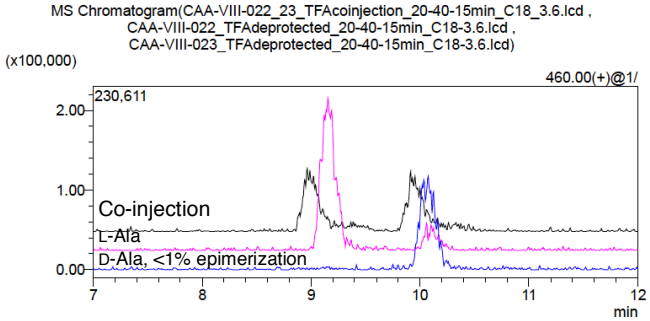


Figure SI-64. Evaluation of C-terminal alanine epimerization with isoleucine. A) L-Ala crude, B) D-Ala crude, and C) co-injection, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min. *For conversion see Figure SI-26.*

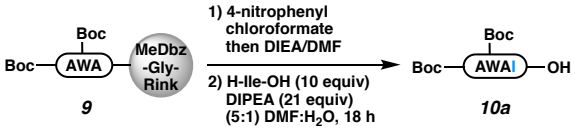


Table 3, entry 5: 10 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH was added directly to the resin followed by (5:1) 300 μL DMF:H_2O. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 18 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-I-OH**.

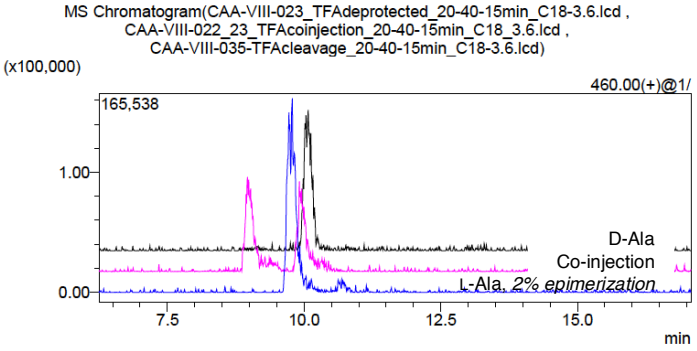


Figure SI-65. Evaluation of C-terminal alanine epimerization with isoleucine. A) L-Ala crude, B) D-Ala crude, and C) co-injection, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

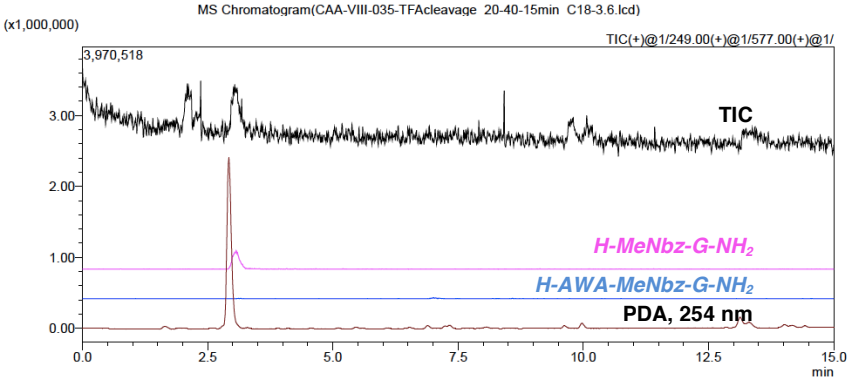


Figure SI-66. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

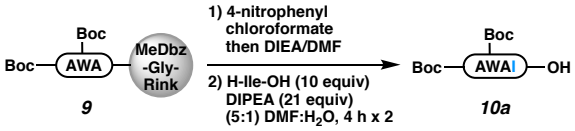


Table 3, entry 5: 10 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH was added directly to the resin followed by (5:1) 300 μL DMF:H_2O. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. The solvent was removed, collected, and the resin was subjected to the same reactions conditions for an additional 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-I-OH**.

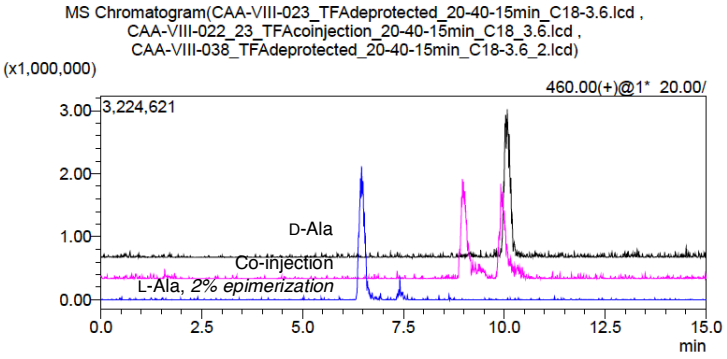


Figure SI-67. Evaluation of C-terminal alanine epimerization with isoleucine. A) L-Ala crude, B) D-Ala crude, and C) co-injection, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

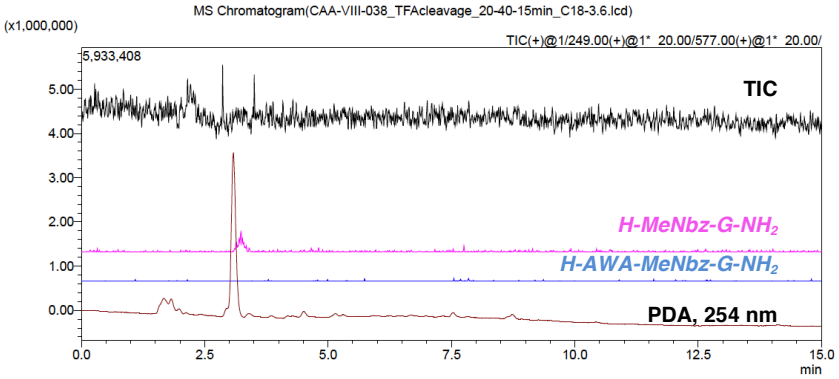


Figure SI-68. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

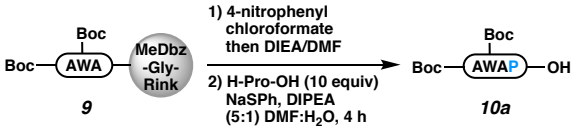


Table 3, entry 6: 20 mg of **9** (4 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH and 1 equiv NaSPh was added directly to the resin followed by (5:1) 600 μL DMF:H_2O. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**.

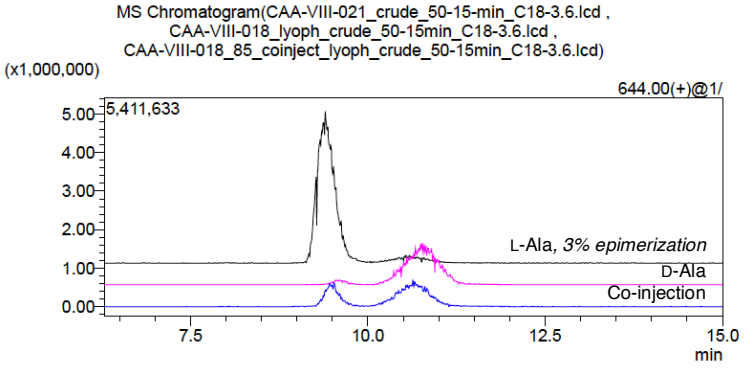


Figure SI-69. Evaluation of C-terminal alanine epimerization with proline. A) L-Ala crude, B) D-Ala crude, and C) co-injection, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min. *For conversion see Figure SI-55.*

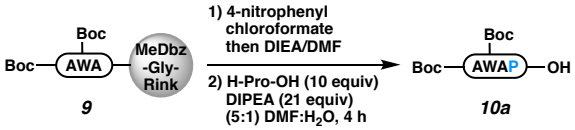


Table 3, entry 7: 10 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin followed by (5:1) 300 μL DMF:H_2O. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**. The ratio of product to acid is 87:17 by PDA (190 nm).

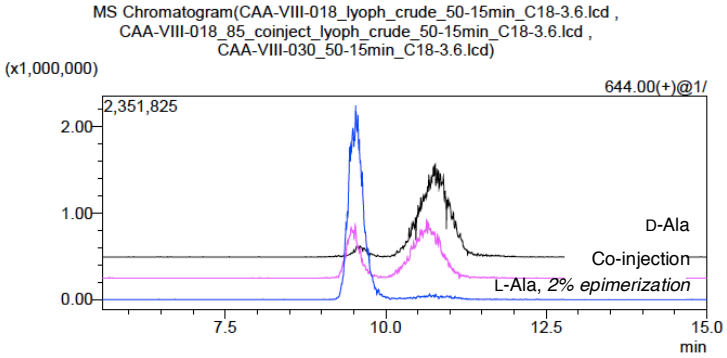


Figure SI-70. Evaluation of C-terminal alanine epimerization with proline. A) L-Ala crude, B) L-Ala purified, C) D-Ala crude, and C) co-injection, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min. *For conversion see Figure SI-49.*

Table 4. LYRAGLRAY elongation to access protected peptide acids.

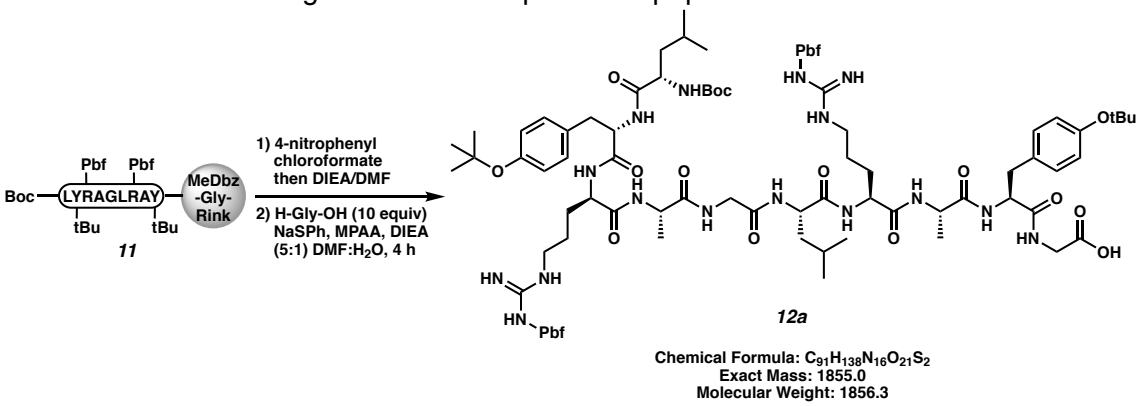


Table 4, entry 1: 20 mg of **11** (7 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Gly-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL (5:1) DMF:modified NCL Buffer (see guanidine-free NCL buffer). Then 21 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 (LN_2), and lyophilized to yield the crude peptide **12a**.

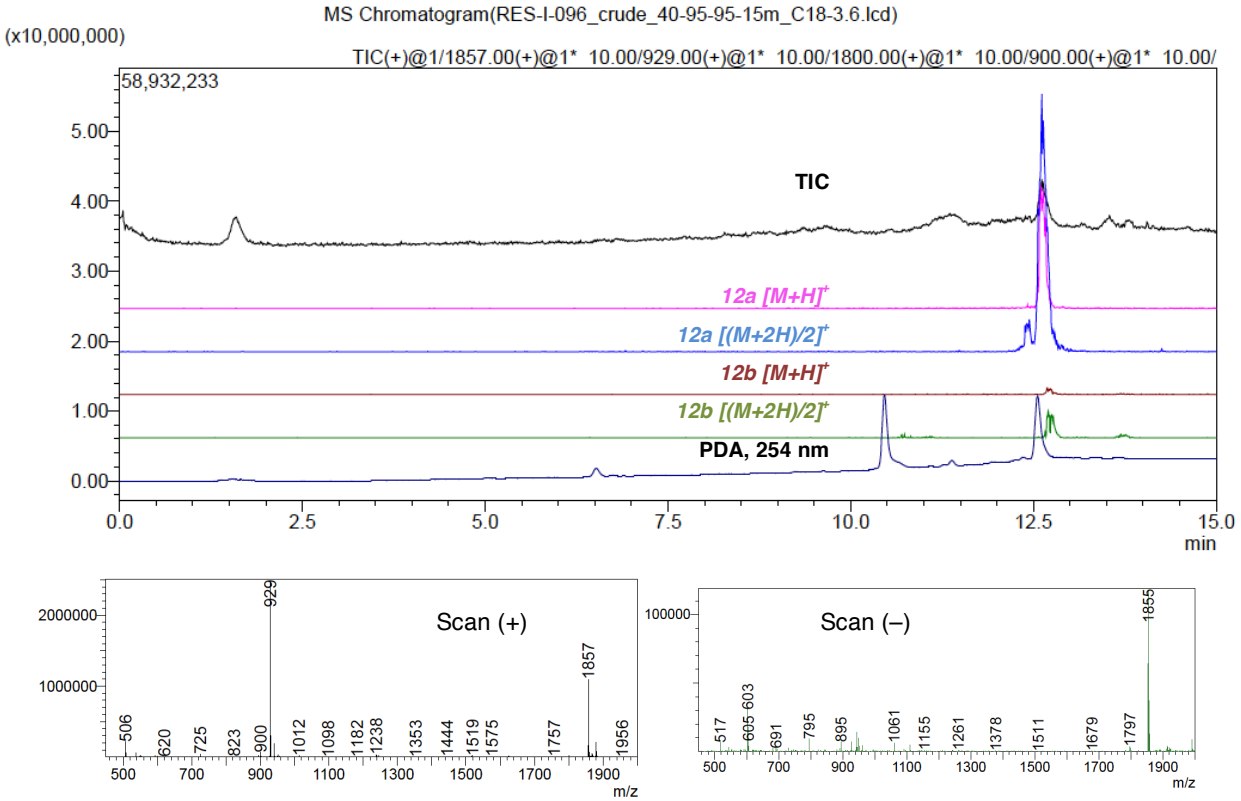


Figure SI-71. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

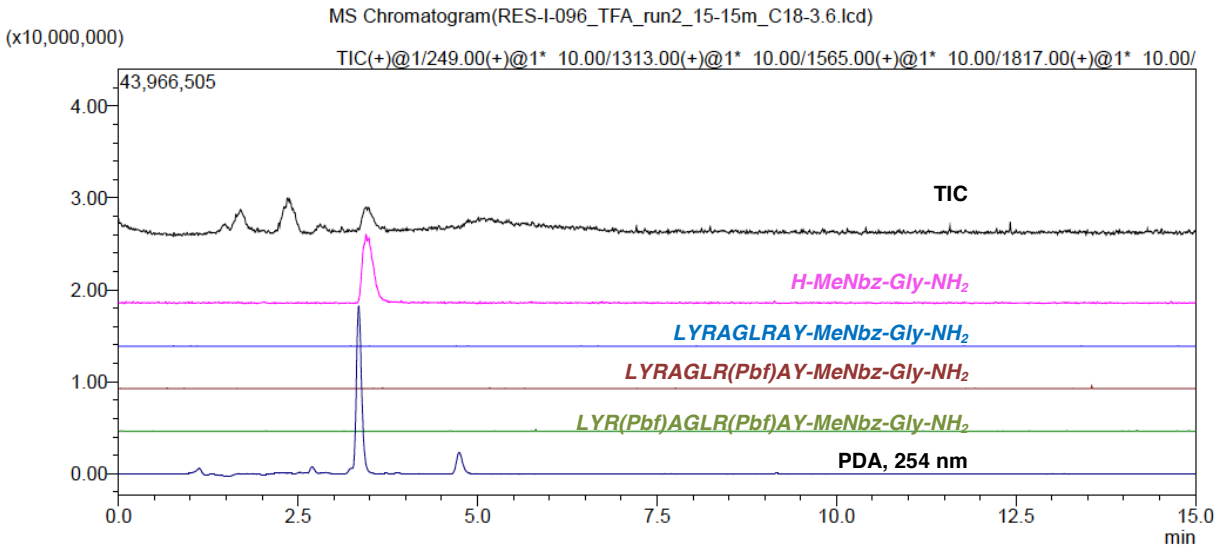


Figure SI-72. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

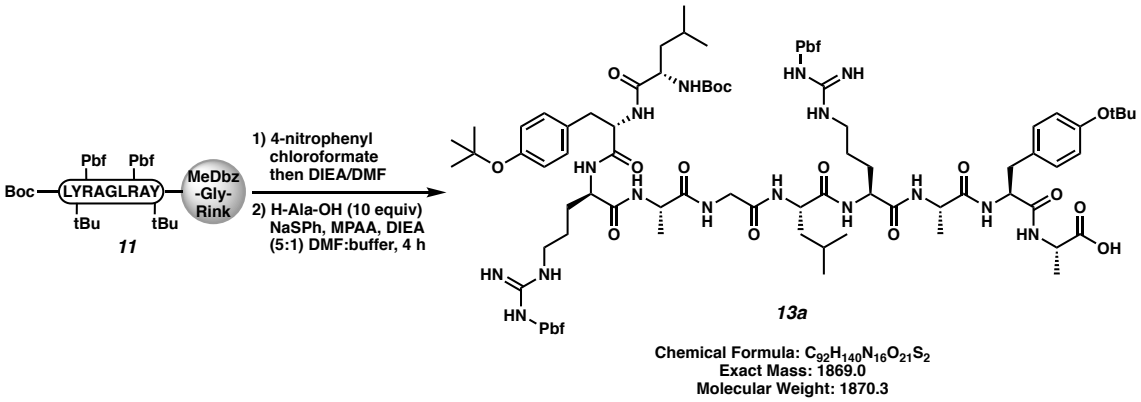


Table 4, entry 2: 20 mg of **11** (7 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ala-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL (5:1) DMF:modified NCL buffer (see guanidine-free NCL buffer). Then 21 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 (LN_2), and lyophilized to yield the crude peptide **13a**.

