An Advance in Proline Ligation

Shiying Shang,[a] Zhongping Tan,[a] Suwei Dong,[a] Samuel J. Danishefsky[a,b,*]

[a]*Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065, USA*

[b]*Department of Chemistry, Columbia University Havemeyer Hall, 3000 Broadway, New York, NY 10027, USA*

s-danishefsky@ski.mskcc.org

Content

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* To whom correspondence should be addressed.

Materials and Methods

All solvents were reagent grade or HPLC grade (Fisher). The following Fmoc amino acids from Sigma, Aldrich, EMD Biosciences, Chem-Impex International and PolyPeptide Laboratories were employed: Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Val-OH, Boc-Ala-OH, Boc-Gln(Trt)-OH, Boc-Phe-OH, Boc-Pro-OH, Boc-Val-OH, (2S, 4R)-Fmoc-Mpt(Trt)-OH, and (2S, 4S)-Fmoc-Mpt(Trt)-OH.

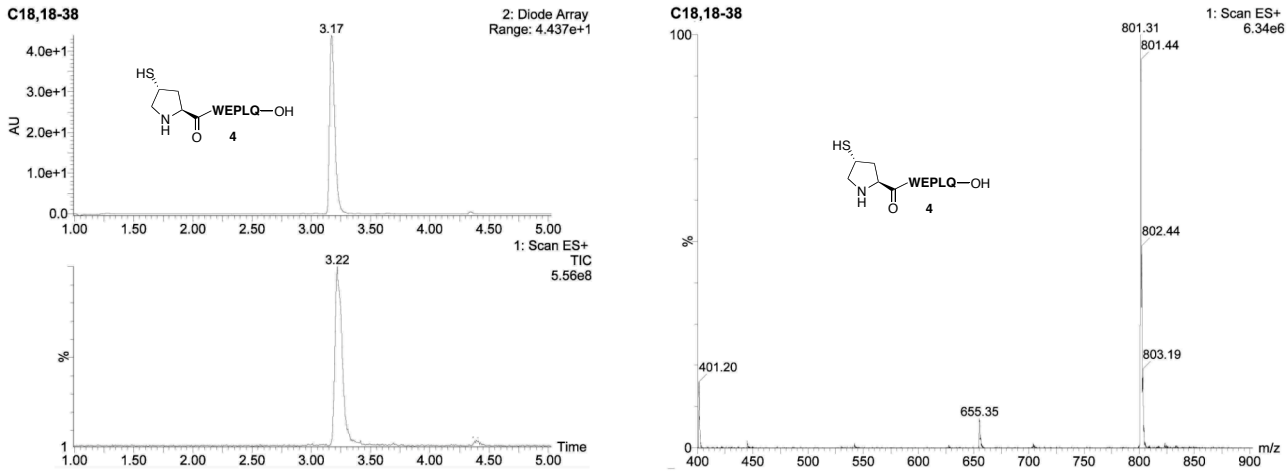
Solid Phase Peptide Synthesis: Automated peptide synthesis was performed on an Applied Biosystems Pioneer continuous flow peptide synthesizer. Peptides were synthesized using the standard automated Fmoc protocol (HATU, DIEA, DMF). The deblocking solution was a mixture of DMF/piperidine/DBU (100/5/5). The NovaSyn® TGT resins from EMD Biosciences were employed for the synthesis.

HPLC: All separations involved a mobile phase of 0.05% TFA (v/v) in water (solvent A)/0.04% TFA in acetonitrile (solvent B). LCMS analyses were performed using a Waters 2695 Separations Module and a Waters 996 Photodiode Array Detector equipped with Varian Microsorb 100-5, C18 150x2.0mm column at a flow rate of 0.2 mL/min. UPLC-MS analyses were performed using a Waters Acquity™ Ultra Preformance LC system equipped with Acquity UPLC® BEH C18, 1.7µl, 2.1 x 100 mm column at a flow rate of 0.3 mL/min. Preparative separations were performed using a Ranin HPLC solvent delivery system equipped with a Rainin UV-1 detector and Varian Dynamax using Varian Microsorb 100-5, C18 250x21.4mm column at a flow rate of 16.0 mL/min.

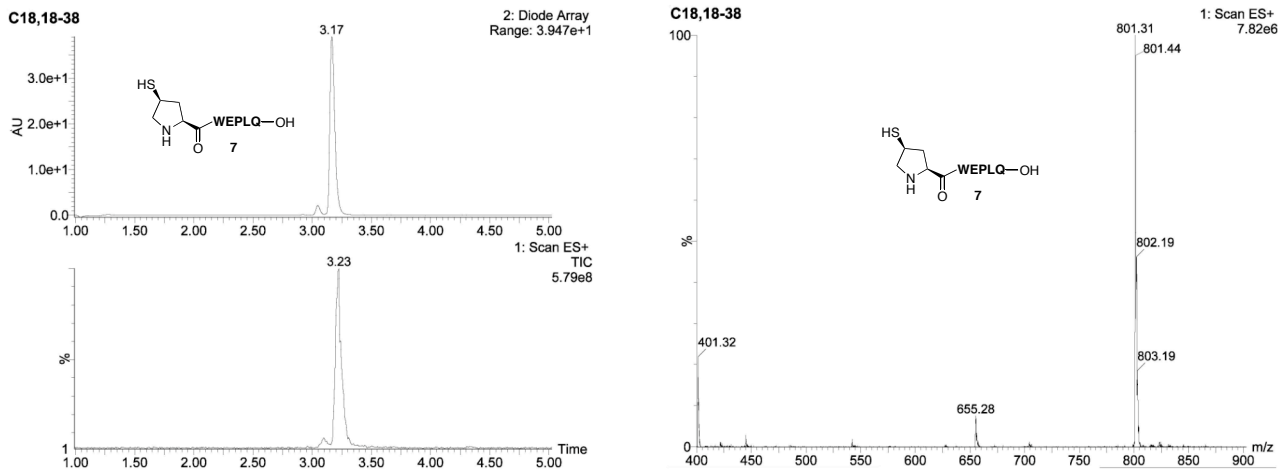
Preparation and Characterization of Peptide Precursors for Ligation

