Supporting Information

Synthesis of β-Thiol Phenylalanine for Applications in One-Pot Ligation-Desulfurization Chemistry

*Lara R. Malins, Andrew M. Giltrap, Luke J. Dowman, Richard J. Payne**

***** School of Chemistry, The University of Sydney, Building F11, Sydney, Australia;
Fax: +61 2 9351 3329; Tel: +61 2 9351 5877
E-mail: richard.payne@sydney.edu.au

Contents

General synthetic experimental

^{1}H NMR spectra were recorded at 300 K or 330 K using a Bruker Avance DPX 400 or DPX 500 spectrometer. Chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: $CDCl_3$ (δ 7.26 [^{1}H]), D_2O (δ 4.79 [^{1}H]), MeOD (δ 3.31 [^{1}H]). ^{1}H NMR data is reported as chemical shift (δ_H), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets), relative integral, coupling constant (*J* Hz) and assignment where possible.

Low-resolution mass spectra were recorded on a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. High resolution ESI mass spectra were measured on a Bruker-Daltonics Apex Ultra 7.0T fourier transform mass spectrometer (FTICR). MALDI-TOF mass spectra were measured on a Bruker autoflex speed MALDI-TOF instrument using a matrix of sinapinic acid in water/acetonitrile (3:7 v/v) containing 0.1 vol.% TFA and analyzed in linear mode. Infrared (IR) absorption spectra were recorded on a Bruker ALPHA Spectrometer with Attenuated Total Reflection (ATR) capability, using OPUS 6.5 software. Optical rotations of enantioenriched compounds were recorded on a Perkin–Elmer 341 polarimeter at 589 nm (sodium D line) with a cell path length of 1 dm, and the concentrations are reported in g/100 mL.

Analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector. Peptides were analyzed using a Waters Sunfire 5 μm (C-18) 2.1 x 150 mm column or an Xbridge BEH300 5 μm (C-18) 2.1 x 150 mm column at a flow rate of 0.2 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B), unless otherwise noted. Results were analyzed with Waters Empower software.

Analytical U-HPLC was performed on a Waters Acquity U-HPLC system equipped with a PDA eλ detector (210–400 nm), Sample Manager FAN, Quaternary Solvent Manager (H-Class) modules and a column heater set to 30 °C. Peptides were analyzed using an Acquity U-HPLC BEH 1.7 μm (C-18) 2.1 x 50 mm column at a flow rate of 0.6 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B). Results were analyzed with Waters Empower software.

Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 230 and 280 nm. Model peptide thioesters and β-thiol phenylalanine-containing peptides were purified on a Waters Sunfire 5 μm (C-18) 19 x 150 mm preparative column operating at a flow rate of 7 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) and a linear gradient as specified. Model peptide ligation and desulfurization products were purified on a Waters Sunfire 5 μm (C-18) semi-preparative column operating at a flow rate of 4 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) and a linear gradient of 0 to 50% B over 40 minutes. Augurin peptides **9**-**11** were purified as specified in the synthetic and analytical data section.

LC-MS was performed on a Shimadzu LC-MS 2020 instrument consisting of a LC-M20A pump and a SPD-20A UV/Vis detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. Separations were performed on a Waters Sunfire 5 μm (C-18) 2.1 x 150 mm column, operating at a flow rate of 0.2 mL min^{-1}. Separations were performed using a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) and a linear gradient of 0-30% B over 30 min, 0-40% B over 30 min, or 0-50% B over 30 min.

Reaction yields for all model ligation reactions are calculated based on the amount of isolated peptide product relative to the theoretical reaction yield. Yields are adjusted to account for the removal of aliquots for reaction monitoring (e.g. LC-MS, measuring pH). The analytical HPLC yield for the construction of augurin **9** using a one-pot ligation-desulfurization protocol was determined by peak integration at 280 nm (see page S44 for details).

Materials

Commercial materials were used as received unless otherwise noted. Amino acids, coupling reagents and resins were obtained from Novabiochem or GL Biochem. Pseudoproline dipeptides were purchased from AAPPTec. Reagents that were not commercially available were synthesized following literature procedures. Dichloromethane (DCM) was distilled from calcium hydride and *N*,*N*-dimethylformamide (DMF) was obtained as peptide synthesis

grade from Merck or Labscan. Trifluoroethanethiol (TFET) was purchased from Sigma and redistilled at atmospheric pressure (b.p 35-36 °C) after opening.

Manual solid-phase peptide synthesis

Preloading Rink amide resin

Rink amide resin was initially washed with DCM (5 x 3 mL) and DMF (5 x 3 mL), followed by removal of the Fmoc group by treatment with 10% piperidine/DMF (3 mL, 2 x 5 min). The resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL). PyBOP (4 eq.) and *N*-methylmorpholine (NMM) (8 eq.) were added to a solution of Fmoc-AA-OH (4 eq.) in DMF (final concentration 0.1 M). After 2 min of pre-activation, the mixture was added to the resin. After 2 h the resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL), capped with acetic anhydride/pyridine (1:9 v/v) (3 mL, 2 x 3 min) and washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL). The resin was subsequently submitted to iterative peptide assembly (Fmoc-SPPS).

Preloading 2-chloro-trityl chloride resin

2-chloro-trityl chloride resin (1.22 mmol/g loading) was swollen in dry DCM for 30 min then washed with DCM (5 x 3 mL) and DMF (5 x 3 mL). A solution of Fmoc-AA-OH (4.0 eq.) and *N*,*N*-diisopropylethylamine (DIPEA) (8.0 eq.) in DMF (final concentration 0.1 M) was added and the resin shaken at rt for 16 h. The resin was washed with DMF (5 x 3 mL) and DCM (5 x 3 mL) and treated with a solution of DCM/CH_3OH/DIPEA (17:2:1 v/v/v, 3 mL) for 0.5 h. The resin washed with DMF (5 x 3 mL), DCM (5 x 3 mL), and DMF (5 x 3 mL) and subsequently submitted to iterative peptide assembly (Fmoc-SPPS).

General iterative peptide assembly (Fmoc-SPPS)

Deprotection: The resin was treated with 10% piperidine/DMF (3 mL, 2 x 3 min) and washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL).

General amino acid coupling: A preactivated solution of protected amino acid (4 eq.), PyBOP (4 eq.) and NMM (8 eq.) in DMF (final concentration 0.1 M) was added to the resin.

After 1 h, the resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL).

Capping: Acetic anhydride/pyridine (1:9 v/v) was added to the resin (3 mL). After 3 min the resin was washed with DMF (5 x 3 mL), DCM (5 x 3 mL) and DMF (5 x 3 mL).

Cleavage: A mixture of TFA, triisopropylsilane (TIS) and water (90:5:5 v/v/v) was added to the resin. After 2 h, the resin was washed with TFA (3 x 2 mL). For peptide thioesters and peptides containing Met residues, a mixture of TFA, triisopropylsilane (TIS), thioanisole and water (85:5:5:5 v/v/v/v) was used instead.

Work-up: The combined solutions were concentrated under a stream of nitrogen. The residue was dissolved in water containing 0.1% TFA, filtered and purified by reverse-phase HPLC and analyzed by LC-MS and ESI mass spectrometry.

Coupling conditions for β-thiol phenylalanine building block (1)

A preactivated solution of β-thiol phenylalanine **1** (1.2 eq.), HATU (1.2 eq.), and *i*Pr_2EtN (2.4 eq.) in DMF (final concentration 0.1 M) was added to the resin (1 eq.). After 6 h, the resin was washed with DMF (5 x 3 mL) and DCM (10 x 3 mL). The peptide was cleaved from the resin using the conditions described above.

Automated solid-phase peptide synthesis

Automated Fmoc-SPPS was carried out on a Biotage Initiator+ Alstra microwave peptide synthesizer equipped with an inert gas manifold. General synthetic protocols for Fmoc deprotection and capping were carried out in accordance with the manufacturer's specifications. Standardized amino acid couplings were performed for 20 min at 50 ^{o}C under microwave irradiation in the presence of amino acid (0.3 M in DMF), Oxyma (0.3 M in DMF) and di*i*sopropylcarbodiimide (0.3 M in DMF). Peptide cleavage and work-up were carried out as described above for manual SPPS.

General Procedures

Peptide Thioesters

Peptide thioesters were prepared according to literature methods.[1]

PhSH-promoted ligation

General Protocol A (PhSH additive): Peptide thioesters (1.1-1.3 eq., 5.5-6.5 mM concentration) were dissolved in degassed buffer (6 M guanidine hydrochloride [Gn•HCl], 100 mM Na_2HPO_4, 50 mM TCEP, adjusted to pH 7.5, 5 mM concentration with respect to the β-thiol phenylalanine-containing peptide fragment). The solution was added to the thiol-containing peptide (~2 mg, 2.9 μmol, 1.0 eq.). Thiophenol (2% v/v) was added to the solution and the reaction gently agitated. The final pH of the solution was measured and adjusted to 7.2-7.4, using 2 M NaOH or 1 M HCl solution, if necessary. The solution was incubated at 37 ^{o}C for 24 h. The reaction was quenched by the addition of 1% TFA in water (0.5 mL) and purified by semi-preparative reverse-phase HPLC as described in the general methods.

Desulfurization

General Protocol B: Water and buffer (6 M Gn•HCl, 100 mM Na_2HPO_4, adjusted to pH 6.5) were degassed with argon for 10 min before use to prepare peptide and reagent stock solutions. The purified ligation product (~2 mg) was dissolved in water (14 μL). Stock solutions of glutathione (40 mM) and VA-044 (200 mM) in water were prepared. A stock solution of 0.5 M TCEP in the buffer solution was prepared. The peptide and stock solutions were flushed further with argon before use. Aliquots of glutathione (13 μL), VA-044 (13 μL) and TCEP (40 μL) were added to the peptide solution (final concentration of peptide ~15-20 mM) and the vessel flushed with argon. The reaction vessel was incubated at 65 ^{o}C for 16 h. The reaction was quenched with 0.1% TFA in water (100 μL) and purified by semi-preparative reverse-phase HPLC as described in the general methods.

TFET-promoted one-pot ligation-desulfurization

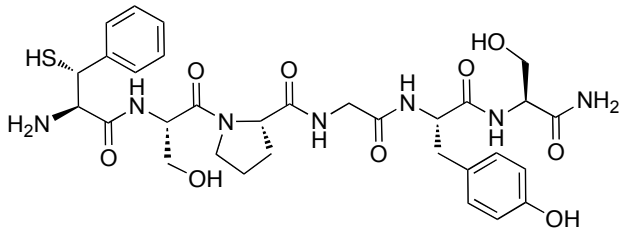
General Protocol C (TFET additive): Peptide thioesters (1.1-1.3 eq., 5.5-6.5 mM concentration) were dissolved in degassed buffer (6 M Gn•HCl, 100 mM Na_2HPO_4, 50 mM TCEP, adjusted to pH 7.5, 5 mM concentration with respect to the β-thiol phenylalanine-containing peptide fragment). The solution was added to the thiol-containing peptide (~2 mg,

2.9 μmol, 1.0 eq.). TFET (2 vol.%) was added to the solution and the reaction gently agitated. The final pH of the solution was measured and adjusted to 7.2-7.4, using 2 M NaOH or 1 M HCl solution, if necessary. The solution was incubated at 30 ^{o}C and monitored *via* LC-MS. Upon consumption of the thiol-containing peptide fragment, the reaction mixture was thoroughly sparged with argon (5 min). The ligation mixture was diluted with a solution of TCEP (0.5 M) and glutathione (80 mM) in buffer (6 M Gn•HCl, 100 mM Na_2HPO_4, pH = 5.0-6.0) to a final concentration of 2.5 mM with respect to the thiol-containing peptide. The mixture was further degassed with argon and radical initiator VA-044 was then added as a solid (20 mM final concentration). The reaction mixture was incubated at 37 ^{o}C for 7-8 h and monitored *via* LC-MS. The reaction was quenched with 0.1% TFA in H_2O (0.5 mL) and purified by semi-preparative reverse-phase HPLC as described in the general methods.

Synthetic details and analytical data

A. Model β-thiol phenylalanine-containing peptides

H-(β-SH)FSPGYS-NH_2 (7)



Peptide **7** was prepared *via* manual Fmoc-strategy SPPS outlined in the general procedures. The crude residue was treated with an aqueous solution of TCEP (1.0 mg/mL) to reduce disulfides and then purified by preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (12.7 mg, 74% yield based on the original 25 μmol resin loading).

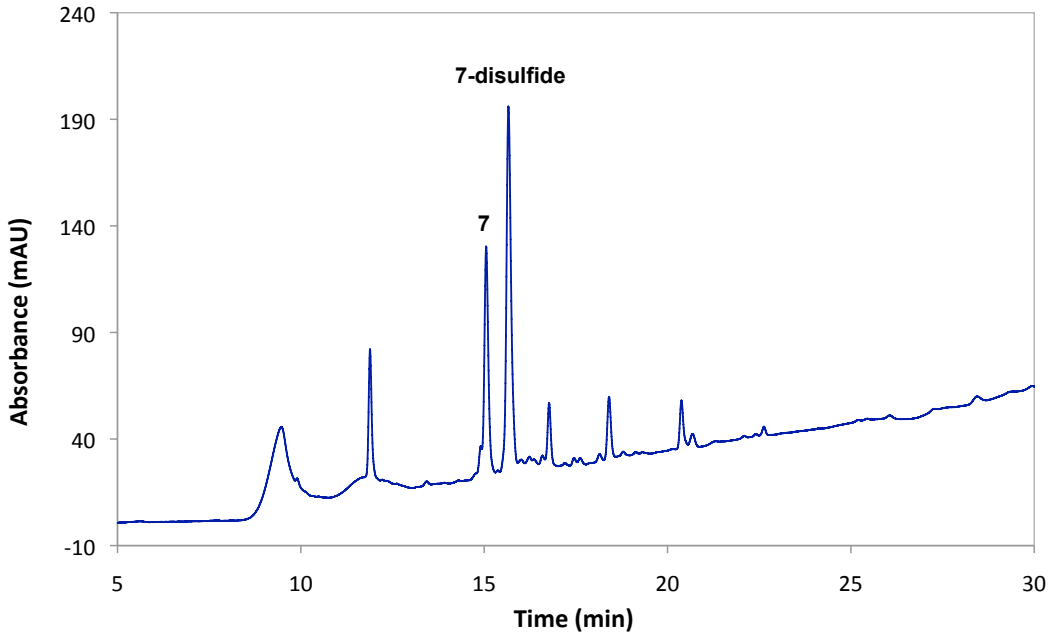
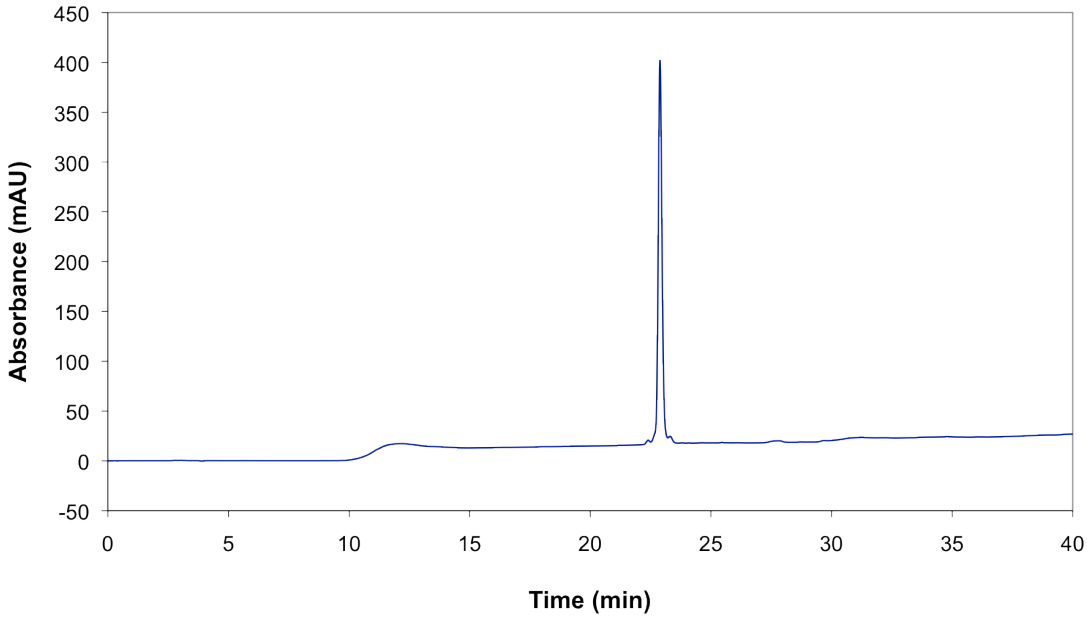
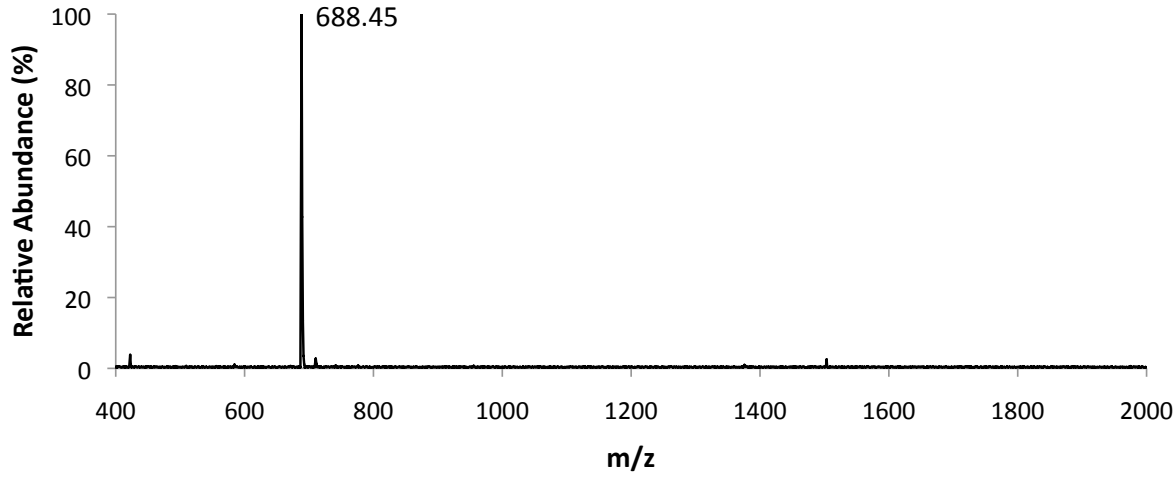


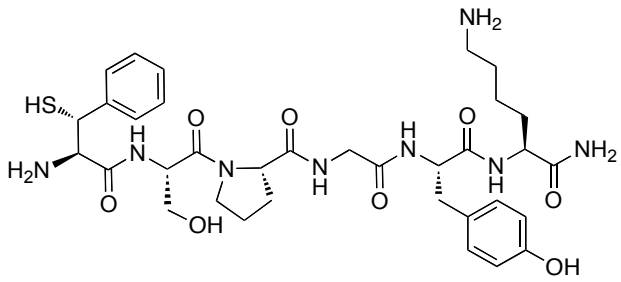
Figure S1. Crude LC-MS trace of peptide **7** (0-40% B over 30 min, 0.1% formic acid, λ = 230 nm) following cleavage from the resin. The crude mixture was treated with TCEP prior to HPLC purification to reduce **7-disulfide** to the target peptide **7**.



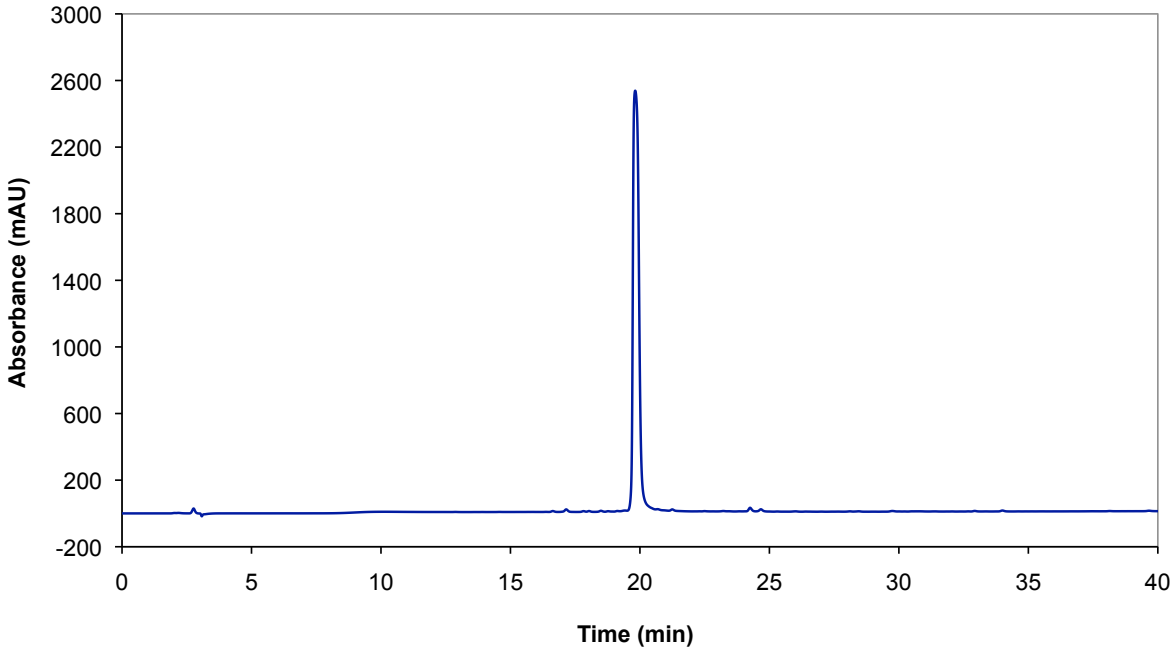
Analytical HPLC: R_t 22.9 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 688.28; Mass Found ESI(+); 688.45 $[M+H]^+$



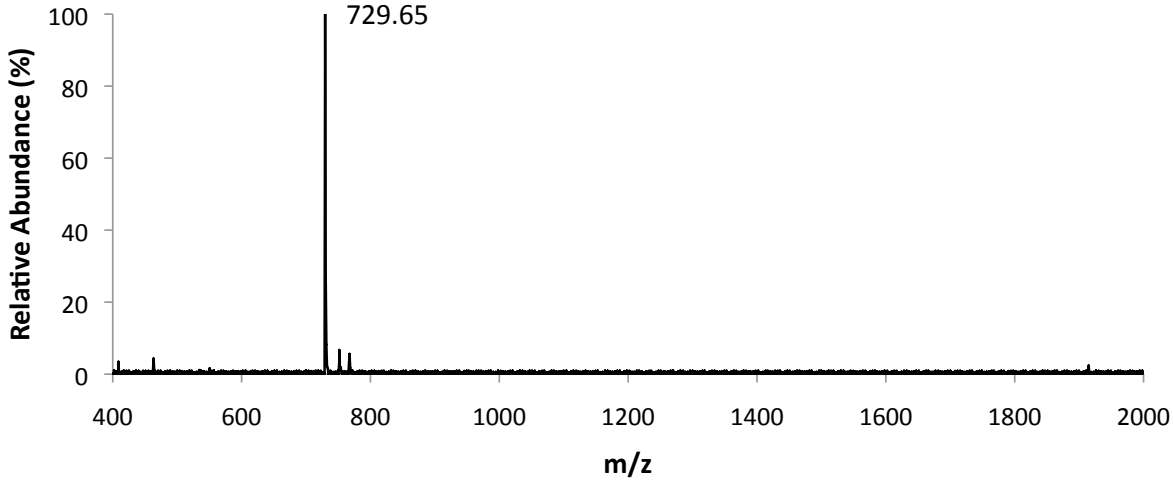
H-(β-SH)FSPGYK-NH_2



The title compound was prepared *via* manual Fmoc-strategy SPPS outlined in the general procedures and purified by preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization.

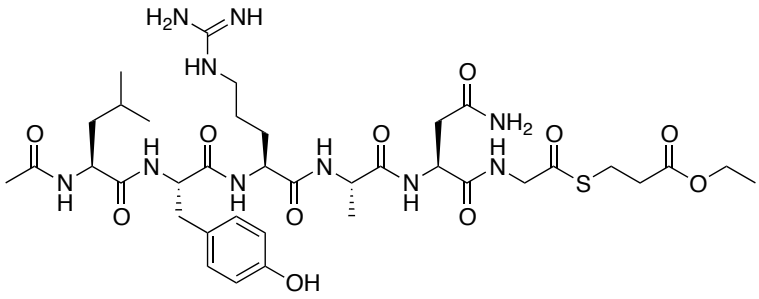


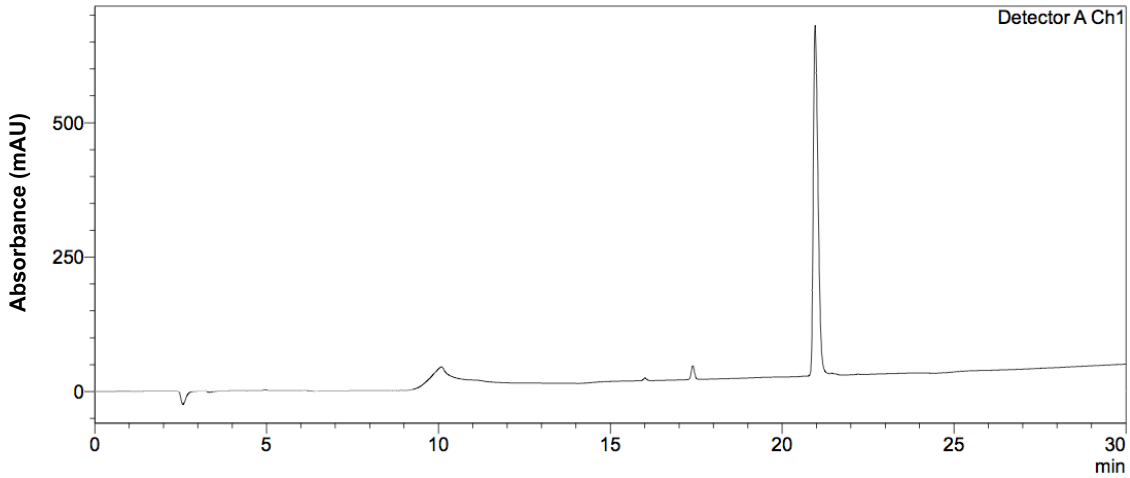
Analytical HPLC: R_t 19.8 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 729.34; Mass Found ESI(+); 729.65 $[M+H]^+$



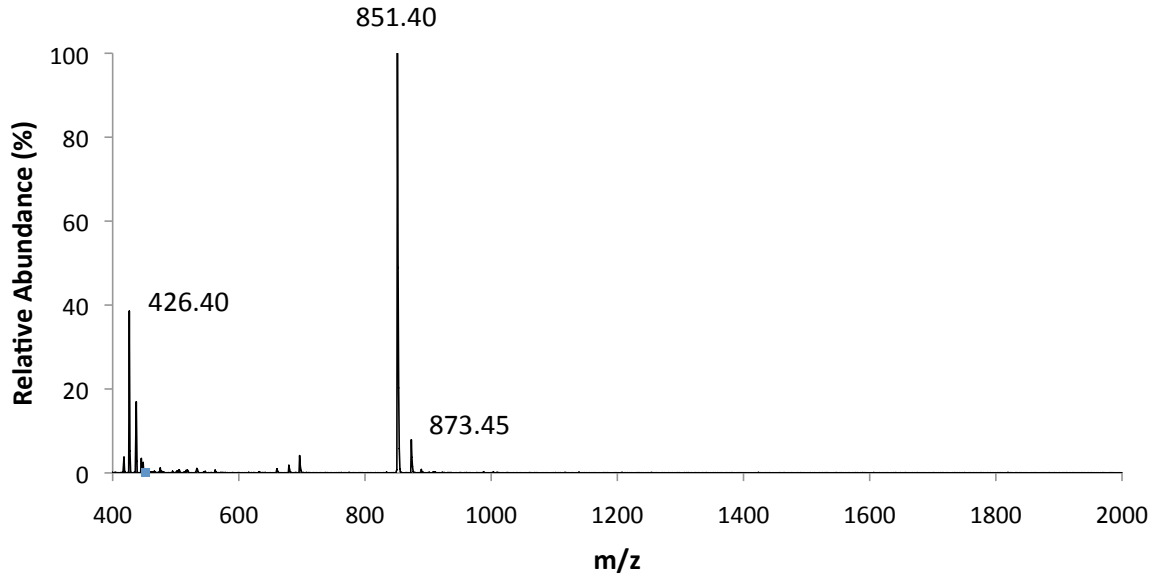
B. Model peptide thioesters

Ac-LYRANG-S$(CH_2)_2CO_2Et$

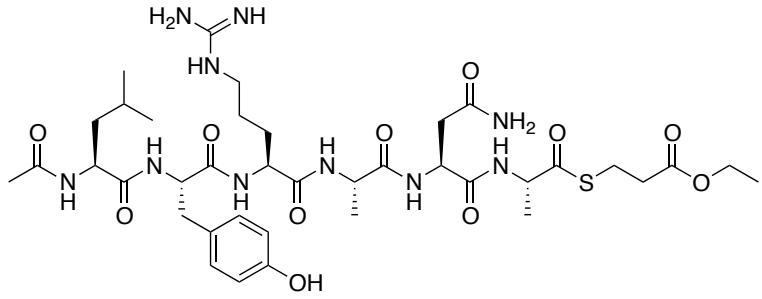


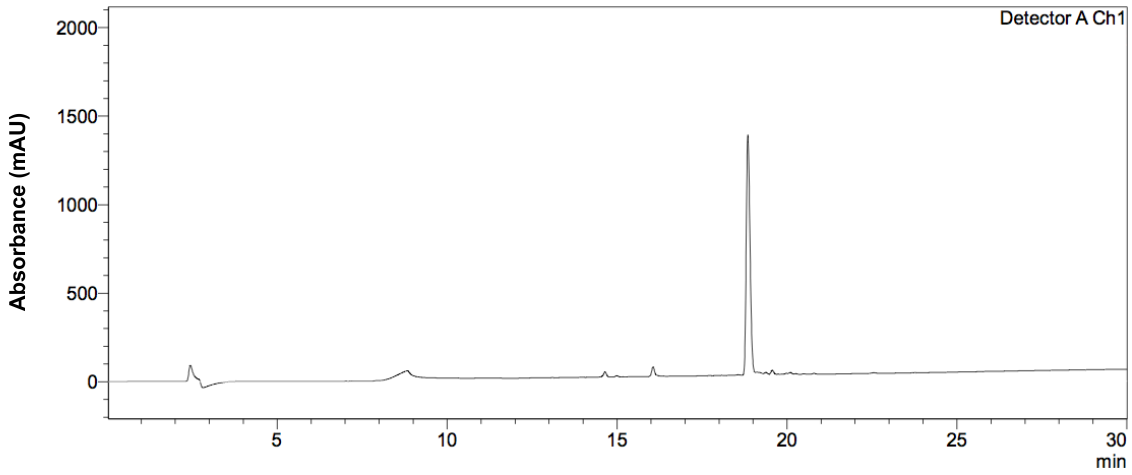


Analytical HPLC: Purified Ac-LYRANG-S$(CH_2)_2CO_2Et$, R_t 20.9 min (0-40% B over 30 min, 0.1% formic acid, λ = 230 nm); Calculated Mass $[M+H]^+$: 851.41, $[M+2H]^{2+}$: 426.21; Mass Found ESI(+); 851.40 $[M+H]^+$, 426.40 $[M+2H]^{2+}$