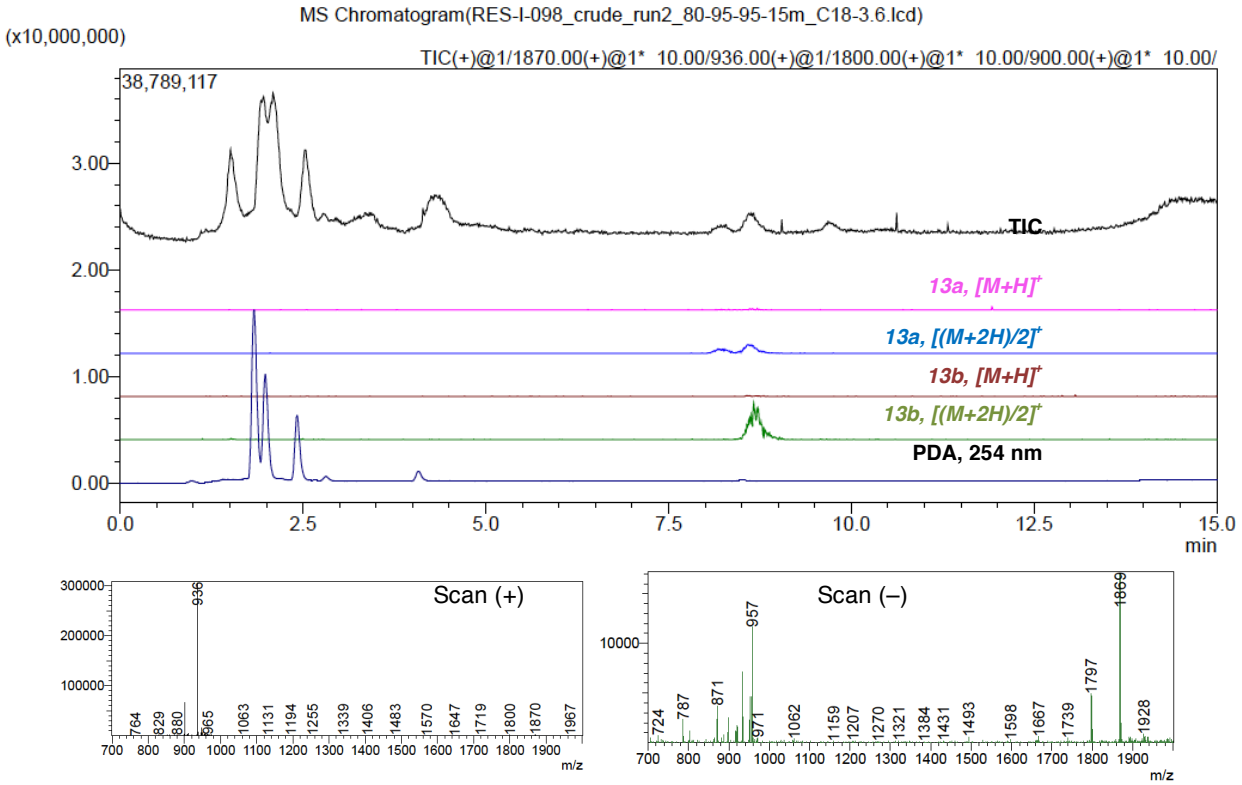


Figure SI-73. Analytical Column PDA of crude peptide at 254 nm. Gradient: 80-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

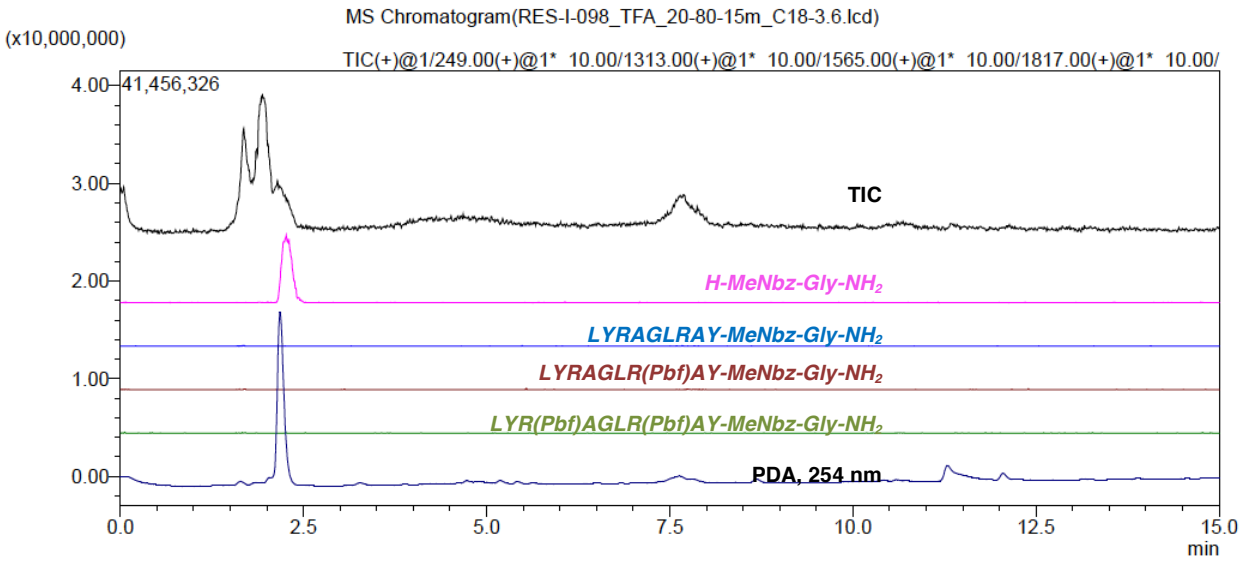


Figure SI-74. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

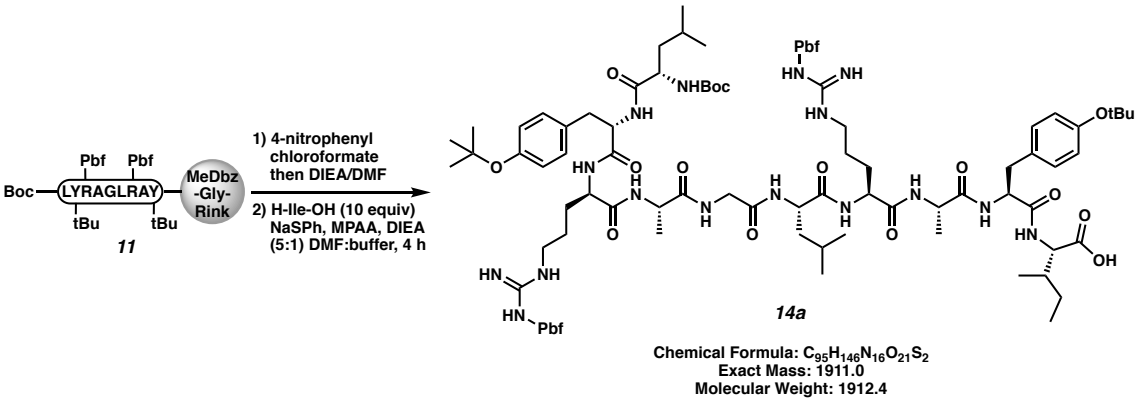


Table 4, entry 3: 20 mg of **11** (7 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Ile-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL (5:1) DMF:modified NCL Buffer (see guanidine-free NCL buffer). Then 21 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 (LN_2), and lyophilized to yield the crude peptide **14a**.

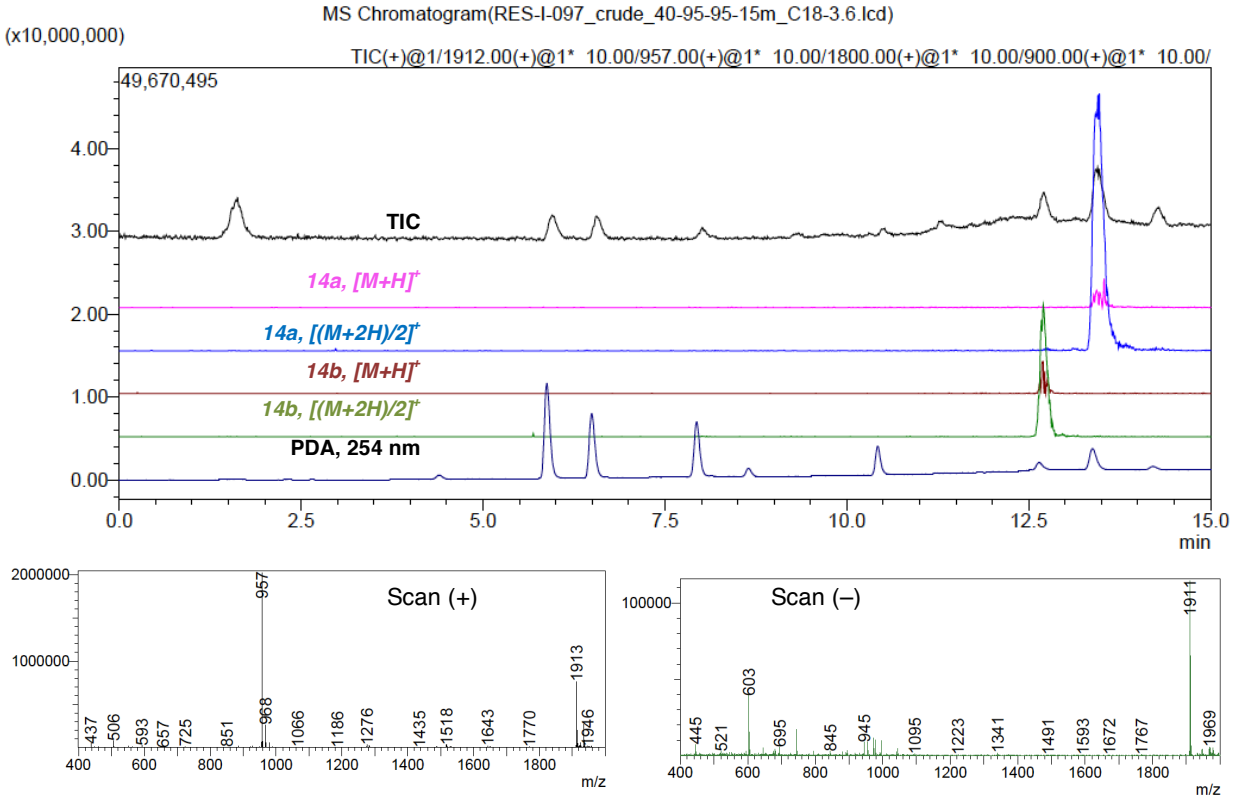


Figure SI-75. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

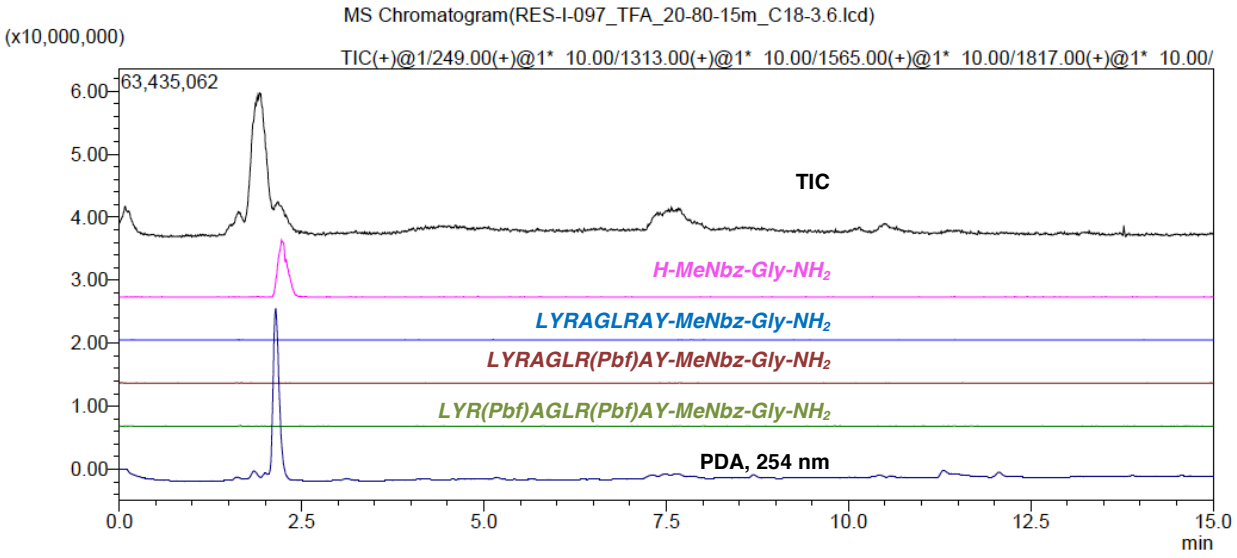


Figure SI-76. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

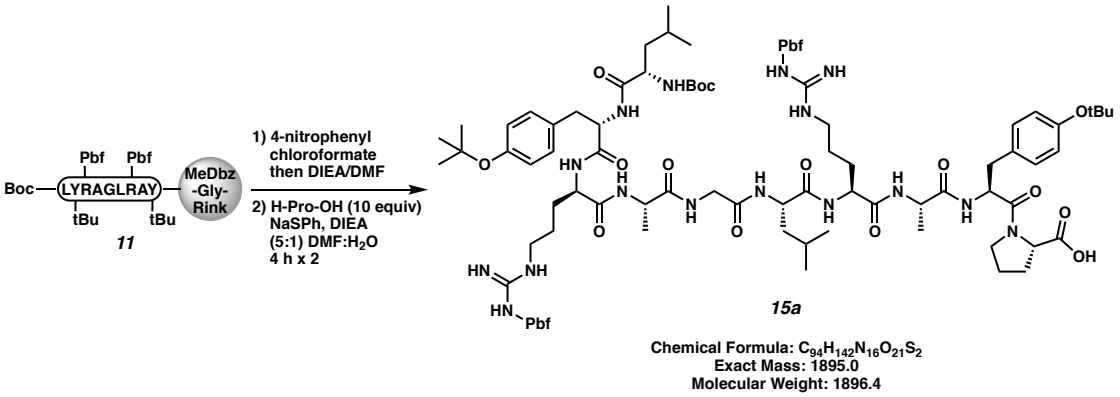


Table 4, entry 4: 100 mg of **11** (35 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH and 1 equiv of NaSPh were added directly to the resin followed by 600 μL DMF:H_2O (5:1). Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. This 4 h cleavage was repeated on the same resin the following day. 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was purified using RP-HPLC-MS to yield the pure white solid (**15a**) in 9% yield (7.5 mg, 70% HPLC purity).