Unprotected Peptides

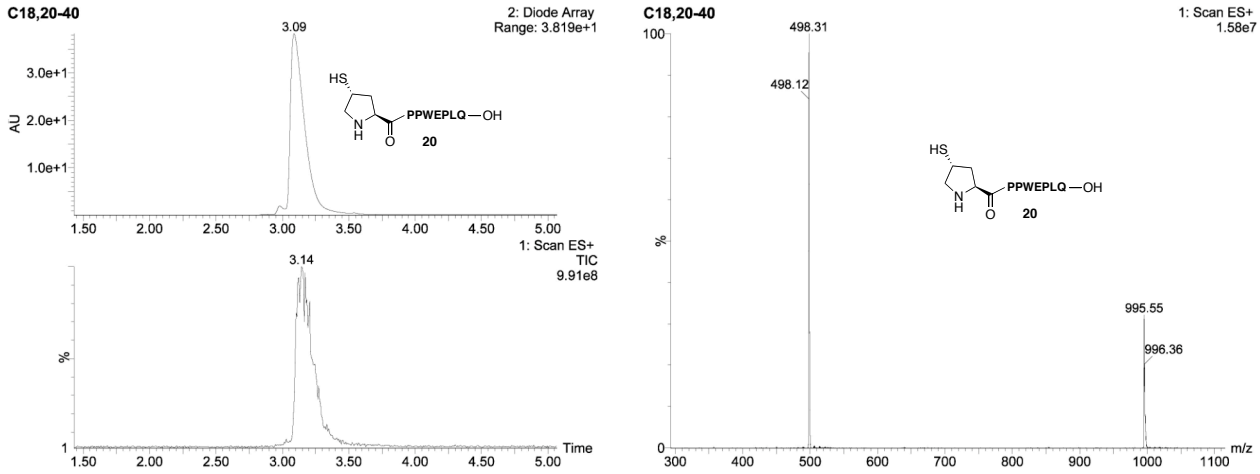
Upon completion of automated synthesis on a 0.05 mmol scale, the peptide resin was washed into a peptide cleavage vessel with DCM. The resin cleavage was performed by the treatment with TFA/H_2O/TIS (95:2.5:2.5) for 45 min to yield the unprotected peptides. TFA was removed by N_2. The oily residue was extracted with diethyl ether and centrifuged to give a white pellet. After the ether was decanted, the solid was dissolved in MeCN/H_2O/AcOH (47.5:47.5:5) for HPLC purification.



Compound 4: $C_{37}H_{52}N_8O_{10}S$ Exact Mass: 800.35, $[M+H]^+$ *m/z* = 801.36, $[M+2H]^{2+}$ *m/z* = 401.18.



Compound 7: $C_{37}H_{52}N_8O_{10}S$ Exact Mass: 800.35, $[M+H]^+$ *m/z* = 801.36, $[M+2H]^{2+}$ *m/z* = 401.18.



Compound 20: $C_{47}H_{66}N_{10}O_{12}S$ Exact Mass: 994.46, $[M+H]^+$ *m/z* = 995.47, $[M+2H]^{2+}$ *m/z* = 498.24.

Figure S1. UPLC-MS analyses of purified unprotected peptides. Left: UV and mass traces of the purified product. Right: ESI mass spectrum of the desired product.

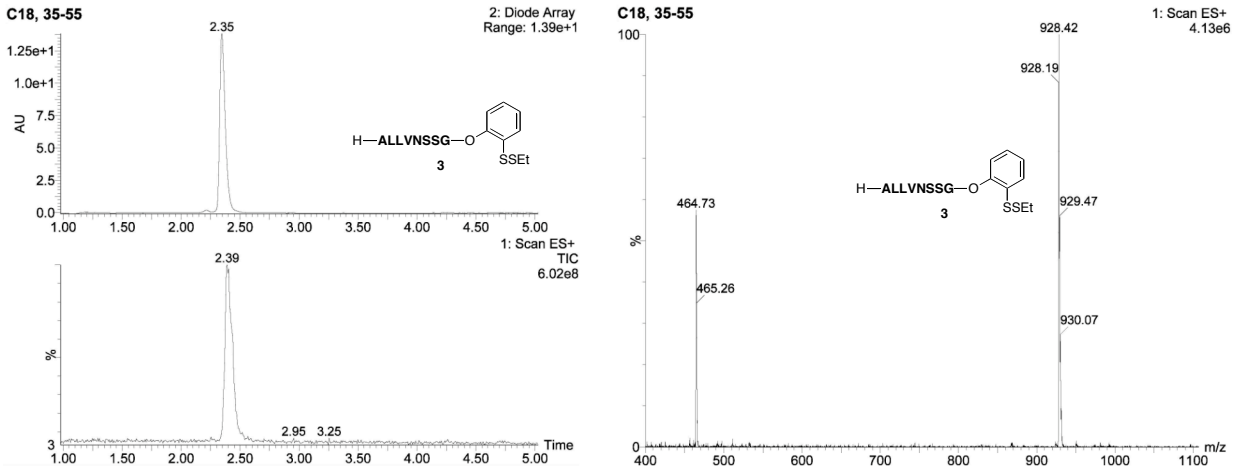
Fully Protected Peptidyl Acids

Upon completion of automated synthesis on a 0.05 mmol scale, the peptide resin was washed into a peptide cleavage vessel with DCM. The resin cleavage was effected by treatment with AcOH/TFE/DCM (1:1:8) for 2 × 1 hour to yield the fully protected peptidyl acids. The solvent was removed by N_2. The oily residue was extracted with diethyl ether and centrifuged to give a white pellet. After the ether was decanted, the solid was resuspended in MeCN/H_2O (1:1) and was lyophilized to dryness.