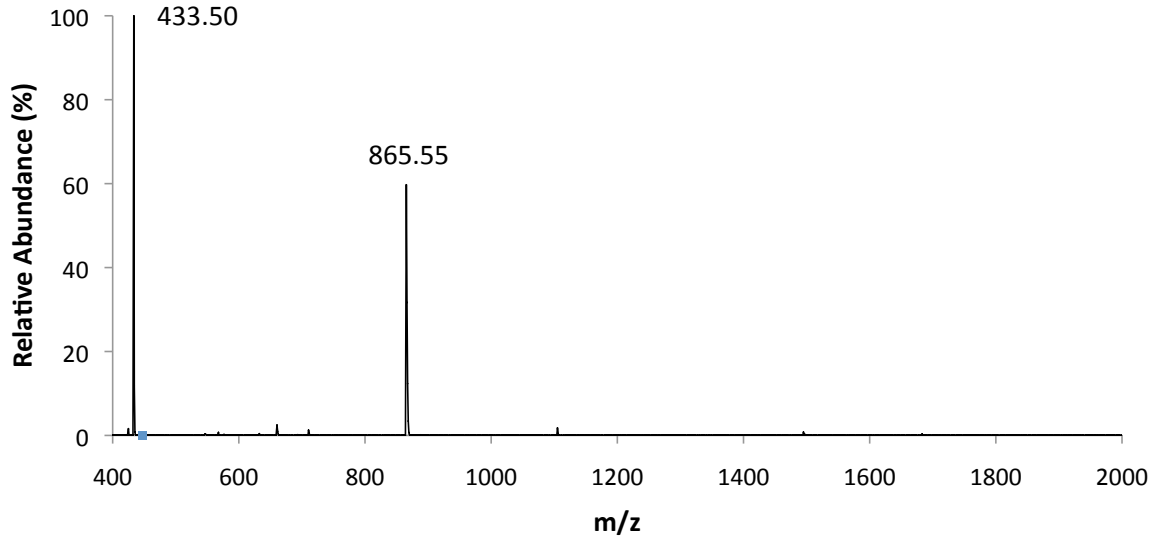


Ac-LYRANA-S$(CH_2)_2CO_2Et$

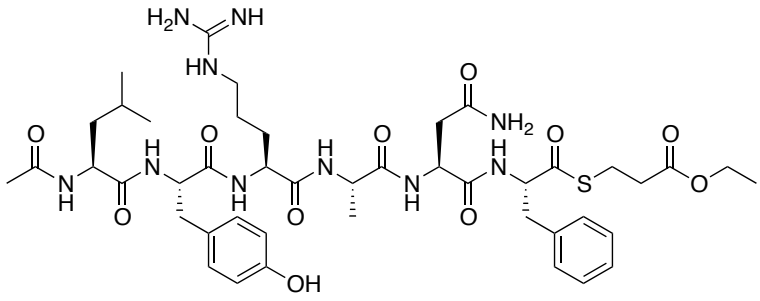


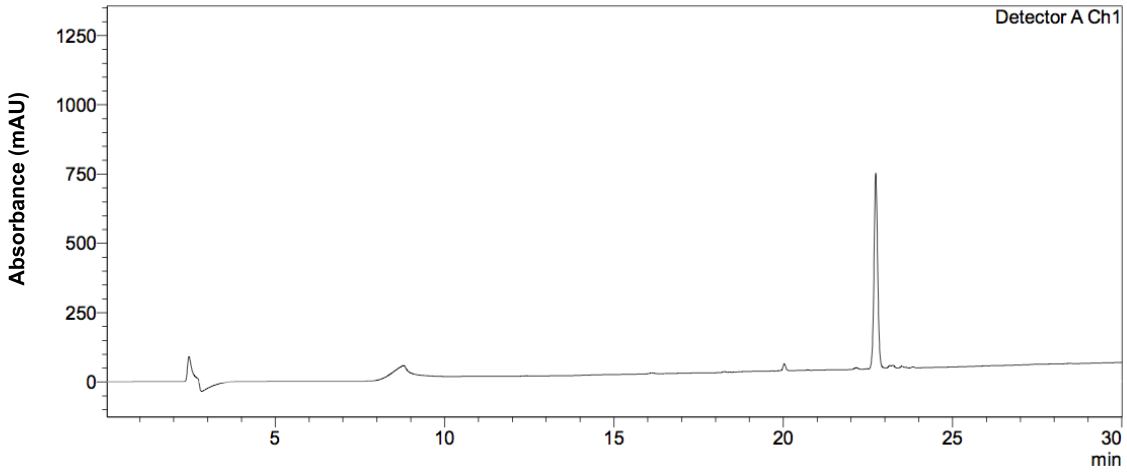


Analytical HPLC: Purified Ac-LYRANA-S$(CH_2)_2CO_2Et$, R_t 18.8 min (0-50% B over 30 min, 0.1% formic acid, λ = 230 nm); Calculated Mass $[M+H]^+$: 865.42, $[M+2H]^{2+}$: 433.22; Mass Found ESI(+); 865.55 $[M+H]^+$, 433.50 $[M+2H]^{2+}$

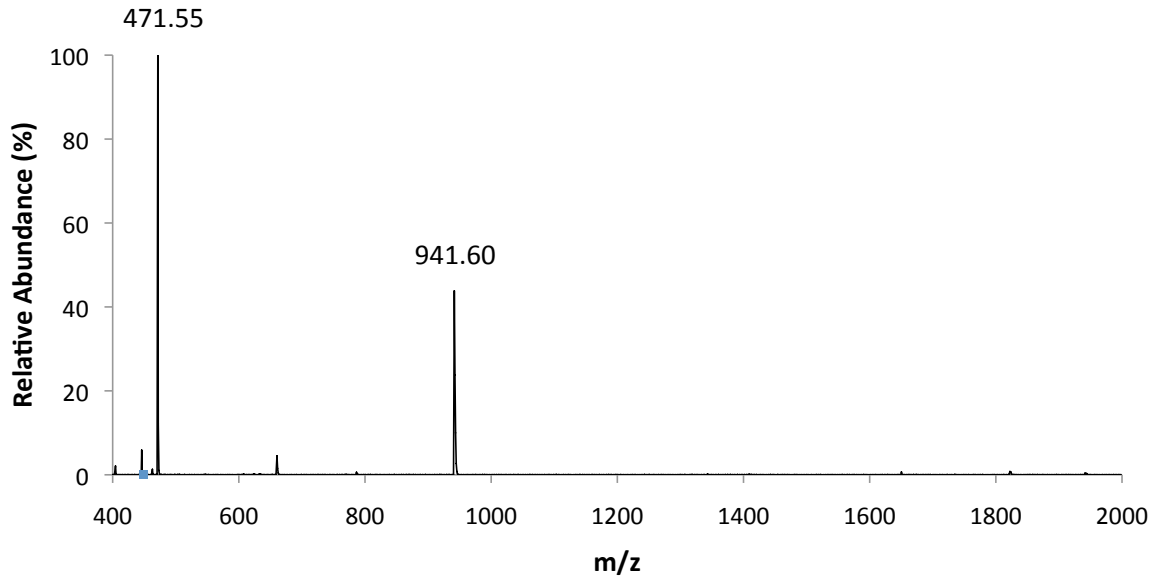


Ac-LYRANF-S$(CH_2)_2CO_2Et$

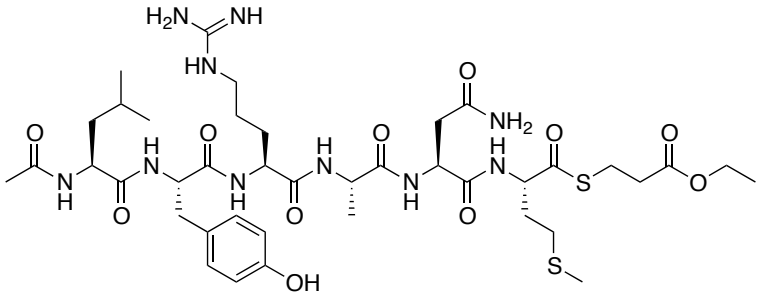


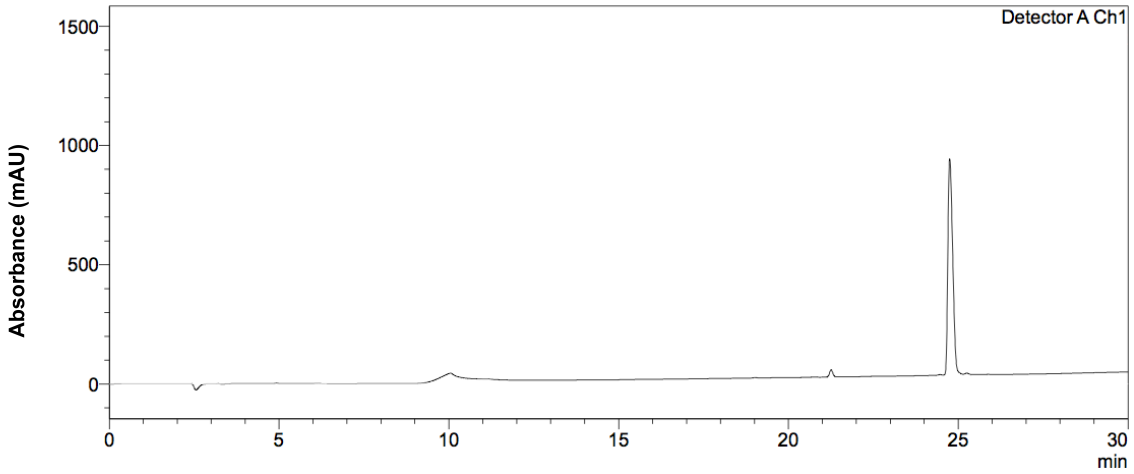


Analytical HPLC: Purified Ac-LYRANF-S$(CH_2)_2CO_2Et$, R_t 22.8 min (0-50% B over 30 min, 0.1% formic acid, λ = 230 nm); Calculated Mass $[M+H]^+$: 941.45, $[M+2H]^{2+}$: 471.23; Mass Found ESI(+); 941.60 $[M+H]^+$, 471.55 $[M+2H]^{2+}$

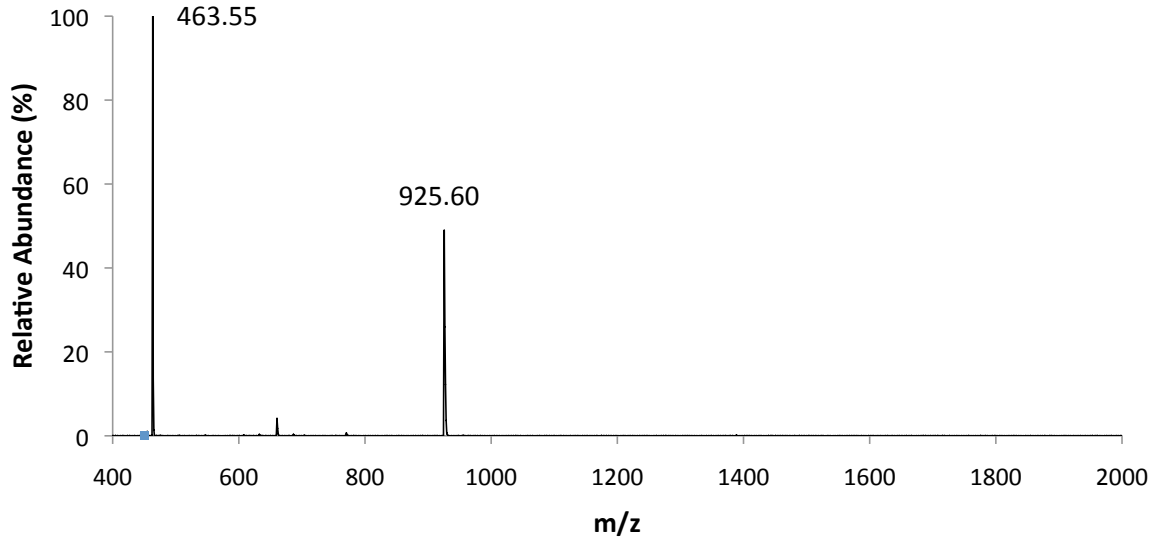


Ac-LYRANM-S$(CH_2)_2CO_2Et$

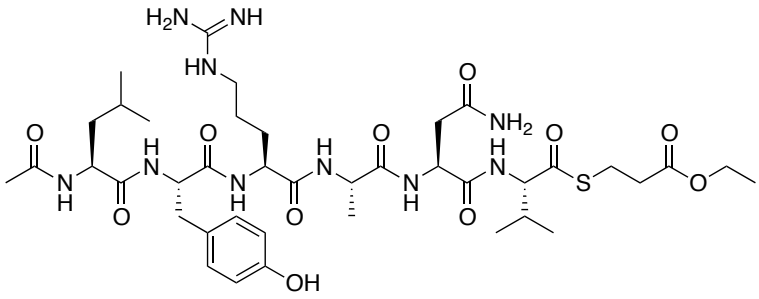


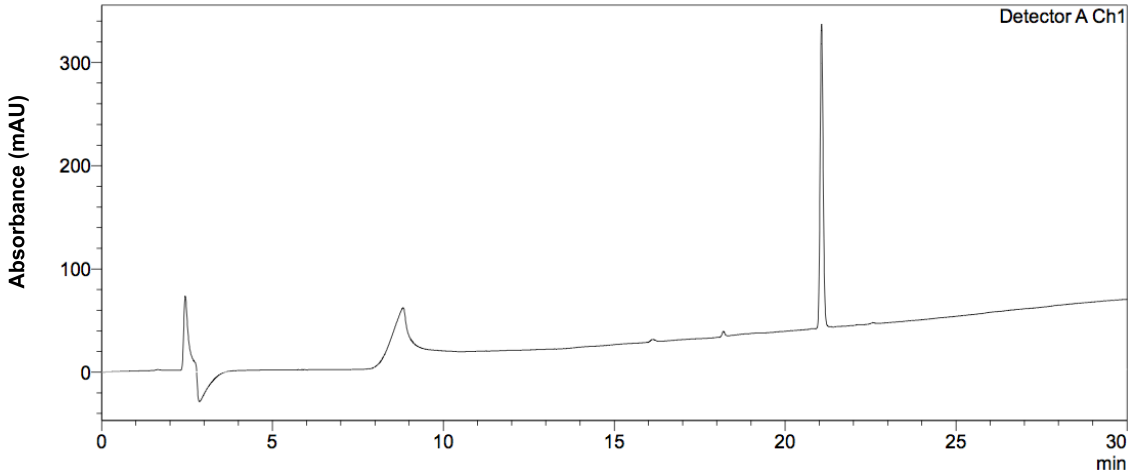


Analytical HPLC: Purified Ac-LYRANM-S$(CH_2)_2CO_2Et$, R_t 24.8 min (0-40% B over 30 min, 0.1% formic acid, λ = 230 nm); Calculated Mass $[M+H]^+$: 925.43, $[M+2H]^{2+}$: 463.22; Mass Found ESI(+); 925.60 $[M+H]^+$, 463.55 $[M+2H]^{2+}$

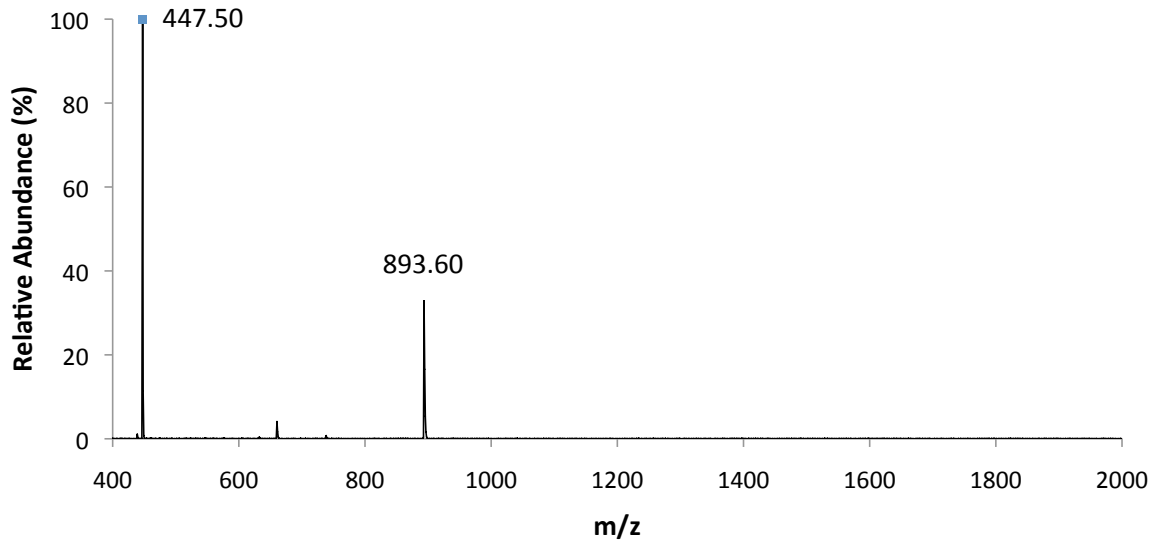


Ac-LYRANV-S$(CH_2)_2CO_2Et$





Analytical HPLC: Purified Ac-LYRANV-S$(CH_2)_2CO_2Et$, R_t 21.1 min (0-50% B over 30 min, 0.1% formic acid, λ = 230 nm); Calculated Mass $[M+H]^+$: 893.45, $[M+2H]^{2+}$: 447.23; Mass Found ESI(+); 893.60 $[M+H]^+$, 447.50 $[M+2H]^{2+}$



C. β-Thiol-mediated ligation products (General Protocol A – PhSH Additive)

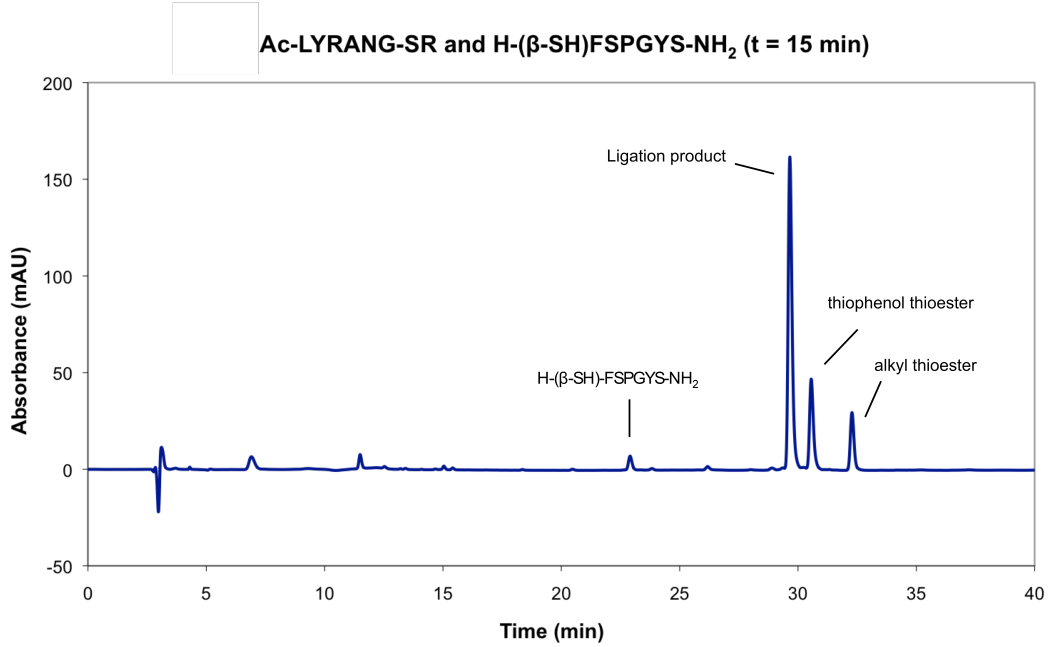
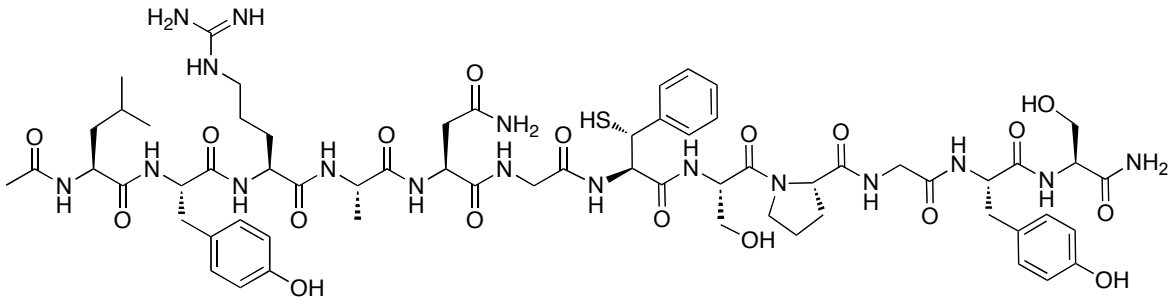
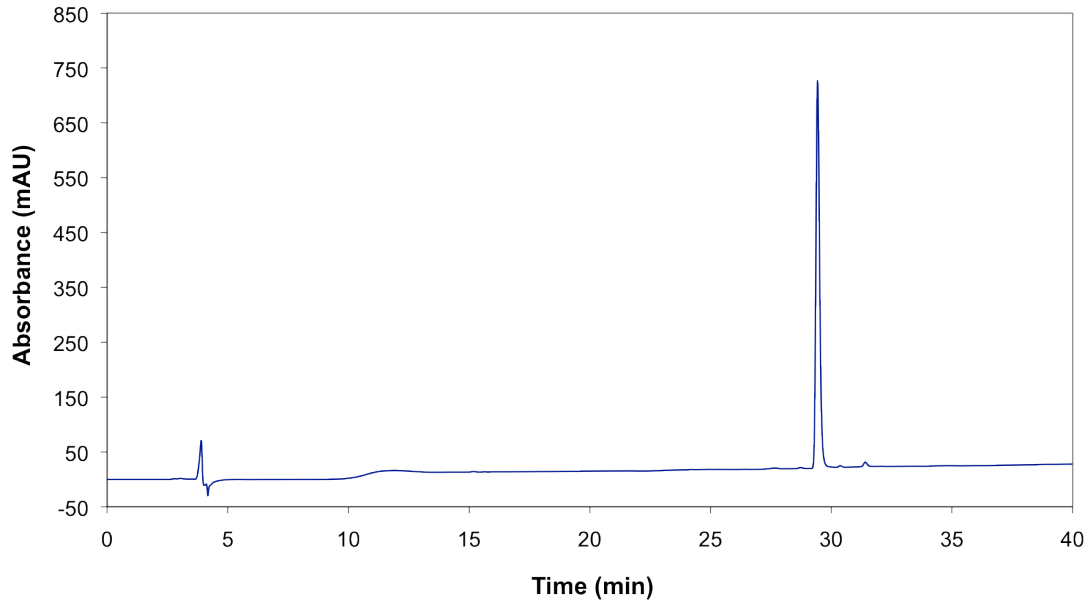


Figure S2. Representative analytical HPLC trace (0 to 50% B over 40 min, λ = 280 nm) of a crude ligation reaction between H-(β-SH)FSPGYS **7** and Ac-LYRANG-S$(CH_2)_2CO_2$Et (t = 15 min).

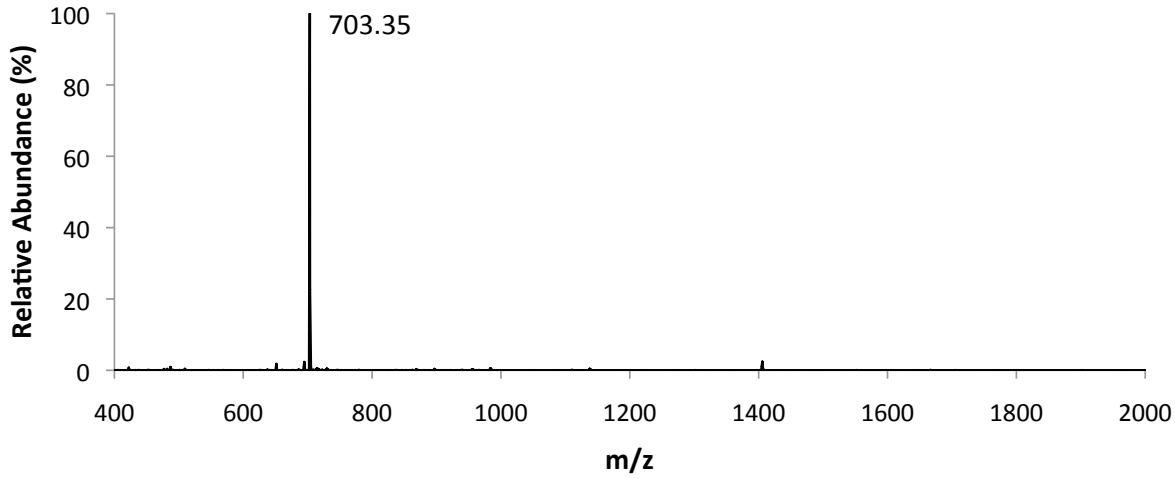
Ac-LYRANG(β-SH)FSPGYS-NH_2



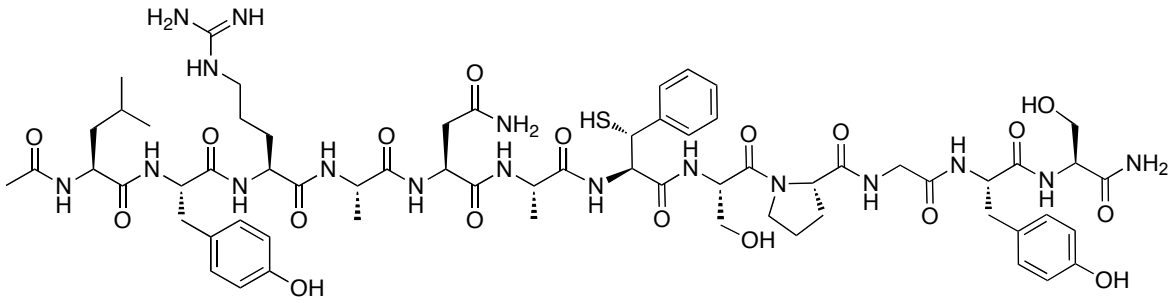
The title compound was prepared *via* ligation of H-(β-SH)FSPGYS-NH_2 **7** (2.0 mg, 2.9 μmol, 5 mM concentration) and Ac-LYRANG-S$(CH_2)_2CO_2$Et (3.2 mg, 3.8 μmol, 6.5 mM concentration) according to General Protocol A and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.1 mg, 79% yield).



Analytical HPLC: R_t 29.4 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1404.64, $[M+2H]^{2+}$: 703.28; Mass Found ESI(+); 703.35 $[M+2H]^{2+}$



Ac-LYRANA(β-SH)FSPGYS-NH_2



The title compound was prepared *via* ligation of H-(β-SH)FSPGYS-NH_2 **7** (1.8 mg, 2.6 μmol, 5 mM concentration) and Ac-LYRANA-S$(CH_2)_2CO_2$Et (2.6 mg, 3.0 μmol, 5.8 mM concentration) according to General Protocol A and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.2 mg, 86% yield).

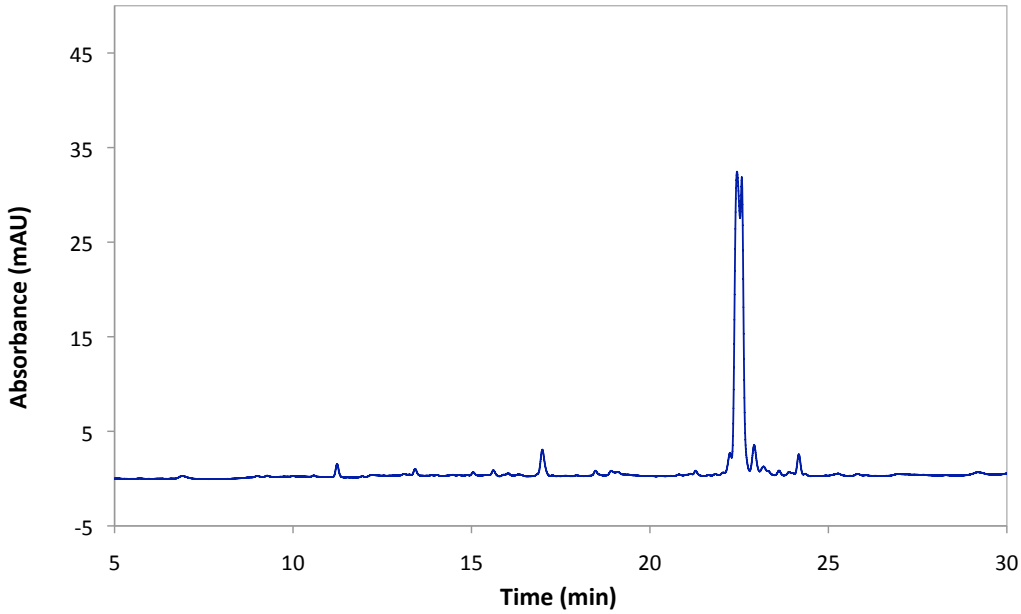
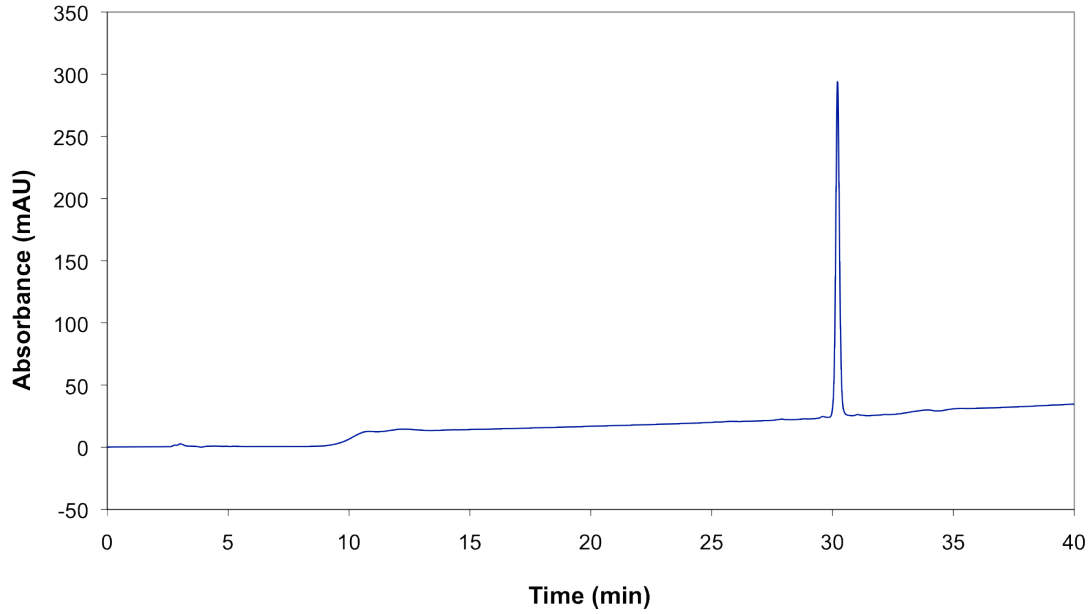
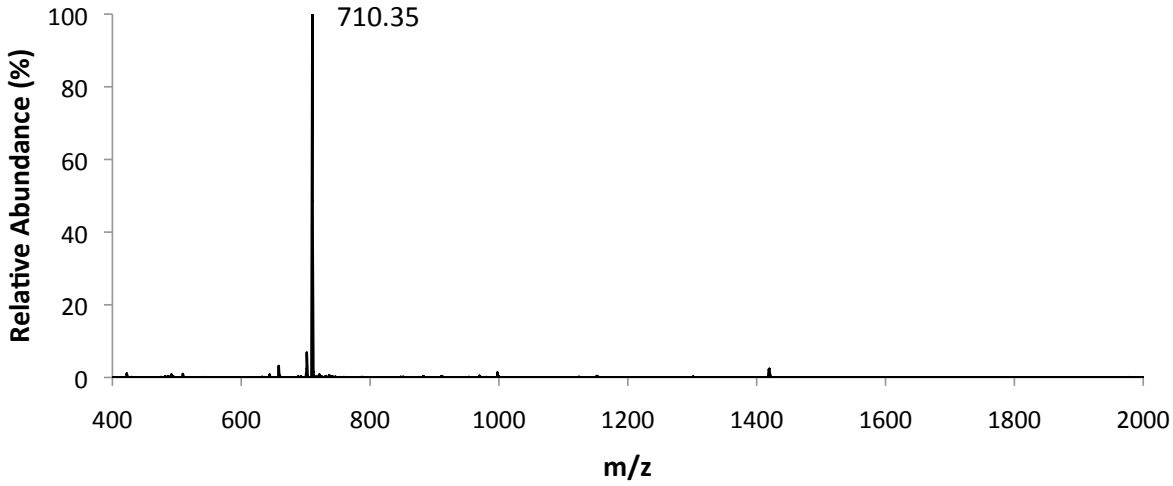


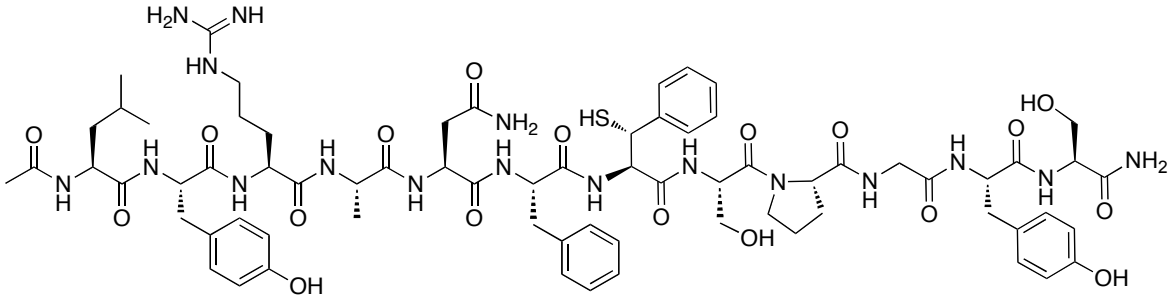
Figure S3. Analytical HPLC-MS trace (0 to 40% B over 30 min, 0.1% formic acid, λ = 280 nm) of the crude ligation reaction between H-(β-SH)FSPGYS **7** and Ac-LYRANA-S$(CH_2)_2CO_2$Et. Note: ligation product co-elutes with the corresponding disulfide dimer ligation product (formed through gradual oxidation in ligation buffer).



Analytical HPLC: R_t 30.2 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1418.65, $[M+2H]^{2+}$: 709.83; ; Mass Found ESI(+); 1418.8 $[M+H]^+$, 710.35 $[M+2H]^{2+}$



Ac-LYRANF(β-SH)FSPGYS-NH_2



The title compound was prepared *via* ligation of H-(β-SH)FSPGYS-NH_2 **7** (2.0 mg, 2.9 μmol, 5 mM concentration) and Ac-LYRANF-S$(CH_2)_2CO_2$Et (3.4 mg, 3.6 μmol, 6.2 mM concentration) according to General Protocol A and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.3 mg, 83% yield).