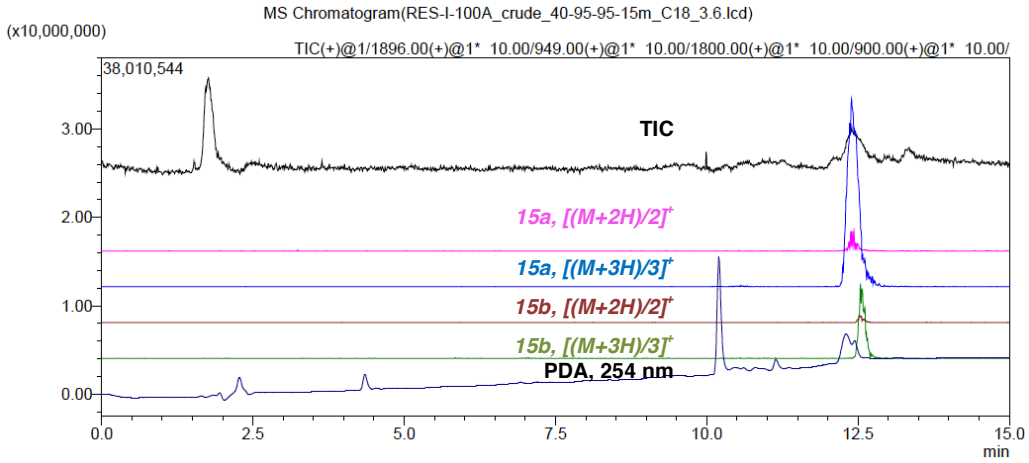


Figure SI-77. Analytical Column PDA of crude peptide at 254 nm. Gradient: 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH).

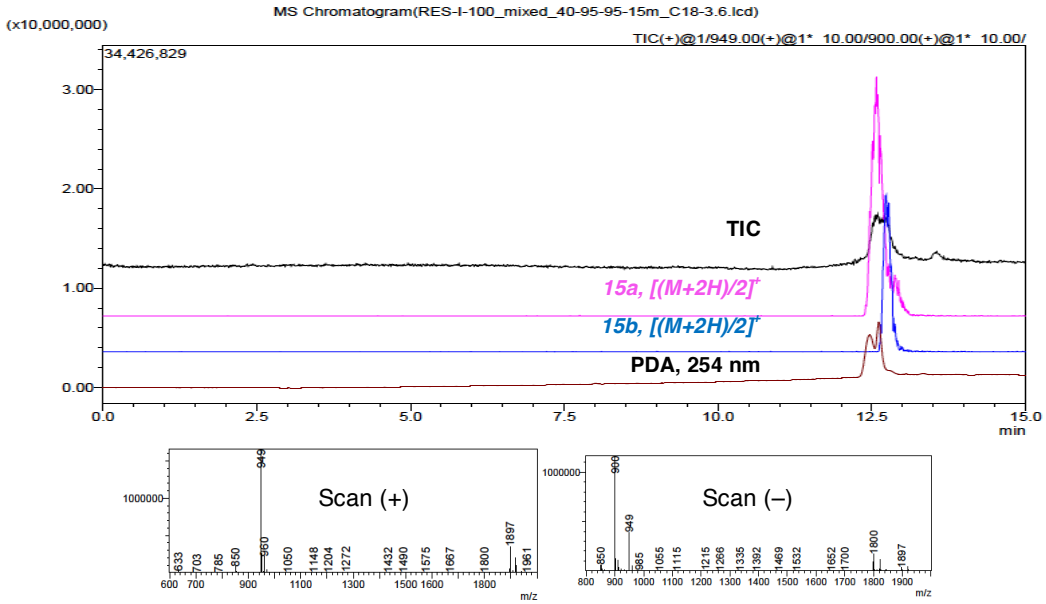


Figure SI-78. Analytical Column PDA of mixed peptides, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

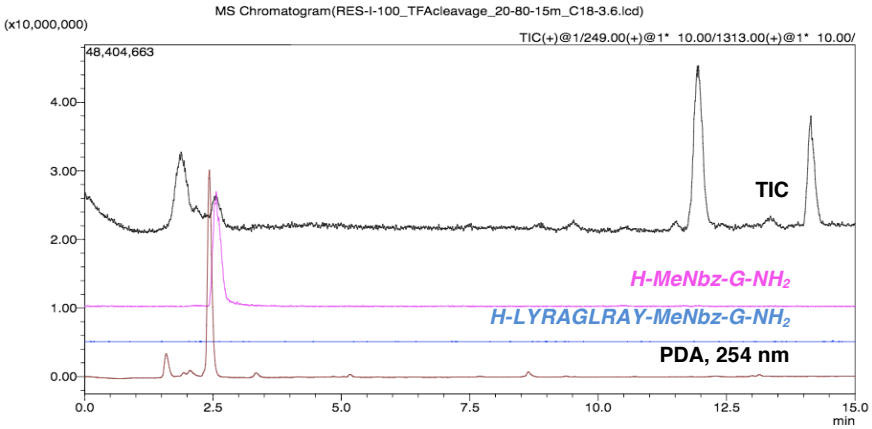


Figure SI-79. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

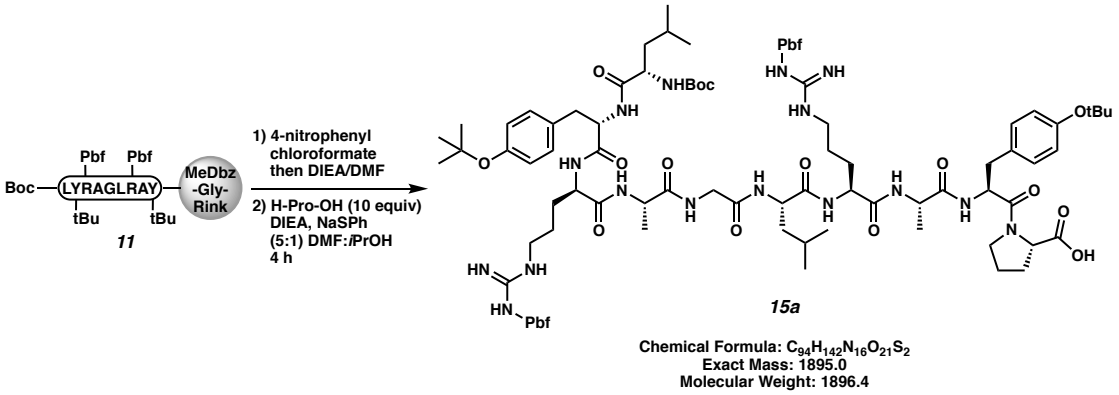


Table 4, entry 5: 20 mg of **11** (7 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin along with 1 equiv NaSPh followed by 600 μL DMF:*i*PrOH (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was analyzed via RP-HPLC-MS.

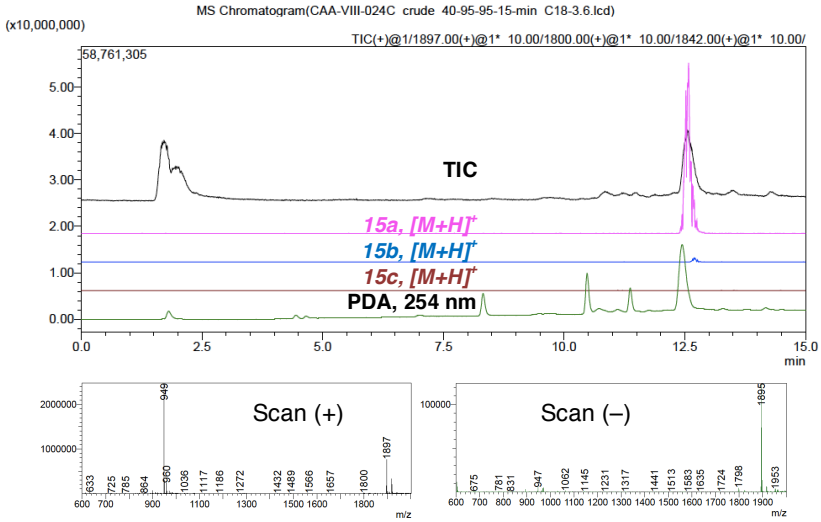


Figure SI-80. Analytical Column PDA of mixed peptides, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

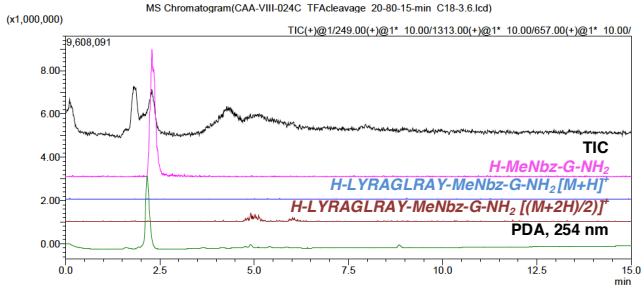


Figure SI-81. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

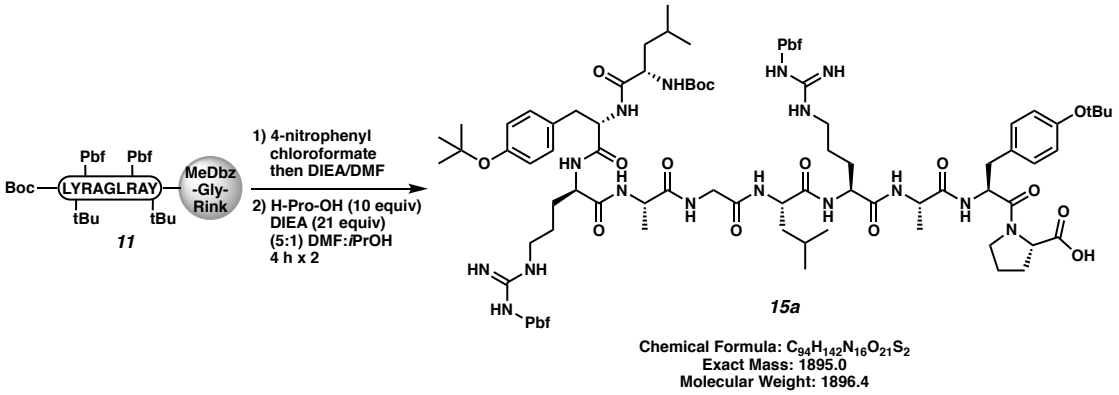


Table 4, entry 6: 100 mg of **11** (35 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin followed by 600 μL DMF:*i*PrOH (5:1). Then 21 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. Then 10 equiv of H-Pro-OH was added directly to the resin followed by 600 μL DMF:*i*PrOH (5:1). Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. The solvent was removed and collected with the initial reaction mixture. 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was purified using RP-HPLC-MS to yield the pure white solid (**15a**) in 11% yield (4.4 mg, >99% HPLC purity and 3.6 mg, 76% HPLC purity).

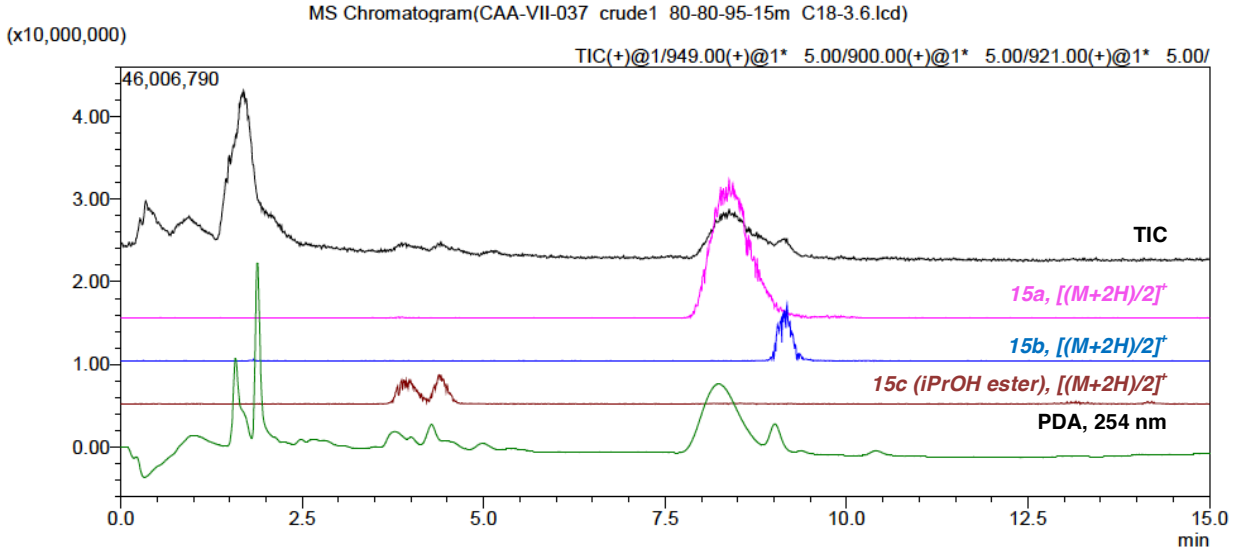


Figure SI-82. Analytical Column PDA of crude reaction mixture, gradient of 80-80(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

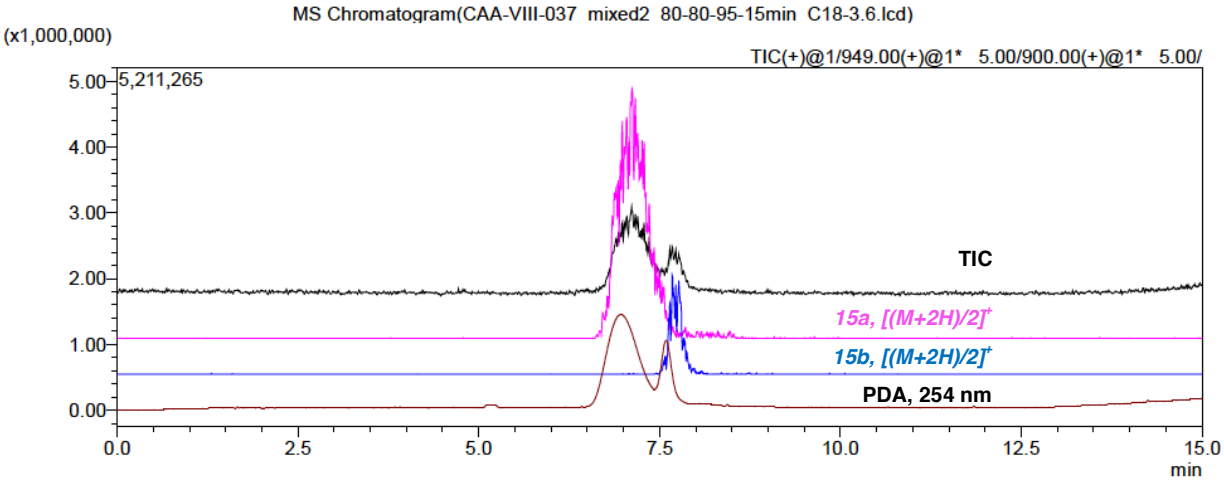


Figure SI-83. Analytical Column PDA of mixed peptides, gradient of 80-80(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

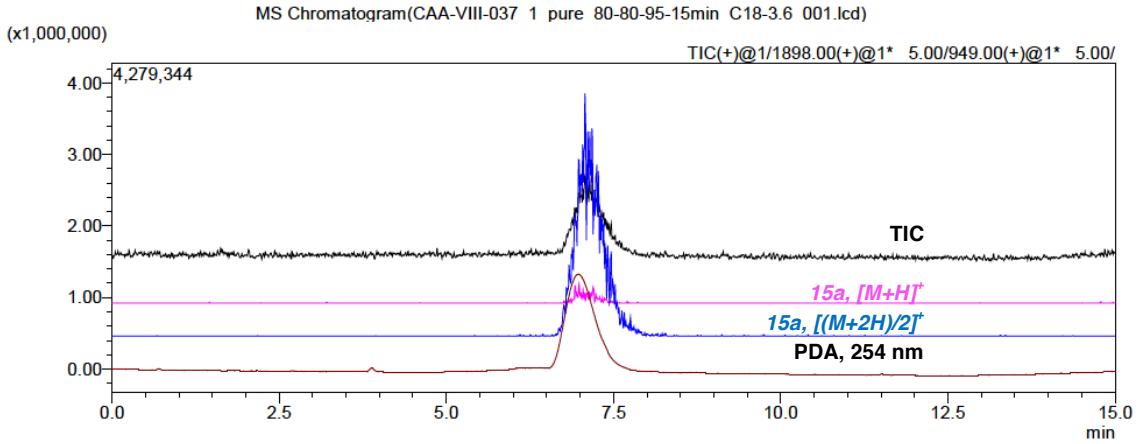


Figure SI-84. Analytical Column PDA of pure peptide, gradient of 80-80(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