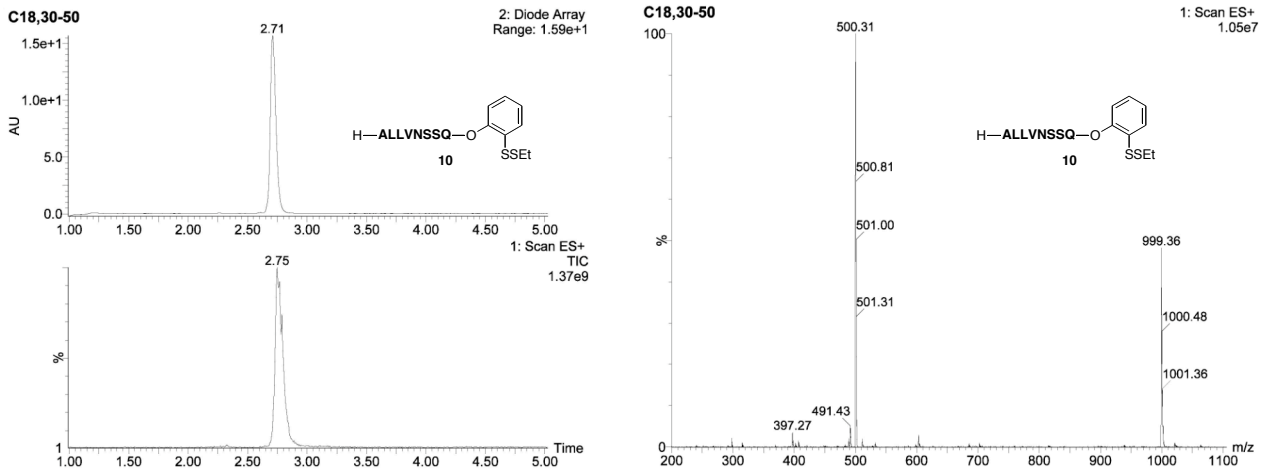
Peptide Phenolic Esters

The fully protected peptidyl acid (43 mM, 1.1 equiv) and HCl·H-AA-O-(2-ethyldithio)-phenyl ester (40 mM, 1.0 equiv) in $CHCl_3$/TFE (v/v = 3/1) was cooled to -10 °C. HOOBt (1.1 equiv) and EDCI (1.1 equiv) were added. The reaction mixture was stirred at room temperature for 2.5 h. The solvent was blown off under a gentle N_2 stream and TFA/H_2O/TIS (95:2.5:2.5, 3 ml) was added. After deprotection

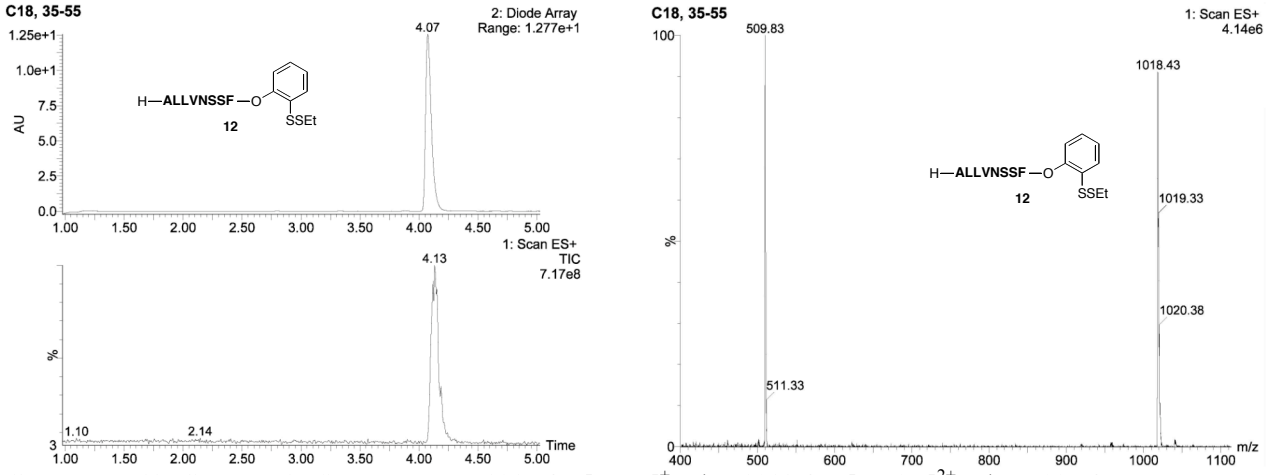
for 20 min, TFA was blown off and the oily residue was extracted with diethyl ether. The precipitate was pelleted and the ether was subsequently decanted. The resulting solid was dissolved in MeCN/H_2O/AcOH (47.5:47.5:5) for HPLC purification.



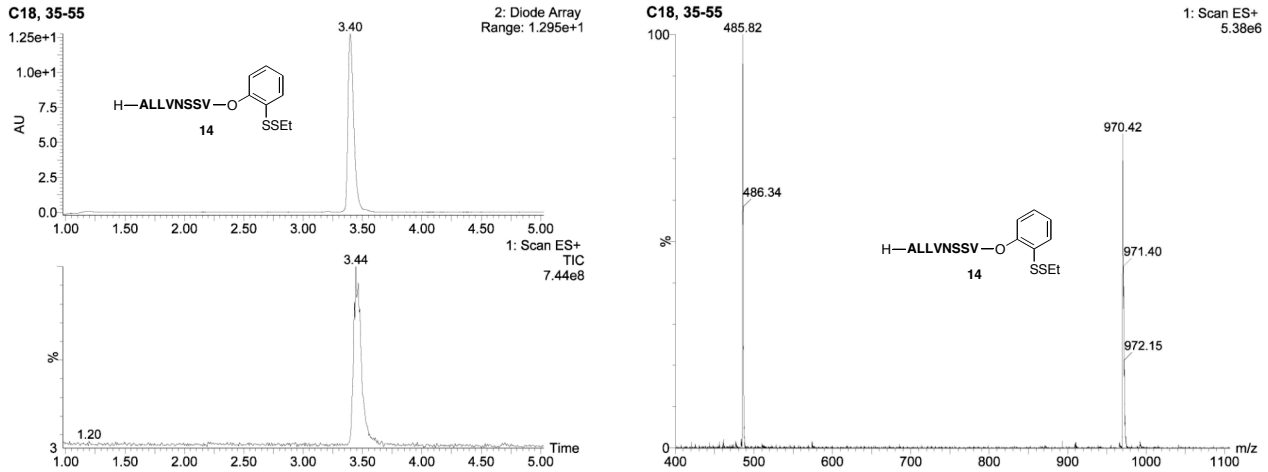
Compound 3: $C_{40}H_{65}N_9O_{12}S_2$ Exact Mass: 927.42, $[M+H]^+$ *m/z* = 928.43, $[M+2H]^{2+}$ *m/z* = 464.72.