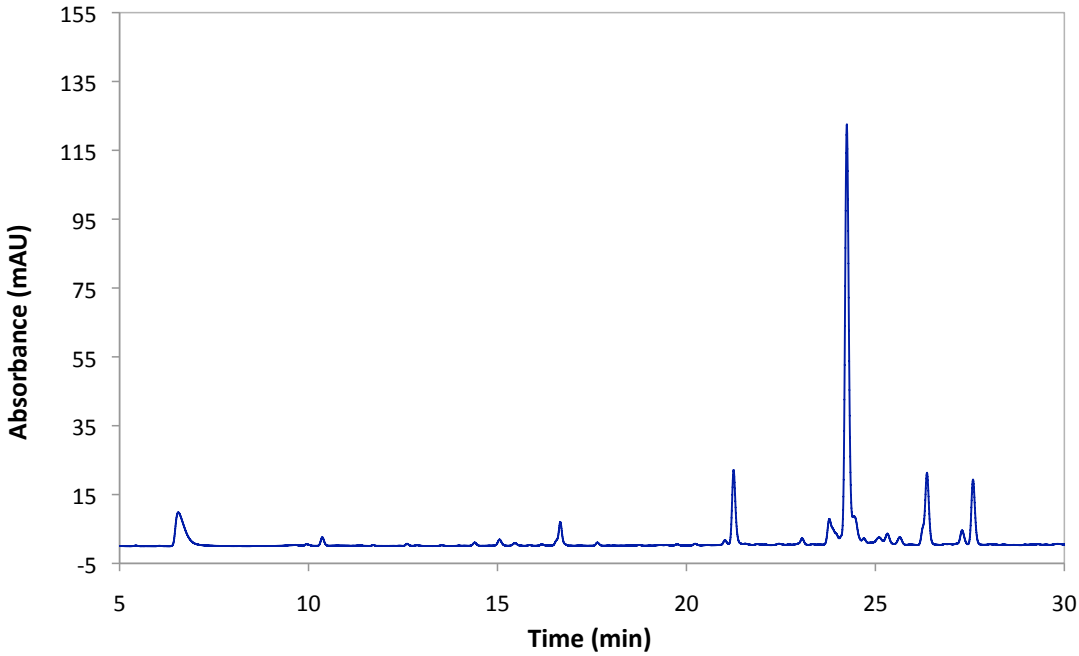
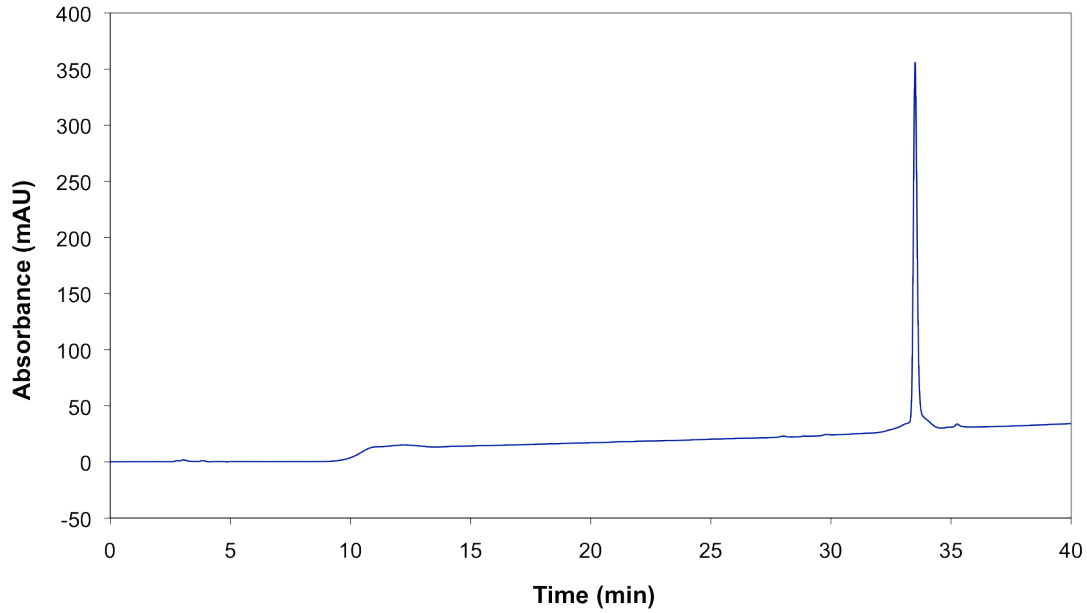
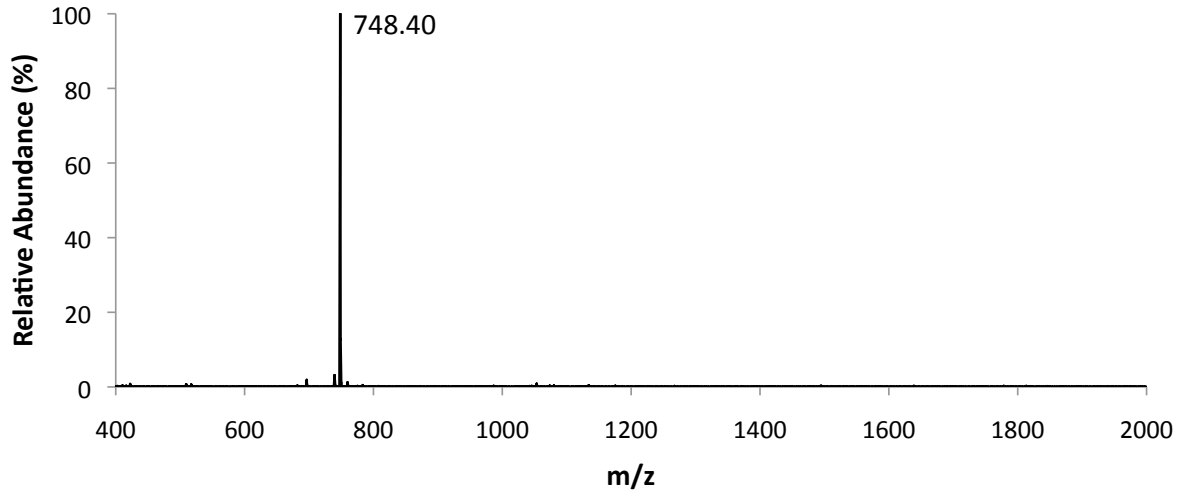


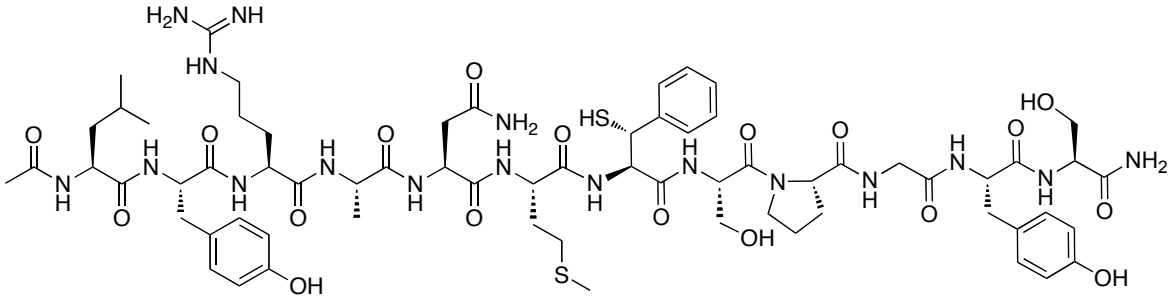
Figure S4. Analytical HPLC-MS trace (0 to 40% B over 30 min, 0.1% formic acid, λ = 280 nm) of the crude ligation reaction between H-(β-SH)FSPGYS **7** and Ac-LYRANF-S$(CH_2)_2CO_2$Et.



Analytical HPLC: R_t 33.5 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1494.68, $[M+2H]^{2+}$: 747.84; Mass Found ESI(+); 748.40 $[M+2H]^{2+}$



Ac-LYRANM(β-SH)FSPGYS-NH_2



The title compound was prepared *via* ligation of H-(β-SH)FSPGYS-NH_2 **7** (2.1 mg, 3.0 μmol, 5 mM concentration) and Ac-LYRANM-S$(CH_2)_2CO_2Et$ (3.6 mg, 3.9 μmol, 6.5 mM concentration) according to General Protocol A and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.0 mg, 72% yield).

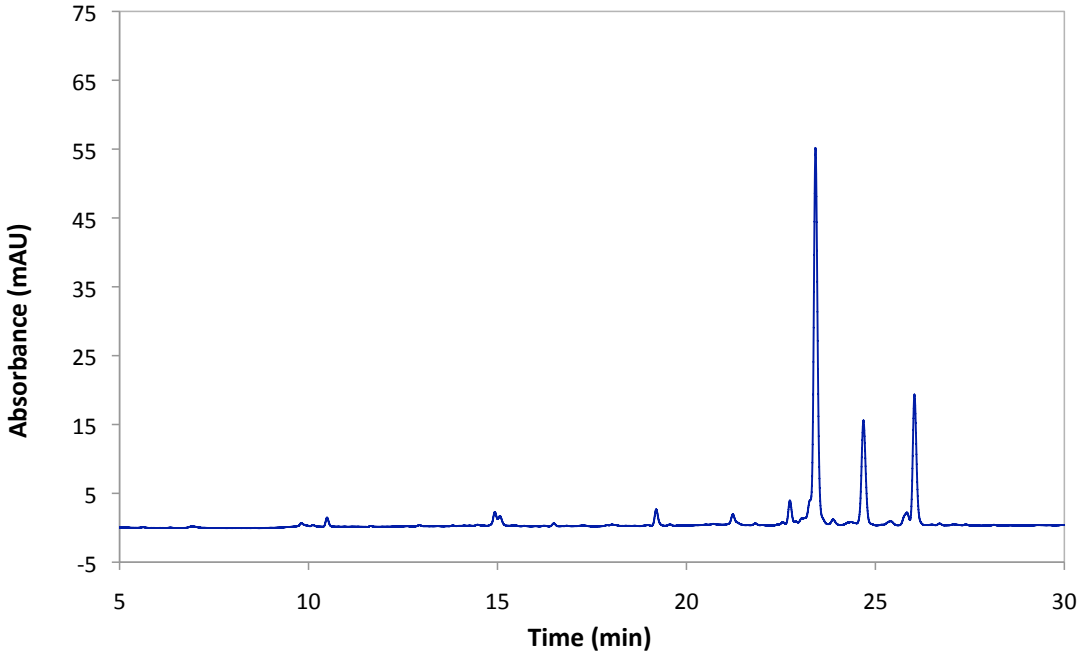
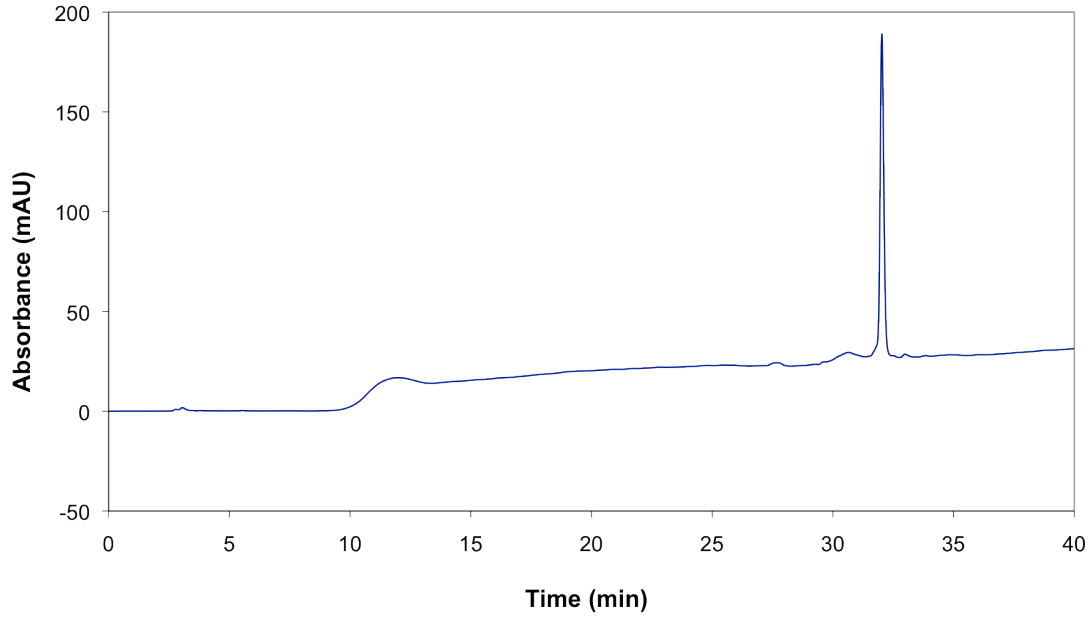
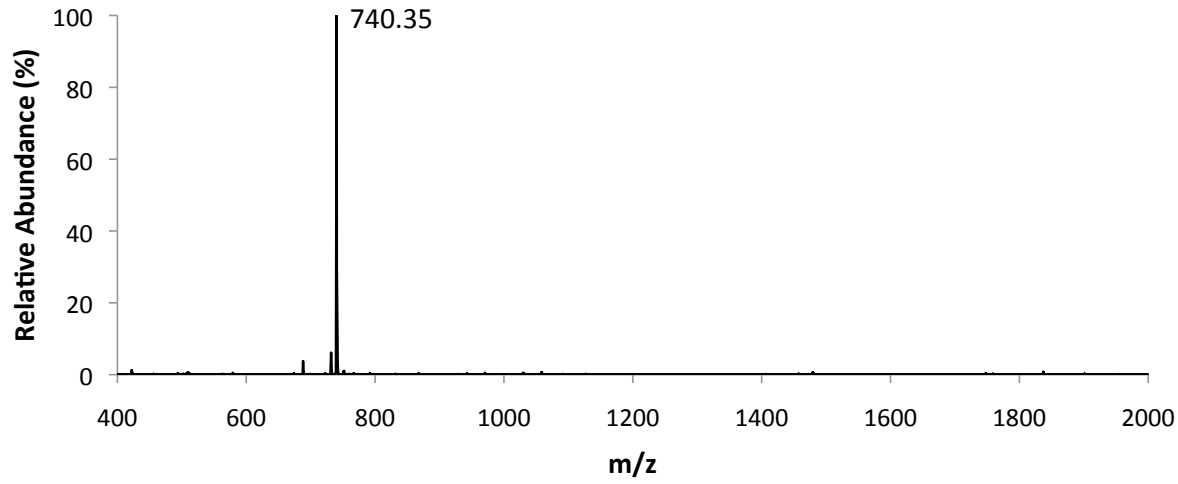


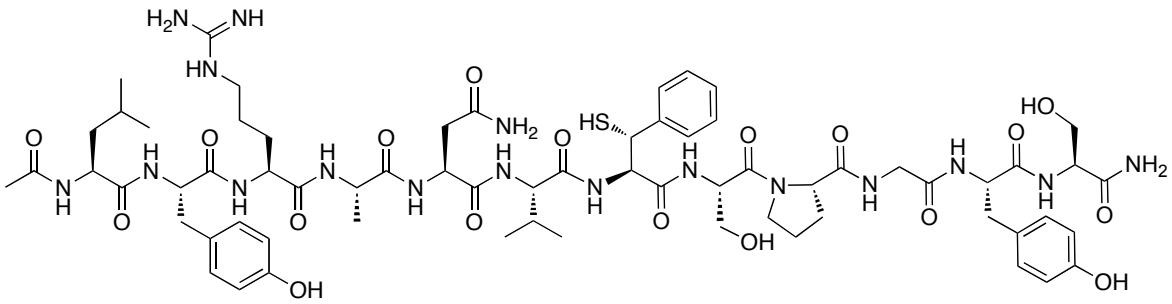
Figure S5. Analytical HPLC-MS trace (0 to 40% B over 30 min, 0.1% formic acid, λ = 280 nm) of the crude ligation reaction between H-(β-SH)FSPGYS **7** and Ac-LYRANM-S$(CH_2)_2CO_2Et$.



Analytical HPLC: R_t 32.0 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1478.66, $[M+2H]^{2+}$: 740.36; Mass Found ESI(+); 740.35 $[M+2H]^{2+}$



Ac-LYRANV(β-SH)FSPGYS-NH_2



The title compound was prepared *via* ligation of H-(β-SH)FSPGYS-NH_2 **7** (2.0 mg, 2.9 μmol, 5 mM concentration) and Ac-LYRANV-S$(CH_2)_2CO_2$Et (3.2 mg, 3.6 μmol, 6.2 mM concentration) according to General Protocol A and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.4 mg, 87% yield).

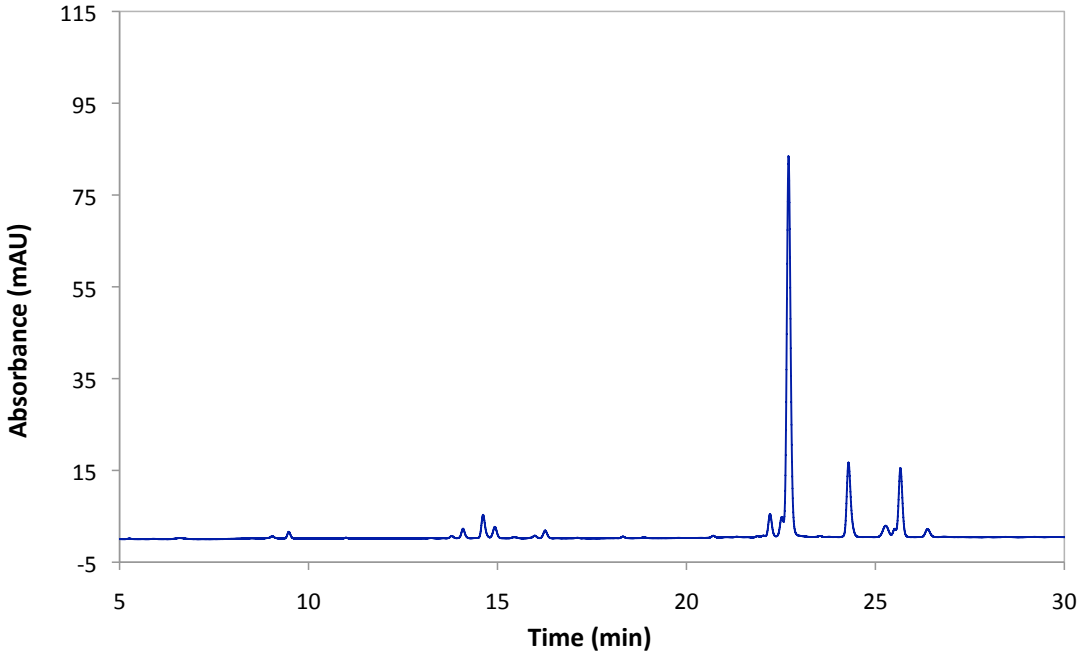
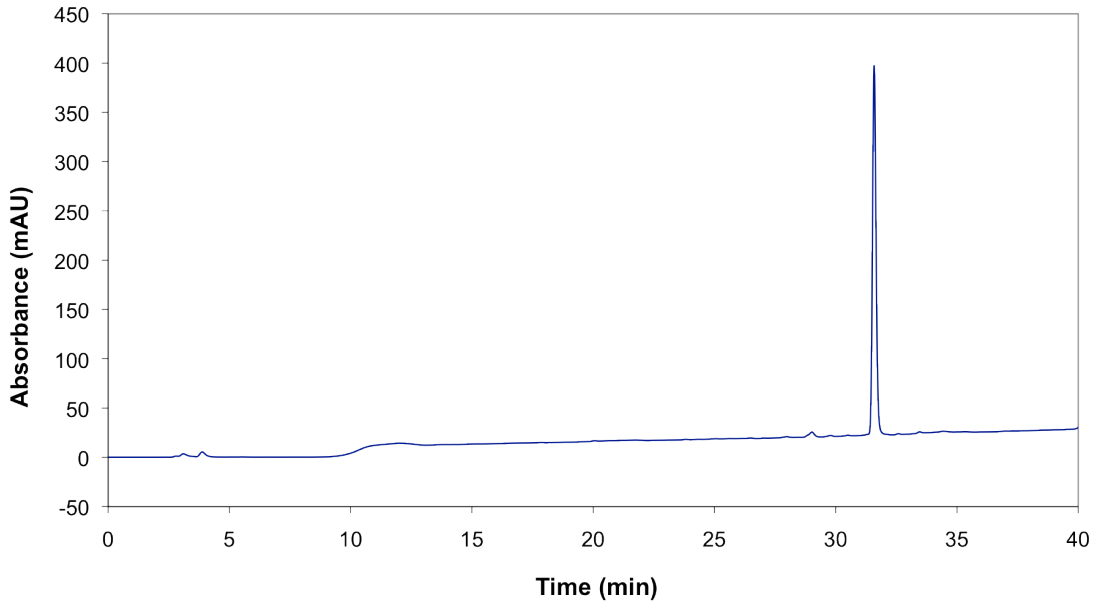
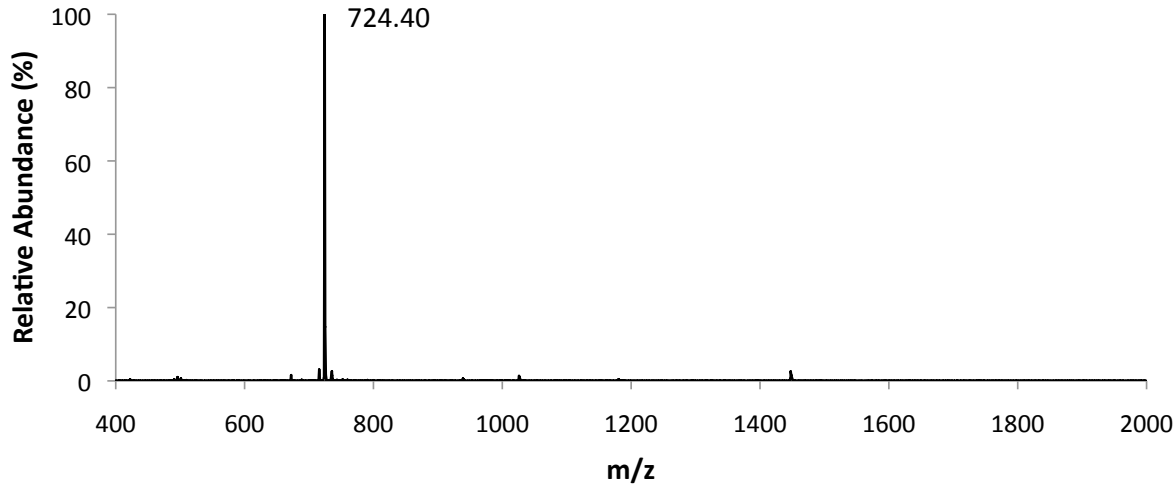


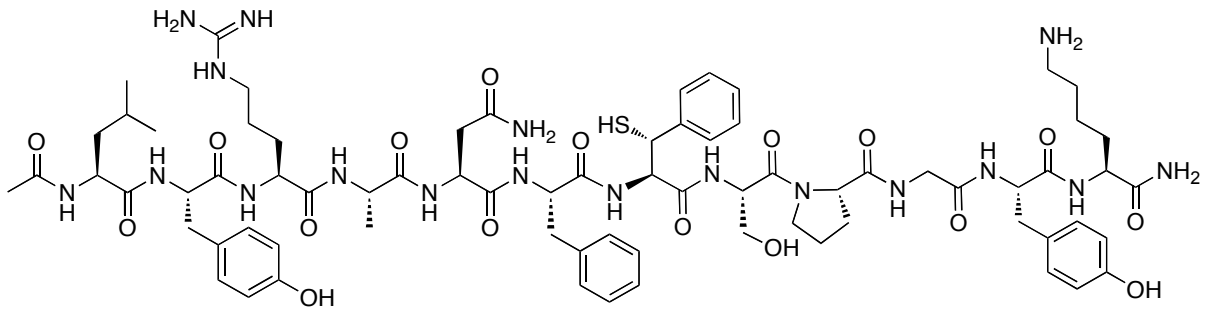
Figure S6. Analytical HPLC-MS trace (0 to 40% B over 30 min, 0.1% formic acid, λ = 280 nm) of the crude ligation reaction between H-(β-SH)FSPGYS **7** and Ac-LYRANV-S$(CH_2)_2CO_2$Et.



Analytical HPLC: R_t 31.6 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1446.68, $[M+2H]^{2+}$: 723.84; Mass Found ESI(+); 1447.6 $[M+H]^+$, 724.40 $[M+2H]^{2+}$



Ac-LYRANF(β-SH)FSPGYK-NH_2



The title compound was prepared *via* ligation of H-(β-SH)FSPGYK-NH_2 and Ac-LYRANF-$S(CH_2)_2CO_2Et$ according to General Protocol A and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (2.5 mg, 68% yield).

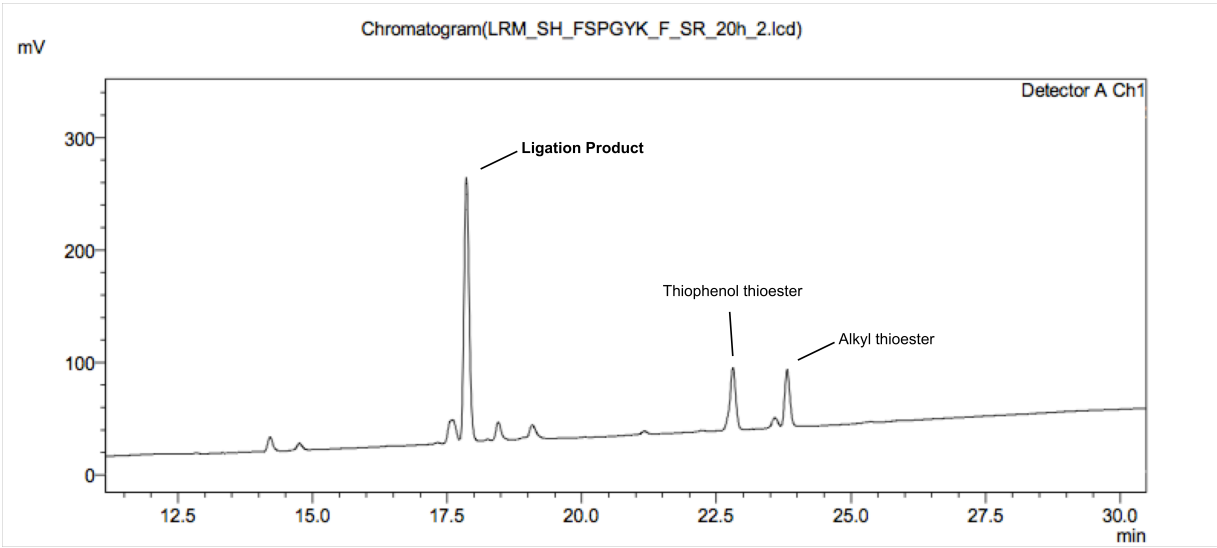
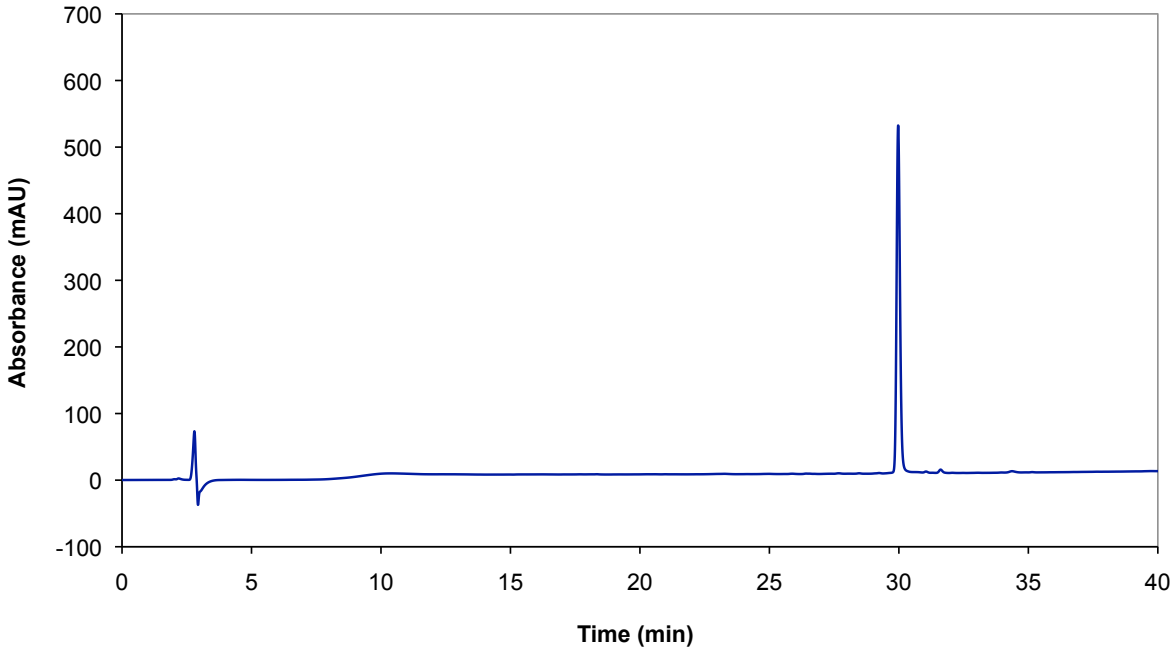
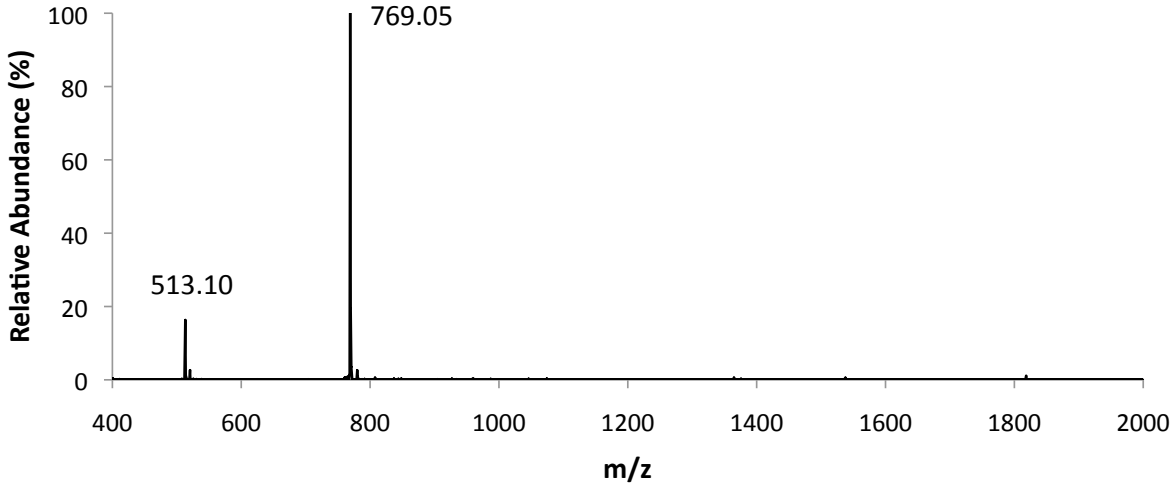


Figure S7. Analytical HPLC trace (0 to 50% B over 30 min, 0.1% formic acid, λ = 230 nm) of crude ligation reaction between H-(β-SH)FSPGYK-NH_2 and Ac-LYRANF-$S(CH_2)_2CO_2Et$ (t = 20 h).



Analytical HPLC: R_t 30.0 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1535.75, $[M+2H]^{2+}$: 768.38; Mass Found ESI(+); 769.05 $[M+2H]^{2+}$, 513.10 $[M+3H]^{3+}$



D. Radical desulfurization products (General Protocol B – Two-Step Method)

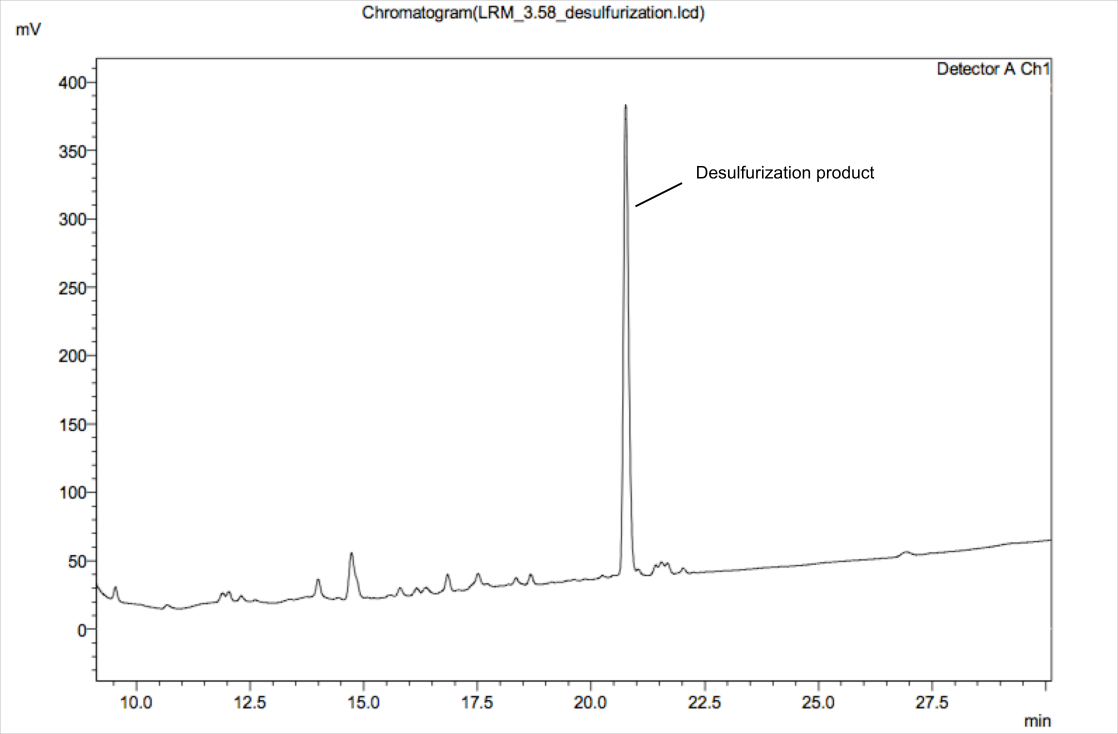
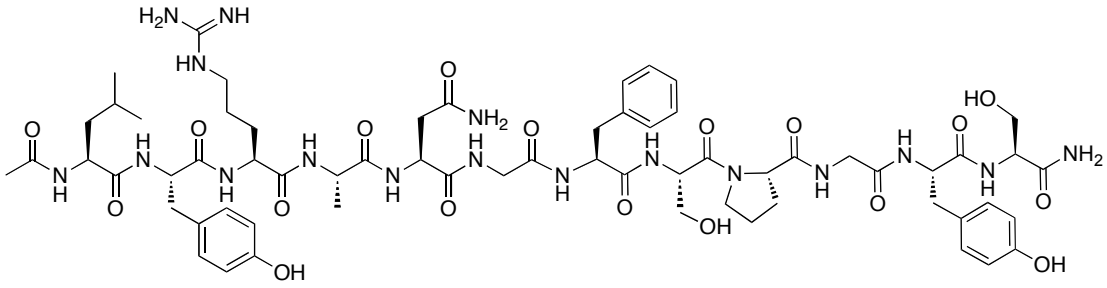


Figure S8. Representative analytical HPLC trace (0 to 40% B over 30 min, 0.1% formic acid, λ = 230 nm) of the crude desulfurization of Ac-LYRANG(β-SH)FSPGYS (t = 20 h).