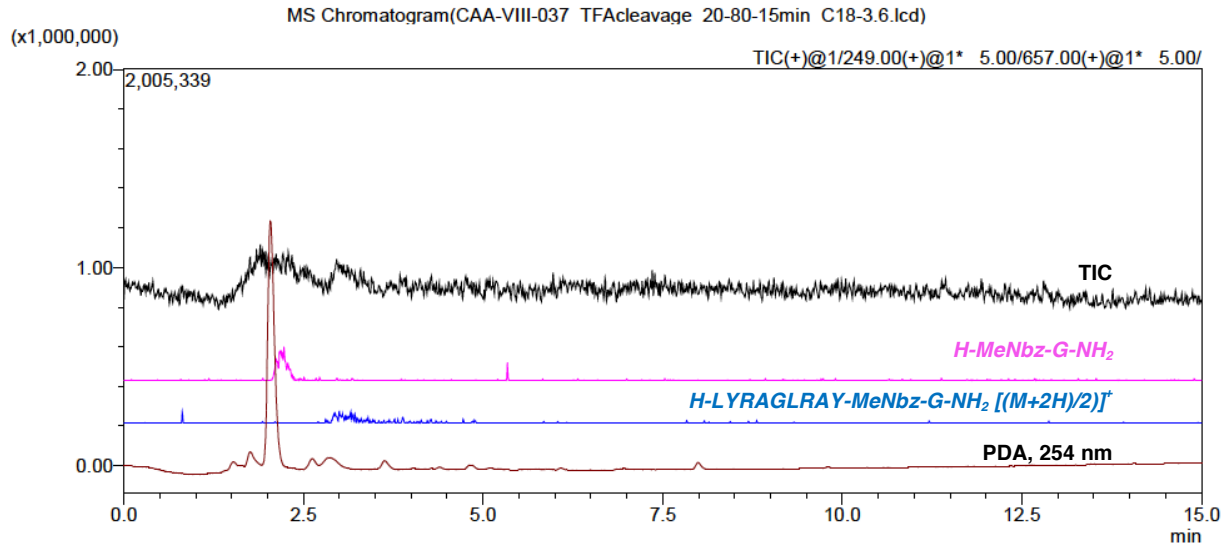


Figure SI-85. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

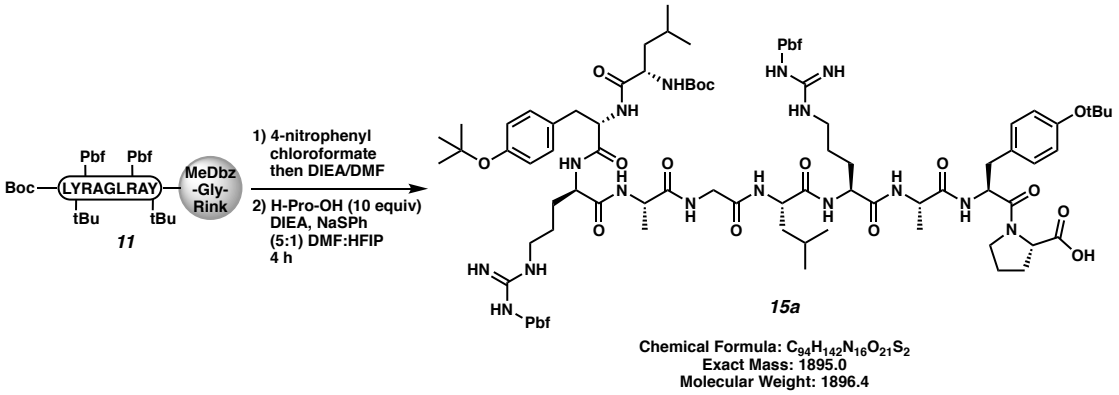


Table 4, entry 7: 20 mg of **11** (7 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin along with 1 equiv NaSPh followed by 600 μL DMF:HFIP (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was analyzed via RP-HPLC-MS.

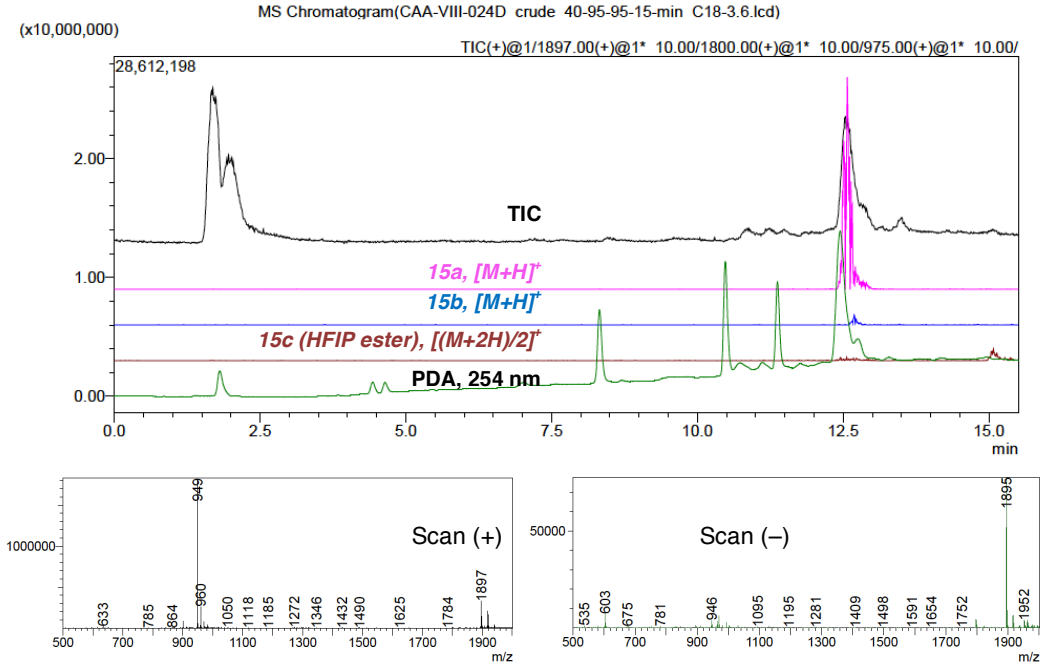


Figure SI-86. Analytical Column PDA of crude reaction mixture, gradient of 40-95(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

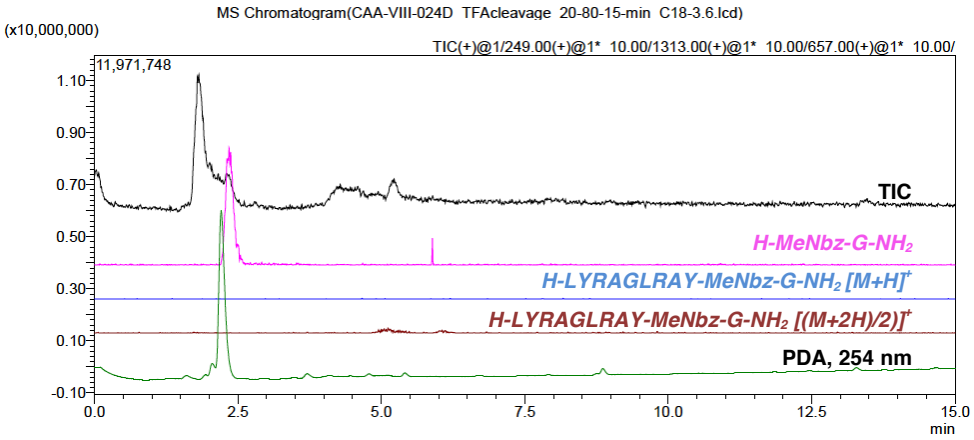


Figure SI-87. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

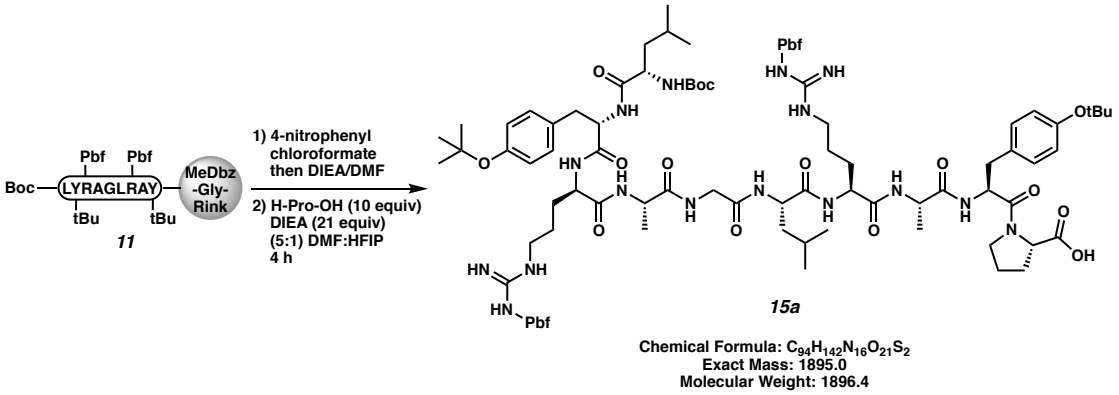


Table 4, entry 8: 100 mg of **11** (35 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin followed by 600 μL DMF:HFIP (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide. The crude peptide was purified using RP-HPLC-MS to yield the pure white solid in 10% yield (7.4 mg, 91% HPLC purity).

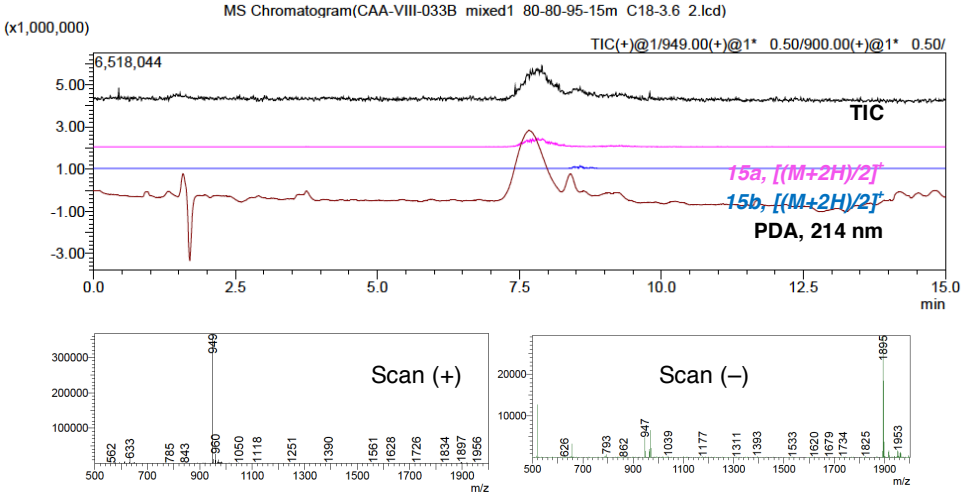


Figure SI-88. Analytical Column PDA of mixed peptides, gradient of 80-80(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

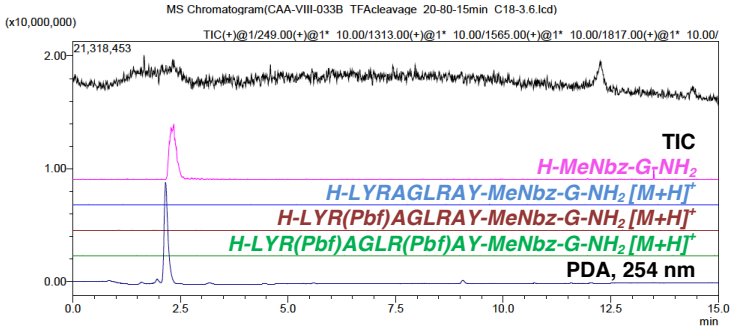
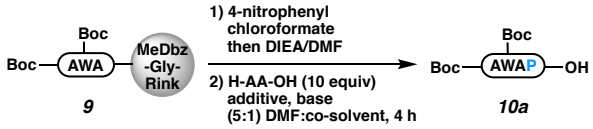


Figure SI-89. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.



entry[a]	DIEA (equiv)	additive	co-solvent	% conversion[b]	epimerization (%D-Ala)[c]
1	21	NaSPh	*i*PrOH	83	4
2	21	NaSPh	HFIP	>99	4
3	21	–	*i*PrOH	74	<1
4	21	–	HFIP	>99	<1

[a] All reactions were conducted on 10 mg of resin in 300 μL (5:1) DMF:solvent, rt = 24 ± 1 °C, [b] Conversion based on integration of remaining peptide on activated linker after nucleophile cleavage in MS data,[c] Relative ratios of MS/HPLC data.

Table SI-01. Evaluation of epimerization of AWA using alcohol co-solvents.

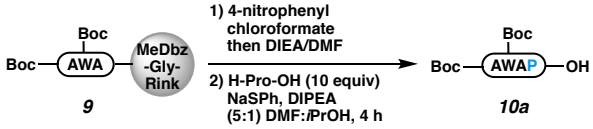


Table SI-01, entry 1: 9 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH and 1 equiv NaSPh was added directly to the resin followed by (5:1) 300 μL DMF:*i*PrOH. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**.

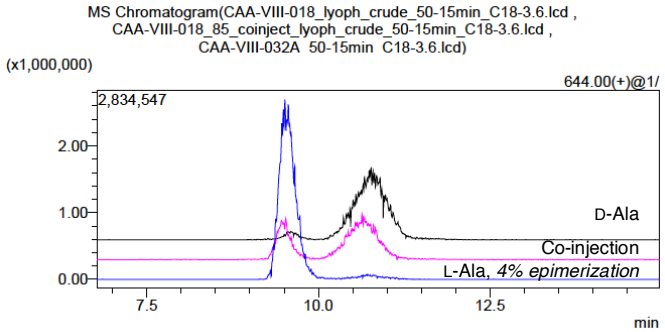


Figure SI-90. Evaluation of C-terminal alanine epimerization with proline. A) D-Ala crude, B) co-injection, and C) L-Ala purified, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

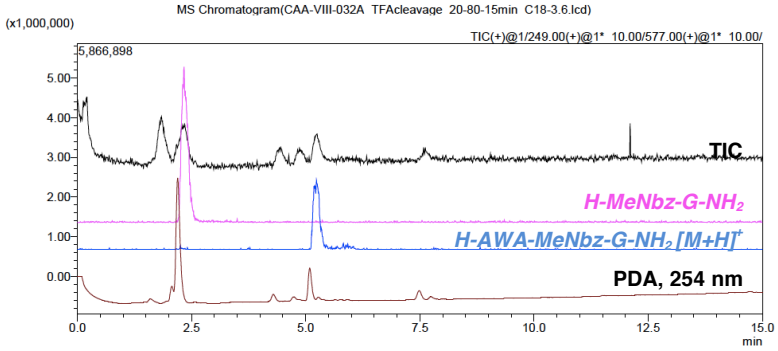


Figure SI-91. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

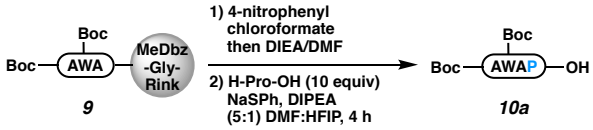


Table SI-01, entry 2: 9 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH and 1 equiv NaSPh was added directly to the resin followed by (5:1) 300 μL DMF:HFIP. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**.