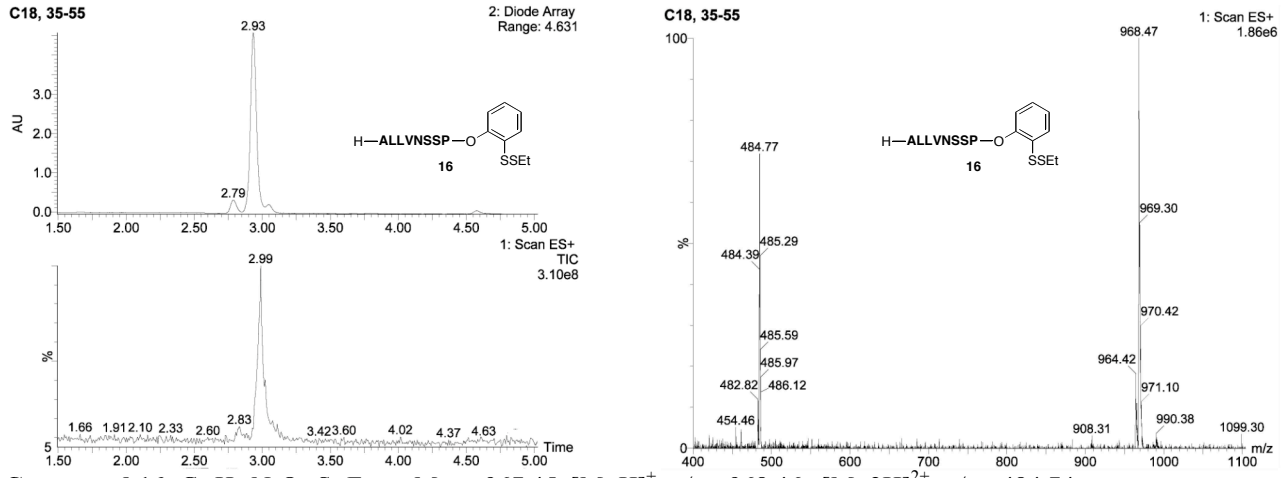
Compound 10: $C_{43}H_{70}N_{10}O_{13}S_2$ Exact Mass: 998.46, $[M+H]^+$ *m/z* = 999.46, $[M+2H]^{2+}$ *m/z* = 500.23.



Compound 12: $C_{47}H_{71}N_9O_{12}S_2$ Exact Mass: 1017.47, $[M+H]^+$ *m/z* = 1018.47, $[M+2H]^{2+}$ *m/z* = 509.74.



Compound 14: $C_{43}H_{71}N_9O_{12}S_2$ Exact Mass: 969.47, $[M+H]^+$ *m/z* = 970.47, $[M+2H]^{2+}$ *m/z* = 485.74.



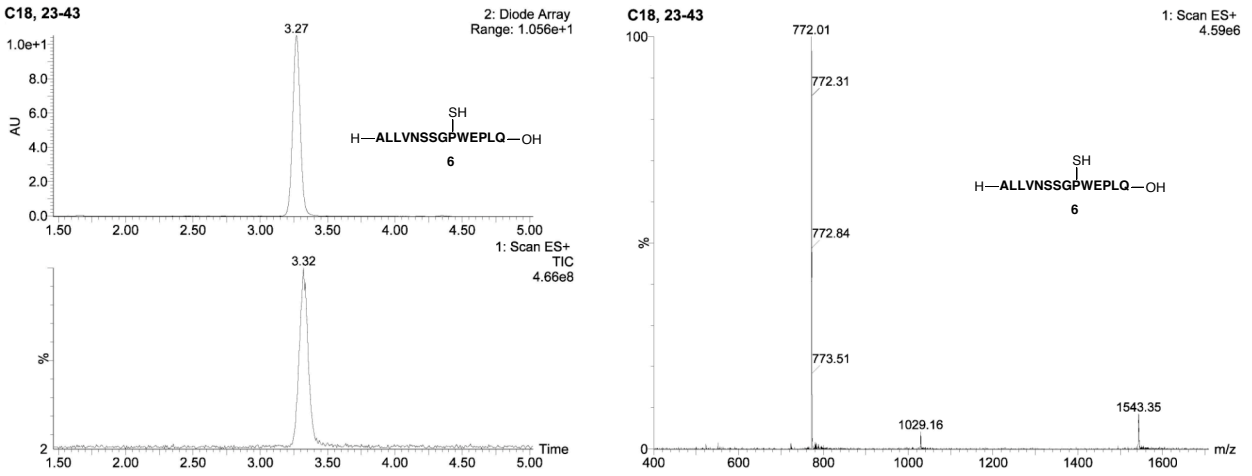
Compound 16: $C_{43}H_{69}N_9O_{12}S_2$ Exact Mass: 967.45, $[M+H]^+$ *m/z* = 968.46, $[M+2H]^{2+}$ *m/z* = 484.74.

Figure S2. UPLC-MS analyses of purified peptide phenolic esters. Left: UV and mass traces of the purified product. Right: ESI mass spectrum of the desired product.

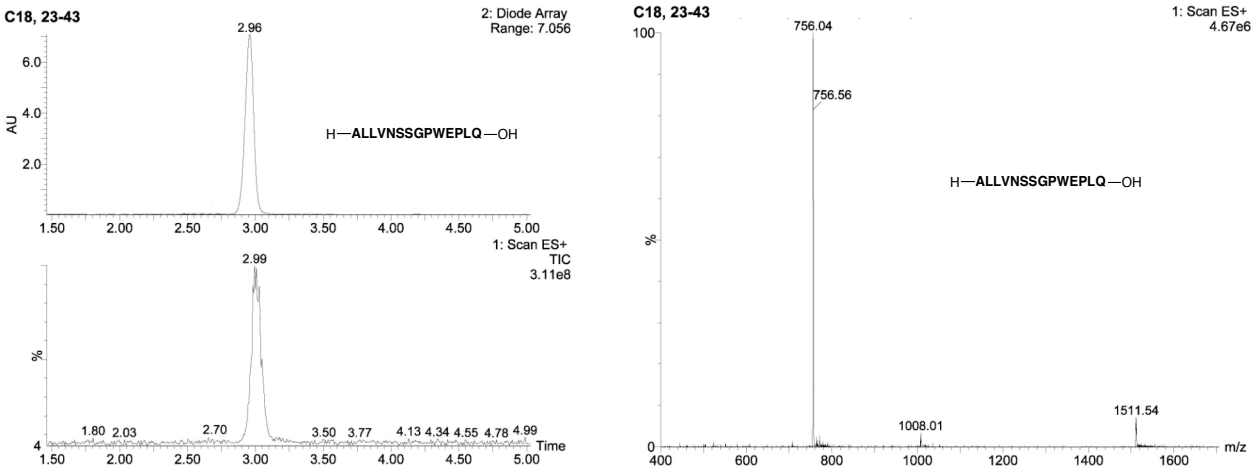
Preparation and Characterization of Ligation/Desulfurization and Direct Aminolysis Products

N-terminal peptide ester (4.1 mM, 1.5 equiv) and C-terminal peptide (2.7 mM, 1.0 equiv) were dissolved in 0.4 ml of ligation buffer (6 M Gdn·HCl, 100 mM Na_2HPO_4, 50 mM TCEP, pH 7.5). The reaction mixture was stirred at room temperature. The reactions were monitored by UPLC-MS and purified directly by HPLC upon consumption of the starting material.