Ac-LYRANGFSPGYS-NH_2



The title compound was prepared by desulfurization of Ac-LYRANG(β-SH)FSPGYS-NH_2 (2.0 mg, 1.4 μmol, 17.5 mM final concentration) according to General Protocol B and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (1.7 mg, 87% yield).

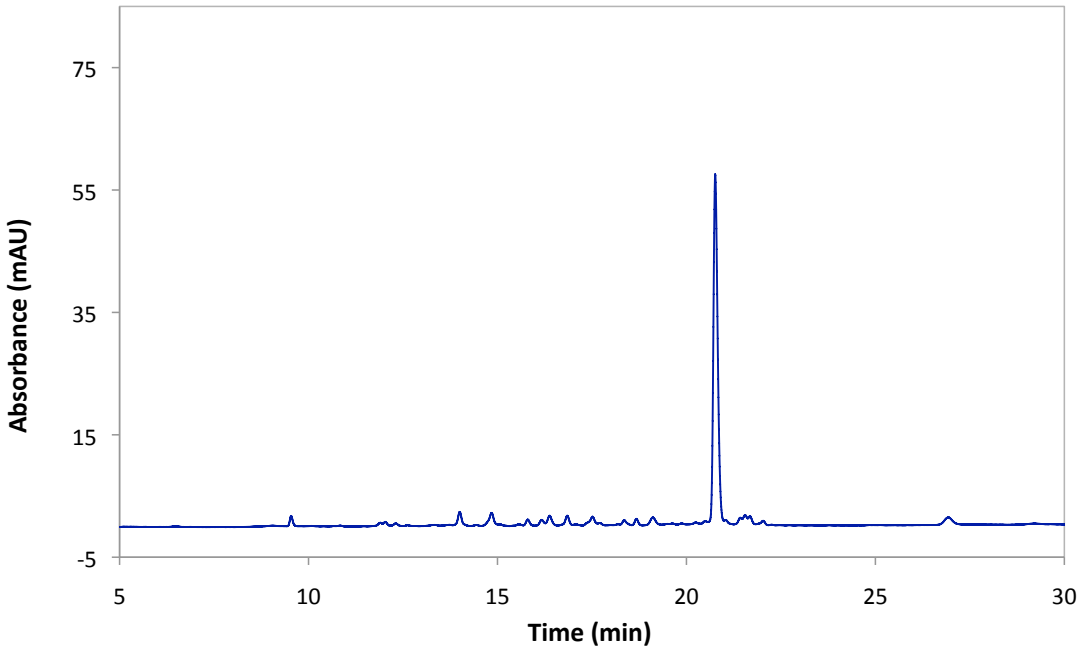
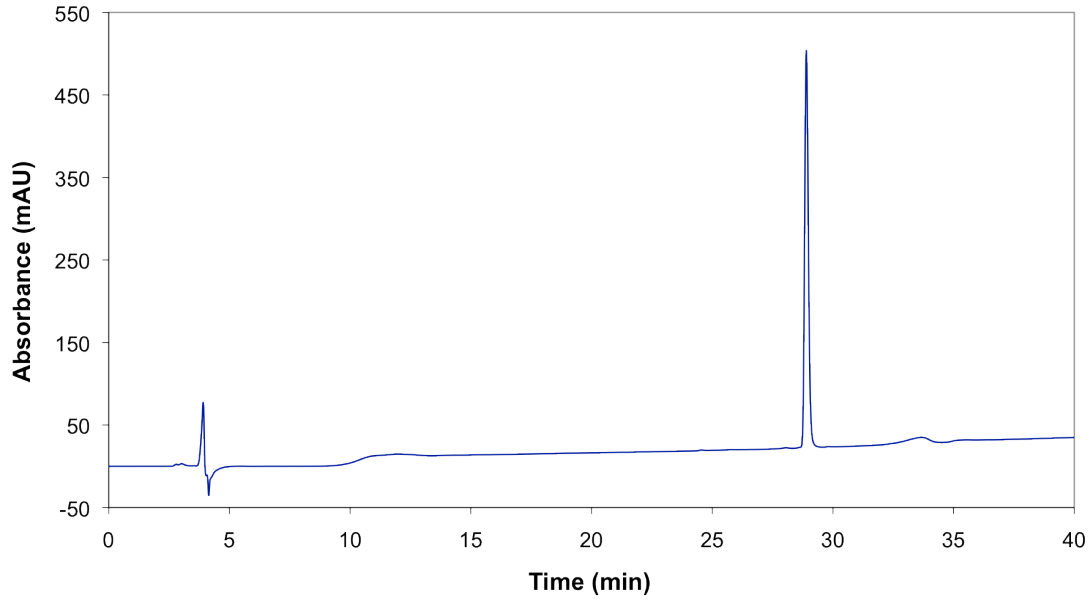
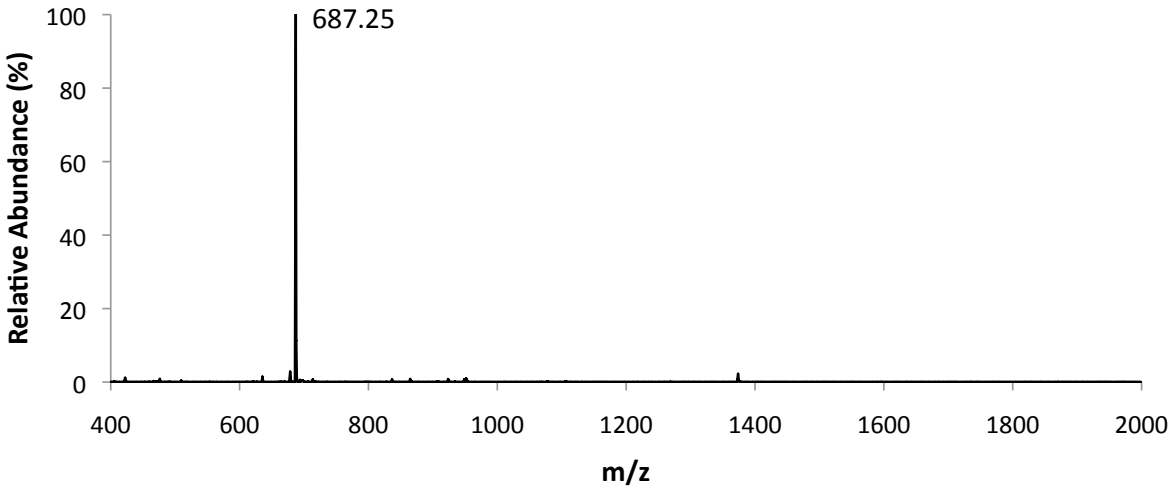


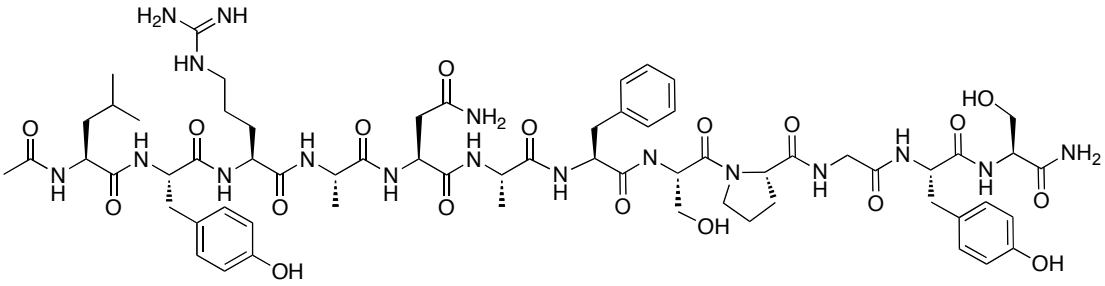
Figure S9. Analytical HPLC-MS trace (0 to 40% B over 30 min, 0.1% formic acid, λ = 280 nm) of the crude desulfurization of Ac-LYRANG(β-SH)FSPGYS.



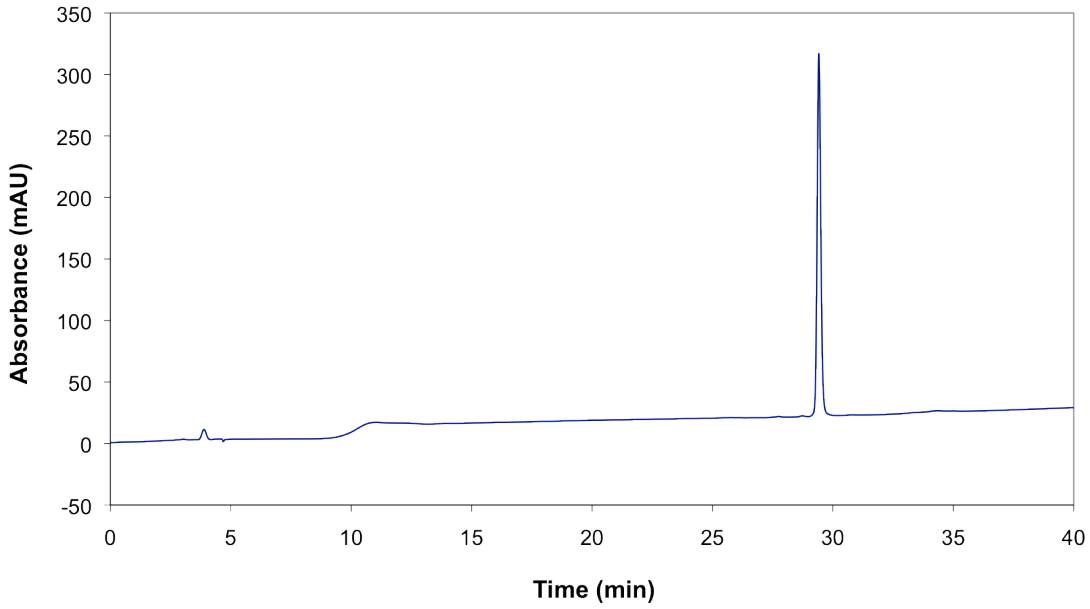
Analytical HPLC: R_t 28.9 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1372.66, $[M+2H]^{2+}$: 686.84; Mass Found ESI(+); 1373.7 $[M+H]^+$, 687.25 $[M+2H]^{2+}$


687.25
Relative Abundance (%)
100
80
60
40
20
0
400
600
800
1000
1200
1400
1600
1800
2000
m/z

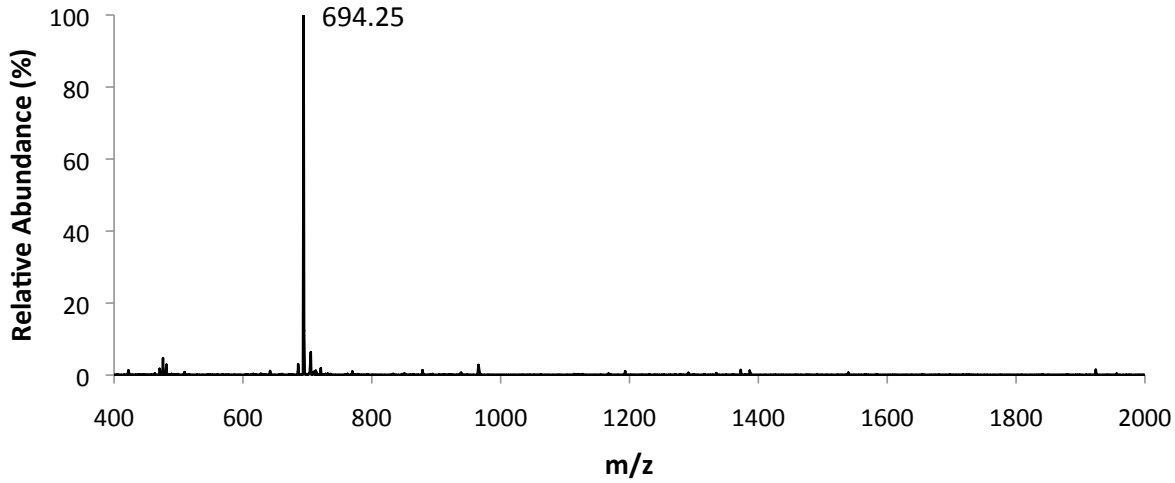
Ac-LYRANAFSPGYS-NH_2



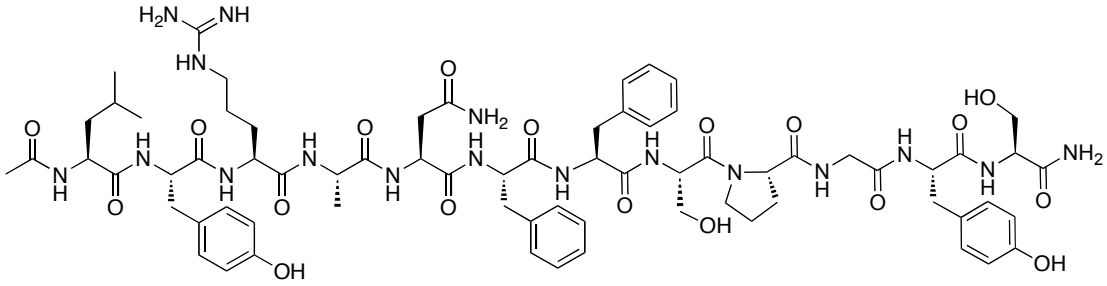
The title compound was prepared by desulfurization of Ac-LYRANA(β-SH)FSPGYS-NH_2 (1.0 mg, 0.70 μmol, 17.5 mM final concentration) according to General Protocol B and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (0.74 mg, 76% yield).



Analytical HPLC: R_t 29.4 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1386.68, $[M+2H]^{2+}$: 693.84; Mass Found ESI(+); 694.25 $[M+2H]^{2+}$



Ac-LYRANFFSPGYS-NH_2



The title compound was prepared by desulfurization of Ac-LYRANF(β-SH)FSPGYS-NH_2 (2.3 mg, 1.5 μmol, 19 mM final concentration) according to General Protocol B and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (1.3 mg, 60% yield).

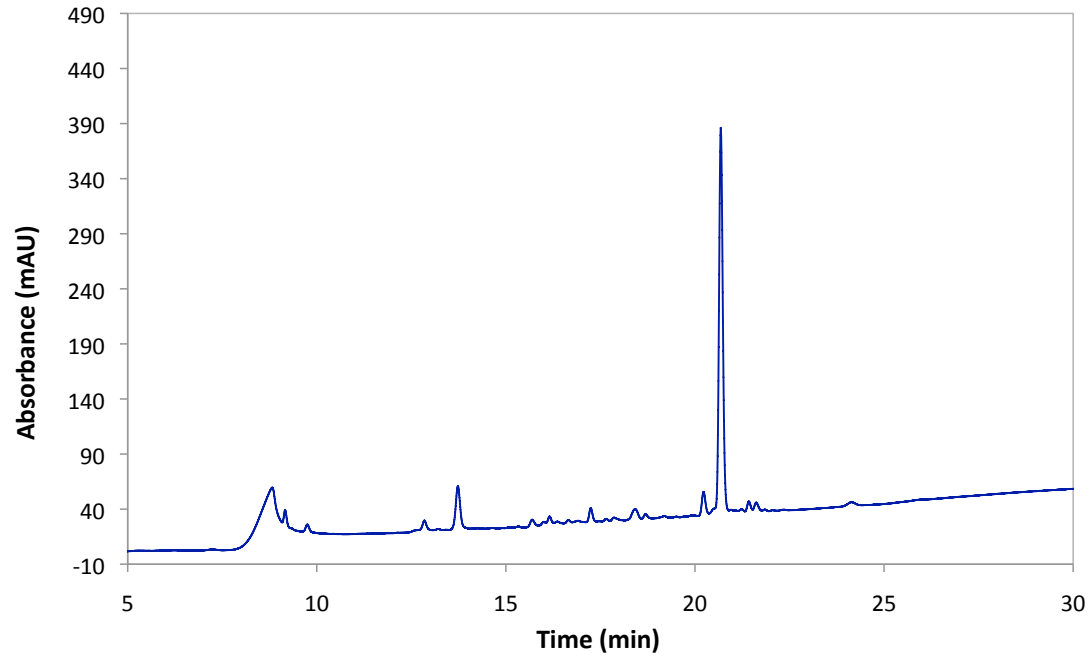
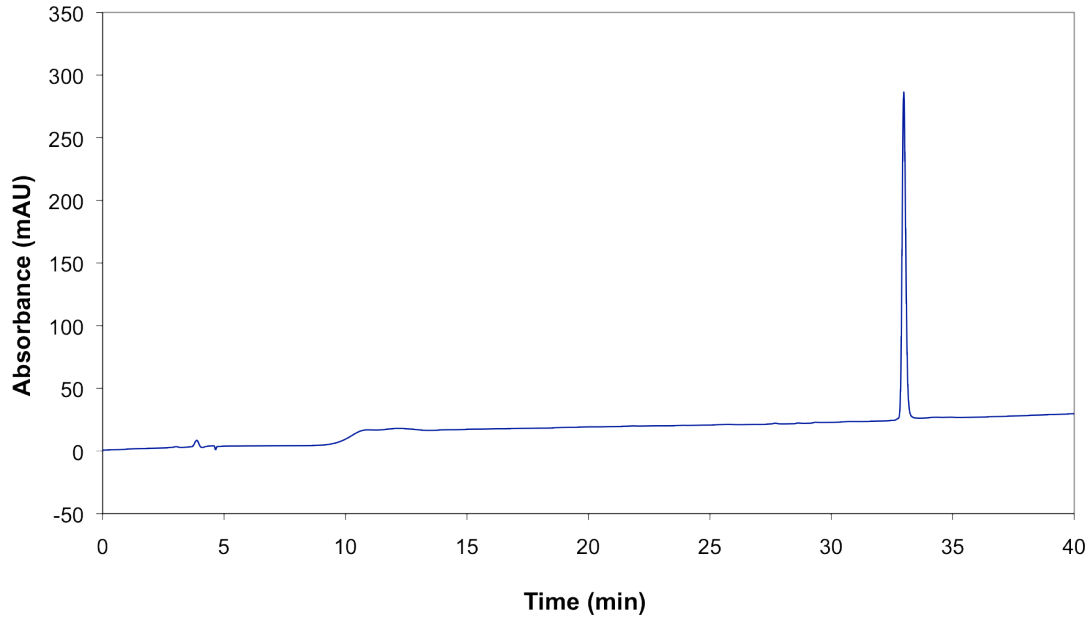
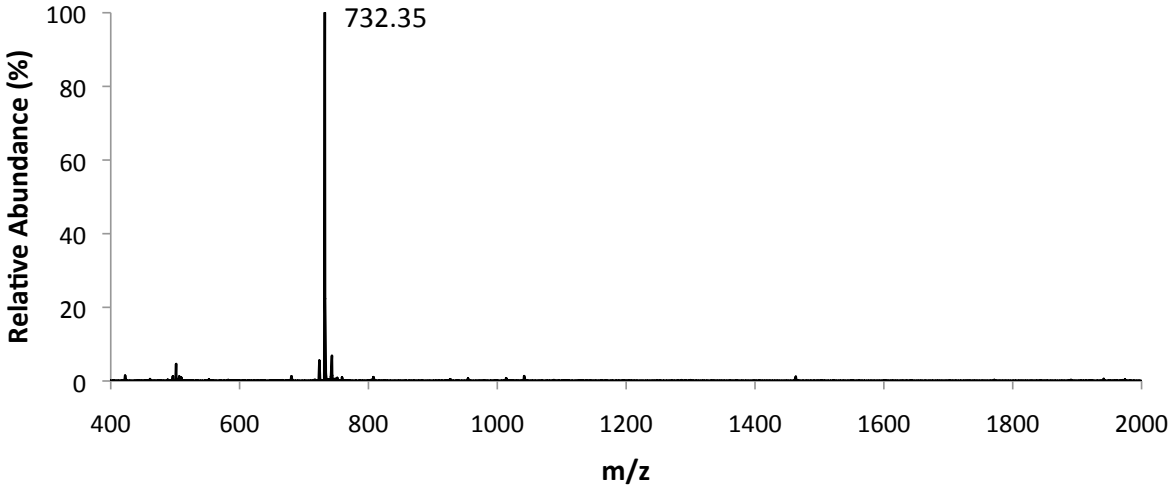


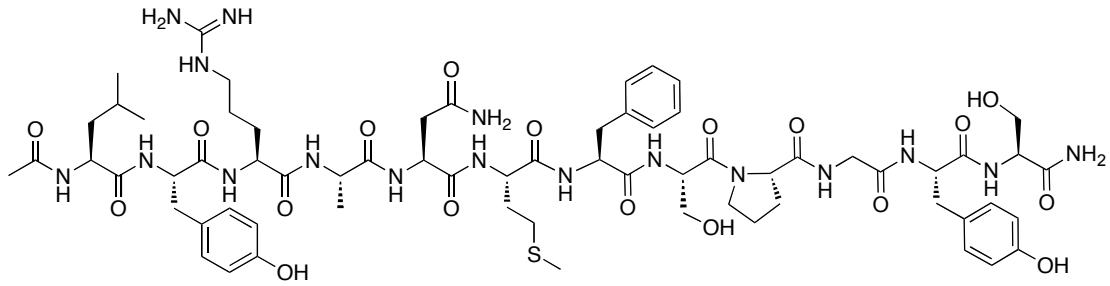
Figure S10. Analytical HPLC-MS trace (0 to 50% B over 30 min, 0.1% formic acid, λ = 230 nm) of the crude desulfurization of Ac-LYRANF(β-SH)FSPGYS.



Analytical HPLC: R_t 33.0 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1462.71; $[M+2H]^{2+}$: 731.86; Mass Found ESI(+); 732.35 $[M+2H]^{2+}$


732.35
Relative Abundance (%)
100
80
60
40
20
0
400
600
800
1000
1200
1400
1600
1800
2000
m/z

Ac-LYRANMFSPGYS-NH_2



The title compound was prepared by desulfurization of Ac-LYRANM(β-SH)FSPGYS-NH_2 (2.1 mg, 1.4 μmol, 17.5 mM final concentration) according to General Protocol B and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (1.0 mg, 52% yield).

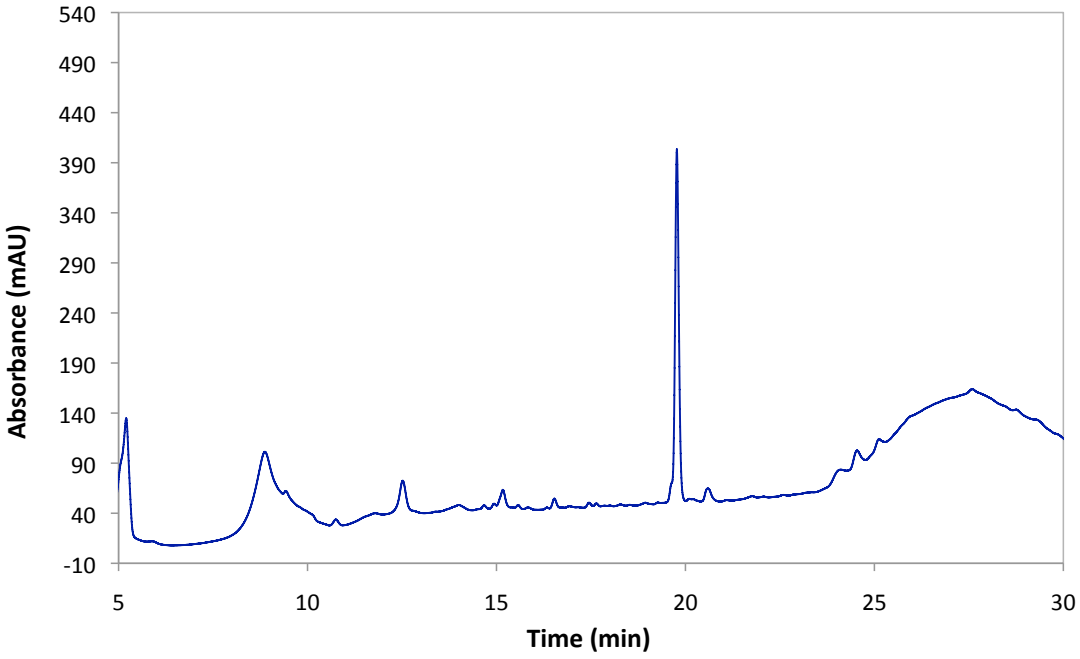
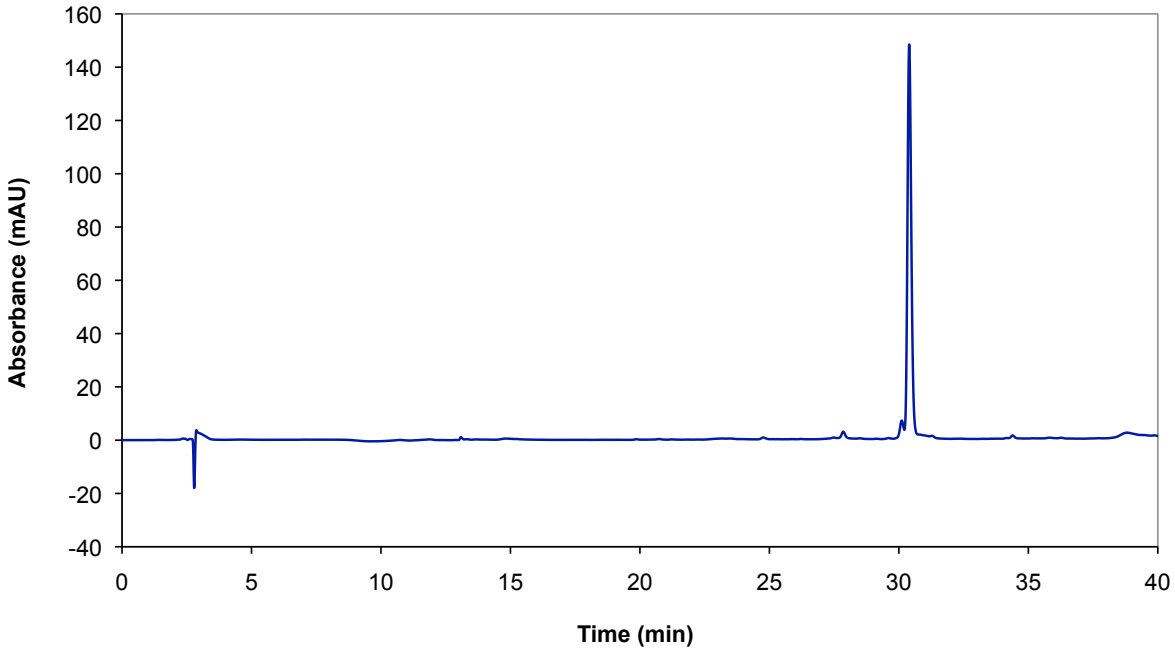
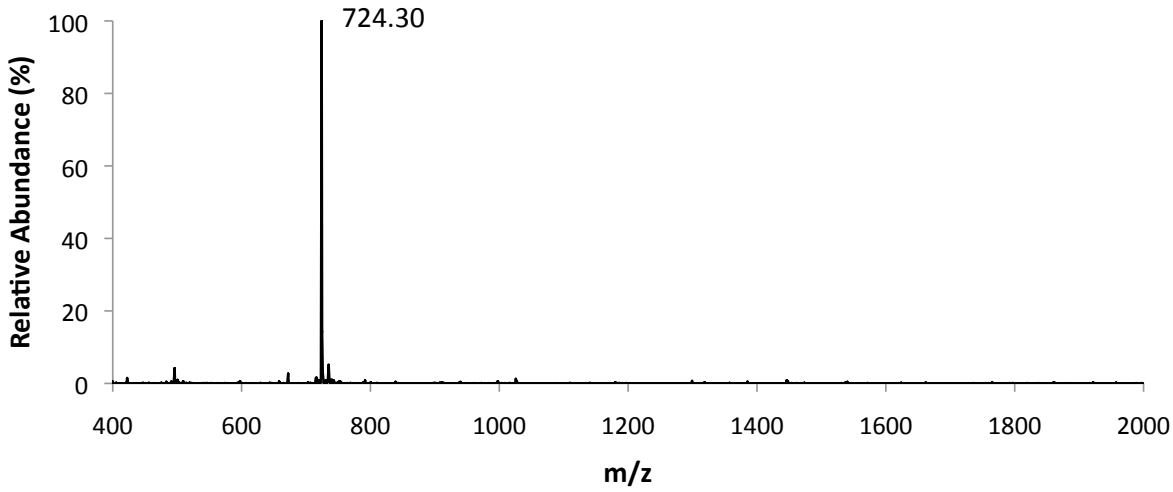


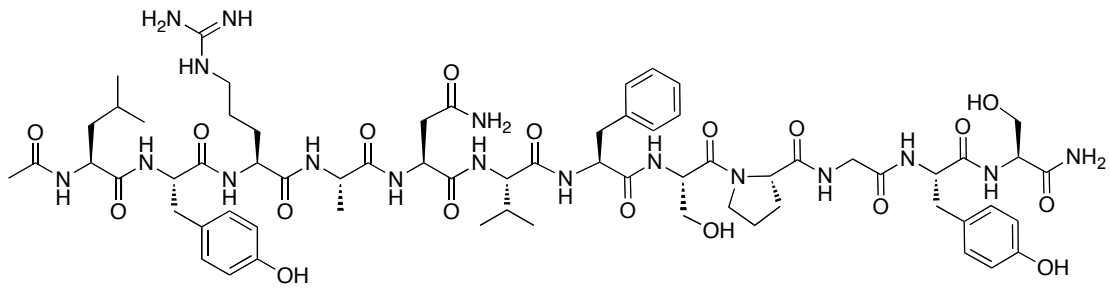
Figure S11. Analytical HPLC-MS trace (0 to 50% B over 30 min, 0.1% formic acid, λ = 230 nm) of the crude desulfurization of Ac-LYRANM(β-SH)FSPGYS.



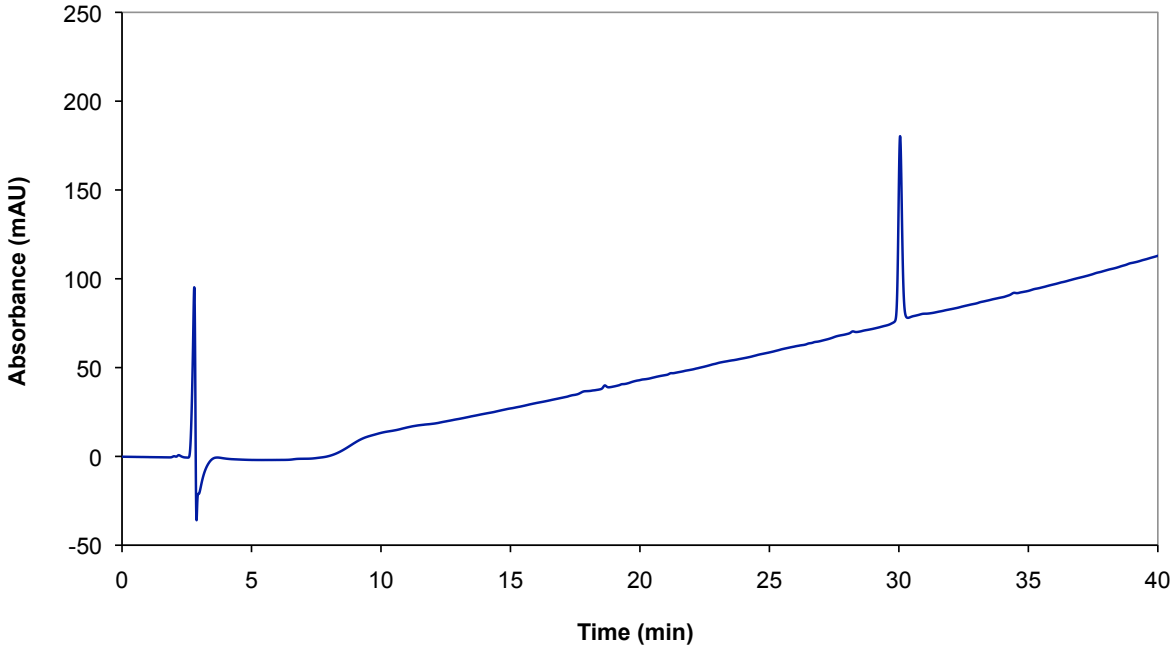
Analytical HPLC: R_t 30.4 min (0-50% B over 40 min, λ = 280 nm); Calculated Mass $[M+H]^+$: 1446.68, $[M+2H]^{2+}$: 723.84; Mass Found ESI(+); 724.3 $[M+2H]^{2+}$



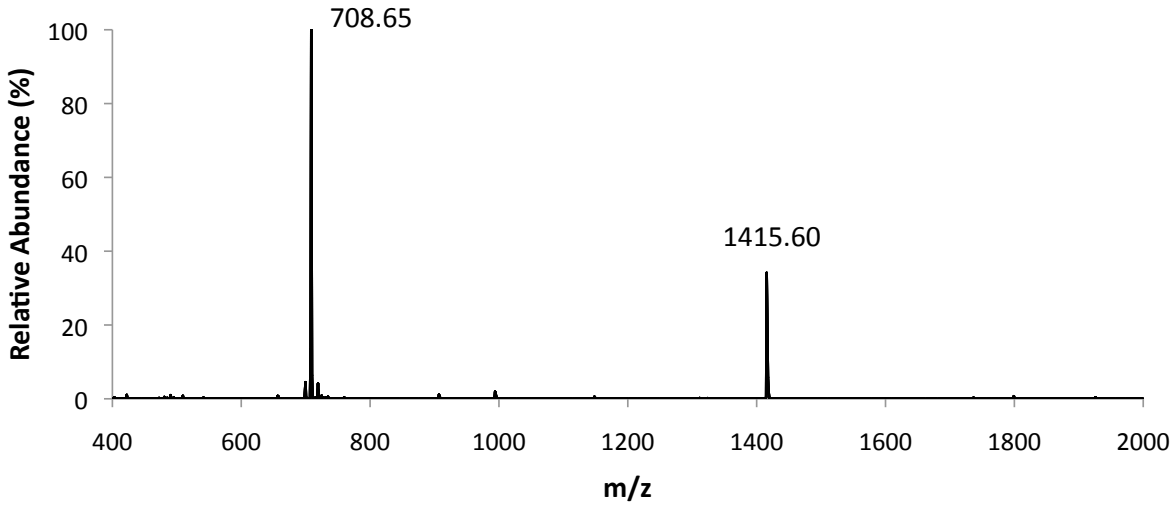
Ac-LYRANVFSPGYS-NH_2



The title compound was prepared by desulfurization of Ac-LYRANV(β-SH)FSPGYS-NH_2 (2.0 mg, 1.4 μmol, 17.5 mM final concentration) according to General Protocol B and purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (1.3 mg, 67% yield).