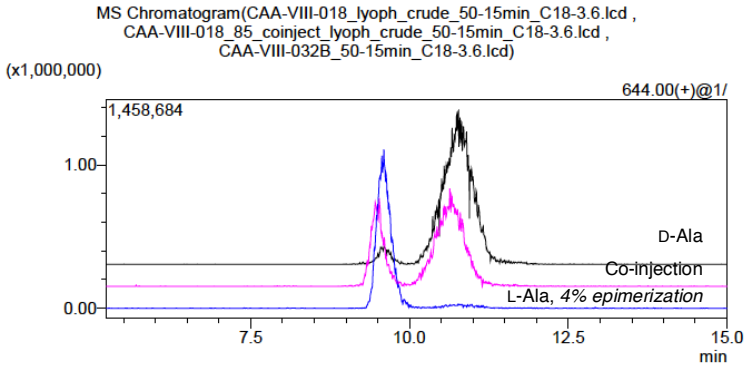


Figure SI-92. Evaluation of C-terminal alanine epimerization with proline. A) D-Ala crude, B) co-injection, and C) L-Ala purified, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

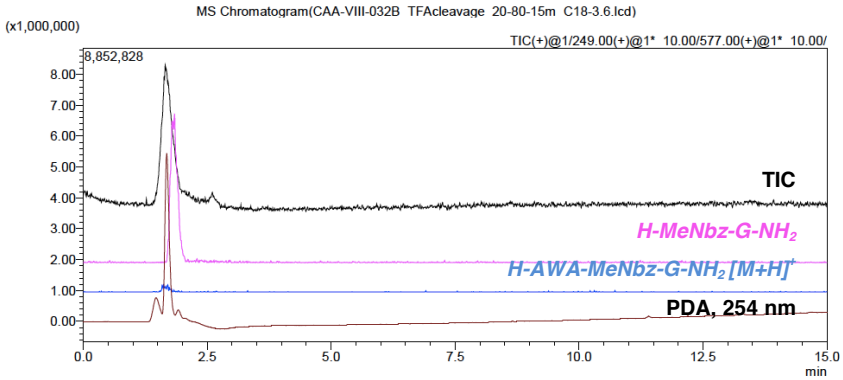


Figure SI-93. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

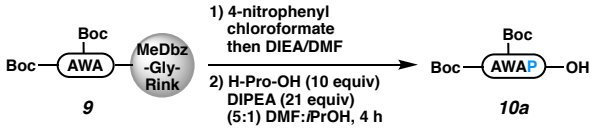


Table SI-01, entry 3: 9 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin followed by (5:1) 300 μL DMF:*i*PrOH. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**. The ratio of product to acid to ester is 87:5:8 by PDA (190 nm).

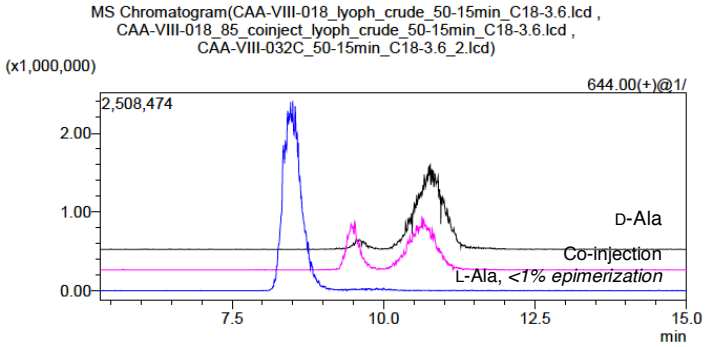


Figure SI-94. Evaluation of C-terminal alanine epimerization with proline. A) D-Ala crude, B) co-injection, and C) L-Ala purified, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

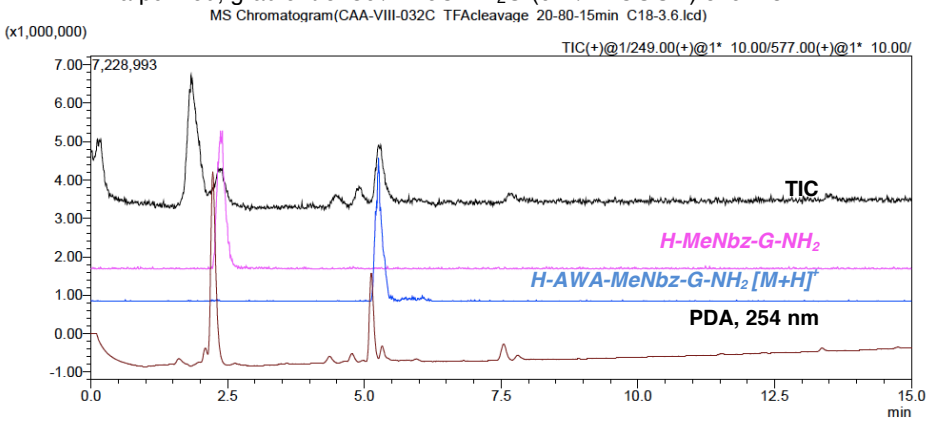


Figure SI-95. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

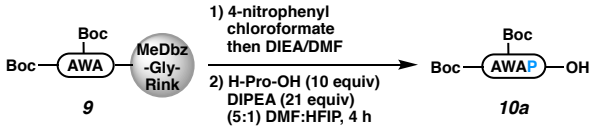


Table SI-01, entry 4: 9 mg of **9** (2 μmol) was weighed out into a 2 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Pro-OH was added directly to the resin followed by (5:1) 300 μL DMF:HFIP. Then 21 equiv of freshly distilled DIEA was added and the reaction vial was agitated at ambient temperature for 4 h. After this time, 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 (LN_2), and lyophilized to yield the crude peptide **10a-P-OH**. The ratio of product to acid to ester is 47:3:50 by PDA (190 nm).

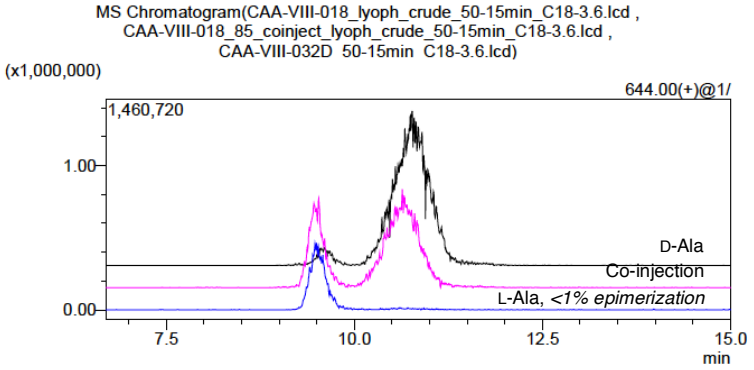


Figure SI-96. Evaluation of C-terminal alanine epimerization with proline. A) D-Ala crude, B) co-injection, and C) L-Ala purified, gradient of 50% MeCN/H_2O (0.1% HCOOH) over 15 min.

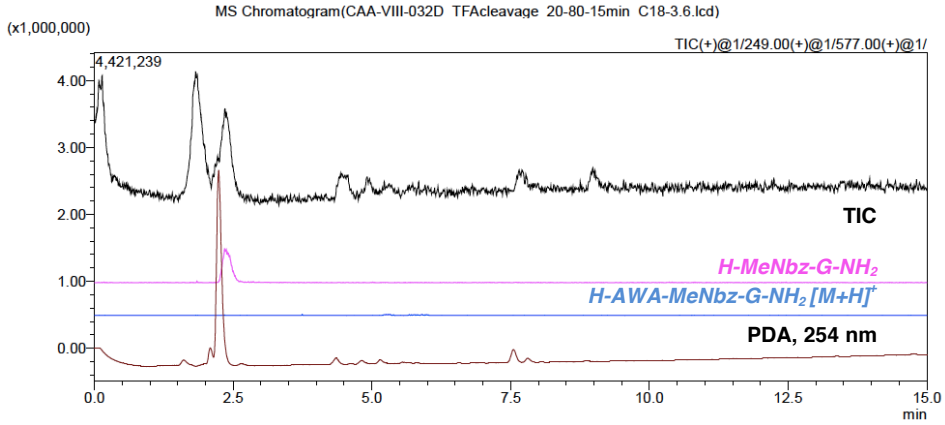


Figure SI-97. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

Table 5. Side-chain protected amino acid elongation of LYRAGLRAY.

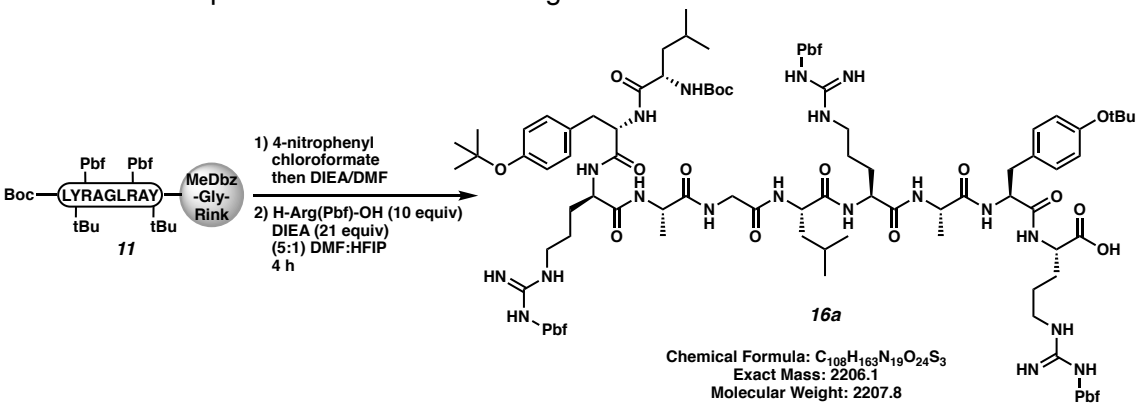


Table 5, entry 1: 50 mg of **11** (18 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Arg(Pbf)-OH was added directly to the resin followed by 600 μL DMF:HFIP (5:1). Then 21 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 (LN_2), and lyophilized to yield the crude peptide **16a**.

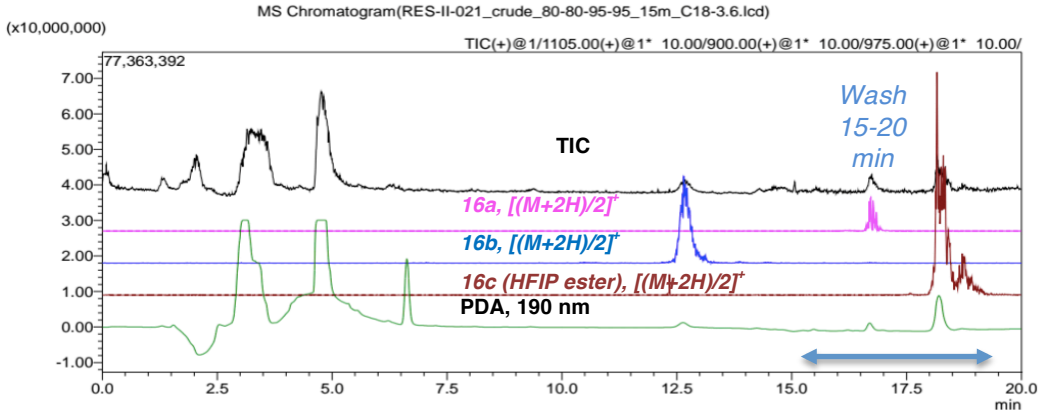


Figure SI-98. Analytical Column PDA of crude reaction mixture, gradient of 80-80(10 min)-95(1 min)-95(4 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

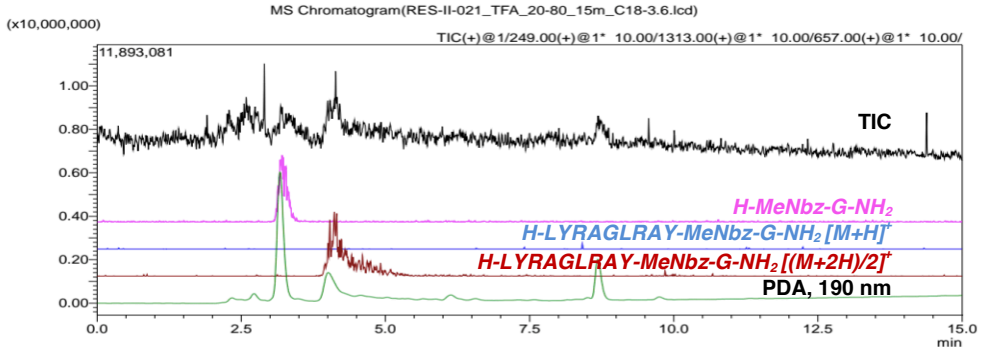


Figure SI-99. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

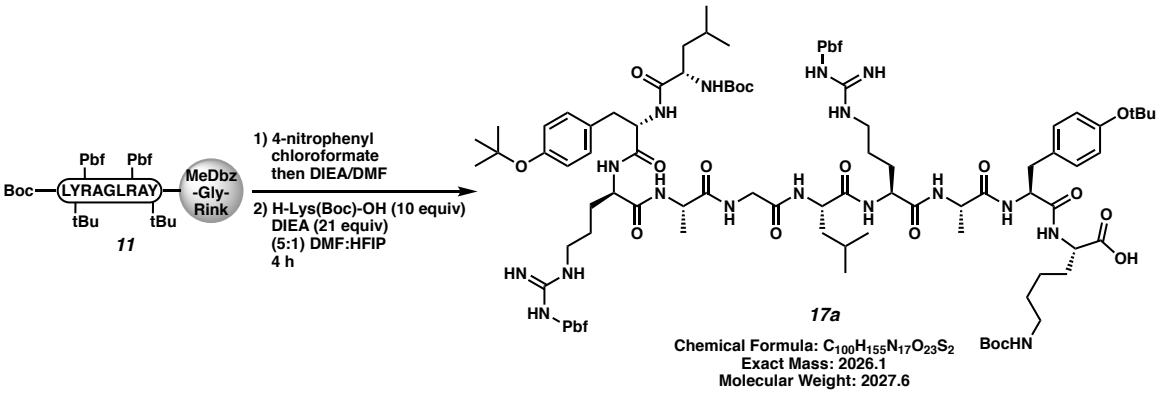


Table 5, entry 1: 50 mg of **11** (18 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Lys(Boc)-OH was added directly to the resin followed by 600 μL DMF:HFIP (5:1). Then 21 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 (1:1:3) TFE:AcOH: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 (LN_2), and lyophilized to yield the crude peptide **17a**. The crude peptide was purified using RP-HPLC-MS to yield the peptide in 5% yield (3.1 mg, 60% HPLC purity).