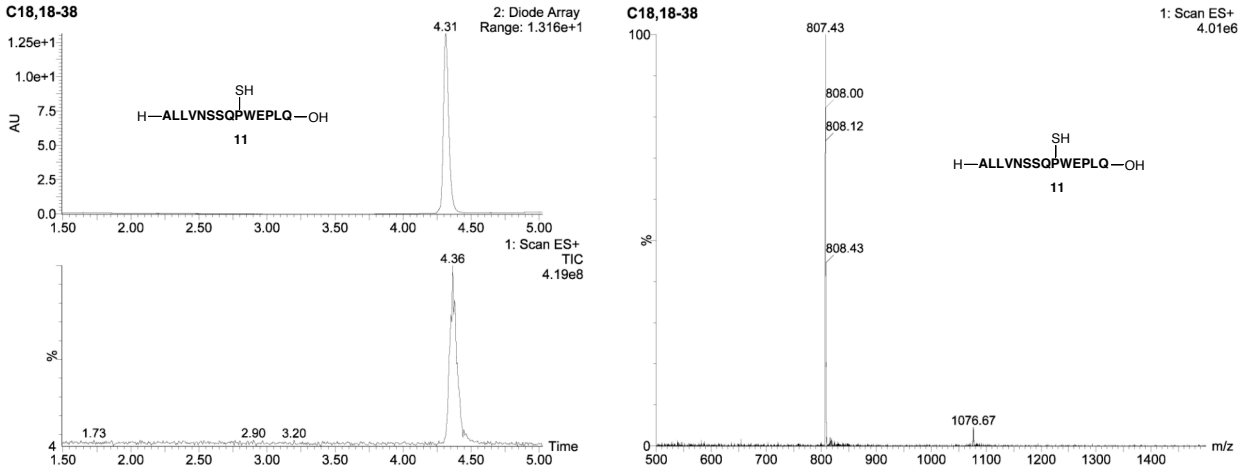
To a solution of the purified ligated peptide in degassed CH_3CN/H_2O (v/v = 1:1, 0.2 ml) were added 0.2 ml of 0.5 M bond-breaker® TCEP solution (Pierce), 0.02 ml of 2-methyl-2-propanethiol and 0.2 ml of radical initiator (VA-044, 0.1 M in H_2O). The reaction mixture was stirred at 37 °C. The reactions were monitored by UPLC-MS and purified directly by HPLC upon disappearance of the starting material.



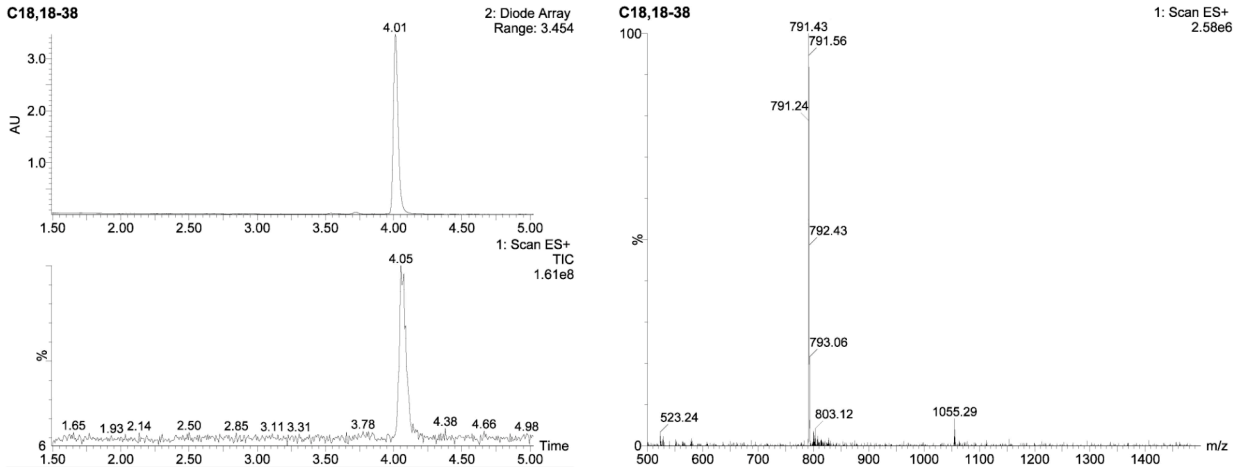
Compound 6: $C_{69}H_{107}N_{17}O_{21}S$ Exact Mass: 1541.75, $[M+H]^+$ *m/z* = 1542.76, $[M+2H]^{2+}$ *m/z* = 771.89.