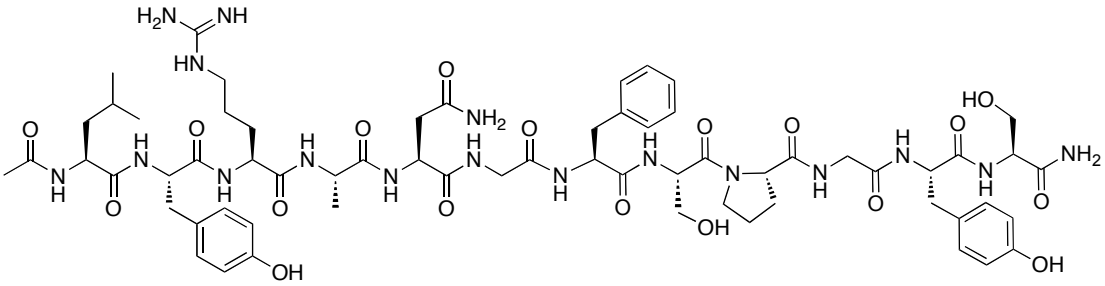


Analytical HPLC: R_t 30.0 min (0-50% B over 40 min, λ = 220 nm); Calculated Mass $[M+H]^+$: 1414.71, $[M+2H]^{2+}$: 707.86; Mass Found ESI(+); 1415.6 $[M+H]^+$, 708.65 $[M+2H]^{2+}$



E. TFET-promoted one-pot ligation-desulfurizations (General Protocol C)

Ac-LYRANGFSPGYS-NH_2



The title compound was prepared *via* TFET-promoted one-pot ligation-desulfurization (General Protocol C) beginning with peptide **7** (2.3 mg, 3.3 μmol, 5 mM concentration) and peptide thioester Ac-LYRANG-$S(CH_2)_2CO_2Et$ (3.0 mg, 3.5 μmol, 5.3 mM concentration). The crude ligation-desulfurization product was purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.0 mg, 68% yield).

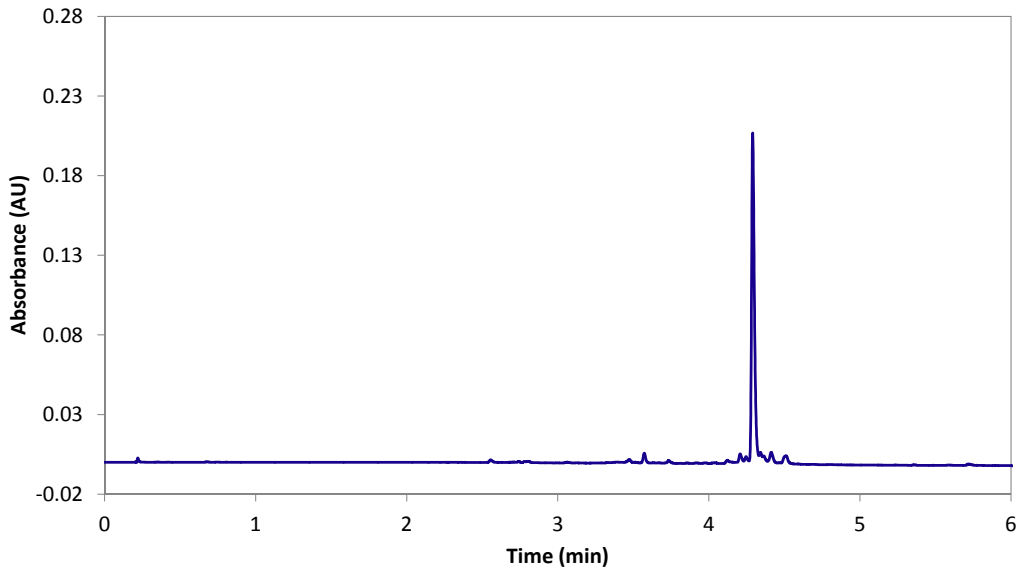
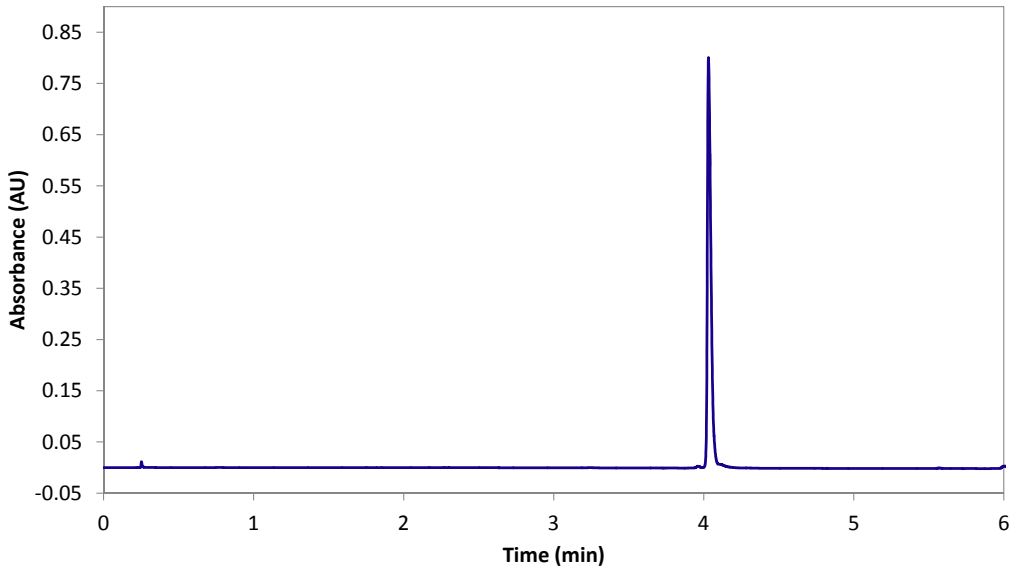
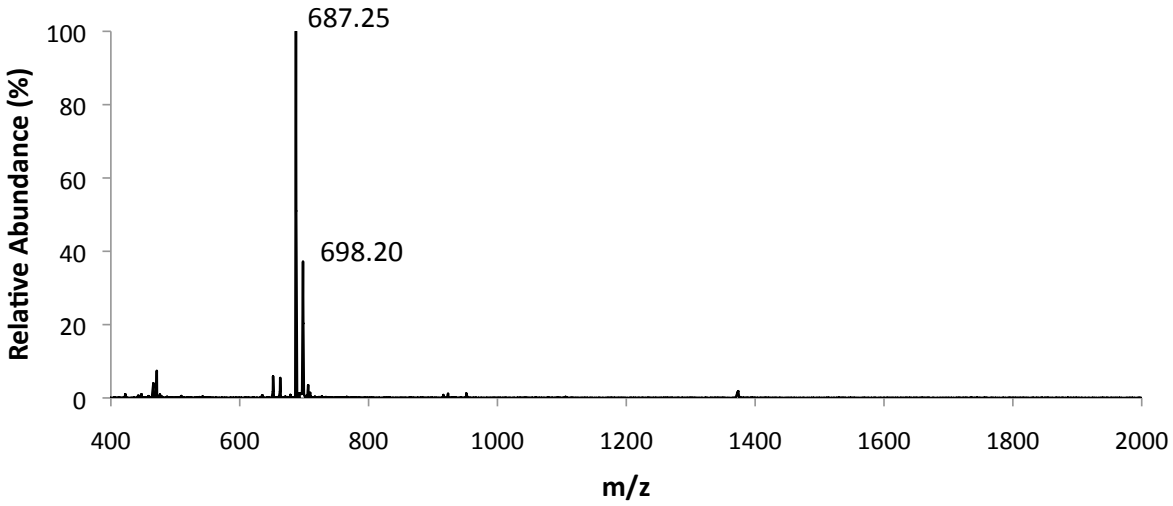


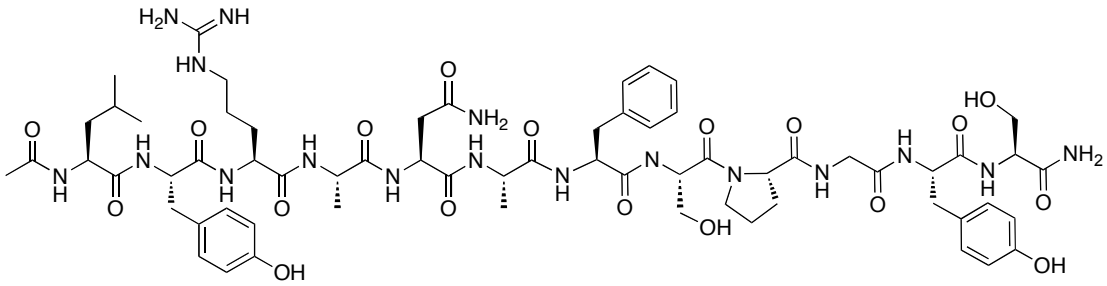
Figure S12. Analytical U-HPLC trace (0 to 50% B over 5 min, λ = 280 nm) of the crude one-pot ligation-desulfurization reaction of peptide **7** and peptide thioester Ac-LYRANG-$S(CH_2)_2CO_2Et$.



Analytical U-HPLC: R_t 4.0 min (0-50% B over 5 min, λ = 230 nm); Calculated Mass $[M+Na+H]^{2+}$: 697.83, $[M+2H]^{2+}$: 686.84; Mass Found ESI(+); 698.20 $[M+Na+H]^{2+}$, 687.25 $[M+2H]^{2+}$



Ac-LYRANAFSPGYS-NH_2



The title compound was prepared *via* TFET-promoted one-pot ligation-desulfurization (General Protocol C) beginning with peptide **7** (1.4 mg, 2.0 μmol, 5 mM concentration) and peptide thioester Ac-LYRANA-S$(CH_2)_2CO_2$Et (2.3 mg, 2.6 μmol, 6.5 mM concentration). The crude ligation-desulfurization product was purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (2.2 mg, 79% yield).

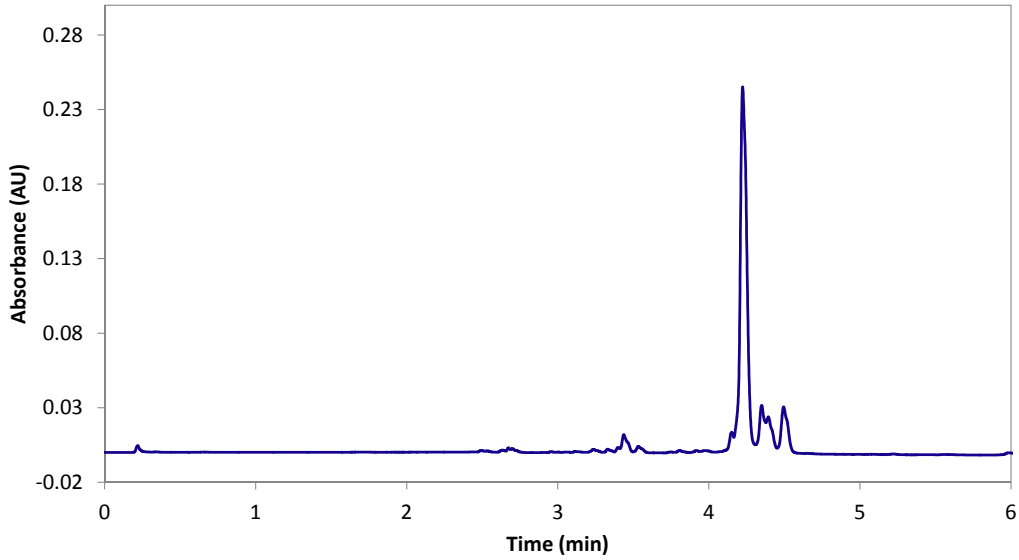
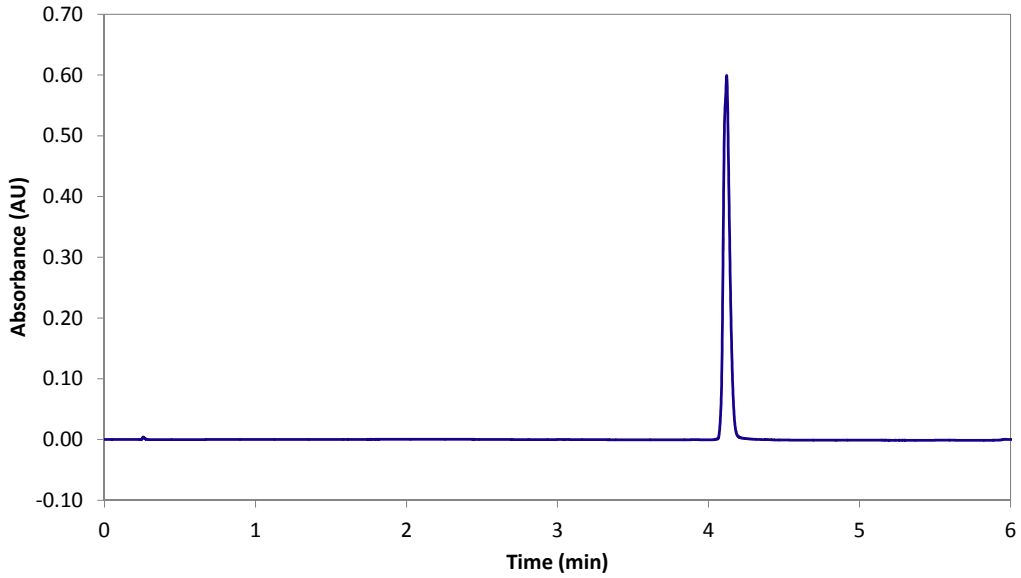
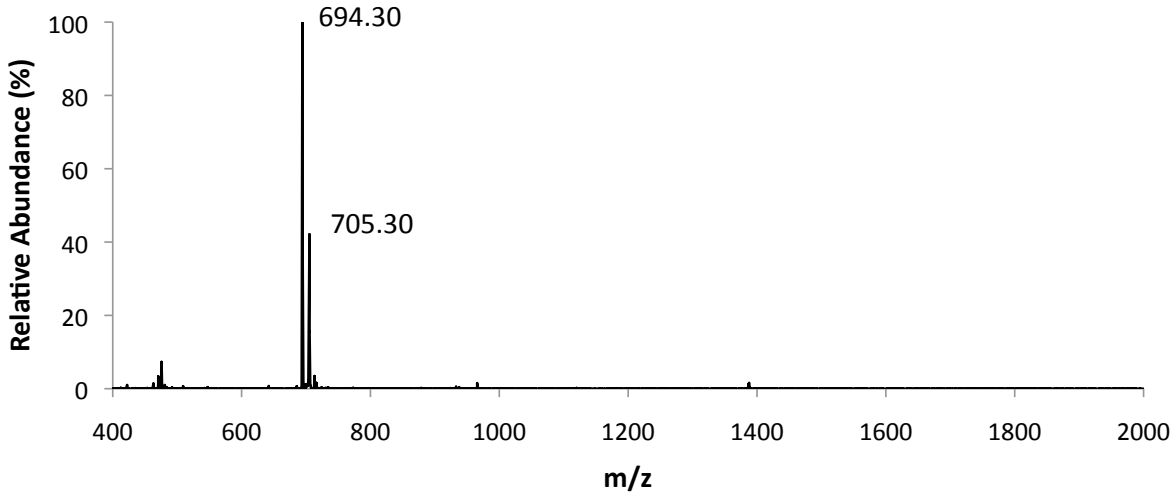


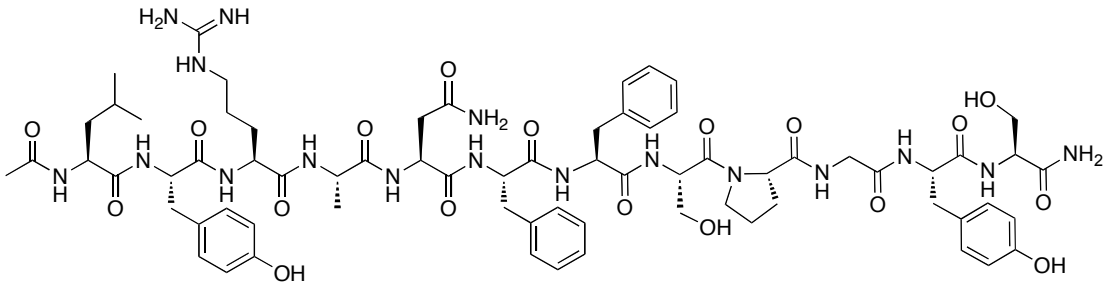
Figure S13. Analytical U-HPLC trace (0 to 50% B over 5 min, λ = 280 nm) of the crude one-pot ligation-desulfurization reaction of peptide **7** and peptide thioester Ac-LYRANA-S$(CH_2)_2CO_2$Et.



Analytical U-HPLC: R_t 4.1 min (0-50% B over 5 min, λ = 230 nm); Calculated Mass $[M+Na+H]^{2+}$: 704.83, $[M+2H]^{2+}$: 693.84; Mass Found ESI(+); 705.30 $[M+Na+H]^{2+}$, 694.30 $[M+2H]^{2+}$



Ac-LYRANFFSPGYS-NH_2



The title compound was prepared *via* TFET-promoted one-pot ligation-desulfurization (General Protocol C) beginning with peptide **7** (1.7 mg, 2.5 μmol, 5 mM concentration) and peptide thioester Ac-LYRANF-S$(CH_2)_2CO_2$Et (2.9 mg, 3.1 μmol, 6.2 mM concentration). The crude ligation-desulfurization product was purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.1 mg, 87% yield).

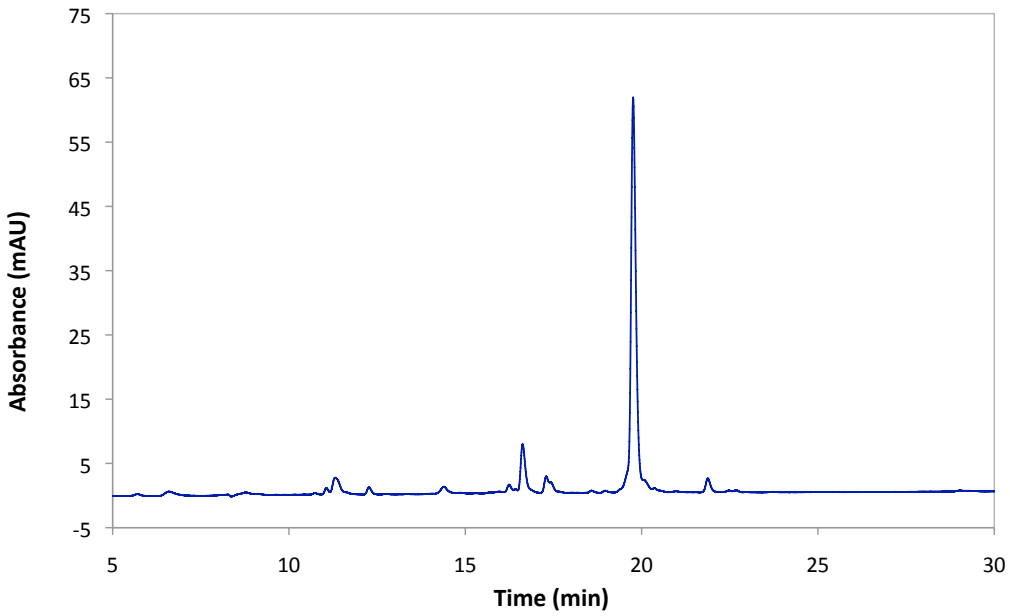
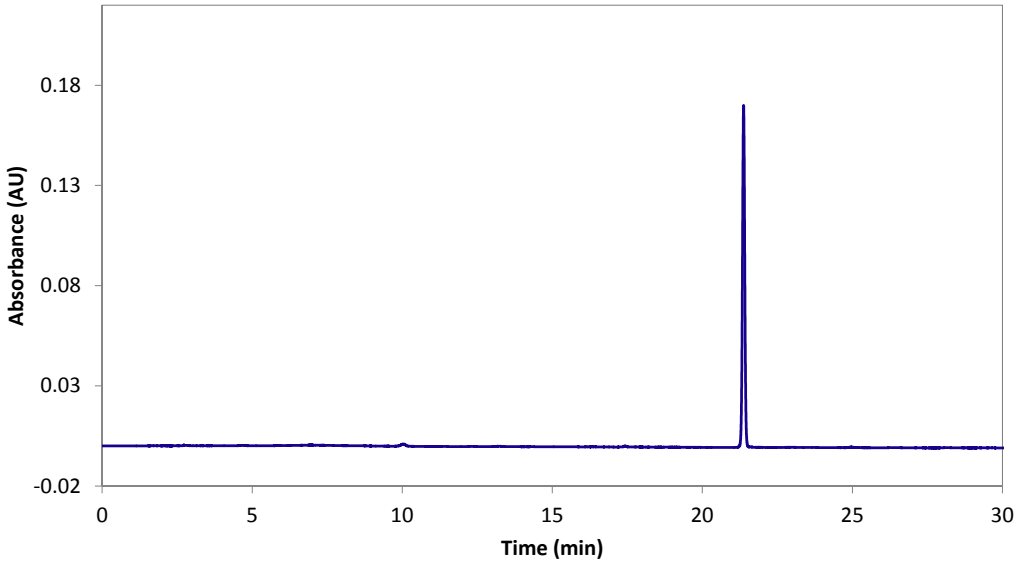
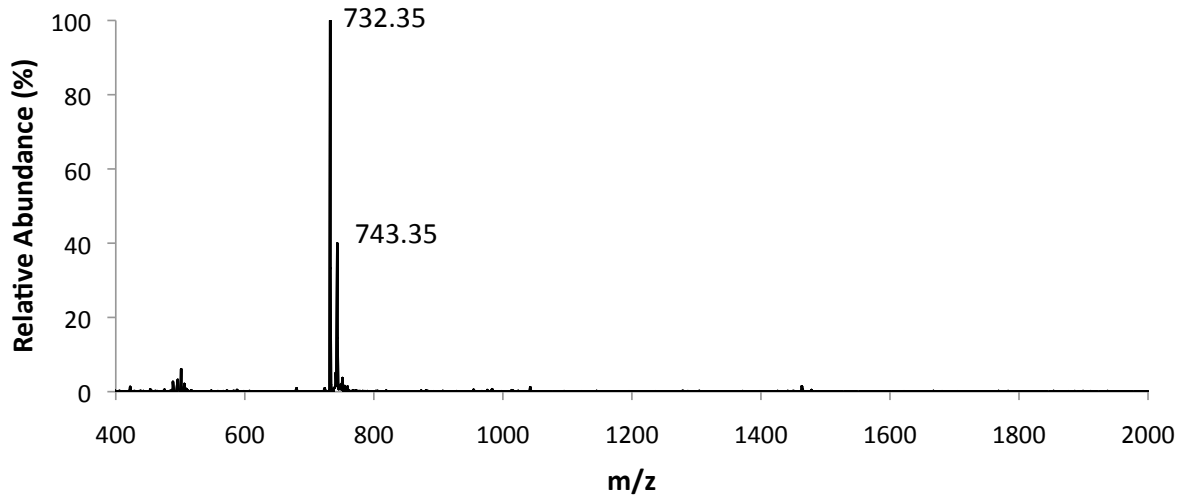


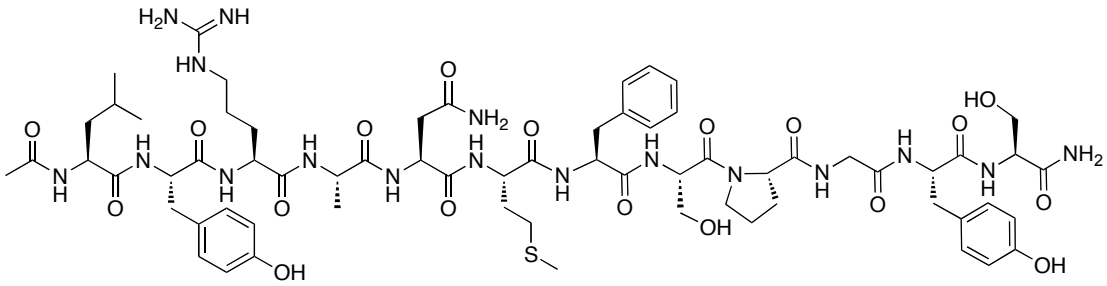
Figure S14. Analytical HPLC-MS trace (0 to 50% B over 30 min, 0.1% formic acid, λ = 280 nm) of the crude one-pot ligation-desulfurization reaction of peptide **7** and peptide thioester Ac-LYRANF-S$(CH_2)_2CO_2$Et.



Analytical HPLC: R_t 21.4 min (0-50% B over 30 min, λ = 280 nm); Calculated Mass $[M+Na+H]^{2+}$: 742.85; $[M+2H]^{2+}$: 731.86; Mass Found ESI(+); 743.35 $[M+Na+H]^{2+}$, 732.35 $[M+2H]^{2+}$



Ac-LYRANMFSPGYS-NH_2



The title compound was prepared *via* TFET-promoted one-pot ligation-desulfurization (General Protocol C) beginning with peptide **7** (2.2 mg, 3.2 μmol, 5 mM concentration) and peptide thioester Ac-LYRANM-S$(CH_2)_2CO_2$Et (3.6 mg, 3.9 μmol, 6.1 mM concentration). The crude ligation-desulfurization product was purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.3 mg, 71% yield).

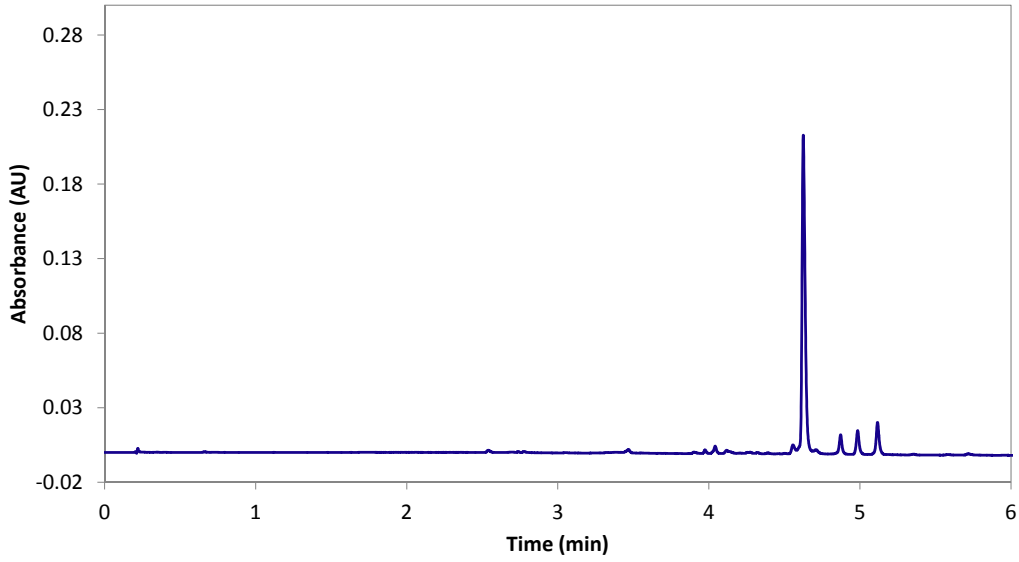
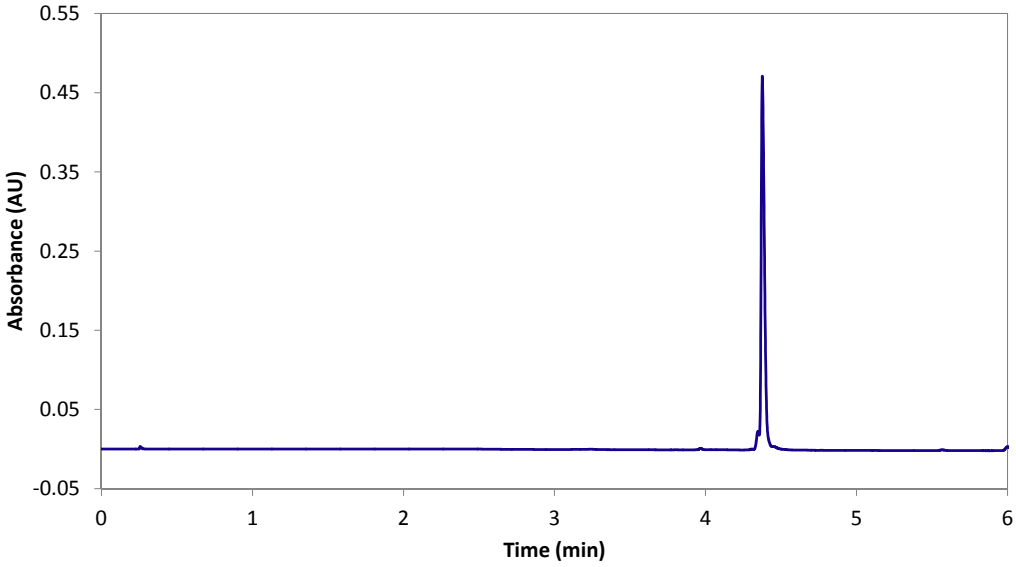
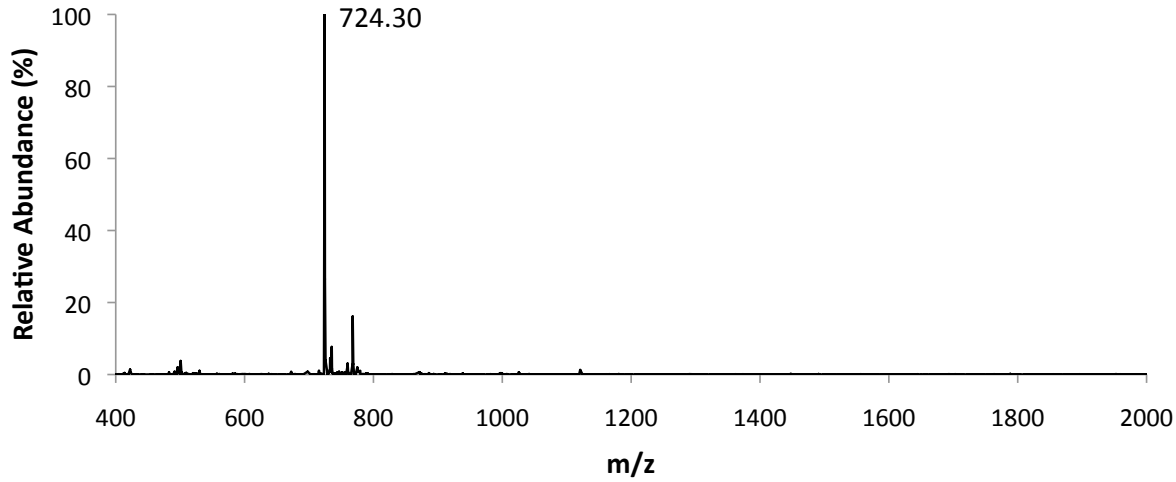


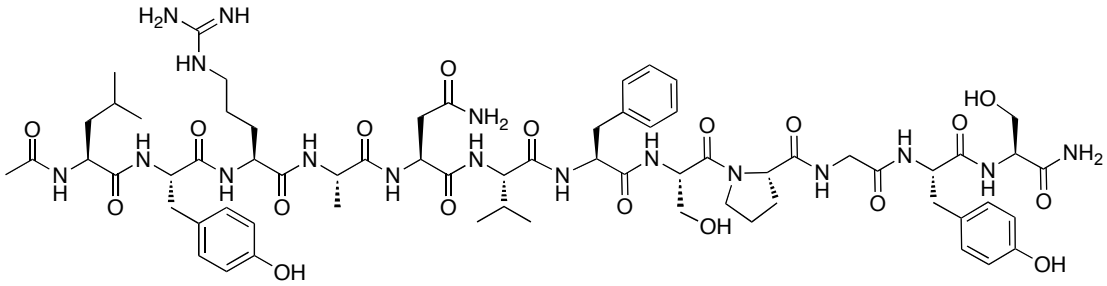
Figure S15. Analytical U-HPLC trace (0 to 50% B over 5 min, λ = 280 nm) of the crude one-pot ligation-desulfurization reaction of peptide **7** and peptide thioester Ac-LYRANM-S$(CH_2)_2CO_2$Et.



Analytical U-HPLC: R_t 4.4 min (0-50% B over 5 min, λ = 230 nm); Calculated Mass $[M+H]^+$: 1446.68, $[M+2H]^{2+}$: 723.84; Mass Found ESI(+); 724.30 $[M+2H]^{2+}$



Ac-LYRANVFSPGYS-NH_2



The title compound was prepared *via* TFET-promoted one-pot ligation-desulfurization (General Protocol C) beginning with peptide **7** (2.4 mg, 3.4 μmol, 5 mM concentration) and

peptide thioester Ac-LYRANV-S$(CH_2)_2CO_2Et$ (4.0 mg, 4.5 μmol, 6.6 mM concentration). The crude ligation-desulfurization product was purified by semi-preparative reverse-phase HPLC (0 to 50% B over 40 min) to afford the target compound as a white solid following lyophilization (3.2 mg, 68% yield).