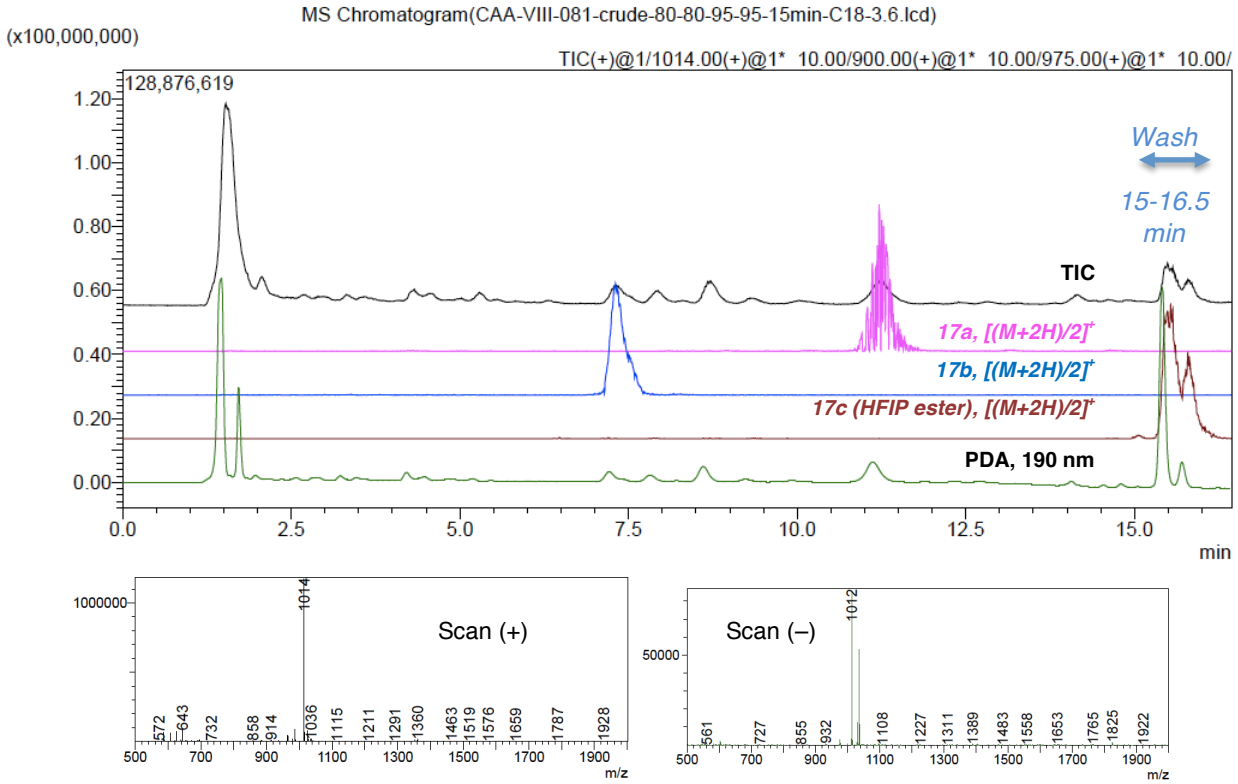


Figure SI-100. Analytical Column PDA of crude reaction mixture, gradient of 80-80(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

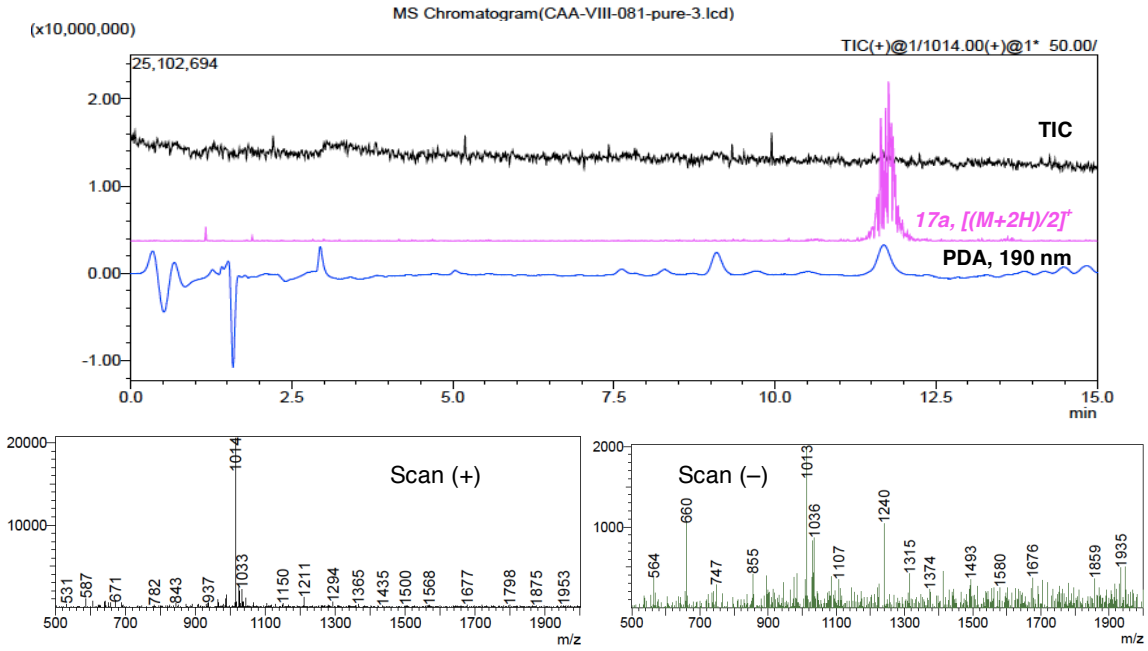


Figure SI-101. Analytical Column PDA of pure peptide, gradient of 80-80(10 min)-95(5 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

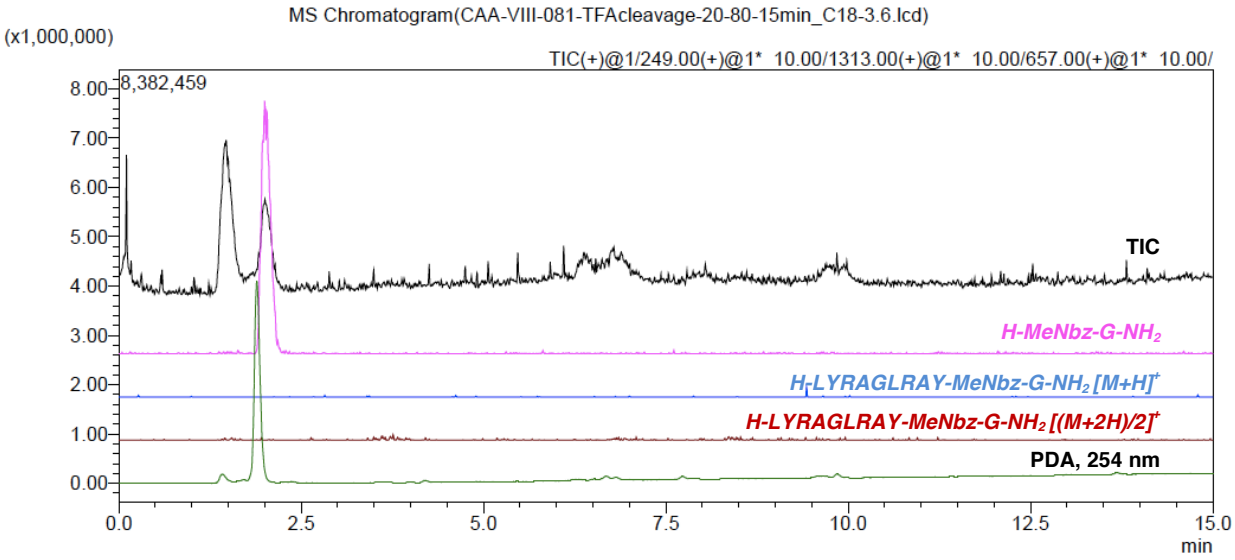


Figure SI-102. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

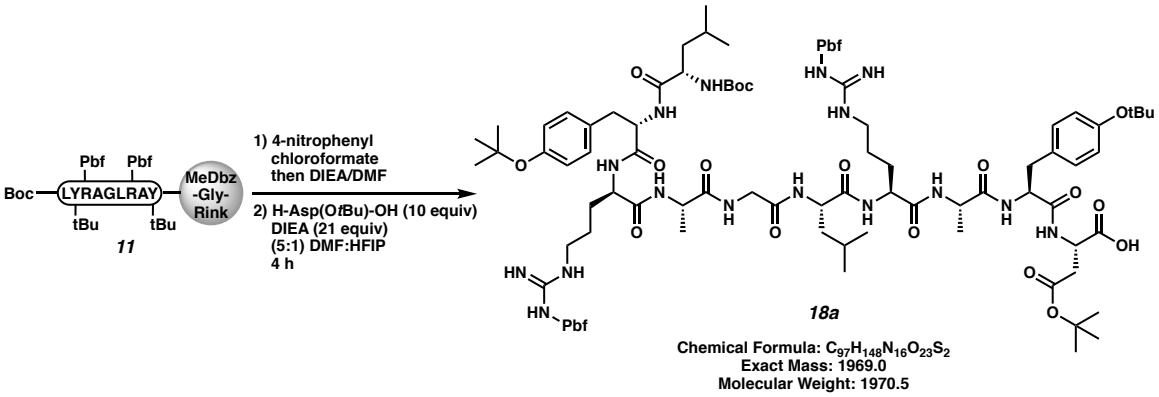


Table 5, entry 3: 50 mg of **11** (18 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-Asp(*t*Bu)-OH was added directly to the resin followed by 600 μL DMF:HFIP (5:1). Then 21 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 (1:1:3) TFE:AcOH: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 (LN_2), and lyophilized to yield the crude peptide **18a**. The peptide was purified by RP-HPLC-MS to produce the pure peptide **18a** in 7% yield (2.7 mg). **note: [M-tBu]$^+$ was observed*

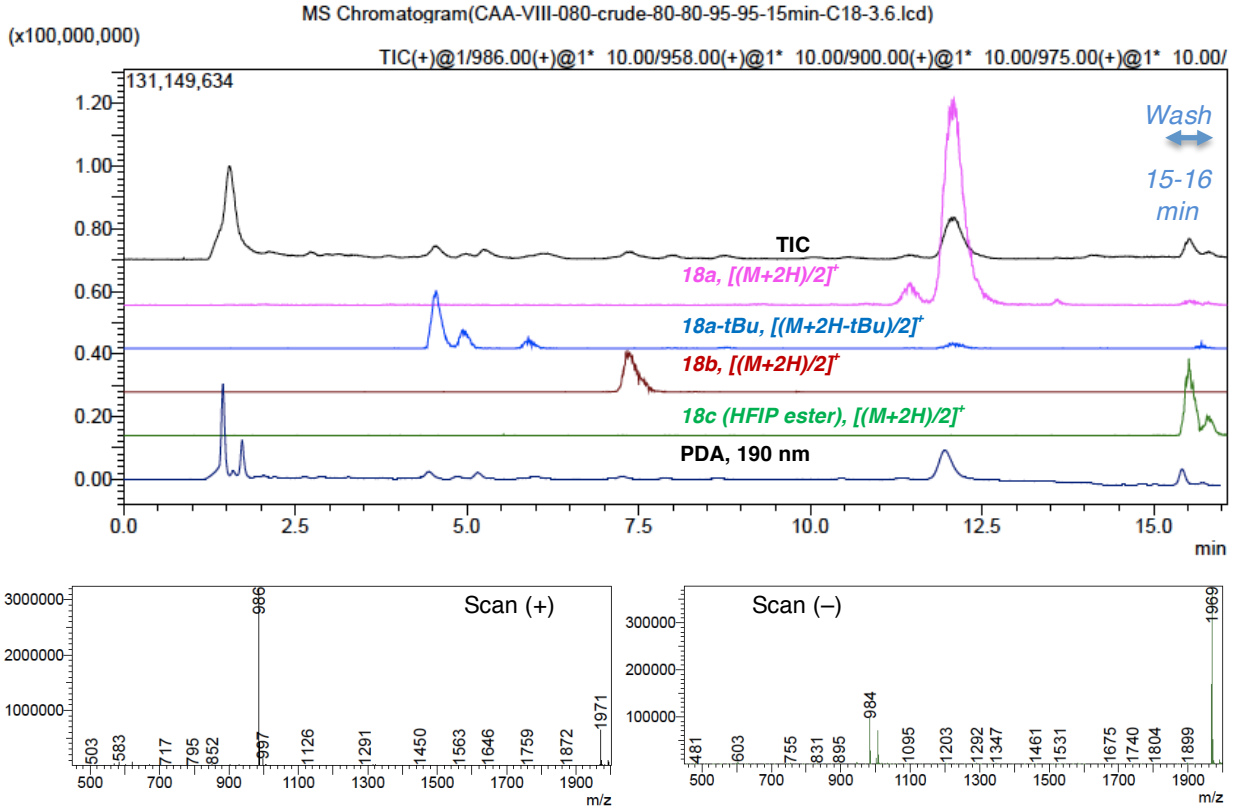


Figure SI-103. Analytical Column PDA of crude reaction mixture, gradient of 80-80(10 min)-95(1 min)-95(4 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

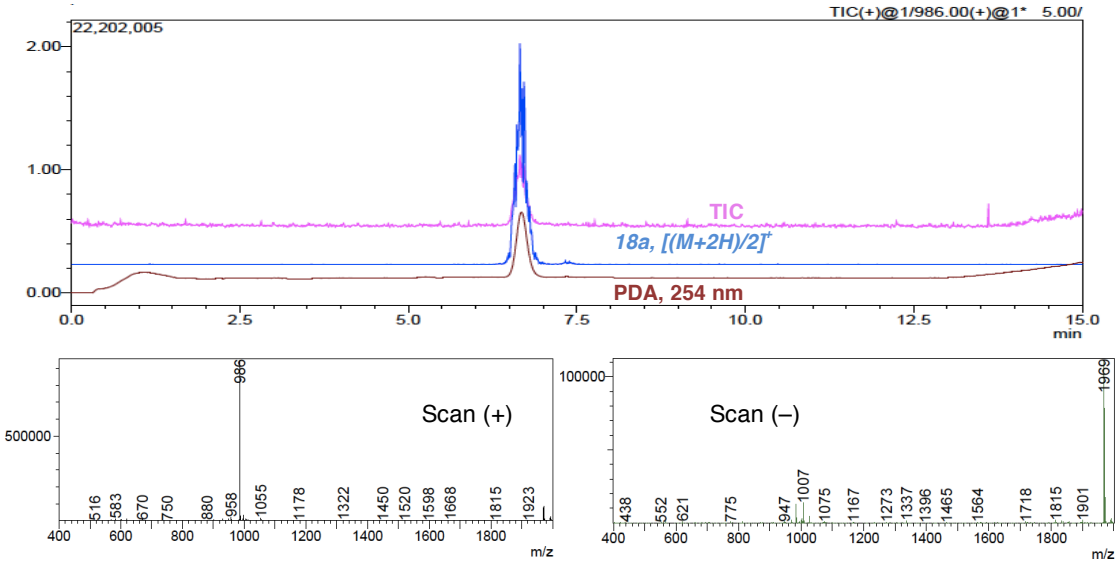


Figure SI-104. Analytical Column PDA of pure peptide, gradient of 80-80(10 min)-95(1 min)-95(4 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

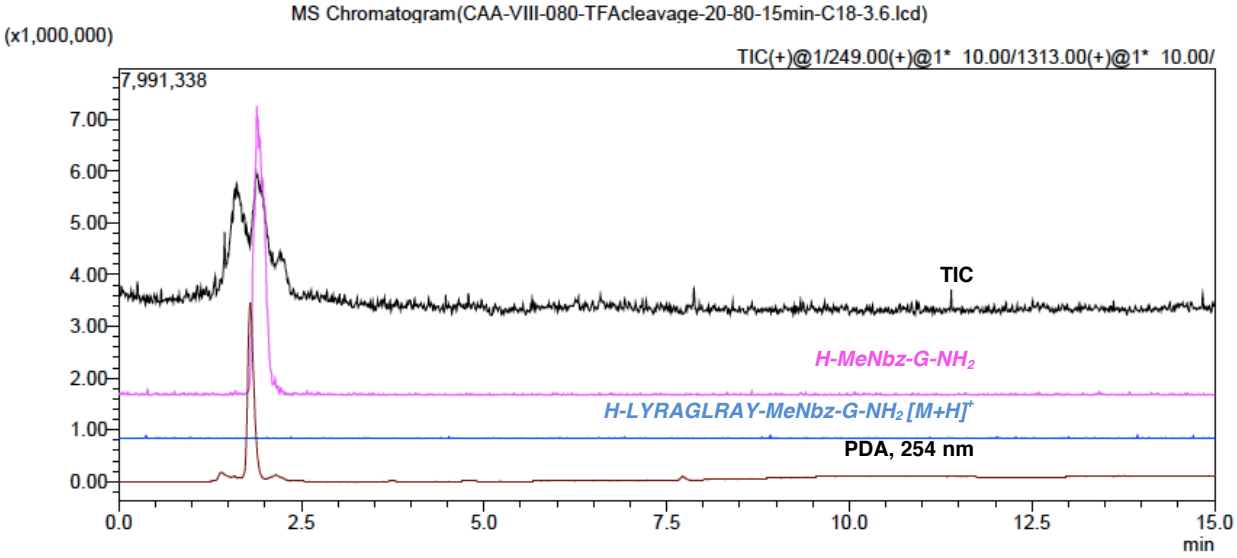


Figure SI-105. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

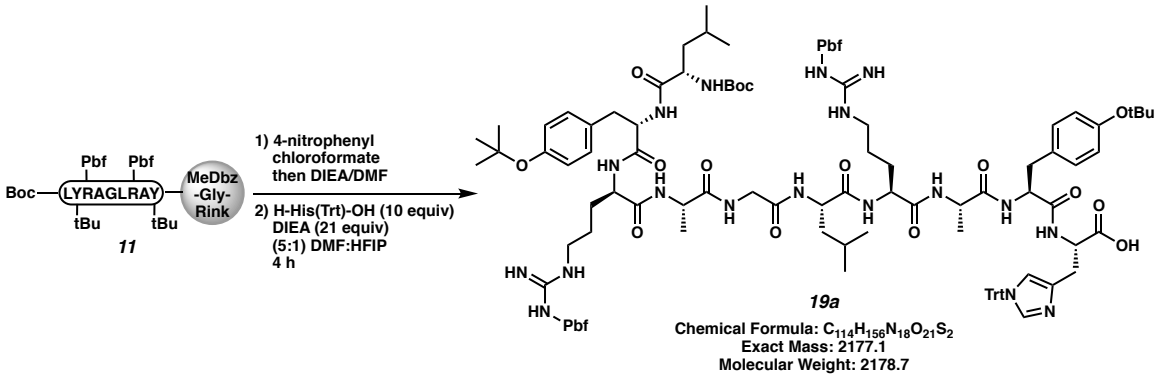


Table 5, entry 4: 50 mg of **11** (18 μmol) was weighed out into a 5 mL reaction vial and swelled in 500 μL CH_2Cl_2 for 30 min. After swelling the solvent was removed and the resin was activated (See General Procedure). 10 equiv of H-His(Trt)-OH was added directly to the resin followed by 600 μL DMF:HFIP (5:1). Then 21 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 (1:1:3) TFE:AcOH: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 (LN_2), and lyophilized to yield the crude peptide **19a**.

