Supporting Information

Photolytic Cleavage of Leader Peptides

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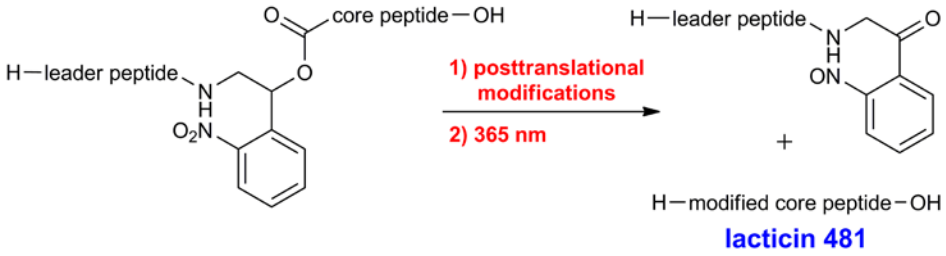
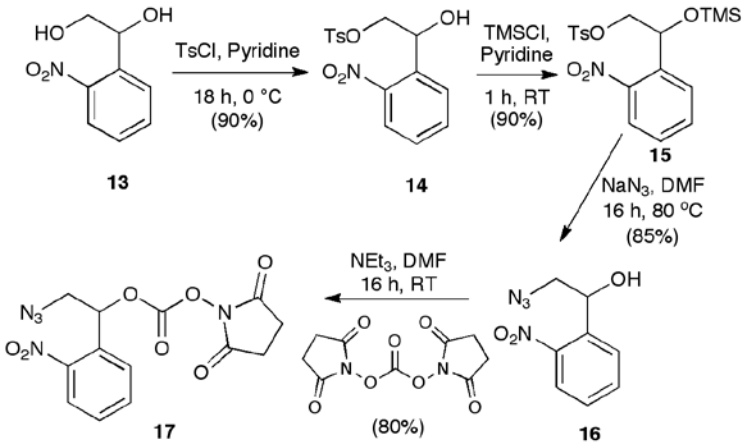


Table of Contents

Materials. Fmoc amino acids and resins were purchased from Advanced ChemTech, Novabiochem, or Chem-Impex. HOBt (1-hydroxybenzotriazole) was purchased from Chem-Impex, and DIC (N,N' diisopropylcarbodiimide) and HCTU (O-(1H-6 chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate) were purchased from Advanced ChemTech. Solvents commonly used in peptide synthesis and purification, including dimethylformamide (DMF), dichloromethane (DCM) trifluoroacetic acid (TFA), and acetonitrile (MeCN) were obtained in HPLC grade or better and used directly without further purification. Tri-n-butylphosphine, piperidine, and N-methyl morpholine were purchased from Acros whereas pyridine, diisopropylethylamine (DIPEA), and β-mercaptoethanol were purchased from Sigma Aldrich. Tris-(2-carboxyethyl)phosphine (TCEP) was obtained from Molecular Probes as the TCEP-HCl salt. The ligand *tris*-(benzyltriazolylmethyl)amine (TBTA), tetrakis(MeCN)copper(I) hexafluorophosphate and hydrazine were purchased from Sigma Aldrich and 3-butyn-1-amine hydrochloride was purchased from AB Chem Inc. For the synthesis of the photocleavable linker, *p*-toluenesulfonyl chloride, trimethylsilyl chloride, sodium azide, and N-hydroxysuccinimidyl carbonate were purchased from Sigma Aldrich. The irradiation used for photochemical reactions was generated using a UVP Blak-Ray lamp (Ultraviolet Products, San Gabriel, CA) with a 365 nm filter.



Synthetic design of photolabile linker **17**.