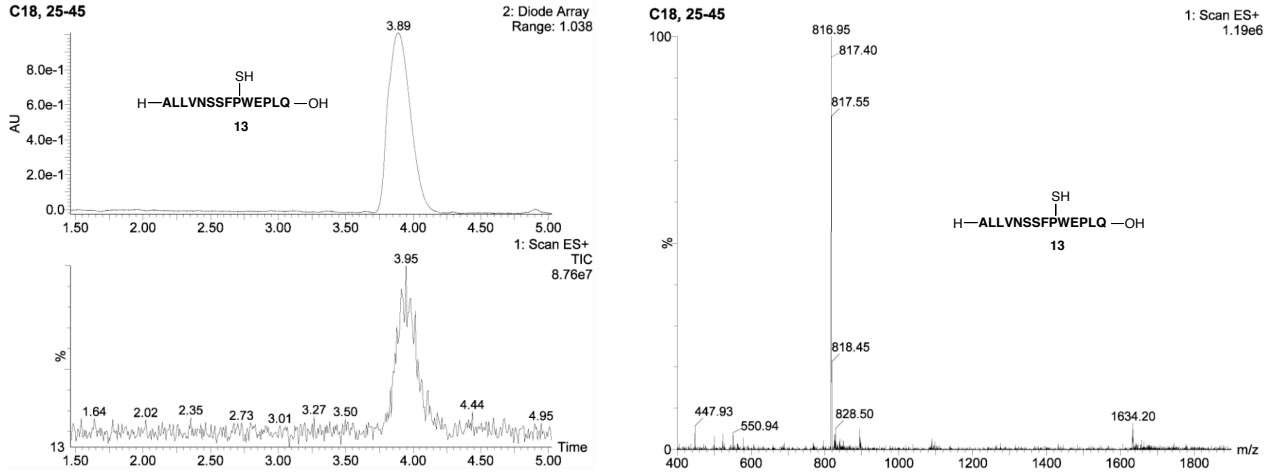
Desulfurized 6: $C_{69}H_{107}N_{17}O_{21}$ Exact Mass: 1509.78, $[M+H]^+$ *m/z* = 1510.79, $[M+2H]^{2+}$ *m/z* = 755.90. Yield: 88%.



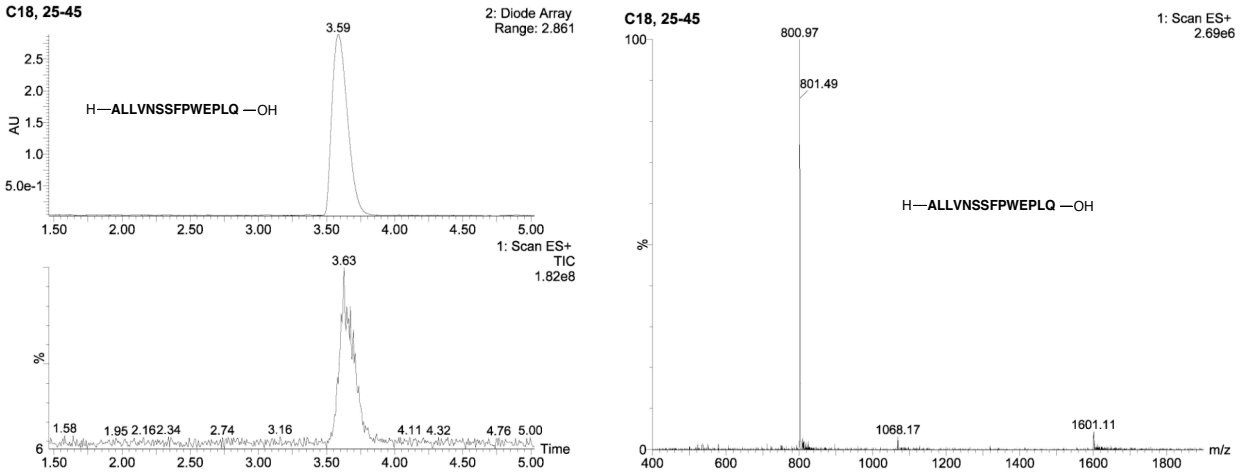
Compound 11: $C_{72}H_{112}N_{18}O_{22}S$ Exact Mass: 1612.79, $[M+2H]^{2+}$ *m/z* = 807.40.



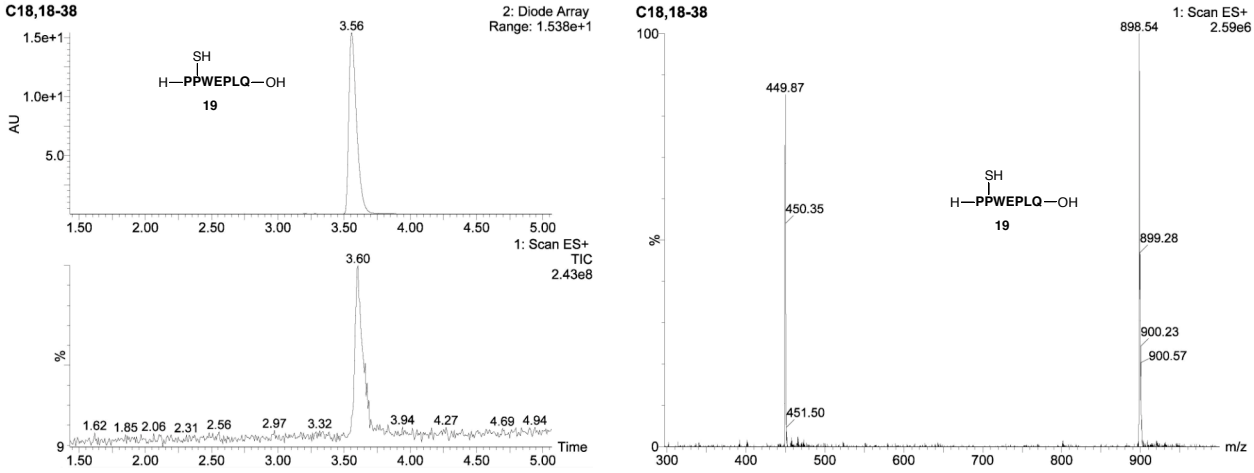
Desulfurized 11: $C_{72}H_{112}N_{18}O_{22}$ Exact Mass: 1580.82, $[M+2H]^{2+}$ *m/z* = 791.42. Yield: 90%.