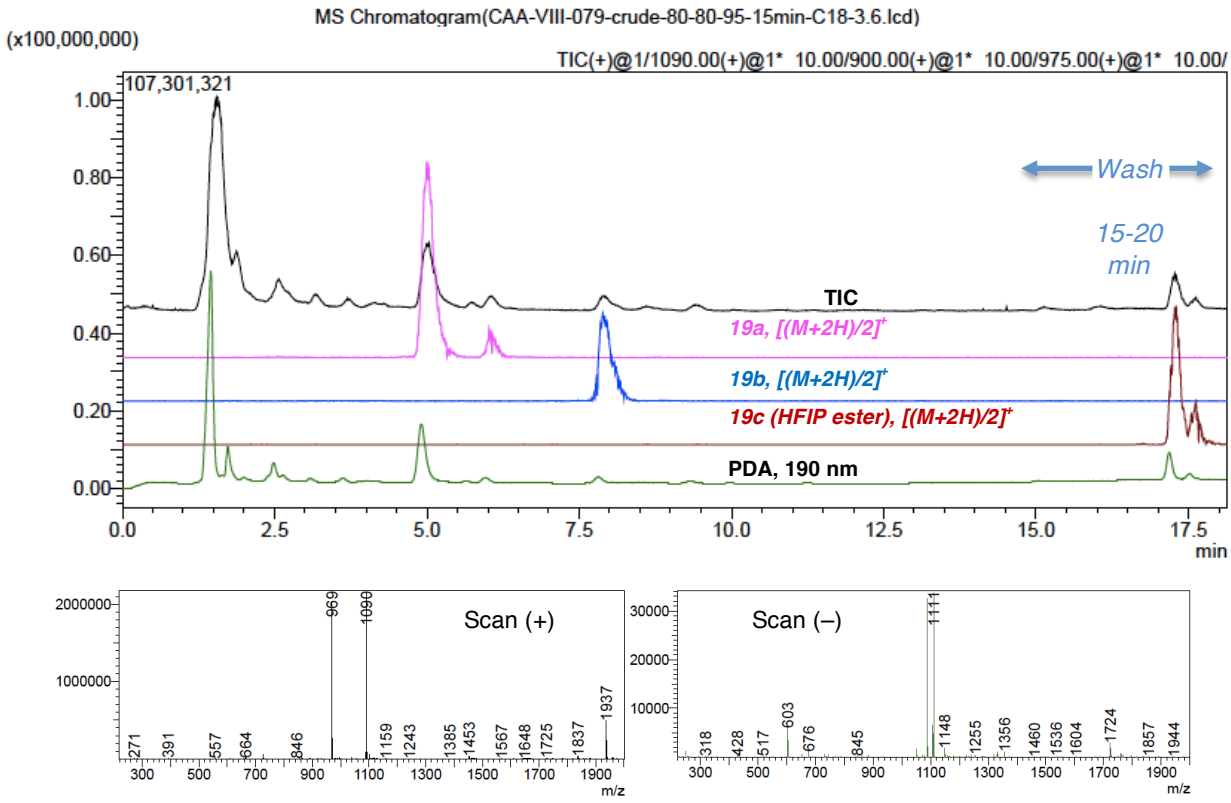


Figure SI-106. Analytical Column PDA of crude reaction mixture, gradient of 80-80(10 min)-95(1 min)-95(4 min)% MeCN/H_2O (0.1% HCOOH) over 15 min.

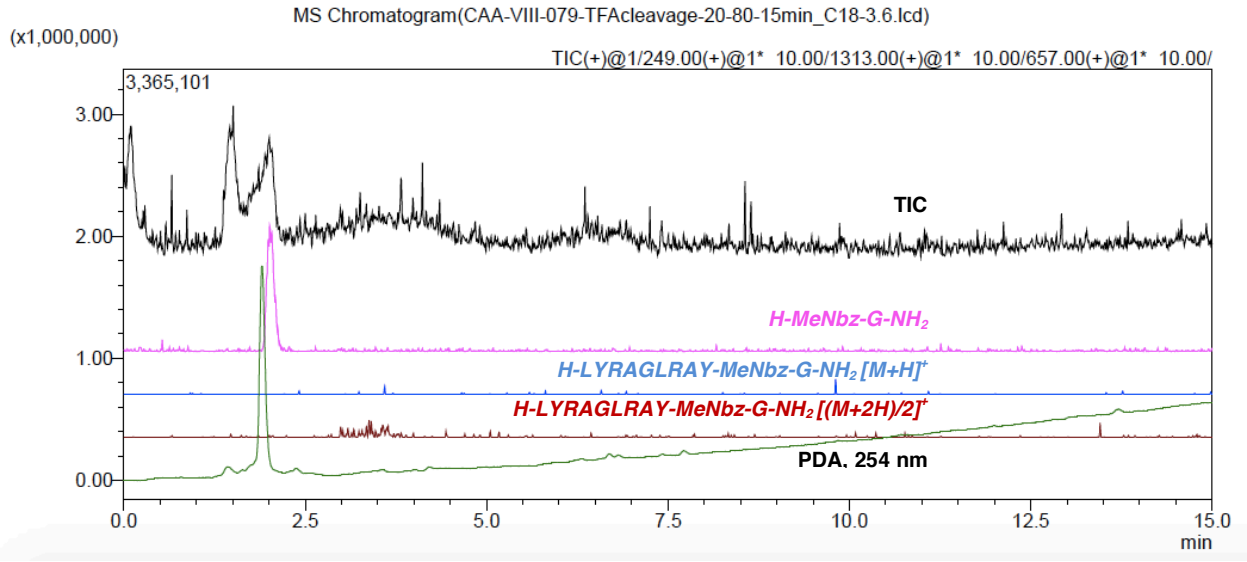
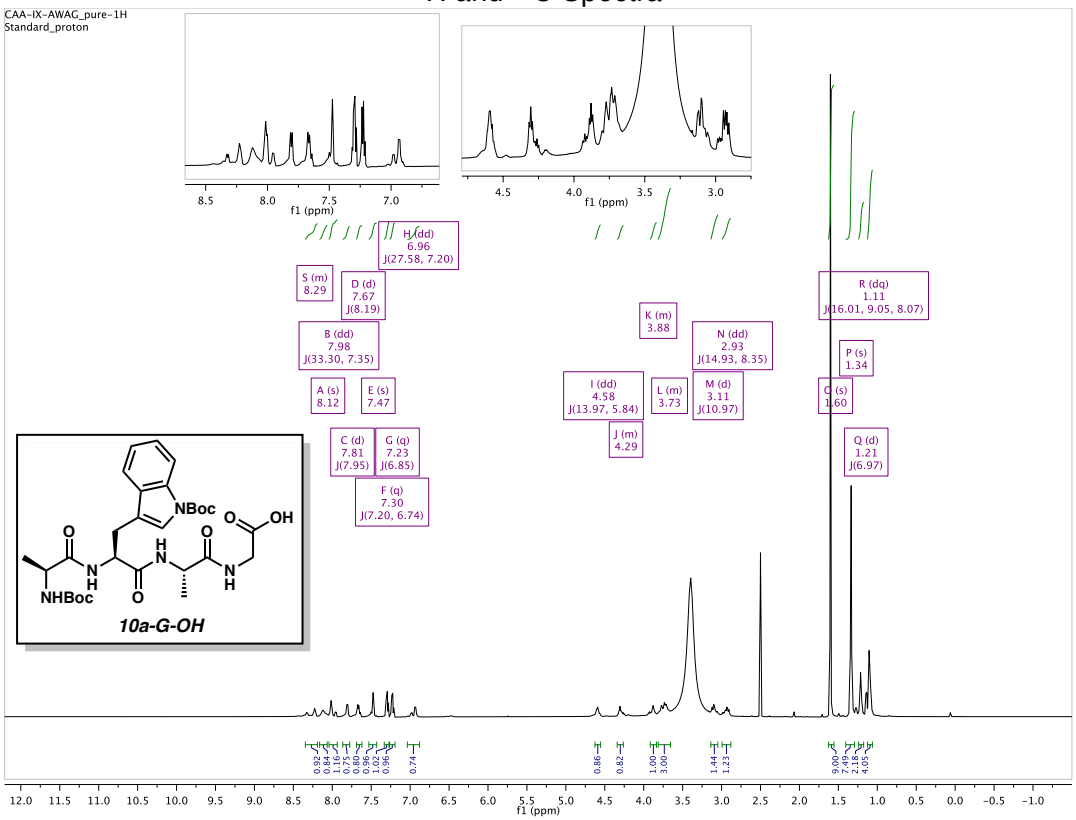
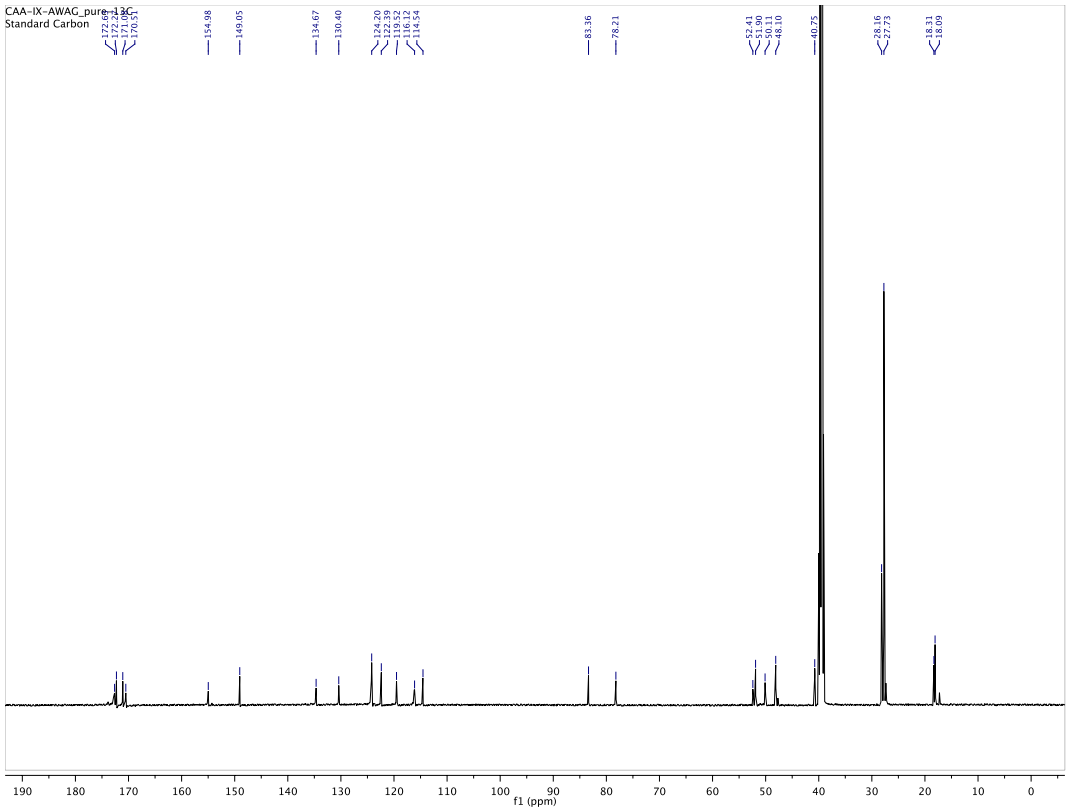
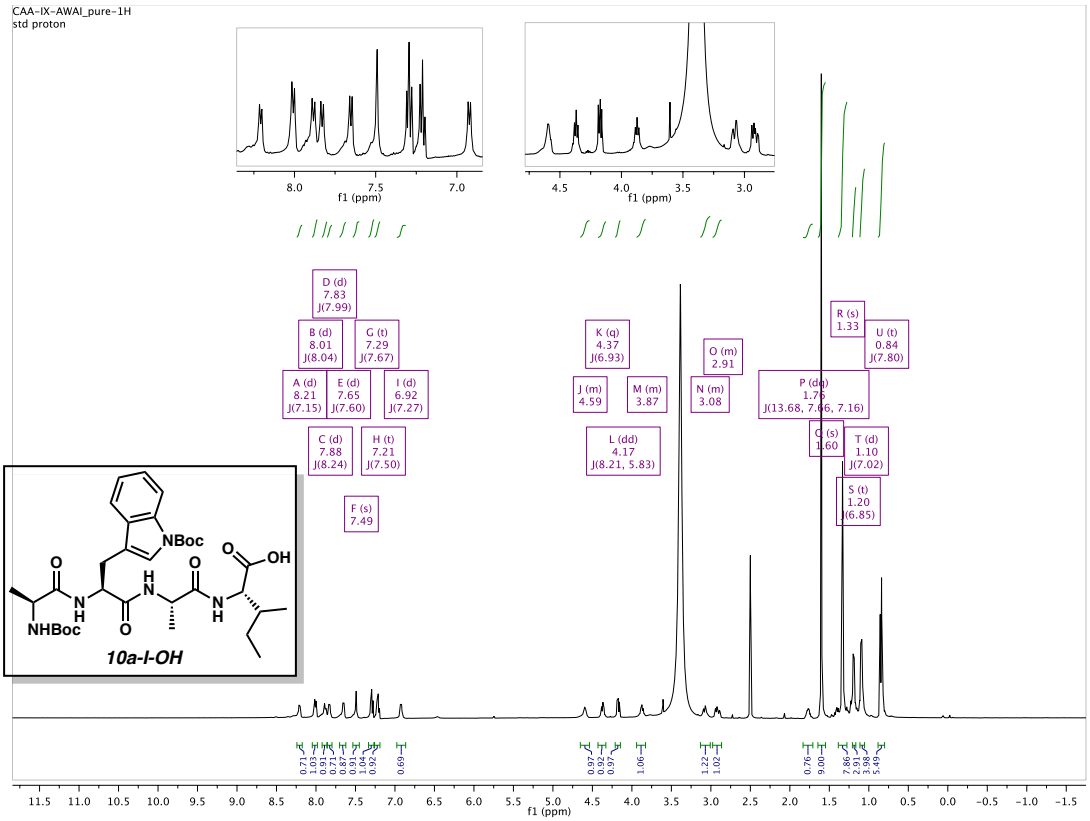


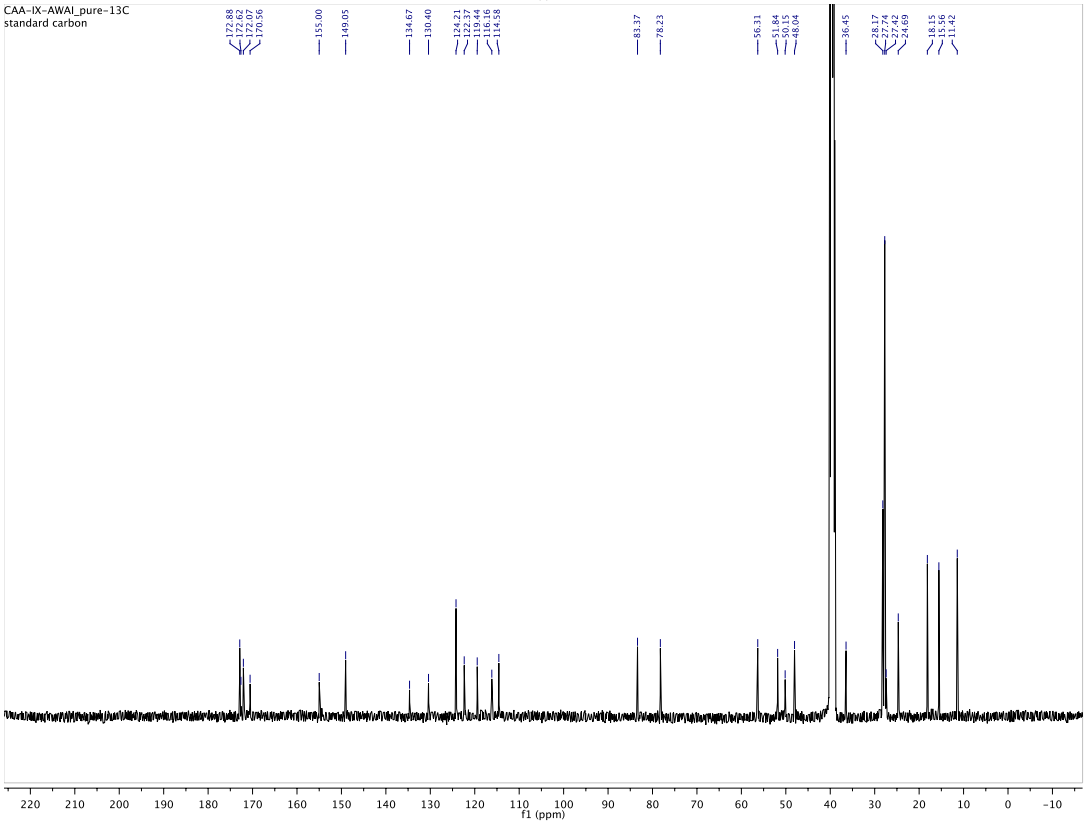
Figure SI-107. Analytical Column PDA for determination of conversion. Gradient: 20-80% MeCN/H_2O (0.1% HCOOH) over 15 min.