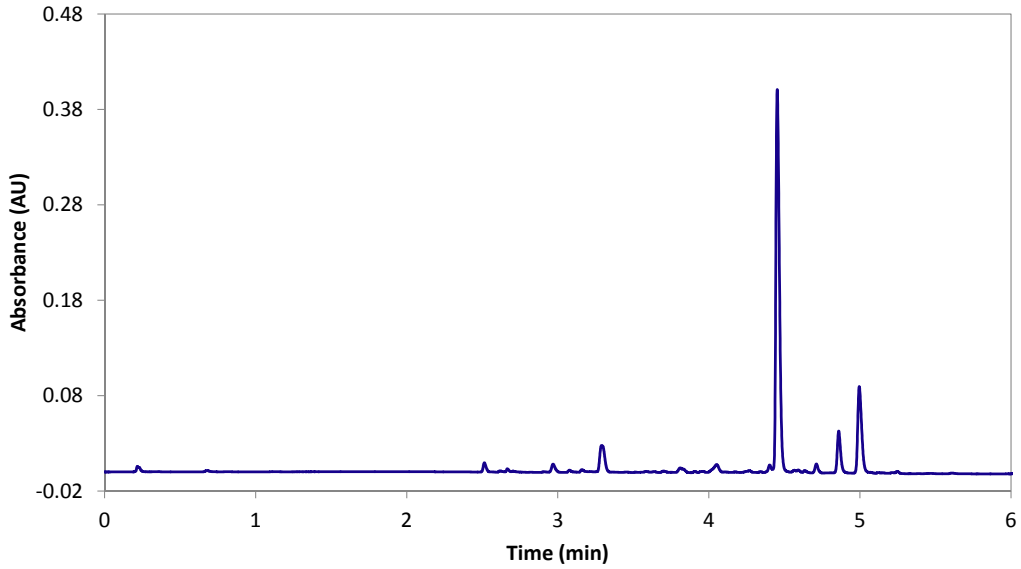
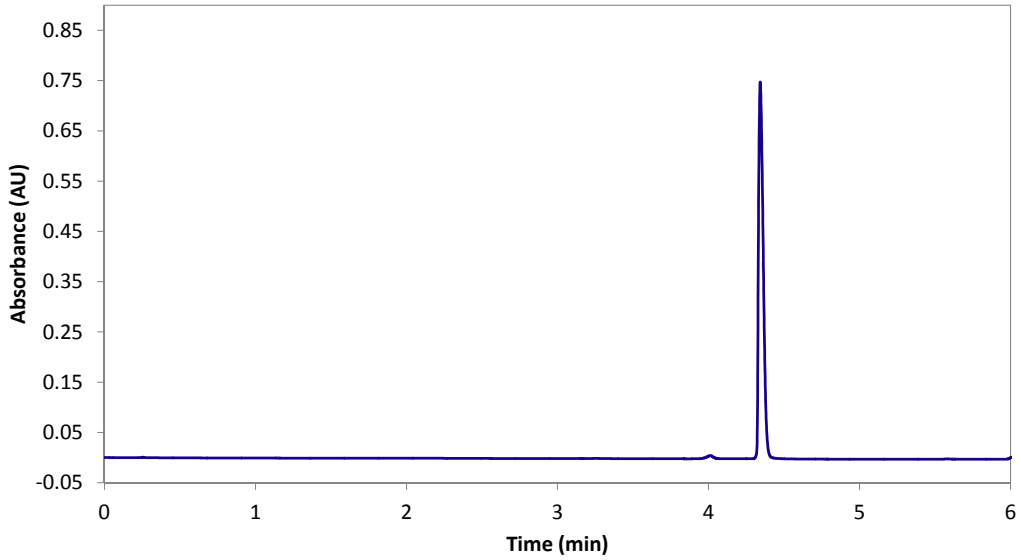
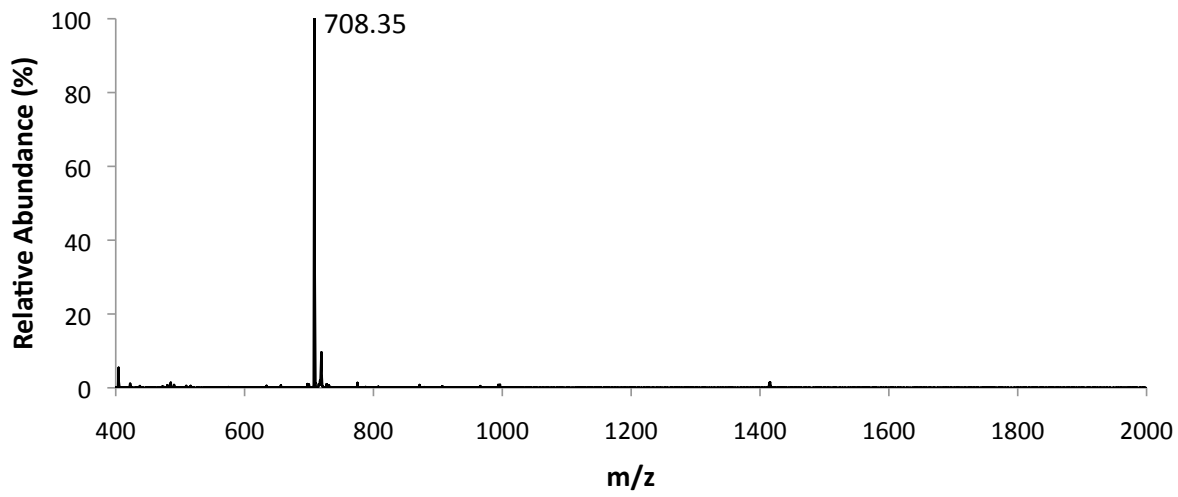


Figure S16. Analytical U-HPLC trace (0 to 50% B over 5 min, λ = 280 nm) of the crude one-pot ligation-desulfurization reaction of peptide **7** and peptide thioester Ac-LYRANV-S$(CH_2)_2CO_2Et$.



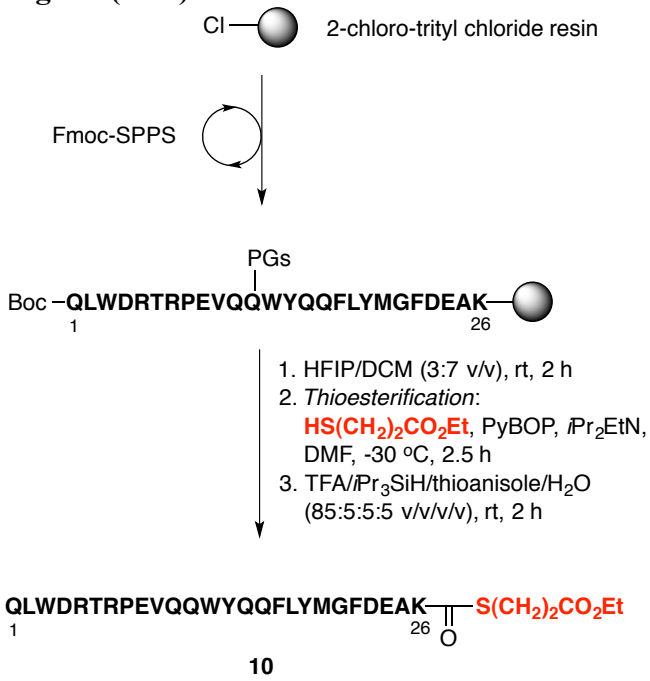
Analytical U-HPLC: R_t 4.3 min (0-50% B over 5 min, λ = 230 nm); Calculated Mass $[M+H]^+$: 1414.71, $[M+2H]^{2+}$: 707.86; Mass Found ESI(+); 708.35 $[M+2H]^{2+}$



Synthesis of peptide hormone augurin 9

Preparation of peptide fragments:

Peptide thioester 10 augurin(1-26)

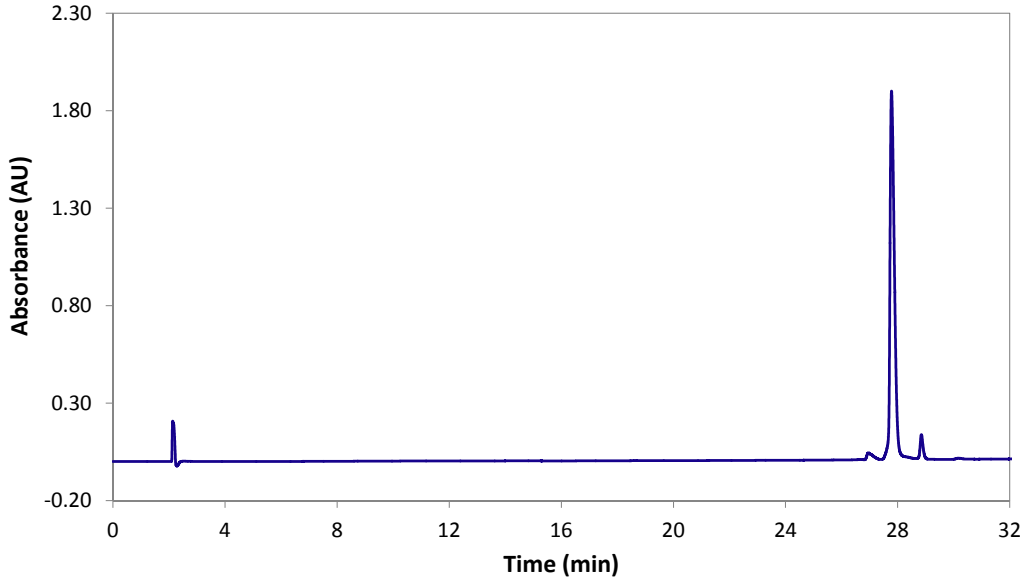


Scheme S1. Synthesis of augurin(1-26) thioester 10

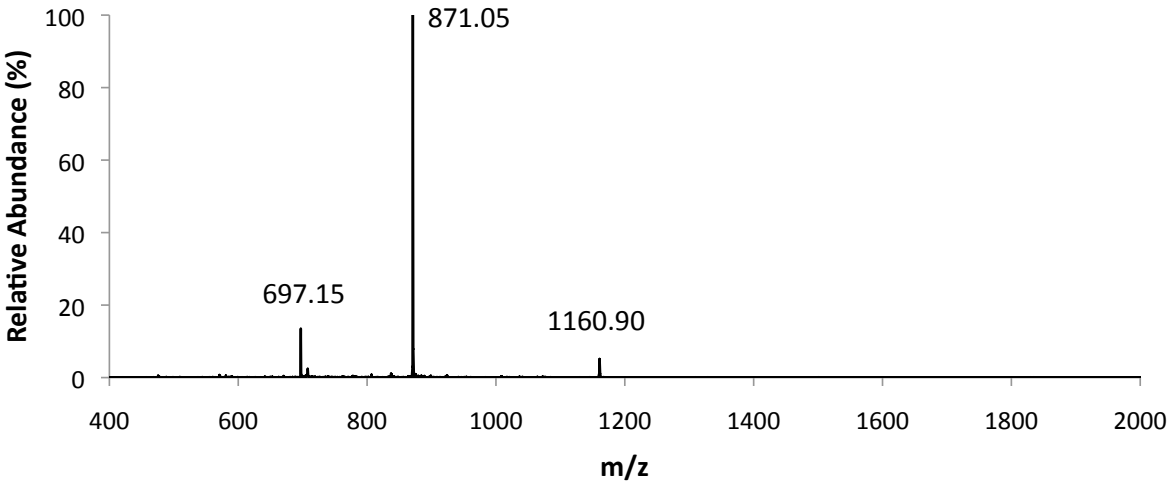
Peptide thioester **10** was prepared on 2-chloro-trityl chloride resin (100 µmol scale). Fmoc-Lys(Boc)-OH was first loaded to the resin according to the general procedures. The peptide was subsequently elongated using automated microwave Fmoc-SPPS. Following coupling of the N-terminal residue, Boc-Gln(Trt)-OH, the resin was split into 4 x 25 µmol portions. A

25 μmol portion of the resin-bound peptide was washed with DCM (10 x 3 mL) and treated with HFIP/DCM (3:7 v/v, 3 mL, 2 h) to cleave the peptide from the resin without cleaving the side chain protecting groups. The resin was then washed with DCM (3 x 2 mL) and the combined washings were concentrated *in vacuo* and dried overnight under vacuum.

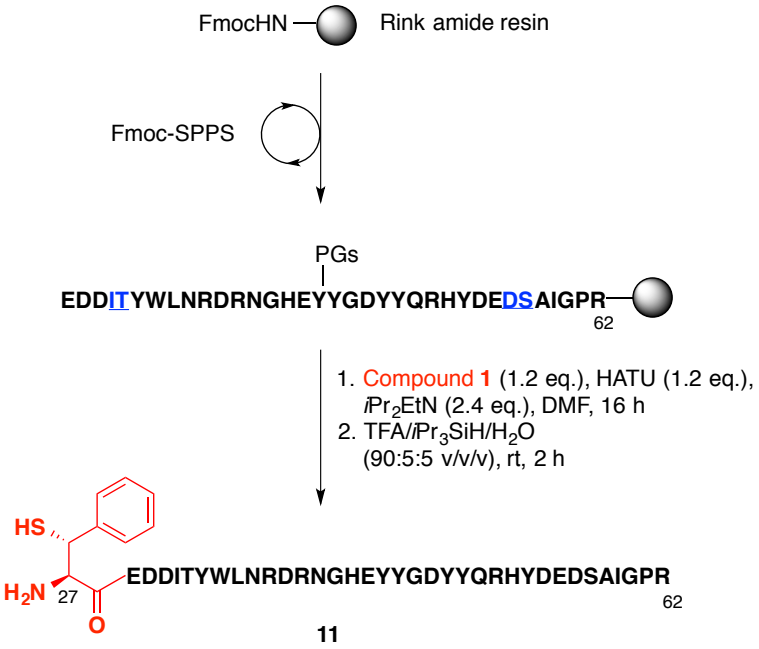
Thioesterification: The crude residue (25 μmol) was placed under an atmosphere of argon, dissolved in dry DMF (0.25 mL) and cooled to -30 ^{o}C. The mixture was treated with ethyl-3-mercaptopropionate (190 μL, 1.50 mmol) and DIPEA (43.5 μL, 250 μmol), followed by PyBOP (130 mg, 250 μmol) and stirred for 2.5 h at -30 ^{o}C. The solvent was removed under a stream of nitrogen and the residue dried under vacuum. After removal of all traces of DMF, the crude mixture was cooled to 0 ^{o}C and treated with a solution of TFA/triisopropylsilane/H_2O/thioanisole (85:5:5:5 v/v/v/v, 3 mL). The reaction was stirred at rt for 2 h and concentrated *in vacuo*. The peptide was precipitated in cold diethyl ether, and the crude product purified by preparative reverse-phase preparative HPLC (*Prep 1*: 0-50% B over 40 min, 0.1% formic acid, XBridge BEH300 C18 preparative column; *Prep 2*: 0 to 50% B over 40 min, 0.1% formic acid, Sunfire C-18 semi-preparative column) to yield the peptide thioester **10** as a white solid following lyophilization (4.2 mg, 5% yield based on the original resin loading). Note: to avoid N-terminal pyroglutamate formation, special care was taken to avoid prolonged exposure of thioester **10** to acidic HPLC buffers.



Analytical HPLC: R_t 27.8 min (0-50% B over 30 min, λ = 230 nm); Calculated Mass $[M+3H]^{3+}$: 1160.55, $[M+4H]^{4+}$: 870.66, $[M+5H]^{5+}$: 696.73; Mass Found ESI(+); 1160.90 $[M+3H]^{3+}$, 871.05 $[M+4H]^{4+}$, 697.15 $[M+5H]^{5+}$

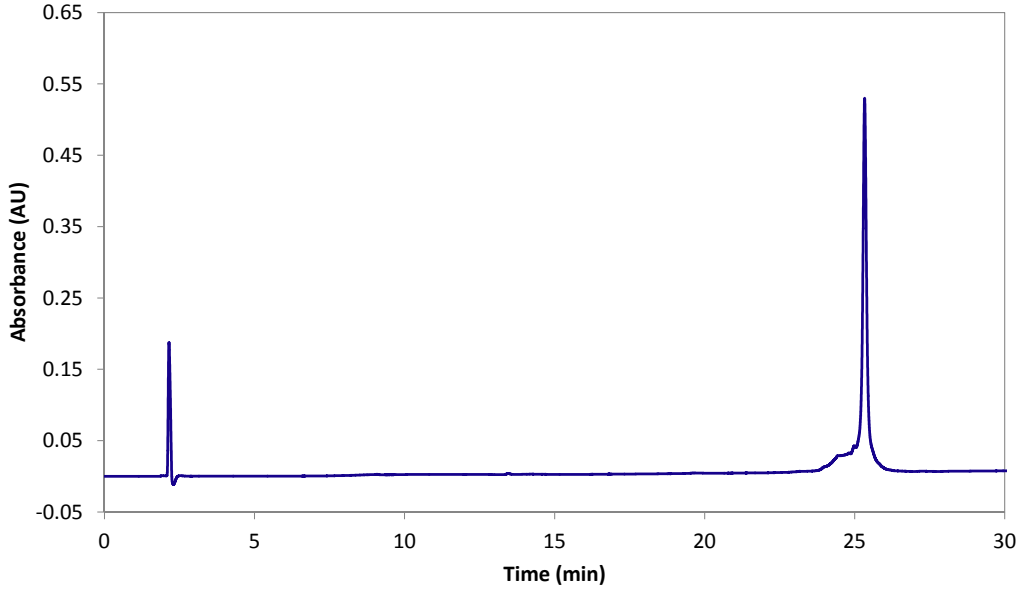

Relative Abundance (%)
100
80
60
40
20
0
871.05
697.15
1160.90
400
600
800
1000
1200
1400
1600
1800
2000
m/z

β-thiol phenylalanine peptide 11 augurin(27-62)

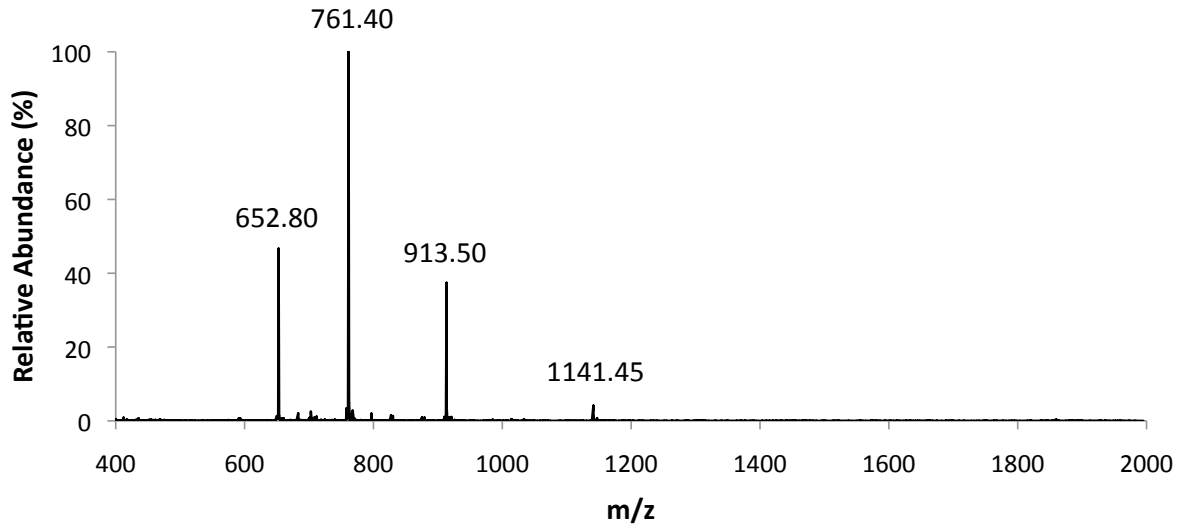


Scheme S2. Synthesis of β-thiol phenylalanine peptide 11 augurin(27-62)

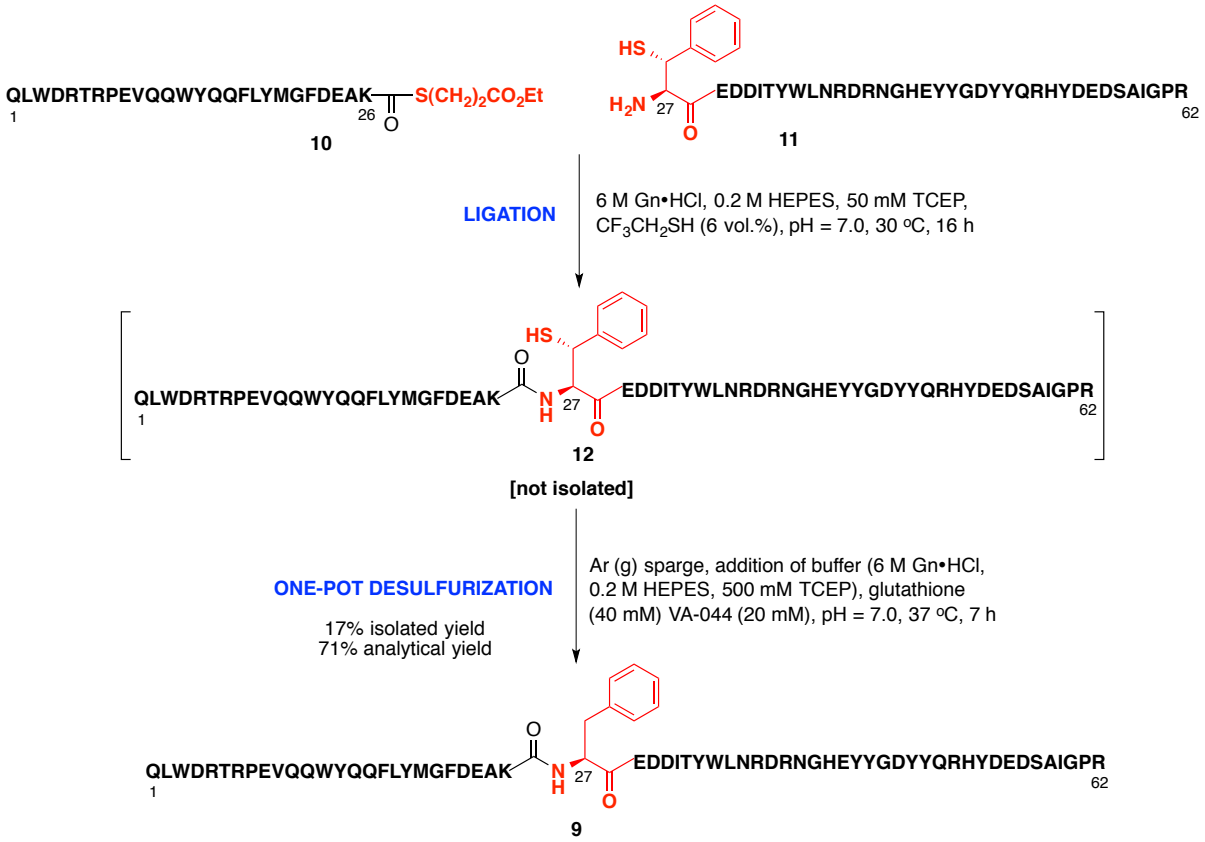
Peptide **11** was prepared on Rink amide resin (100 μmol). Fmoc-Arg(Pbf)-OH was first loaded to the resin according to the general procedures. The peptide was subsequently elongated using automated microwave Fmoc-SPPS, with the incorporation of two pseudoproline dipeptides (highlighted in scheme S2) to facilitate the efficient construction of the aggregation-prone target peptide fragment. Following elongation to the penultimate amino acid residue (Glu28), the peptide was dried and split into multiple portions. To a 12.5 μmol portion of the resin-bound peptide was coupled β-thiol phenylalanine derivative **1** using the conditions outlined in the general procedures. Following cleavage from the resin, the crude residue was precipitated in cold diethyl ether and the crude product purified by preparative reverse-phase preparative HPLC (*Prep 1*: 0-40% B over 80 min, 0.1% formic acid, Sunfire C-18 preparative column; *Prep 2*: 0 to 40% B over 60 min, 0.1% formic acid (buffered to pH = 5.0 with ammonium hydroxide), XBridge BEH300 C-18 semi-preparative column) to yield the β-thiol phenylalanine peptide **11** as a white solid following iterative lyophilization (1.1 mg, 2% yield based on the original resin loading). Note: Poor HPLC recovery was observed for peptide **11**, likely owing to the aggregation-prone nature of this fragment.



Analytical HPLC: R_t 25.3 min (0-40% B over 30 min, λ = 230 nm); Calculated Mass $[M+4H]^{4+}$: 1141.00, $[M+5H]^{5+}$: 913.00, $[M+6H]^{6+}$: 761.00, $[M+7H]^{7+}$: 652.43; Mass Found ESI(+); 1141.45 $[M+4H]^{4+}$, 913.50 $[M+5H]^{5+}$, 761.40 $[M+6H]^{6+}$, 652.80 $[M+7H]^{7+}$



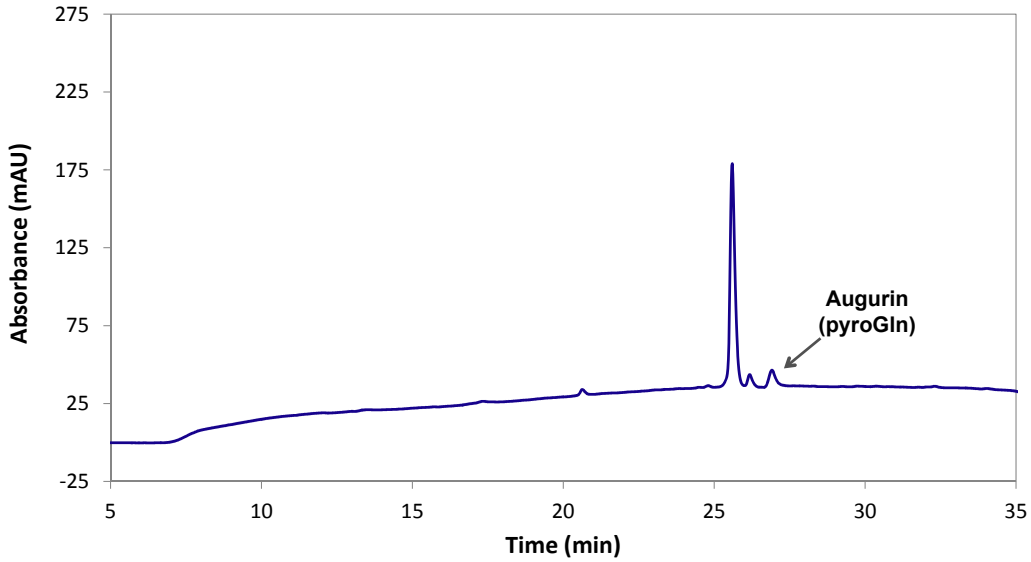
One-pot ligation-desulfurization reaction:



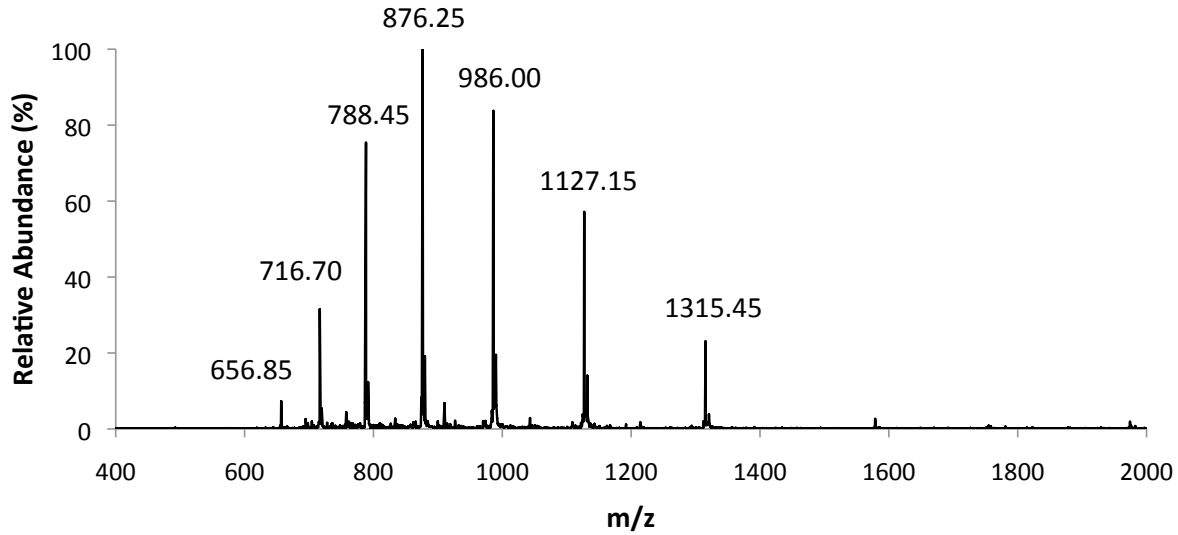
Scheme S3. One-pot synthesis of augurin 9

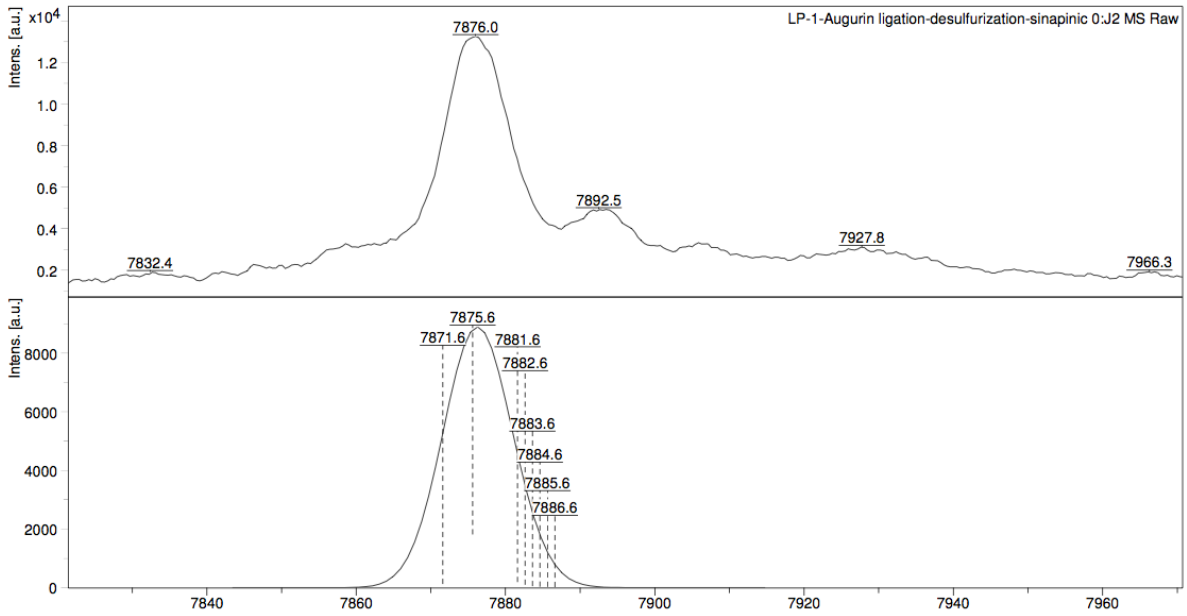
The synthesis of augurin **9** was carried out using a TFET-promoted one-pot ligation-desulfurization reaction. Peptide thioester **10** (1.5 mg, 0.43 μmol, 1.4 eq., 5.7 mM final concentration) and β-thiol phenylalanine peptide **11** (1.4 mg, 0.31 μmol, 1.0 eq.) were dissolved in degassed ligation buffer (78 μL, 6 M Gn•HCl/0.2 M HEPES, 50 mM TCEP, pH = 7.1-7.2, 4 mM with respect to peptide **11**). Following the addition of TFET (5 μL, 6 vol.%), the peptide was incubated at 30 °C and a final pH = 7.0 for 16 h, at which point HPLC-MS analysis indicated complete consumption of peptide **11** and formation of intermediate ligation product **12**. Without isolation, the crude reaction mixture was sparged with argon (5 min) and diluted first with degassed buffer (78 μL, 6 M Gn•HCl/0.2 M HEPES, pH = 7.0), followed by a degassed TCEP solution (156 μL, 6 M Gn•HCl/0.2 M HEPES, 500 mM TCEP, pH = 7.0) and MeCN (10 μL) to give a final concentration of peptide of approximately 1 mM. The solution was further sparged with argon prior to the addition of glutathione (3.9 mg, 40 mM final concentration) and VA-044 (2.0 mg, 20 mM final concentration) as solids. The crude mixture was incubated at 37 °C for 7 h, at which point HPLC-MS analysis indicated complete consumption of ligation product **12** to afford the desired peptide target. Pure augurin **9** was

isolated (0.36 mg, 17%) following semi-preparative reverse-phase HPLC purification (10-50% B over 60 min, 0.1% formic acid, XBridge BEH300 C-18 semi-preparative column).