Experimental:

2-Hydroxy-2-(2-nitrophenyl)ethyl 4-methylbenzenesulfonate *(14)*:
Into a 50 mL round-bottomed flask was placed *p*-toluenesulfonyl chloride (1.46 g, 7.64 mmol, 1.4 equiv) and 1-(2-nitrophenyl)-1,2-ethanediol **13** (1.0 g, 5.46 mmol, 1 equiv). The flask was then sparged with N_2 for five min. Distilled pyridine (25 mL) was added and the stirred reaction was placed in a 0 ^oC icebath. The mixture was stirred for 18 h at 0 ^oC, was allowed to warm to room temperature, and was then quenched with H_2O (80 mL). Crude product was extracted with Et_2O (2 × 50 mL) and combined extracts were washed successively with 1 M $KHSO_4$ (3 × 30 mL), sat. aqueous $NaHCO_3$ (3 × 30 mL) and sat. aqueous NaCl (2 × 50 mL). The organic layer was dried over $MgSO_4$, concentrated by rotary evaporation, and then purified by SiO_2 gel column

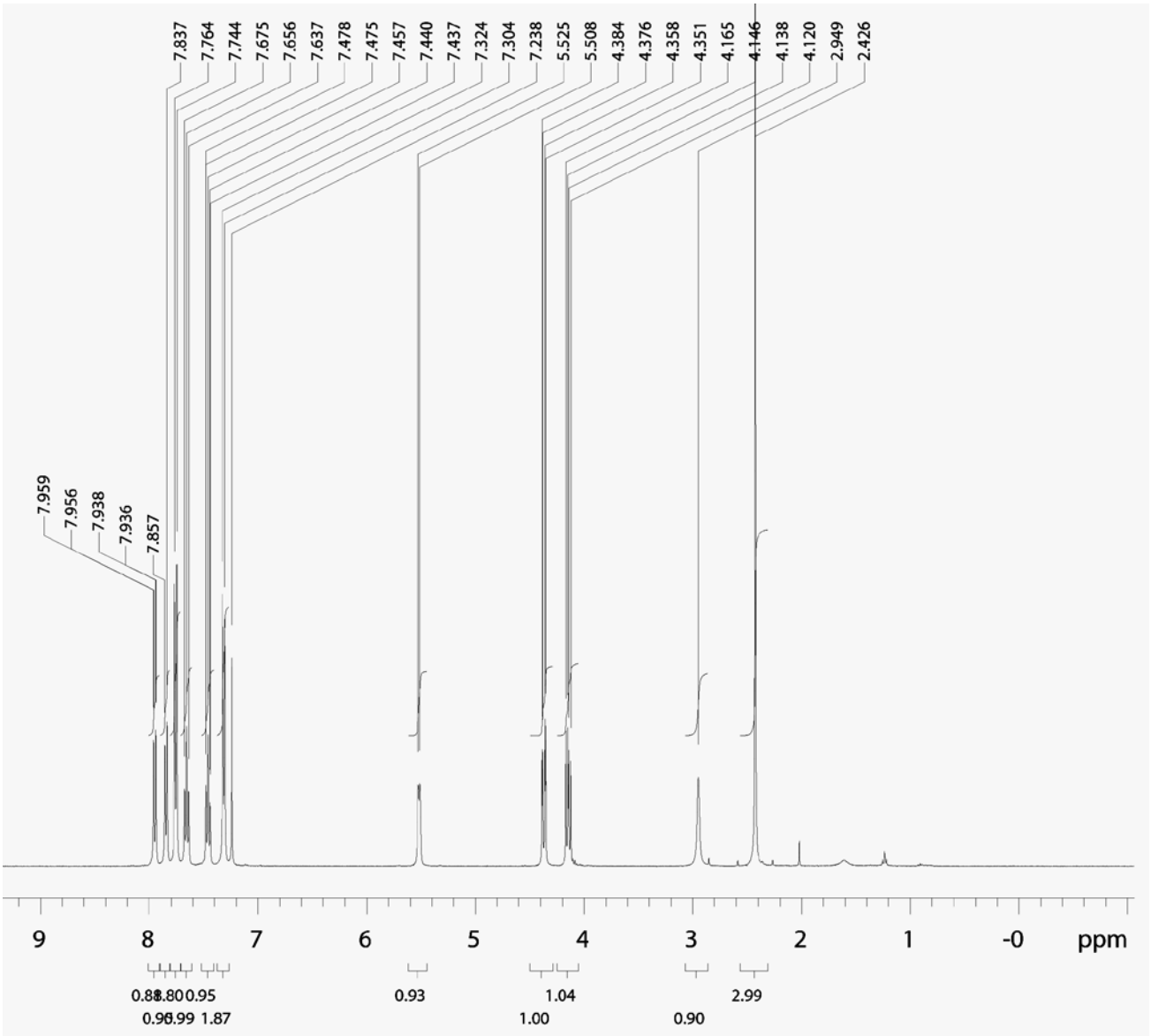
Supplementary Material (ESI) for Chemical Communications

chromatography (eluted with hexanes/EtOAc (4:1); Rf = 0.065) yielding compound **14** (1.65 g, 90%) as an orange oil.