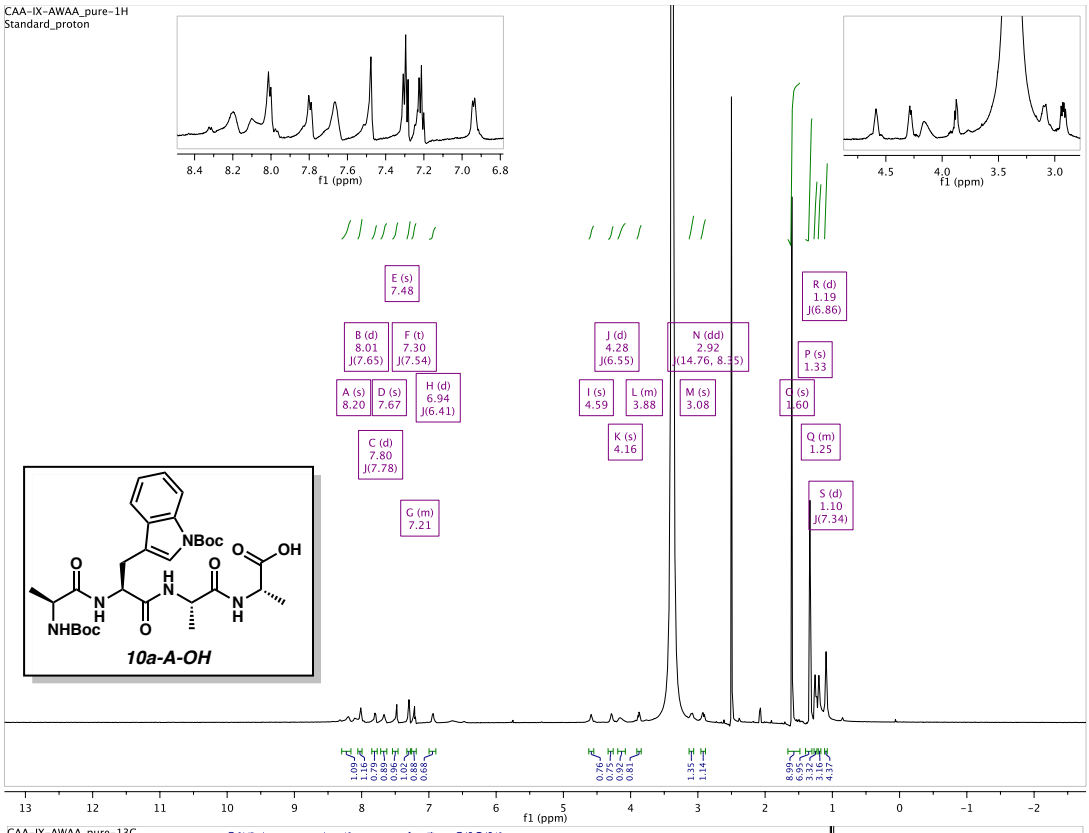
^{1}H and ^{13}C Spectra

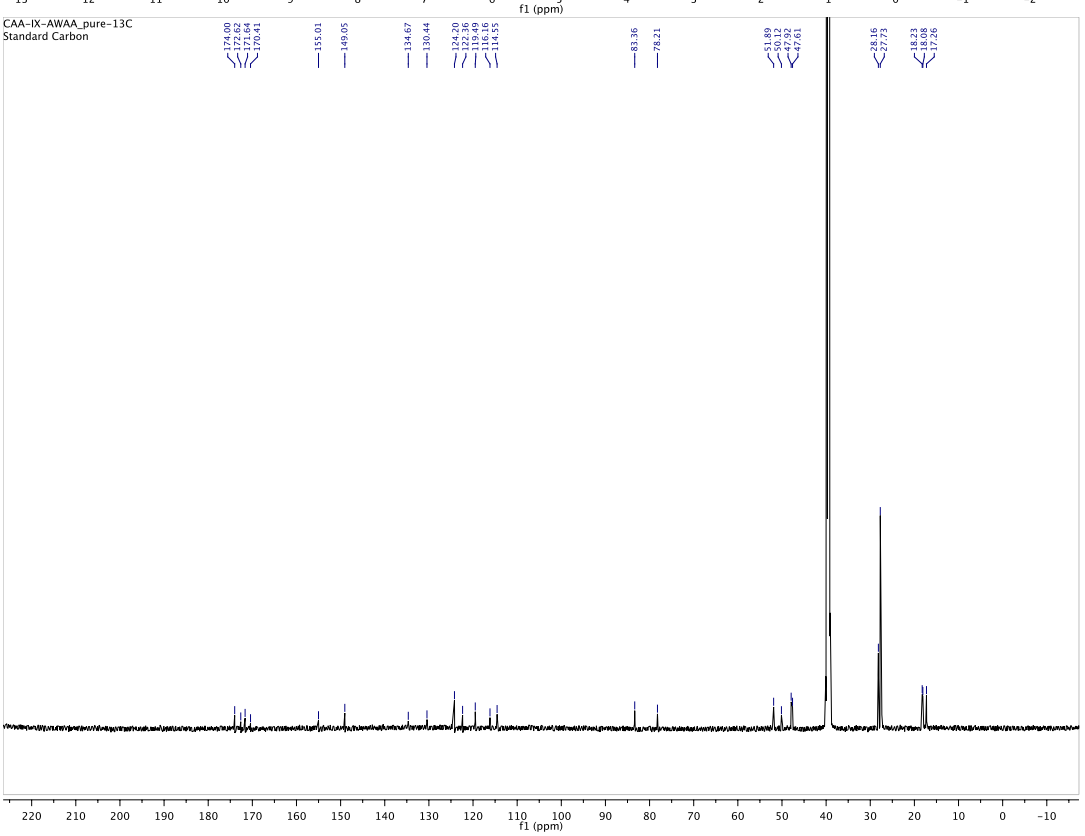


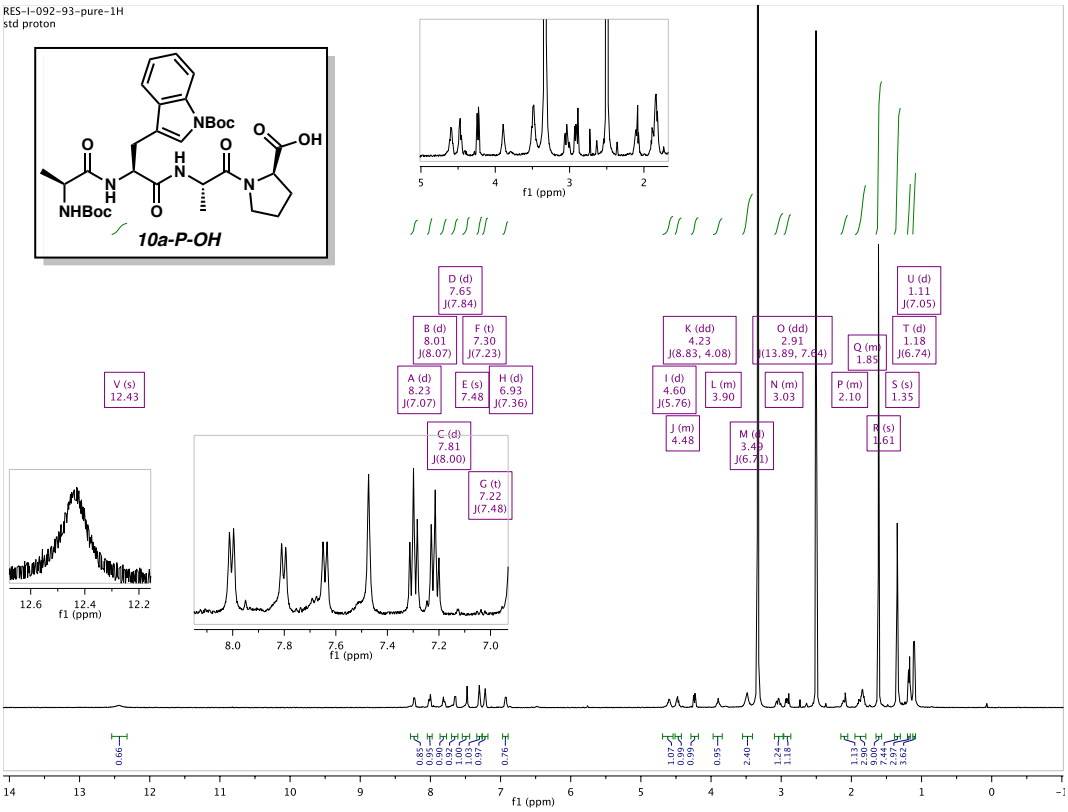


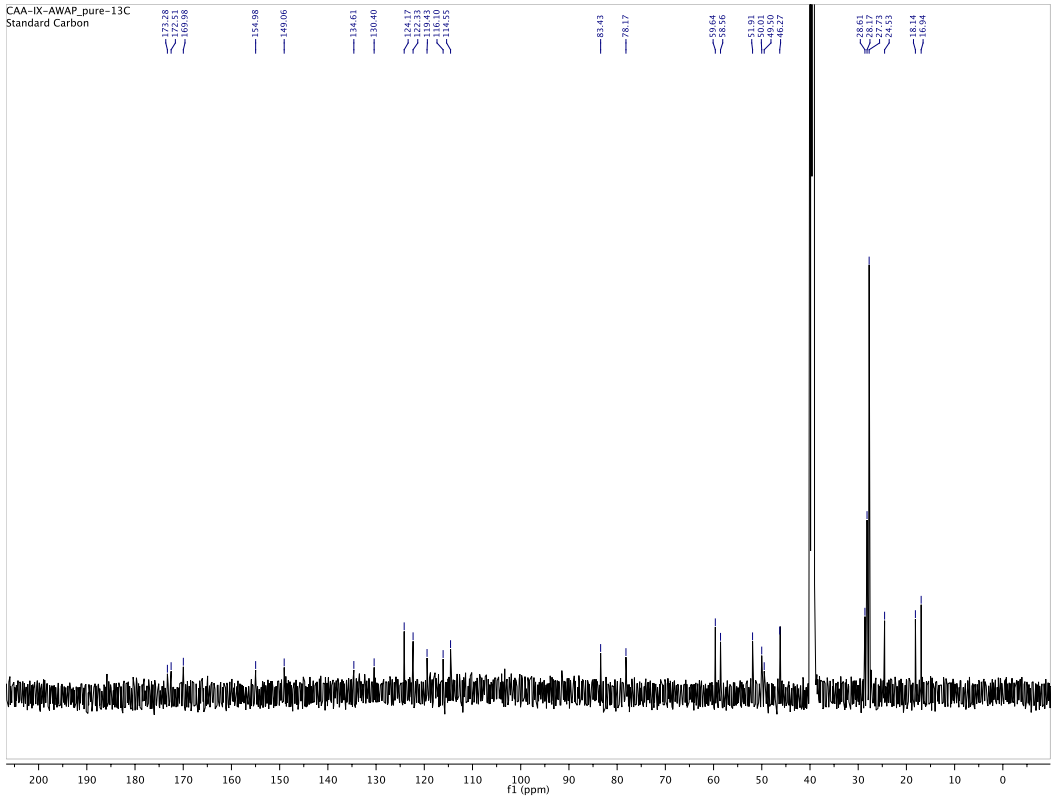

CAA-IX-AWAI_pure-1H
std proton
D (d) 7.83 J(7.99)
B (d) 8.01 J(8.04)
G (t) 7.29 J(7.67)
A (d) 8.21 J(7.15)
E (d) 7.65 J(7.60)
I (d) 6.92 J(7.27)
C (d) 7.88 J(8.24)
H (t) 7.21 J(7.50)
F (s) 7.49
K (q) 4.37 J(6.93)
J (m) 4.59
M (m) 3.87
O (m) 2.91
N (m) 3.08
L (dd) 4.17 J(8.21, 5.83)
R (s) 1.33
U (t) 0.84 J(7.80)
P (dq) 1.76 J(13.68, 7.66, 7.16)
Q (s) 1.60
T (d) 1.10 J(7.02)
S (t) 1.20 J(6.85)
NBoc
NHBoc
OH
10a-I-OH
f1 (ppm)


CAA-IX-AWAI_pure-13C
standard carbon
172.88
172.62
172.07
170.96
155.00
149.05
134.67
130.40
124.21
122.57
119.44
116.16
114.58
83.37
78.23
56.31
51.84
50.15
48.04
36.45
28.17
27.74
27.42
24.69
18.15
15.56
11.42
f1 (ppm)


CAA-IX-AWAA_pure-1H
Standard_proton
E (s) 7.48
B (d) 8.01 J(7.65)
F (t) 7.30 J(7.54)
A (s) 8.20
D (s) 7.67
H (d) 6.94 J(6.41)
C (d) 7.80 J(7.78)
G (m) 7.21
J (d) 4.28 J(6.55)
N (dd) 2.92 J(14.76, 8.35)
I (s) 4.59
L (m) 3.88
M (s) 3.08
K (s) 4.16
R (d) 1.19 J(6.86)
P (s) 1.33
O (s) 1.60
Q (m) 1.25
S (d) 1.10 J(7.34)
NBoc
OH
NHBoc
10a-A-OH
f1 (ppm)


CAA-IX-AWAA_pure-13C
Standard Carbon
f1 (ppm)


RES-I-092-93-pure-1H
std proton
NBoc
OH
NHBoc
10a-P-OH
D (d) 7.65 J(7.84)
B (d) 8.01 J(8.07)
F (t) 7.30 J(7.23)
A (d) 8.23 J(7.07)
E (s) 7.48
H (d) 6.93 J(7.36)
C (d) 7.81 J(8.00)
G (t) 7.22 J(7.48)
V (s) 12.43
K (dd) 4.23 J(8.83, 4.08)
O (dd) 2.91 J(13.89, 7.64)
Q (m) 1.85
U (d) 1.11 J(7.05)
T (d) 1.18 J(6.74)
I (d) 4.60 J(5.76)
L (m) 3.90
N (m) 3.03
P (m) 2.10
S (s) 1.35
J (m) 4.48
M (d) 3.49 J(6.71)
R (s) 1.61
f1 (ppm)


CAA-IX-AWAP_pure-13C
Standard Carbon
173.28
172.51
169.98
154.98
149.06
134.61
130.40
124.17
122.33
119.43
116.10
114.55
83.43
78.17
59.64
58.56
51.91
51.01
49.50
46.27
28.61
28.17
27.73
24.53
18.14
16.94
f1 (ppm)