Analytical HPLC: R_t 25.6 min (0-60% B over 30 min, 0.1% TFA, λ = 214 nm); Note: Acid-catalyzed pyroglutamate formation leads to the minor by-product augurin (pyroGln) upon HPLC purification and analysis; Calculated Mass $[M+5H]^{5+}$: 1575.73, $[M+6H]^{6+}$: 1313.27, $[M+7H]^{7+}$: 1125.81, $[M+8H]^{8+}$: 985.21, $[M+9H]^{9+}$: 875.85, $[M+10H]^{10+}$: 788.37, $[M+11H]^{11+}$: 716.79, $[M+12H]^{12+}$: 657.14; Mass Found ESI(+); 1315.45 $[M+6H]^{6+}$, 1127.15 $[M+7H]^{7+}$, 986.00 $[M+8H]^{8+}$, 876.25 $[M+9H]^{9+}$, 788.45 $[M+10H]^{10+}$, 716.70 $[M+11H]^{11+}$, 656.85 $[M+12H]^{12+}$





MALDI-TOF: Calculated Mass $[M+H]^+$: 7876.50; Mass Found 7876.0 $[M+H]^+$

Analytical HPLC – One-pot ligation-desulfurization:

An analytical HPLC yield for the preparation of augurin **9** was determined using a slight modification to the one-pot ligation-desulfurization protocol described above. The relative concentrations of starting materials and peptide ligation-desulfurization products were determined using HPLC analysis *via* integration of the corresponding peak area at 280 nm, taking into account the calculated extinction coefficients for each peptide at 280 nm. These calculations were performed based on the relative number of tyrosine (ε_{280} = 1280 $M^{-1}cm^{-1}$) and tryptophan (ε_{280} = 5690 $M^{-1}cm^{-1}$) residues in each peptide fragment:

ε (peptide thioester **10**): ε_{280} = (2 x 5690 $M^{-1}cm^{-1}$) + (2 x 1280 $M^{-1}cm^{-1}$) = 13940 $M^{-1}cm^{-1}$

ε (β-thiol Phe peptide **11**): ε_{280} = (1 x 5690 $M^{-1}cm^{-1}$) + (6 x 1280 $M^{-1}cm^{-1}$) = 13370 $M^{-1}cm^{-1}$

ε (Augurin **9**): ε_{280} = (3 x 5690 $M^{-1}cm^{-1}$) + (8 x 1280 $M^{-1}cm^{-1}$) = 27310 $M^{-1}cm^{-1}$

Reaction conditions: A solution of peptide thioester **10** (~2.0 eq.) and β-thiol phenylalanine peptide **11** (1.0 eq.) was prepared in degassed ligation buffer (15 µL, 6 M Gn•HCl/0.2 M HEPES, 50 mM TCEP, pH = 7.1-7.2). [Note: excess thioester **10** (~2.0 eq.) was used due to a competing intramolecular lactamization reaction at the C-terminal lysine thioester moiety.] An aliquot (1 µL) of the solution was diluted in a mixture of H_2O/MeCN (18 µL, 15:3 v/v) and analyzed using reverse-phase analytical HPLC (XBridge BEH300 5 µm (C-18) 2.1 x 150

mm column, 0 to 50% B over 30 min, 0.1% formic acid buffer) at a wavelength of 280 nm to quantify the relative amounts of peptide starting materials. The mixture was then treated with TFET (2 μL, 13 vol.%) and incubated at 30 ^{o}C for 15.5 h, at which point another aliquot (1 μL) was removed and diluted in H_2O/MeCN (18 μL, 15:3 v/v) for HPLC analysis. The ligation mixture was then diluted with a degassed TCEP/glutathione buffer (30 μL, 6 M Gn•HCl/0.2 M HEPES, 500 mM TCEP, 80 mM glutathione, pH = 7.0). The resulting ligation mixture was sparged with argon (5 min) prior to the addition of a degassed buffer solution containing VA-044 (15 μL, 6 M Gn•HCl/0.2 M HEPES, 80 mM VA-044). The reaction mixture was subsequently sealed and incubated at 37 ^{o}C for 8 h. A final aliquot (4 μL) was removed and diluted in H_2O/MeCN (18 μL, 15:3 v/v) for quantification of the reaction product.

Taking into account the relative extinction coefficients of each peptide, the calculated yield of augurin **9** was determined to be 71% and the yield of a minor ligation byproduct consistent with pyroglutamate formation was determined to be 20% based on the limiting reagent (peptide **11**, 1.0 eq.). Desulfurization of starting peptide **11** to afford augurin(27-62) bearing a native phenylalanine residue at the N-terminus (see Figure S17) accounts for the remainder of the reaction yield (9%).

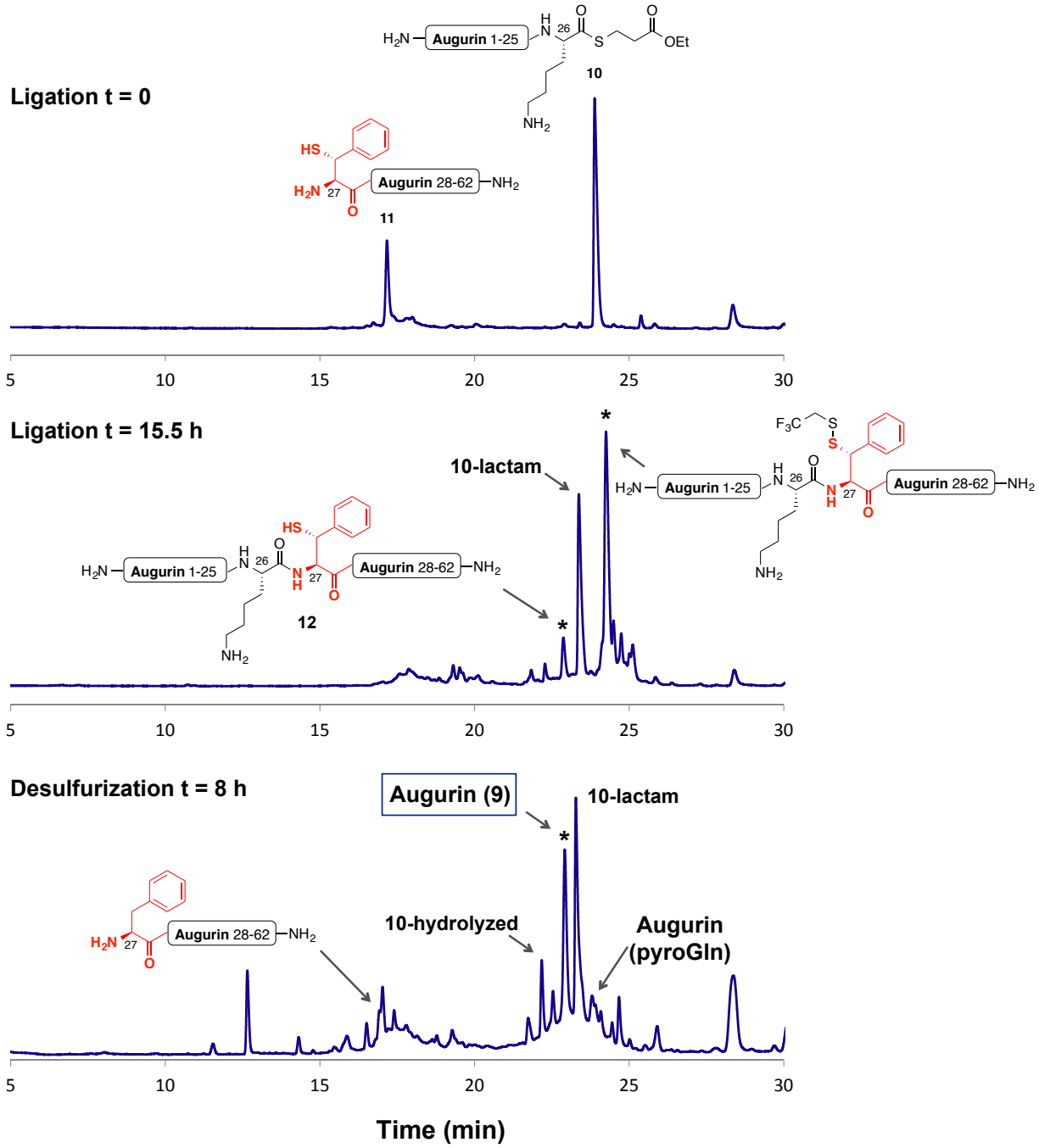


Figure S17. Analytical HPLC traces (0 to 50% B over 30 min, λ = 280 nm, 0.1% formic acid, XBridge BEH300 5 µm (C-18) 2.1 x 150 mm column) of the synthesis of augurin **9** *via* a TFET-promoted one-pot ligation-desulfurization protocol beginning with peptide **10** (~2 eq.) and peptide **11** (1.0 eq., limiting reagent). Note: excess thioester **10** (~2.0 eq.) was used due to a competing intramolecular lactamization reaction at the C-terminal lysine thioester moiety to afford **10-lactam**. As such, minor unlabelled peaks can be attributed primarily to thioester-derived byproducts (including pyroglutamate formation) and/or excess desulfurization reagents.

Kinetic Studies (Cys vs. β-thiol Phenylalanine)

Ligation time-courses were plotted for the reaction of H-CSPGYS-NH_2 with Ac-LYRANG-S$(CH_2)_2CO_2$Et and for compound **7** (H-(β-SH)FSPGYS-NH_2) with Ac-LYRANG-S$(CH_2)_2CO_2$Et. Ligation experiments were carried out according to General Protocol A with a final concentration of 5 mM for the thiol-containing peptide and a final concentration of 6 mM for the peptide thioester. Aliquots of 10 µL were taken from the reaction mixture at various time intervals and quenched with 50 µL of 1% TFA in water and analyzed *via* analytical HPLC. Yield estimations are based upon the relative peak areas of the thiol-containing starting material versus the desired ligation product at λ = 280 nm, taking into account the corresponding extinction coefficients based on the presence of tyrosine residues (ε_{280}(peptide thioester) = ε_{280}(thiol-containing peptide) = 1280; ε_{280}(ligation product) = 2560).

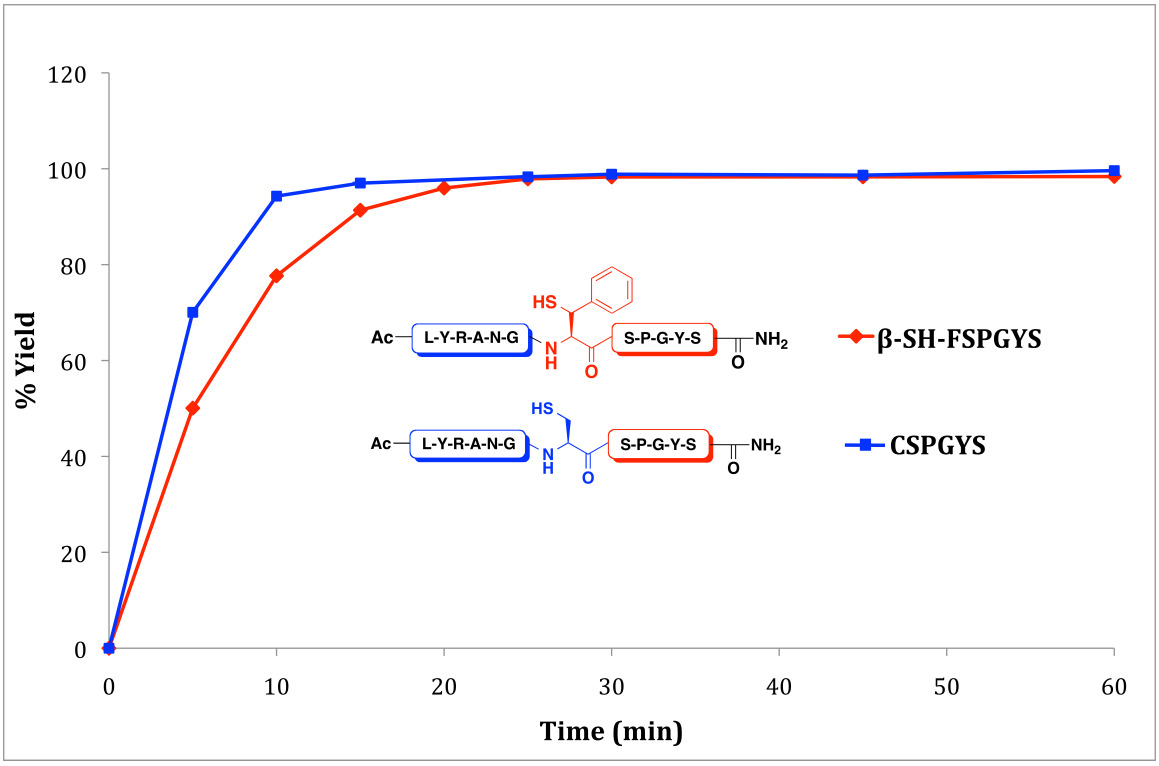


Figure S18. Comparative rates of ligation with Ac-LYRANG-S$(CH_2)_2CO_2$Et: H-CSPGYS-NH_2 versus H-(β-SH)FSPGYS-NH_2 (**7**).

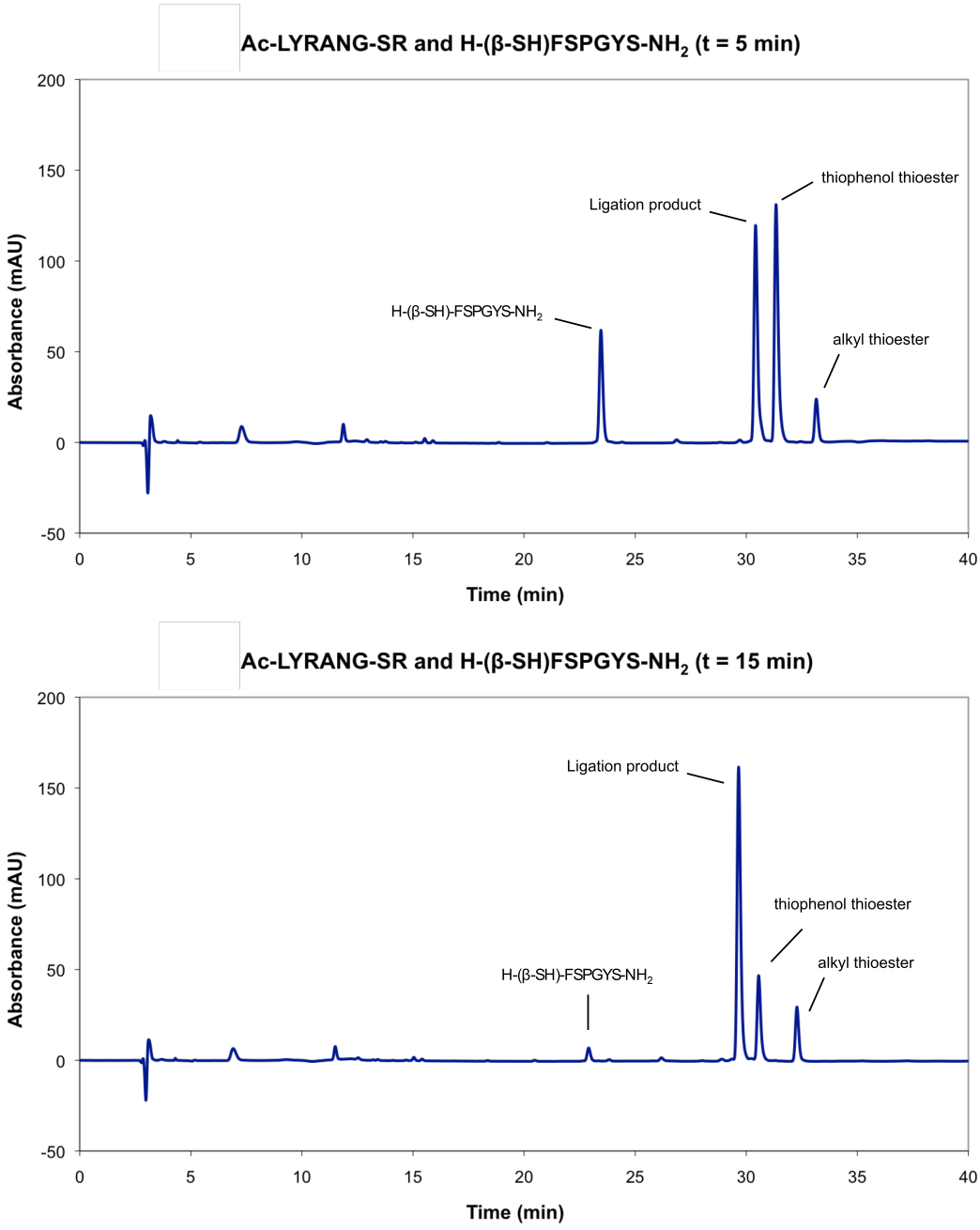


Figure S19. Representative analytical HPLC traces at various time points for the ligation of compound **7** with Ac-LYRANG-S$(CH_2)_2CO_2$Et (0 to 50% B over 40 min, λ = 280 nm).

Competition experiment (peptide 7 vs. peptide S1):

The relative ligation rate of β-thiol phenylalanine containing peptide **7** (H-(β-SH)FSPGYS-NH_2) and a β-selenophenylalanine containing peptide dimer **S1** (H-(β-Se)FSPGYS-NH_2 dimer) were evaluated in a competition reaction with Ac-LYRANG-S$(CH_2)_2CO_2$Et. The peptide thioester (0.9 mg, 1.0 μmol, 0.75 eq., 3.75 mM concentration) was dissolved in degassed buffer (0.27 mL, 6 M guanidine hydrochloride, 100 mM Na_2HPO_4, 200 mM 4-mercaptophenylacetic acid (MPAA), adjusted to pH 7.5). The solution was added to a

mixture of thiol peptide **7** (0.94 mg, 1.36 µmol, 1.0 eq., 5 mM concentration) and selenopeptide **S1** (1.0 mg, 1.36 µmol, 1.0 eq., 5 mM concentration). The final pH of the solution was measured to be 7.45. The solution was flushed with argon, placed on an orbital shaker at room temperature and monitored over a period of 24 h. LC-MS results revealed formation of the thiol-mediated ligation product (Ac-LYRANG(β-SH)FSPGYS-NH_2 as the asymmetric MPAA-disulfide), significant amounts of unreacted selenopeptide (as the H-(β-Se)FSPGYS-NH_2 dimer **S1** and the corresponding selenyl-MPAA sulfide adduct), and no evidence of the selenium-mediated ligation product (see Figure S20).

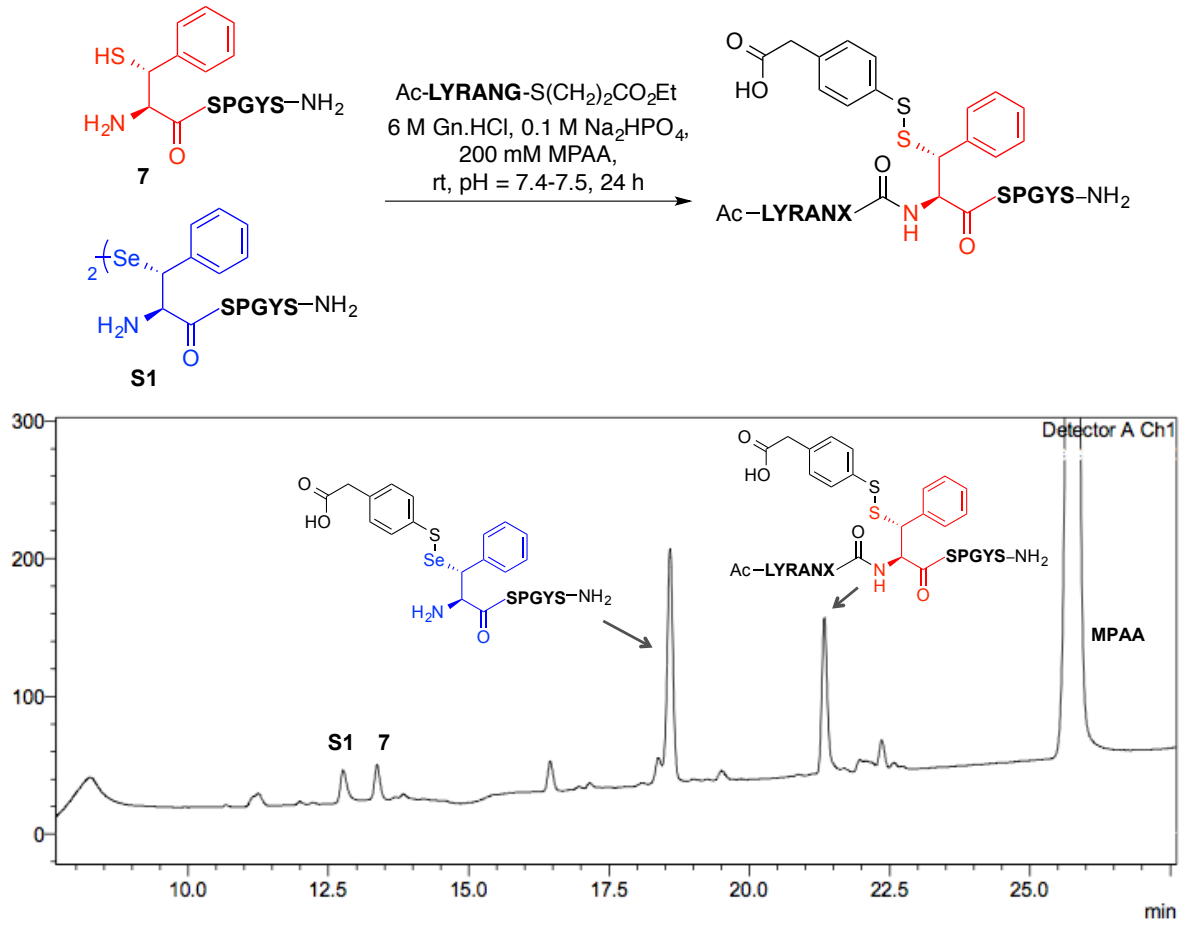
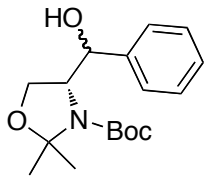


Figure S20. Analytical HPLC trace (0 to 50% B over 30 min, 0.1% formic acid, λ = 230 nm) of the competitive ligation of **7** (H-(β-SH)FSPGYS-NH_2) and **S1** (H-(β-Se)FSPGYS-NH_2 dimer) with Ac-LYRANG-S$(CH_2)_2CO_2$Et) at t = 24 h.

Synthesis of β-thiol phenylalanine 1



(4*R*)-*tert*-butyl 4-(hydroxy(phenyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (3).

A dry 2-neck flask was charged with a magnetic stirrer, magnesium granules (230 mg, 9.46 mmol), and THF (2 mL) and fitted with a reflux condenser. A spatula tip of iodine was added and the reaction mixture was stirred vigorously at room temperature for 25 minutes. A solution of bromobenzene (0.52 mL, 4.94 mmol) in THF (3 mL) was added dropwise to the magnesium granules. The resultant yellow-brown solution was heated at 40 ^{o}C for 1 h to complete formation of the Grignard reagent. The reaction mixture was cooled to 0 ^{o}C and a solution of Garner's aldehyde **2** (843 mg, 3.68 mmol) in THF (5 mL) was added dropwise. The reaction was stirred for 20 minutes at 0 ^{o}C before warming to room temperature and stirring an additional 1 h. Upon completion, the reaction was quenched with saturated aqueous NH_4Cl (50 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (1:4 EtOAc/hexanes) to yield the title compound (2:3 *syn*: *anti* diastereomeric ratio) as a clear oil which solidified upon cooling (907 mg, 80% yield): IR (neat) 3375, 2983, 1651, 1398, 1365, 1168, 1106, 1073 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, rotamers) *major diastereomer (anti)*: δ 7.40-7.21 (m, 5H, Ar-H), 5.09 (m, 1H, benzylic C*H* + rotamer), 4.41 (br s, 1H, O*H*), 4.28 (br s, 1H, CH_α), 4.07-3.85 (m, 2H, CH_{2a}, CH_{2b}), 1.6-1.45 (m, 15H, Boc, 2 x CH_3); *minor diastereomer (syn)*: δ 7.25-7.40 (m, 5H, Ar-H), 5.30 (br s, 1H, O*H*), 4.74 (d, 1H, J = 8.0 Hz, benzylic C*H*), 4.20 (t, 1H, J = 6.8 Hz, CH_α), 3.69 (m, 1H, CH_{2a}), 3.61 (m, 1H, CH_{2b}), 1.60-1.45 (m, 15H, Boc, 2 x CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz, diastereomers, rotamers) δ 155.9, 155.0, 154.1, 142.0, 141.1, 128.6, 128.2, 127.5, 127.4, 126.4, 125.9, 125.8, 94.7, 81.9, 81.2, 78.2, 75.0, 73.7, 64.9, 64.3, 63.8, 62.0, 28.5, 28.3, 27.3, 26.0, 24.4, 22.8; HRMS ESI(+) calcd for $C_{17}H_{25}NO_4Na$: $[M+Na]^+$, 330.16758, found $[M+Na]^+$, 330.16763.

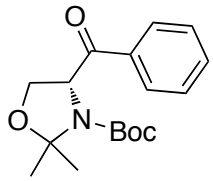
Notes:

1. Experimental results agree with previously reported literature data.[2]

2. The *syn*:*anti* ratio was determined by ^{1}H NMR integration of the benzylic C*H* proton[3]: (*syn*: δ 4.74 (d, 1H, J = 8.0 Hz), *anti:* δ 5.09 (m, 1H)), see attached spectra.

3. Compound **3** (2:3 *syn*:*anti* ratio) was either submitted directly to mesylation and inversion with potassium thiocyanate or submitted to an oxidation-reduction cascade to yield *anti*-enriched **3** (see below).

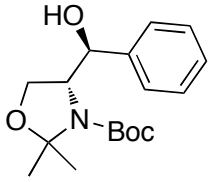
4. Broad peaks in the ^{13}C NMR spectrum may be attributed to the highly rotameric nature of compound **3**.



(*R*)-*tert*-butyl 4-benzoyl-2,2-dimethyloxazolidine-3-carboxylate.

To a solution of oxalyl chloride (0.53 mL, 6.12 mmol) in CH_2Cl_2 (19 mL) at -78 ^{o}C was added DMSO (0.91 mL, 12.8 mmol). After 5 min of stirring, a solution of alcohol **3** (1.64 g, 5.33 mmol) in CH_2Cl_2 (6.3 mL) was added dropwise, keeping the reaction temperature below -65 ^{o}C. The solution was stirred for 45 min at -78 ^{o}C and excess triethylamine (3.8 mL) was added. The reaction was stirred for 10 min at which point TLC indicated consumption of starting material. The reaction was quenched *via* the addition of water (30 mL). The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (1:4 EtOAc/hexanes) to afford the desired compound as a white solid (1.58 g, 97% yield): mp 115.2-115.7 ^{o}C; IR (ATR Zn/Se, neat) 2916, 1696, 1389, 1365, 1218, 1169, 1156, 1093, 1060, 1044, 843, 767, 690, 629 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, rotamers) *major rotamer:* δ 7.89-7.87 (m, 2H, Ar-H), 7.58-7.40 (m, 3H, Ar-H), 5.36-5.33 (m, 1H, CH_{α}), 4.13-4.25 (m, 1H, CH_{2a}), 3.93-3.88 (m, 1H, CH_{2b}), 1.75-1.20 (m, 15H, Boc, 2 x CH_3); *minor rotamer*: δ 7.89-7.87 (m, 2H, Ar-H), 7.58-7.40 (m, 3H, Ar-H), 5.46-5.44 (m, 1H, CH_{α}), 4.13-4.25 (m, 1H, CH_{2a}), 3.93-3.88 (m, 1H, CH_{2b}), 1.75-1.20 (m, 15H, Boc, 2 x CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) *major rotamer:* δ 195.9, 151.3, 135.1, 133.5, 128.9, 128.1, 95.2, 80.2, 66.0, 61.6, 28.2, 25.9, 24.8; *minor rotamer:* δ 195.2, 152.1,

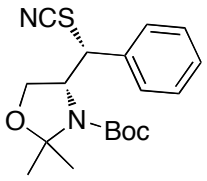
134.9, 133.4, 128.8, 128.4, 94.6, 80.7, 65.6, 61.9, 28.4, 25.4, 24.6; HRMS ESI(+) calcd for $C_{17}H_{23}NO_4Na$: $[M+Na]^+$, 328.1519, found $[M+Na]^+$, 328.1515.



(*R*)-*tert*-butyl 4-((*S*)-hydroxy(phenyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (3).

To a solution of the above oxidation product (0.73 g, 2.38 mmol) in THF (70 mL) at 0 ^oC under argon atmosphere was added DIBAL-H (5.05 mL, 1.0 M solution in hexanes, 5.05 mmol) dropwise over 15 min. The reaction was stirred at 0 ^oC for 30 min, quenched with cold MeOH (3.5 mL) and poured into cold 1 M HCl (100 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (50 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash column chromatography (1:4 EtOAc/hexanes) afforded the title compound as a white solid (0.70 g, 96% yield). HRMS ESI(+) calcd for $C_{17}H_{25}NO_4Na$: $[M+Na]^+$, 330.1676, found $[M+Na]^+$, 330.1672.

For full characterization, see initial synthesis of compound **3**. However, in order to confirm the diastereoselectivity of the reduction step, additional NMR experiments were performed at elevated temperatures (C_6D_6 solvent, T = 330 K) to resolve rotamers. Under these conditions, integration of the benzylic CH (*syn*: δ 4.78 (d, 1H, J = 10.8 Hz), *anti:* δ 4.96 (s, 1H)) enabled determination of the ratio of diastereomers (see attached NMR spectra). ^1H NMR (C_6D_6, 300 MHz, T = 330 K) *major diastereomer (anti):* δ 7.28 (d, 2H, J = 7.5 Hz, Ar-H), 7.08-6.93 (m, 3H, Ar-H), 4.96 (br s, 1H, benzylic C*H*), 3.96 (m, 1H, CH_α), 3.80 (d, 1H, J = 8.7 Hz, CH_{2a}), 3.36 (dd, 1H, J = 9.0 Hz, 6.9 Hz, CH_{2b}), 1.40-1.27 (m, 15H, Boc, 2 x CH_3); *minor diastereomer (syn)*: δ 7.21 (d, 2H, J = 6.9 Hz, Ar-H), 7.08-6.93 (m, 3H, Ar-H), 4.78 (d, 1H, J = 10.8 Hz, benzylic C*H*), 4.03 (m, 1H, CH_α), 3.55 (m, 1H, CH_{2a}), 3.30 (m, 1H, CH_{2b}), 1.40-1.24 (m, 15H, Boc, 2 x CH_3); ^{13}C NMR (C_6D_6, 300 MHz, T = 330 K) *major diastereomer (anti):* δ 142.8, 127.9, 127.1 (additional aromatic carbons obscured by benzene signal), 95.2, 80.5, 74.8, 64.5, 28.9, 27.0; *minor diastereomer (syn):* δ 142.8, 127.9, 127.1 (additional aromatic carbons obscured by benzene signal), 95.2, 80.5, 74.9, 65.3, 28.9, 24.5.

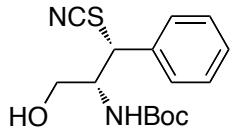


(*R*)-*tert*-butyl 2,2-dimethyl-4-((*S*)-phenyl(thiocyanato)methyl)oxazolidine-3-carboxylate (4).

Synthesis A (direct mesylation and inversion): Compound **3** (2:3 *syn*:*anti* ratio) (570 mg, 1.86 mmol) was dissolved in CH_2Cl_2 (8 mL) and the mixture cooled to 0 ^oC. Triethylamine (376 μL, 2.78 mmol) and mesyl chloride (167 μL, 2.23 mmol) were added dropwise and the resultant solution was stirred at 0 ^oC for 1 h. The reaction was diluted with CH_2Cl_2 (25 mL) and poured into saturated aqueous NH_4Cl (40 mL). The organic layer was washed with water (1 x 40 mL) and brine (1 x 40 mL), dried (Na_2SO_4), filtered, and concentrated to yield a pale yellow oil (668 mg). The compound was utilized immediately without further purification. To a solution of crude mesylate in dry acetonitrile (16.4 mL) was added oven-dried potassium thiocyanate (903 mg, 9.29 mmol). The reaction mixture was stirred at room temperature for 2 d and concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (50 mL) and poured into water (50 mL); the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL), and the combined organic layers were washed with brine (1 x 40 mL), dried (Na_2SO_4), filtered, and concentrated. The resultant crude oil was purified by flash column chromatography (1:9 → 1:3 EtOAc/hexanes) to afford the title compound as a white solid (246 mg, 38% yield over two steps) as a single diastereomer. Unreacted *syn*-mesylate (204 mg, 30%) was also recovered following flash column chromatography.

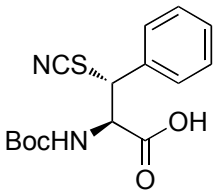
Synthesis B (from oxidation-reduction cascade): Compound **3** (*anti*-enriched, 1:8 *syn*:*anti* ratio) (167 mg, 0.54 mmol) was dissolved in CH_2Cl_2 (2.4 mL) and the mixture cooled to 0 ^oC. Triethylamine (110 μL, 0.78 mmol) and mesyl chloride (49 μL, 0.63 mmol) were added dropwise and the resultant solution was stirred at 0 ^oC for 1 h. The reaction was diluted with CH_2Cl_2 (15 mL) and poured into saturated aqueous NH_4Cl (30 mL). The organic layer was washed with water (1 x 20 mL) and brine (1 x 20 mL), dried (Na_2SO_4), filtered, and concentrated to yield a pale yellow oil. The compound was utilized immediately without further purification. To a solution of crude mesylate in dry acetonitrile (4.8 mL) was added oven-dried potassium thiocyanate (265 mg, 2.72 mmol). The reaction mixture was stirred at room temperature for 2 d and concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (20 mL) and poured into water (40 mL); the aqueous layer was extracted with CH_2Cl_2 (2 x 20

mL), and the combined organic layers were washed with brine (1 x 40 mL), dried (Na_2SO_4), filtered, and concentrated. The resultant crude oil was purified by flash column chromatography (1:9 → 1:3 EtOAc/hexanes) to afford the title compound as a white solid (97 mg, 51% yield over two steps) as a single diastereomer: $[\alpha]_D^{25}$ –2.1 (*c* 0.27, CH_2Cl_2); IR (ATR Zn/Se, neat) 2979, 2155 (SCN), 1689, 1453, 1365, 1245, 1166, 1091, 1029, 840, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, rotamers) *major*: δ 7.45-7.31 (m, 5H, Ar-H), 5.24 (d, 1H, J = 2.8 Hz, benzylic C*H*), 4.40 (m, 1H, CH_α), 4.12-4.09 (m, 1H, CH_{2a}), 3.91-3.86 (m, 1H, CH_{2b}), 1.73-1.43 (m, 15H, Boc, 2 x CH_3); *minor*: δ 7.45-7.31 (m, 5H, Ar-H), 5.06 (m, 1H, benzylic C*H*), 4.27 (m, 1H, CH_α), 4.12-4.09 (m, 1H, CH_{2a}), 3.91-3.86 (m, 1H, CH_{2b}), 1.73-1.43 (m, 15H, Boc, 2 x CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) *major*: δ 152.6, 136.1, 129.2, 129.0, 128.1, 112.1 (S*C*N), 94.8, 81.4, 63.7, 61.8, 55.6, 28.4, 26.5, 24.0; *minor*: δ 151.5, 136.2, 128.9, 128.7, 127.9, 112.1 (S*C*N), 95.5, 81.4, 63.7, 61.4, 56.6, 28.5, 25.9, 22.5; HRMS ESI(+) calcd for $C_{18}H_{24}N_2O_3SNa$: $[M+Na]^+$, 371.13998, found $[M+Na]^+$, 371.13997.