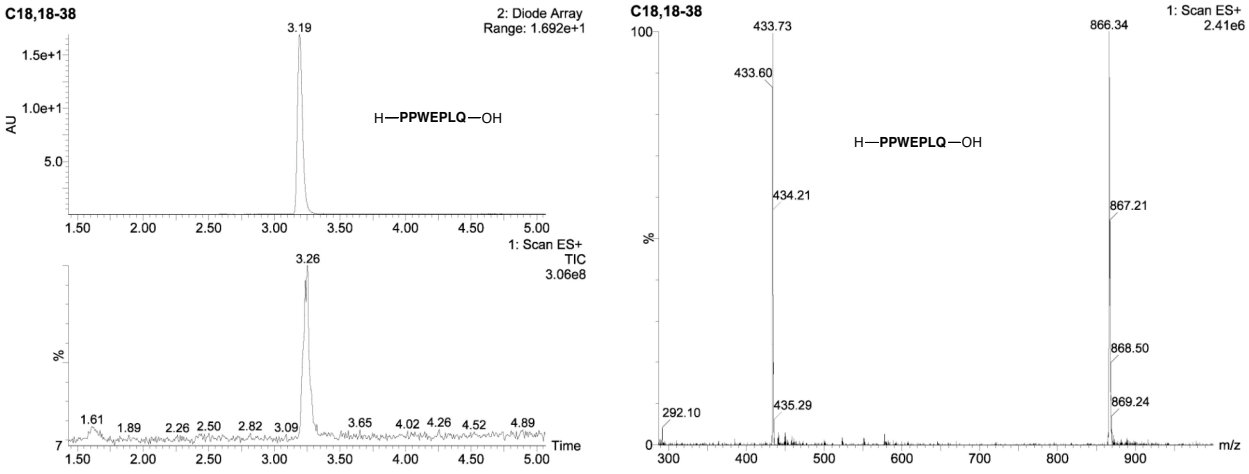
Compound 13: $C_{76}H_{113}N_{17}O_{21}S$ Exact Mass: 1631.80, $[M+H]^{+}$ *m/z* = 1632.81, $[M+2H]^{2+}$ *m/z* = 816.91.



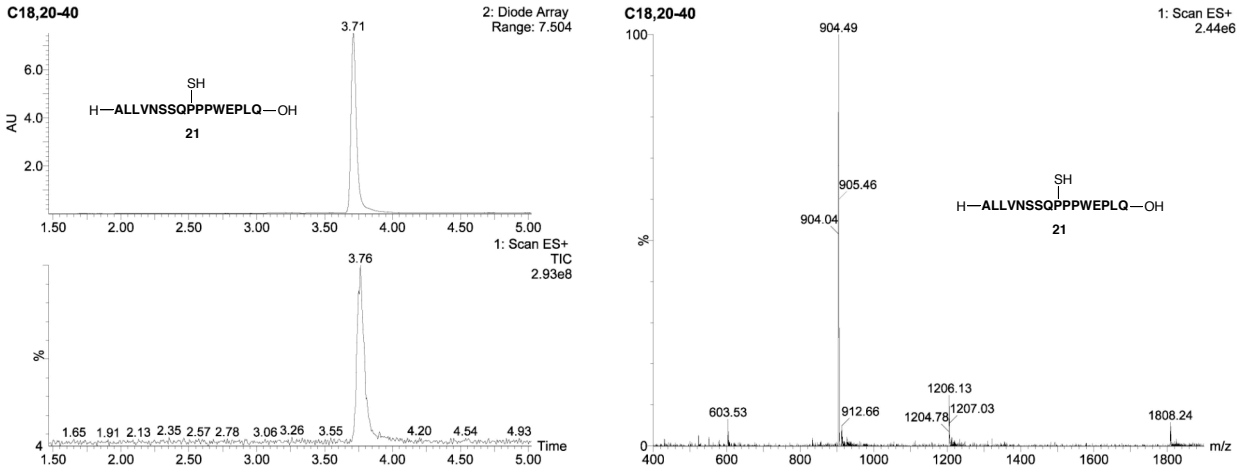
Desulfurized 13: $C_{76}H_{113}N_{17}O_{21}$ Exact Mass: 1599.83, $[M+H]^+$ *m/z* = 1600.84, $[M+2H]^{2+}$ *m/z* = 800.93. Yield: 83%.



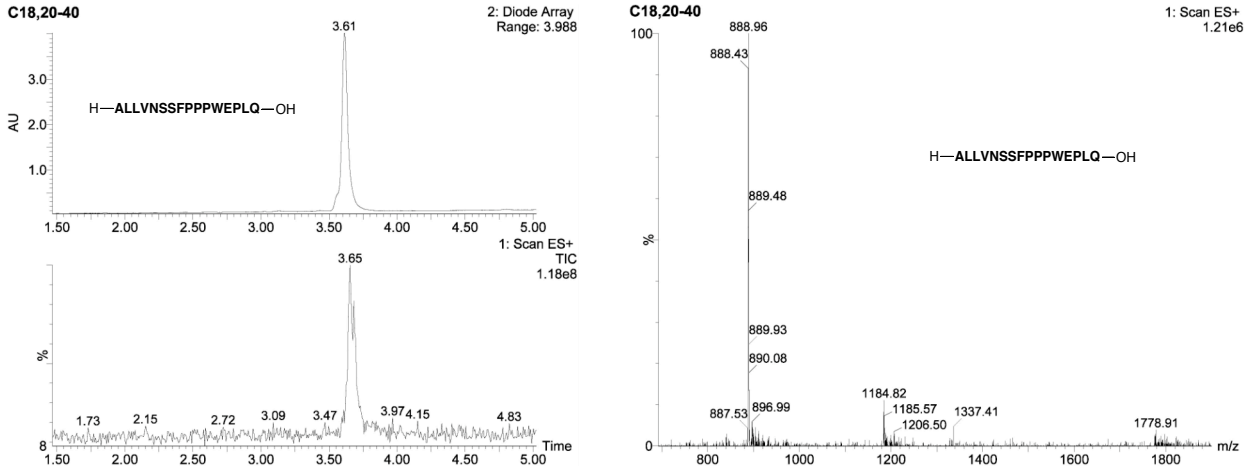
Compound 19: $C_{45}H_{59}N_9O_{11}S$ Exact Mass: 897.41, $[M+H]^+$ *m/z* = 898.41, $[M+2H]^{2+}$ *m/z* = 449.71.



Desulfurized 19: $C_{42}H_{59}N_9O_{11}$ Exact Mass: 865.43, $[M+H]^+$ *m/z* = 866.44, $[M+2H]^{2+}$ *m/z* = 433.73. Yield: 89%.