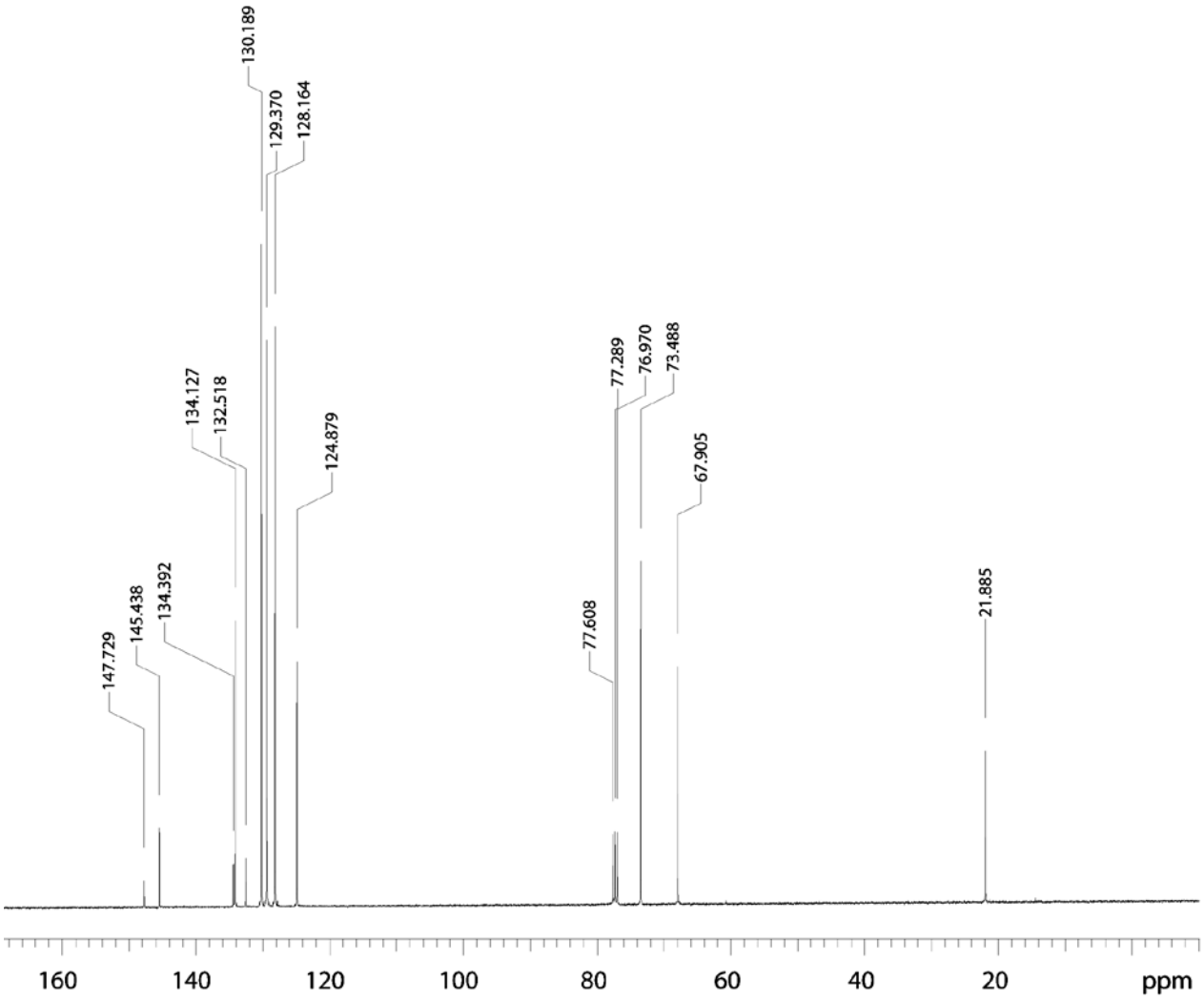
^{1}H NMR: (399.947 MHz, $CDCl_3$) δ=2.43 (s, 3H, CH_3), 3.95 (bs, 1H, OH) 4.14 (dd, J = 7.3, 3.2 Hz, 1H, CH_2), 4.36 (dd, J = 2.7, 7.8 Hz, 1H, CH_2), 5.51 (dd, J = 2.7, 4.7 Hz, 1H, CH), 7.31 (d, J = 8.1 Hz, 2H, CH_{tosyl}), 7.44 (t, J = 7.8 Hz, 1H, CH_{phenyl}), 7.65 (t, J = 7.3 Hz, 1H, CH_{phenyl}), 7.75 (d, J = 8.3 Hz, 2H, CH_{tosyl}), 7.85 (d, J = 7.8 Hz, 1H, CH_{phenyl}), 7.95 (d, J = 8.1 Hz, 1H, CH_{phenyl}).