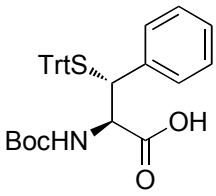
***tert*-butyl ((1*R*,2*R*)-3-hydroxy-1-phenyl-1-thiocyanatopropan-2-yl)carbamate (5).**

To a solution of **4** (133 mg, 0.38 mmol) in 1,4-dioxane (6.3 mL) was added *p*-toluenesulfonic acid (166 mg, 0.87 mmol) in one portion. The reaction mixture was stirred at rt for 3 h before the addition of a second aliquot of *p*-toluenesulfonic acid (82 mg, 0.43 mmol). The reaction was stirred at rt for an addition 3 h when TLC showed consumption of starting material. The mixture was poured into saturated aqueous $NaHCO_3$ (20 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (3:7 → 1:1 EtOAc/hexanes) to afford the title compound as a colorless oil (93 mg, 79% yield): $[\alpha]_D^{25}$ +70.7 (*c* 0.21, CH_2Cl_2); IR (ATR Zn/Se, neat) 3373, 2927, 2154 (S*CN* stretch), 1694, 1504, 1455, 1367, 1247, 1160, 1047, 853, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.35 (m, 5H, Ar-H), 4.90 (d, 1H, J = 9.2 Hz), 4.80 (d, 1H, J = 6.4 Hz), 4.25 (m, 1H, CH_α), 3.94 (m, 1H, CH_{2a}), 3.64 (m, 1H, CH_{2b}), 2.51 (br s, 1H, OH), 1.34 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 135.7, 129.0, 128.3, 111.8 (S*C*N), 80.5, 62.2, 55.0, 53.7, 28.2. HRMS ESI(+) calcd for $C_{15}H_{20}N_2O_3SNa$: $[M+Na]^+$, 331.1087, found $[M+Na]^+$, 331.1082.



(2*R*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-phenyl-3-thiocyanatopropanoic acid (6)

To a solution of **5** (93 mg, 0.30 mmol) in DMF (5.8 mL) was added pyridinium dichromate (1.14 g, 3.0 mmol) in one portion. The reaction mixture was stirred at rt for 20 h then poured into a 1 M solution of citric acid (20 mL). The aqueous layer was extracted with ethyl acetate (3 x 15 mL) and the combined organic layers were washed with water (3 x 20 mL) and brine (1 x 20 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification *via* flash column chromatography (1:99 → 5:95 MeOH/CH_2Cl_2) afforded the title compound as a clear oil (61 mg, 62%): $[\alpha]_D^{25}$ +203.1 (*c* 0.07, CH_2Cl_2); IR (ATR Zn/Se, neat) 2925, 2854, 2156 (S*CN* stretch), 1713, 1505, 1455, 1368, 1248, 1155, 1056, 698 cm^{-1}; ^1H NMR (9:1 $CDCl_3$/MeOD, 300 MHz) δ 7.35-7.24 (m, 5H, Ar-H), 4.93 (d, 1H, J = 5.6 Hz, benzylic C*H*), 4.88 (d, 1H, J = 4.8 Hz, CH_α), 1.32 (s, 9H, Boc); ^{13}C NMR (9:1 $CDCl_3$/MeOD, 75 MHz) δ 170.1, 155.5, 134.2, 129.2, 128.7, 128.2, 127.9, 111.5 (S*C*N), 80.8, 56.7, 54.7, 27.9. HRMS ESI(+) calcd for $C_{15}H_{18}N_2O_4SNa$: $[M+Na]^+$, 345.08795, found $[M+Na]^+$, 345.08826.



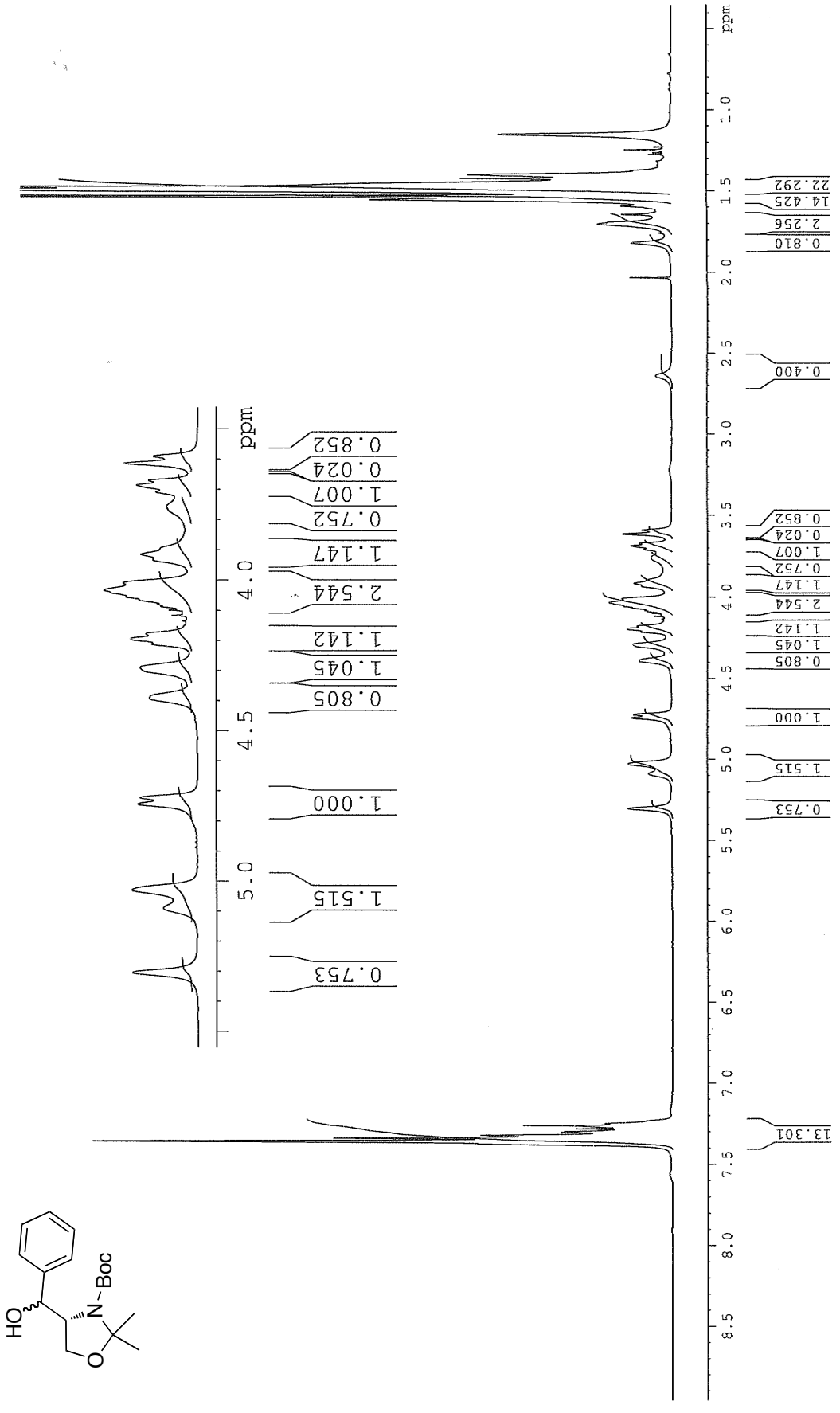
(2*R*,3*R*)-2-((*tert*-butoxycarbonyl)amino)-3-phenyl-3-(tritylthio)propanoic acid (1)

To a stirred solution of sodium borohydride (17 mg, 0.46 mmol) in 95% ethanol (1.0 mL) was added dropwise a solution of **6** (30 mg, 0.09 mmol) in 95% ethanol (1.0 mL). The resultant heterogeneous mixture was stirred at rt for 1 h before the addition of DTT (3.5 mg, 0.23 mmol). The reaction mixture was stirred for an additional 1 h then poured into cold, degassed 2 M HCl (15 mL). The aqueous layer was quickly extracted with ethyl acetate (3 x 10 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude residue was co-evaporated with DCM (1 x 10 mL) and dried for 1 h under

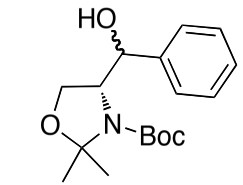
vacuum. The mixture was subsequently dissolved in dry ether (2.8 mL) and triphenylmethanol (53 mg, 0.20 mmol) was added followed by the dropwise addition of BF_3•OEt_2 (26 μL, 0.21 mmol). The reaction mixture was stirred at rt for 1 h before the solvent was evaporated and the residue washed with 5% $NaHCO_3$ (1 x 15 mL). The aqueous layer was extracted with ethyl acetate (3 x 10 mL) and the combined organic layers were dried (Na_2SO_4), filtered and concentrated. The crude material was purified *via* flash column chromatography (1:9 EtOAc/Hex → 1:9 MeOH/EtOAc) to yield the desired product as a white solid (33 mg, 0.062 mmol, 73%): IR (ATR Zn/Se, $CHCl_3$) 3432, 3057, 3027, 2978, 2930, 1722, 1492, 1445, 1394, 1368, 1163, 1052, 1034, 742, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.00 (m, 20H, Ar-H), 6.16 (br s, 1H, COO*H*), 4.75 (d, 1H, J = 9.6 Hz, N*H*), 4.43 (d, 1H, J = 6.0 Hz, CH_α), 3.90 (m, 1H, benzylic C*H*), 1.40 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0, 155.8, 144.0, 138.0, 129.7, 129.3, 128.6, 128.4, 127.9, 127.5, 126.8, 80.6, 69.3, 58.9, 50.5, 28.2; HRMS ESI(+) calcd for $C_{33}H_{33}NO_4SNa$: $[M+Na]^+$, 562.20225, found $[M+Na]^+$, 562.20294.

NMR Spectra

Compound 3 (2:3 *syn:anti*)



Compound 3 (2:3 *syn:anti)*



1.734
1.698
1.569
1.532
1.473
1.462
1.259
1.250
3.879
3.885
3.888
3.902
3.907
3.924
3.929
4.252
4.262
4.271
4.282
4.291
4.302
4.313
5.330
5.339
5.349
5.358
5.438
5.449
5.457
7.250
7.399
7.417
7.435
7.454
7.472
7.486
7.507
7.525
7.545
7.562
7.579
7.871
7.876
7.888
O
N
Boc
O
ppm
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1.98
1.02
1.96
0.42
0.57
1.00
1.01
5.34
3.97
3.14
3.06

195.86
195.19
152.08
151.25
135.08
134.90
133.51
133.42
128.88
128.75
128.39
128.12
95.22
94.57
80.70
80.22
77.40
77.08
76.76
66.02
65.65
61.85
61.62
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25.36
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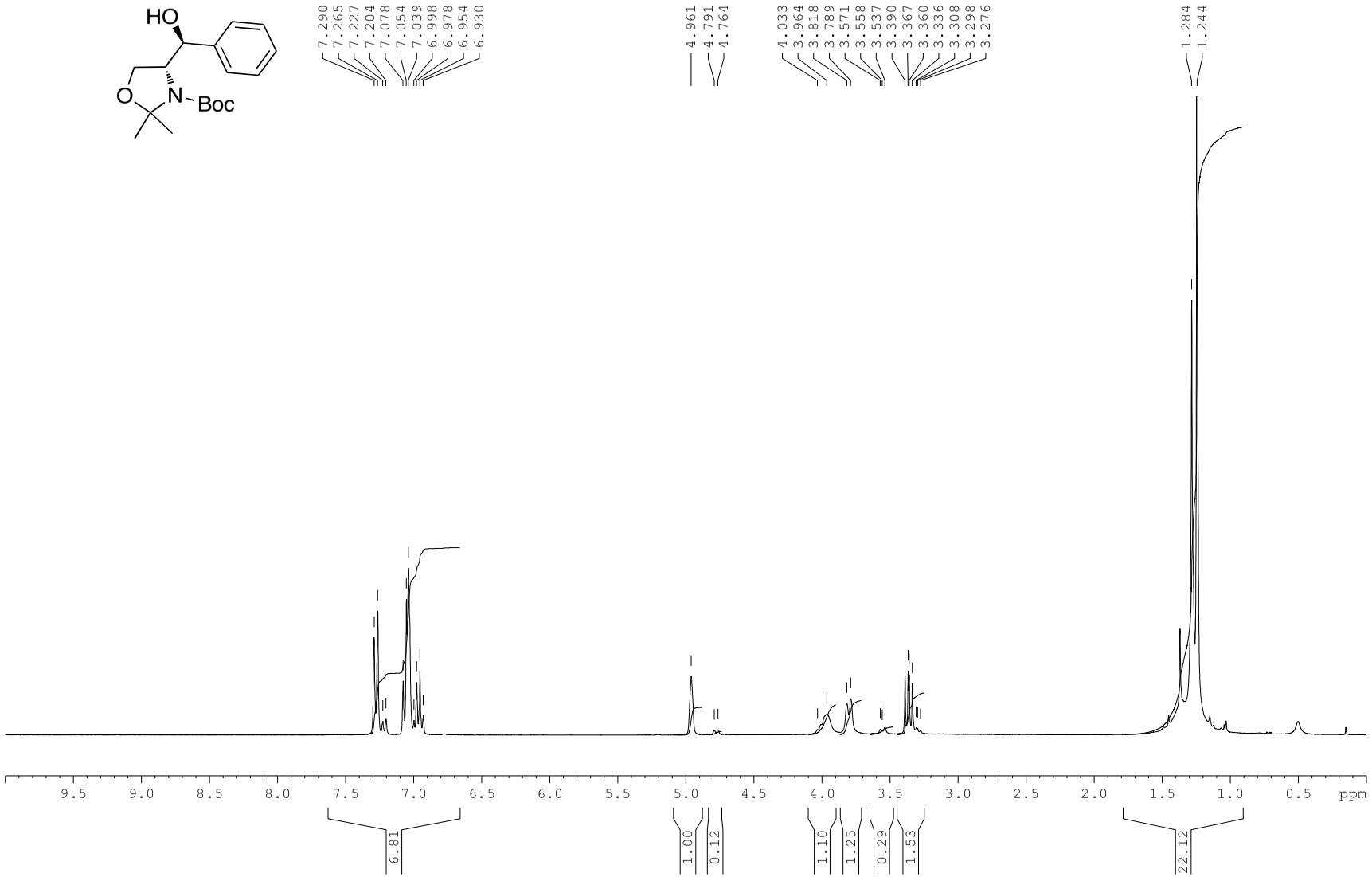
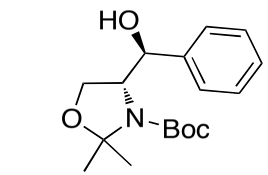
24.60
O
N-Boc
O
ppm

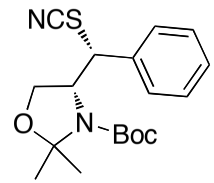
Compound 3 (*anti*-enriched), T = 330 K



Compound 3 (*anti*-enriched), T = 330 K



Compound 4


NCS
O
N
Boc

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7.396
7.376
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5.239
5.058
4.396
4.267
4.115
4.090
3.907
3.883
3.866
1.728
1.685
1.626
1.495
1.434
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
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1.0
0.5
ppm
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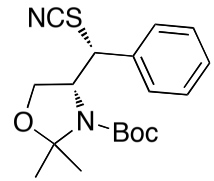
1.00
1.03
15.61

Compound 4


NCS
O
N
Boc

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136.24
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128.88
128.73
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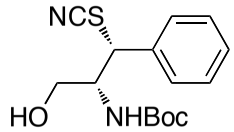
112.11
95.48
94.81
81.37
77.40
77.08
76.76
63.71
61.76
61.40
56.58
55.55
28.47
28.36
26.51
25.89
23.97
22.47
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

Compound 5


NCS
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NHBoc

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7.348
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4.805
4.789
4.277
4.266
4.256
4.248
4.243
4.237
4.225
4.215
3.949
3.926
3.702
3.701
3.696
3.673
2.506
1.357
9.5
9.0
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8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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ppm
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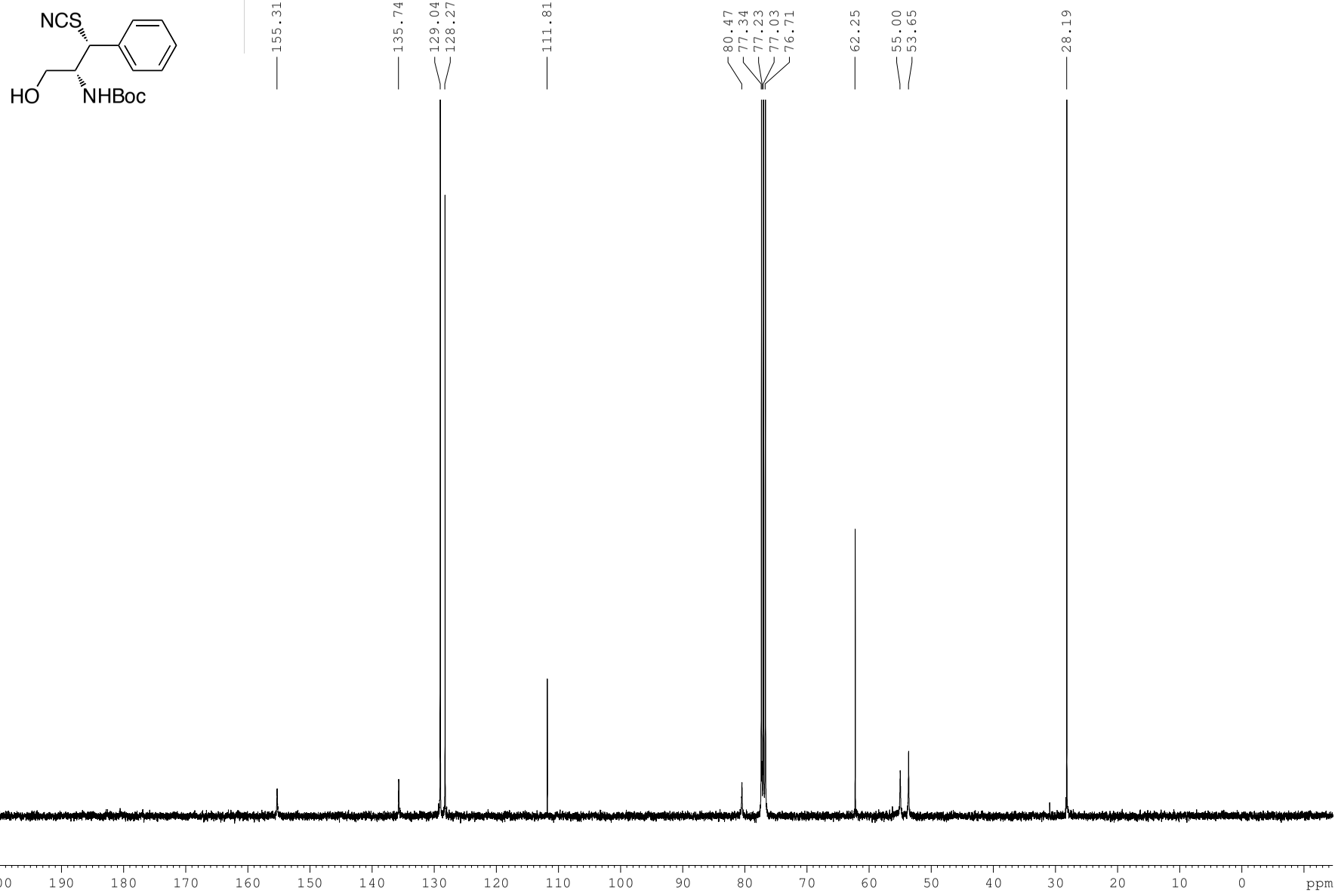
1.00
0.97
1.00
0.93
8.85

Compound 5


NCS
HO
NHBoc
155.31
135.74
129.04
128.27
111.81
80.47
77.34
77.23
77.03
76.71
62.25
55.00
53.65
28.19
200
190
180
170
160
150
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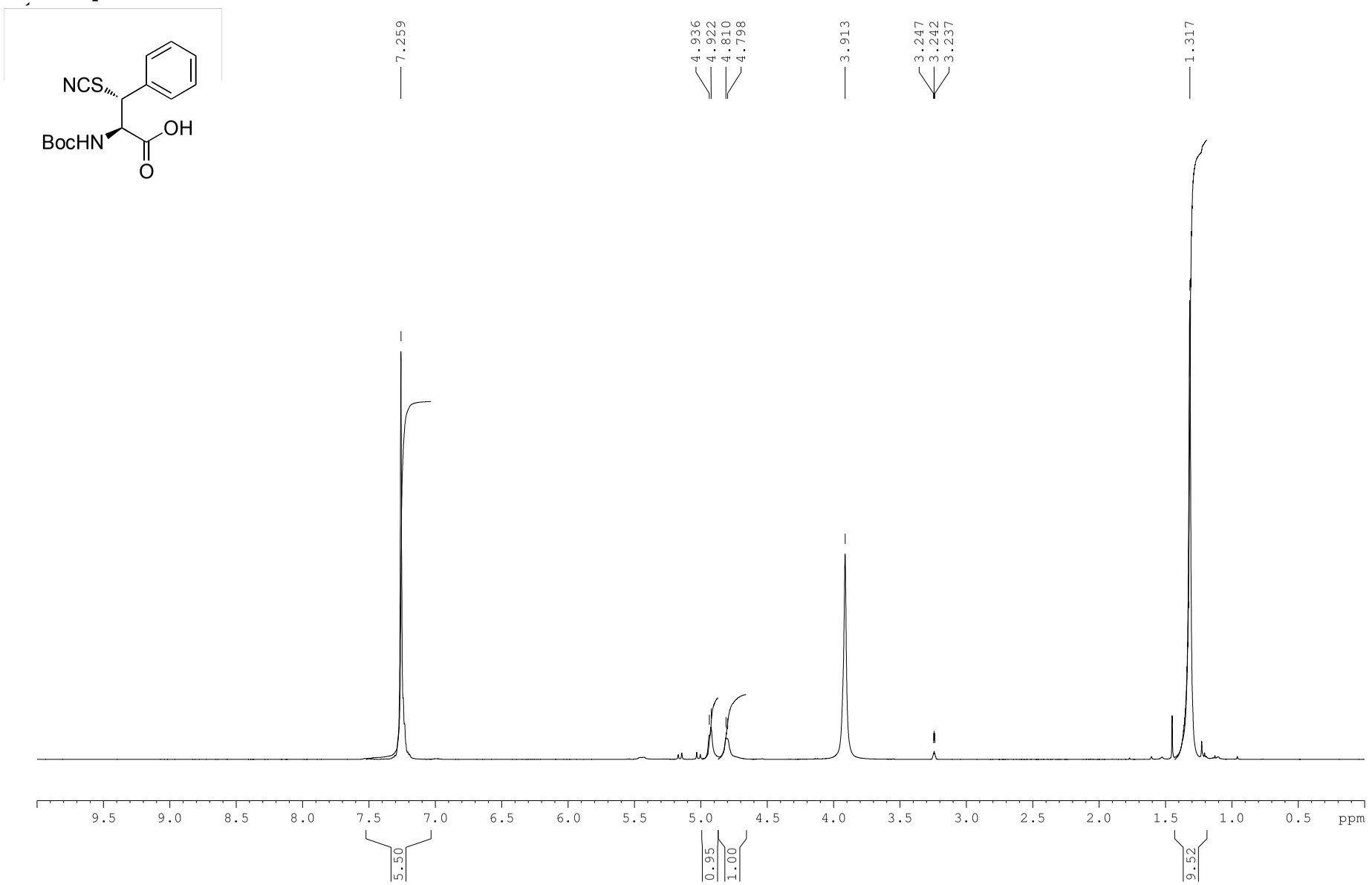
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

Compound 6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9.5
9.0
8.5
8.0
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7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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5.50
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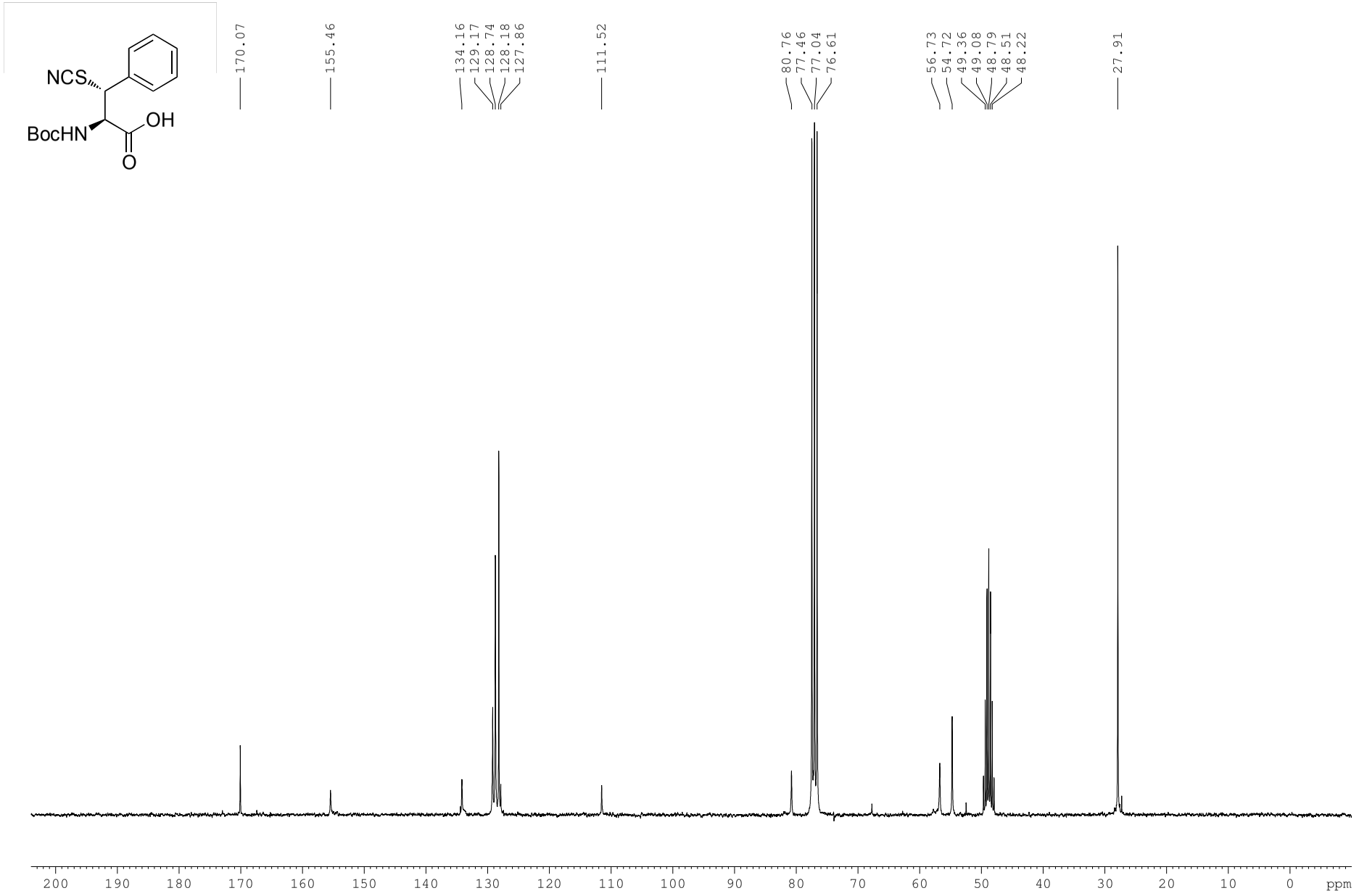
9.52

Compound 6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111.52
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77.04
76.61
56.73
54.72
49.36
49.08
48.79
48.51
48.22
27.91
200
190
180
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120
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90
80
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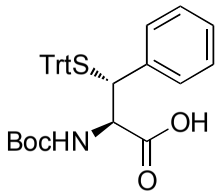
50
40
30
20
10
0
ppm

Compound 1


TrtS
BocHN
OH
O

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7.227
7.197
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7.186
7.176
7.157
7.136
7.125
7.118
7.010
6.995
6.160
4.766
4.742
4.436
4.421
3.897
1.399
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
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0.5
ppm
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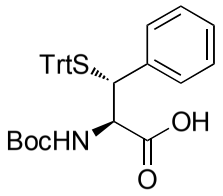
0.79
0.81
0.95
9.00

Compound 1


TrtS
OH
BocHN
O

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144.03
137.96
129.74
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128.57
128.35
127.86
127.52
126.80
80.61
77.32
77.00
76.68
69.27
58.94
50.52
28.22
200
190
180
170
160
150
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60
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40
30
20
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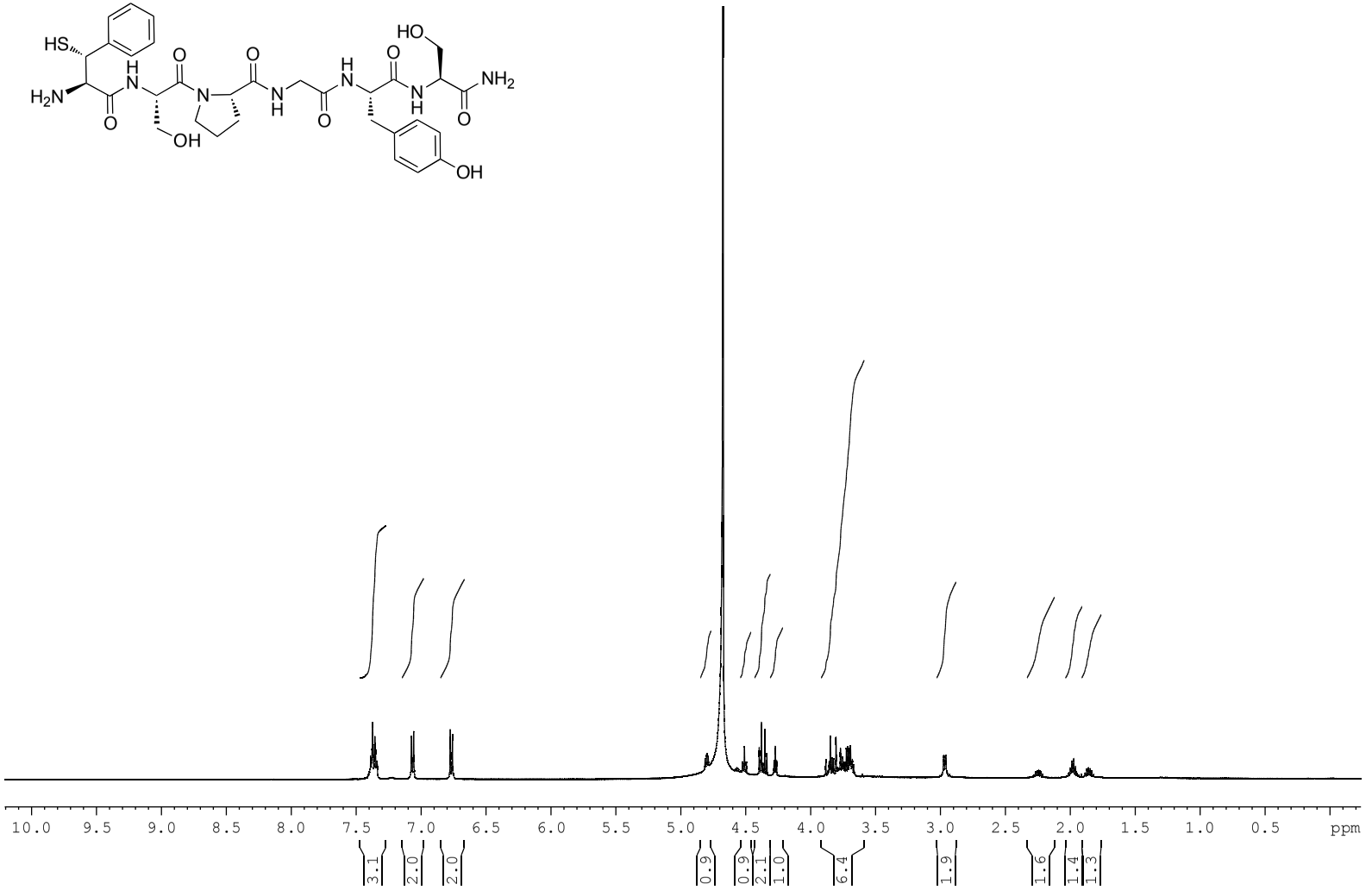
0
ppm

Peptide 7 (in D_2O)



References:

1. a) Ficht, S.; Payne, R.J.; Guy, R.T.; Wong, C.-H. *Chem. Eur. J.* **2008**, *14*, 3620-3629; b) Kajihara, Y.; Yoshihara, A.; Hirano, K.; Yamamoto, N. *Carbohydr. Res.* **2006**, *341*, 1333-1340.

2. For *anti*-**4** characterization data, see: Williams, L.; Zhang, Z. D.; Shao, F.; Carroll, P. J.; Joullie, M. M. *Tetrahedron* **1996**, *52*, 11673-11694.

3. For *syn*:*anti* determination, see: Nishida, A.; Sorimachi, H.; Iwaida, M.; Matsumizu, M.; Kawate, T.; Nakagawa, M. *Synlett* **1998**, 389-390.