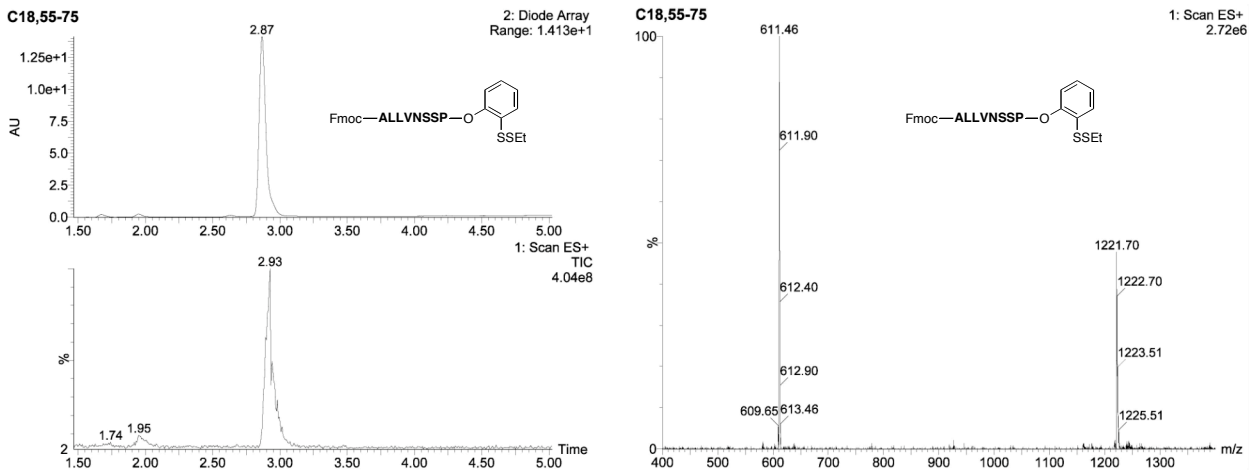


Compound 21: $C_{82}H_{126}N_{20}O_{24}S$ Exact Mass: 1806.90, $[M+H]^+$ *m/z* = 1807.91, $[M+2H]^{2+}$ *m/z* = 904.46.

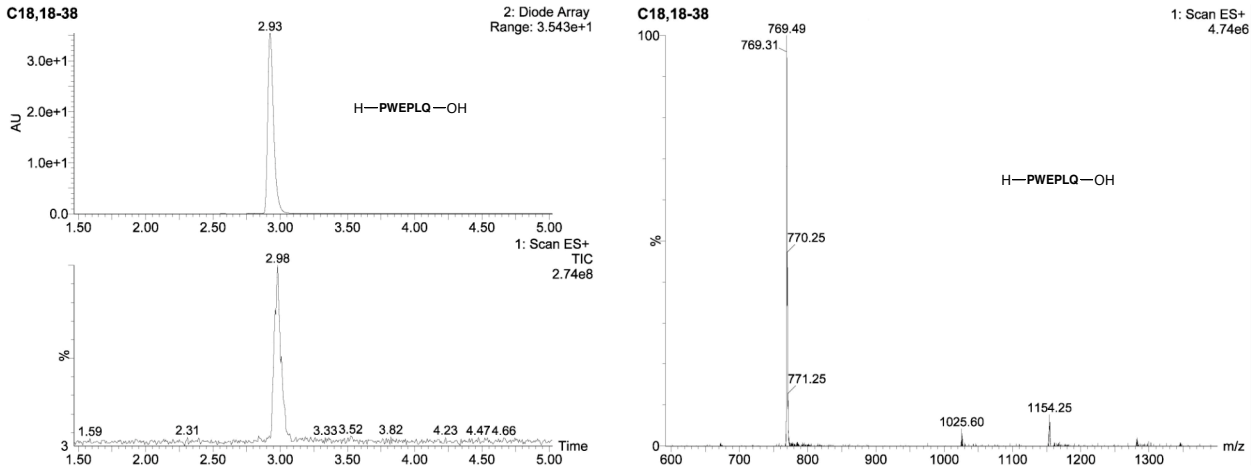


Desulfurized 21: $C_{82}H_{126}N_{20}O_{24}$ Exact Mass: 1774.93, $[M+H]^+$ *m/z* = 1775.93, $[M+2H]^{2+}$ *m/z* = 888.47. Yield: 85%.

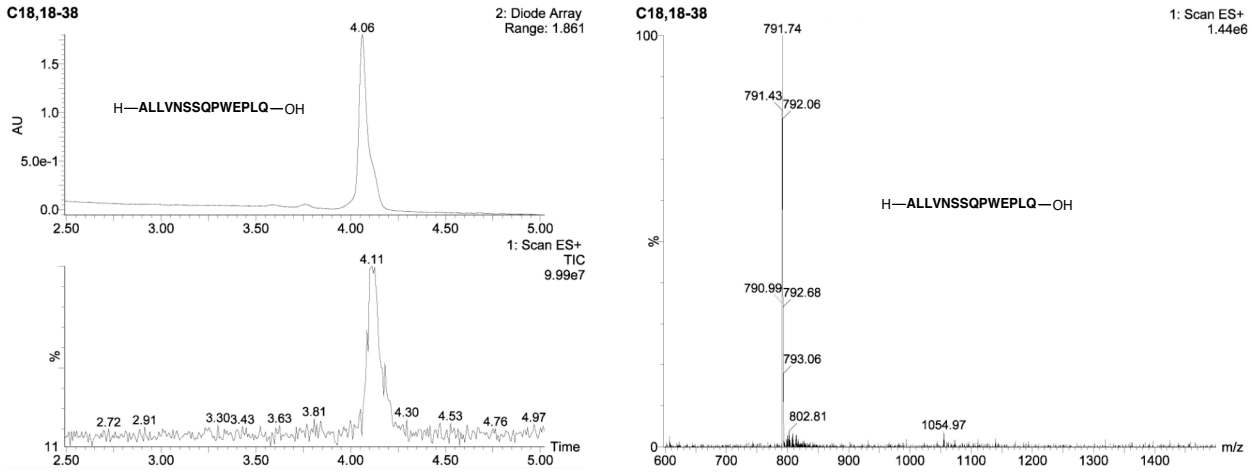
Figure S3. UPLC-MS analysis of purified ligation and desulfurization products. Left: UV and mass traces of the purified product. Right: ESI mass spectrum of the desired product.



$C_{58}H_{80}N_{10}O_{15}S_2$ Exact Mass: 1220.52, $[M+H]^+$ *m/z* = 1221.53, $[M+2H]^{2+}$ *m/z* = 611.27.



$C_{37}H_{52}N_8O_{10}$ Exact Mass: 768.38, $[M+H]^+$ *m/z* = 769.39.



Desulfurized 11: $C_{72}H_{112}N_{18}O_{22}$ Exact Mass: 1580.82, $[M+2H]^{2+}$ *m/z* = 791.42. Yield: 80%.

Figure S4. UPLC-MS analysis of the starting materials for the direct aminolysis reaction and the product. Left: UV and mass

traces of the purified product. Right: ESI mass spectrum of the desired product.