^{13}C NMR: (100.527 MHz, $CDCl_3$) δ=21.9, 67.9, 73.5, 124.9, 128.2, 129.4, 130.3, 132.5, 134.1, 134.4, 145.4, 147.7.

HRMS $[M+H]^+$ $C_{15}H_{16}NO_6S$ calc'd = 338.0698, found = 338.0704.



^{1}H NMR of **14**.



^{13}C NMR of **14.**

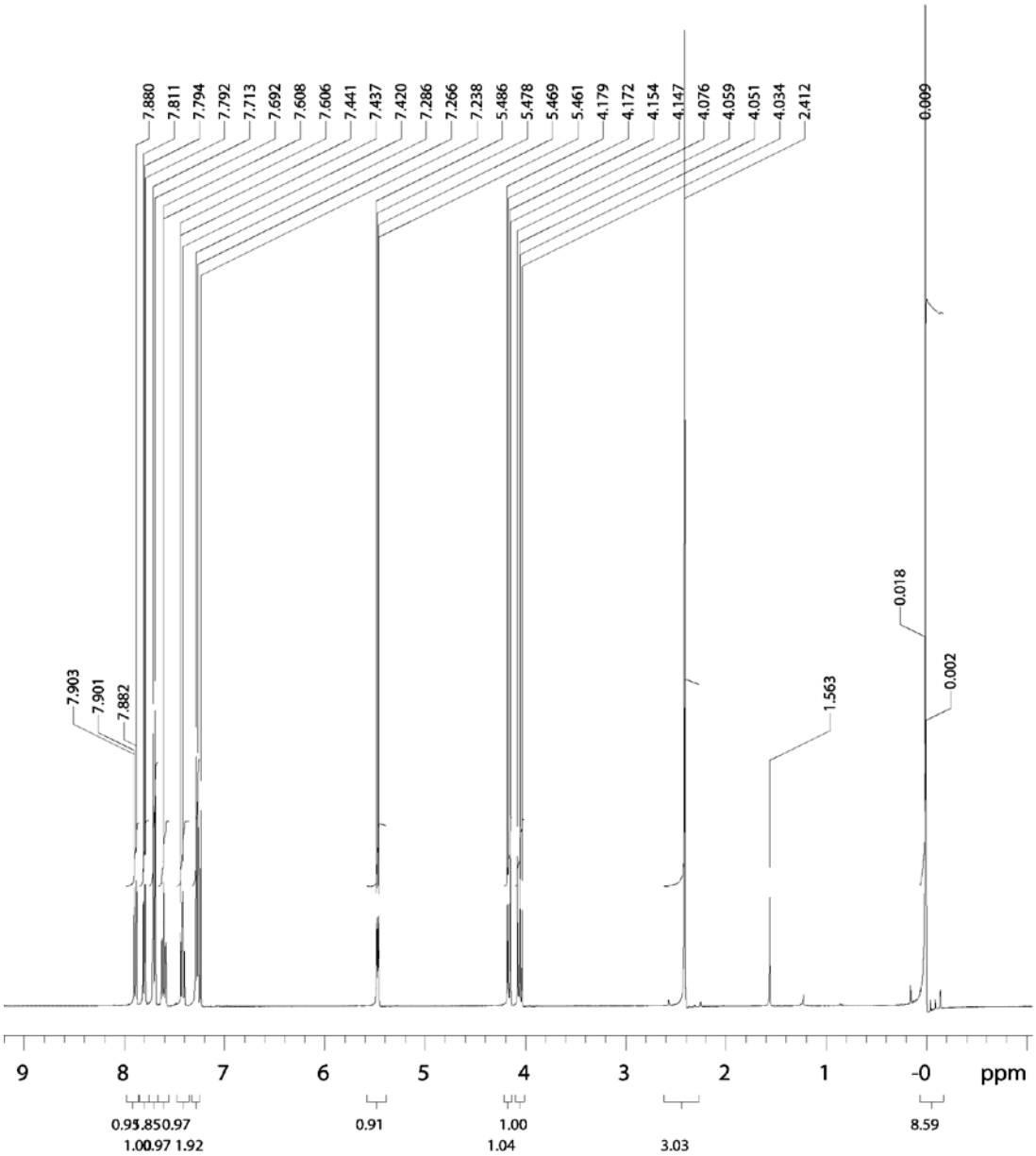
2-(2-Nitrophenyl)-2-((trimethylsilyl)oxy)ethyl 4-methylbenzenesulfonate *(15)*

Into a 50 mL round-bottomed flask was placed a solution of *p*-toluenesulfonyl derivative **14** (1.69 g, 5.0 mmol, 1 equiv). The flask was purged with a nitrogen stream for five min followed by the addition of distilled pyridine (25 mL). To this flask was added TMSCl (2.17 g, 20 mmol, 4 equiv) dropwise and the solution was stirred for 1 h at room temperature. At this time the reaction was quenched with H_2O (80 mL), crude product was extracted with Et_2O (2 × 50 mL), and the combined extracts were washed successively with 1 M $KHSO_4$ (3 × 30 mL), sat. aqueous $NaHCO_3$ (3 × 30 mL), and sat. aqueous NaCl (2 × 50 mL). The organic layer was dried over $MgSO_4$ and concentrated by rotary evaporation, yielding compound **15** (1.84 g, 90%) as a tan solid.

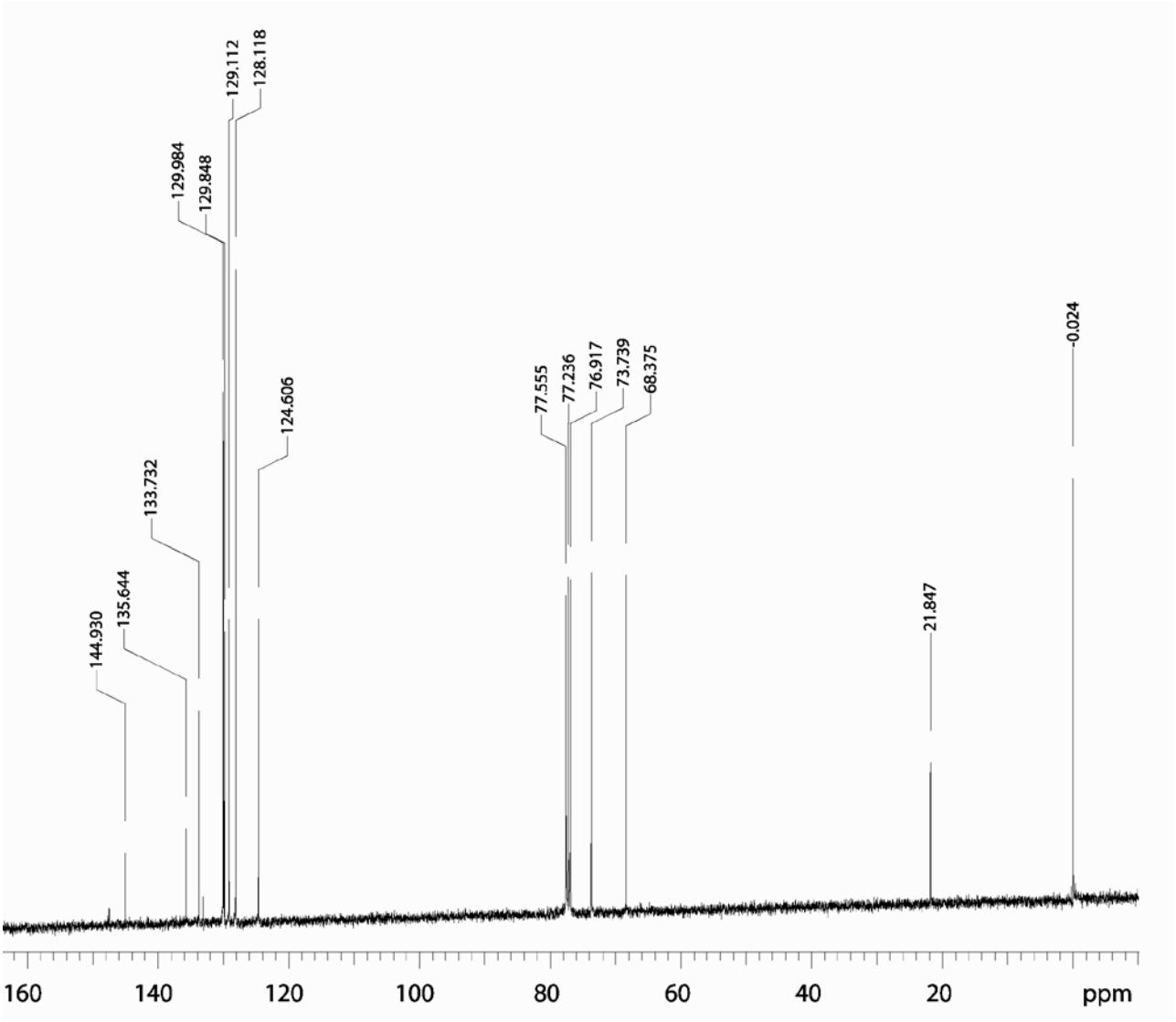
^{1}H NMR: (399.746 MHz, $CDCl_3$) δ=0.01 (s, 9H, CH_3), 2.41 (s, 3H, CH_3), 4.05 (dd, J = 6.6, 4.4 Hz, 1H, CH_2), 4.16 (dd, J = 2.9, 6.8 Hz, 1H, CH_2), 5.47 (dd, J = 6.6, 6.6 Hz, 1H, CH), 7.27 (d, J = 8.1 Hz, 2H, CH_{tosyl}), 7.43 (t, J = 7.8 Hz, 1H, CH_{phenyl}), 7.61 (t, J = 7.7 Hz, 1H, CH_{phenyl}), 7.70 (d, J = 8.3 Hz, 2H, CH_{tosyl}), 7.79 (d, J = 7.9 Hz, 1H, CH_{phenyl}), 7.89 (d, J = 8.3 Hz, 1H, CH_{phenyl}).

^{13}C NMR: (100.527 MHz, $CDCl_3$) δ=-0.02, 21.8, 68.4, 73.7, 124.6, 128.1, 129.1, 129.8, 130.0, 133.1, 133.7, 135.6, 144.9, 147.8.

HRMS $[M+Na]^+$ $C_{18}H_{23}N_6NaSSi$ calc'd = 432.0913, found = 432.0917.
M.P. = 83-87 ^{o}C.



^{1}H NMR of **15**.



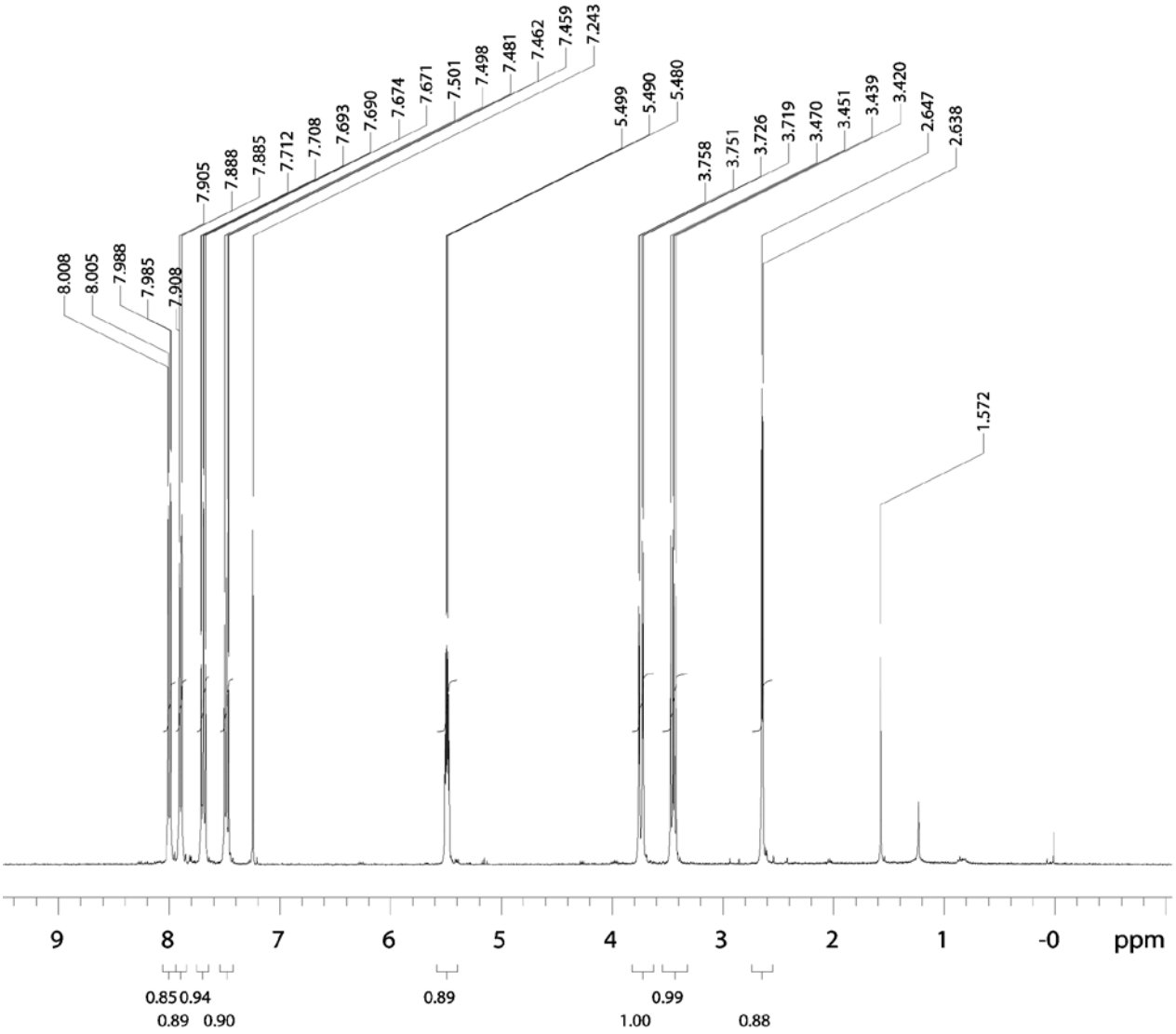
^{13}C NMR of **15**.

2-Azido-1-(2-nitrophenyl) ethanol *(16)*:
Into a 25 mL round-bottomed flask was placed sodium azide (0.52 g, 2.52 mmol, 2 equiv). The flask was purged with a nitrogen stream for five min. To this flask was added trimethylsilyl ether derivative **15** (0.52 g, 1.26 mmol, 1 equiv) in DMF (9 mL) and the reaction was stirred at 80 °C for 10 h. At this time the reaction was quenched with H_2O (80 mL), crude product was extracted with Et_2O (2 × 50 mL), and the combined extracts were washed with sat. aqueous NaCl (2 × 50 mL). The organic layer was dried with $MgSO_4$, concentrated by rotary evaporation, and purified by SiO_2 gel column chromatography (eluted with hexanes/EtOAc (4:1); Rf = 0.29) yielding **16** (0.23 g, 85%) as a red oil.

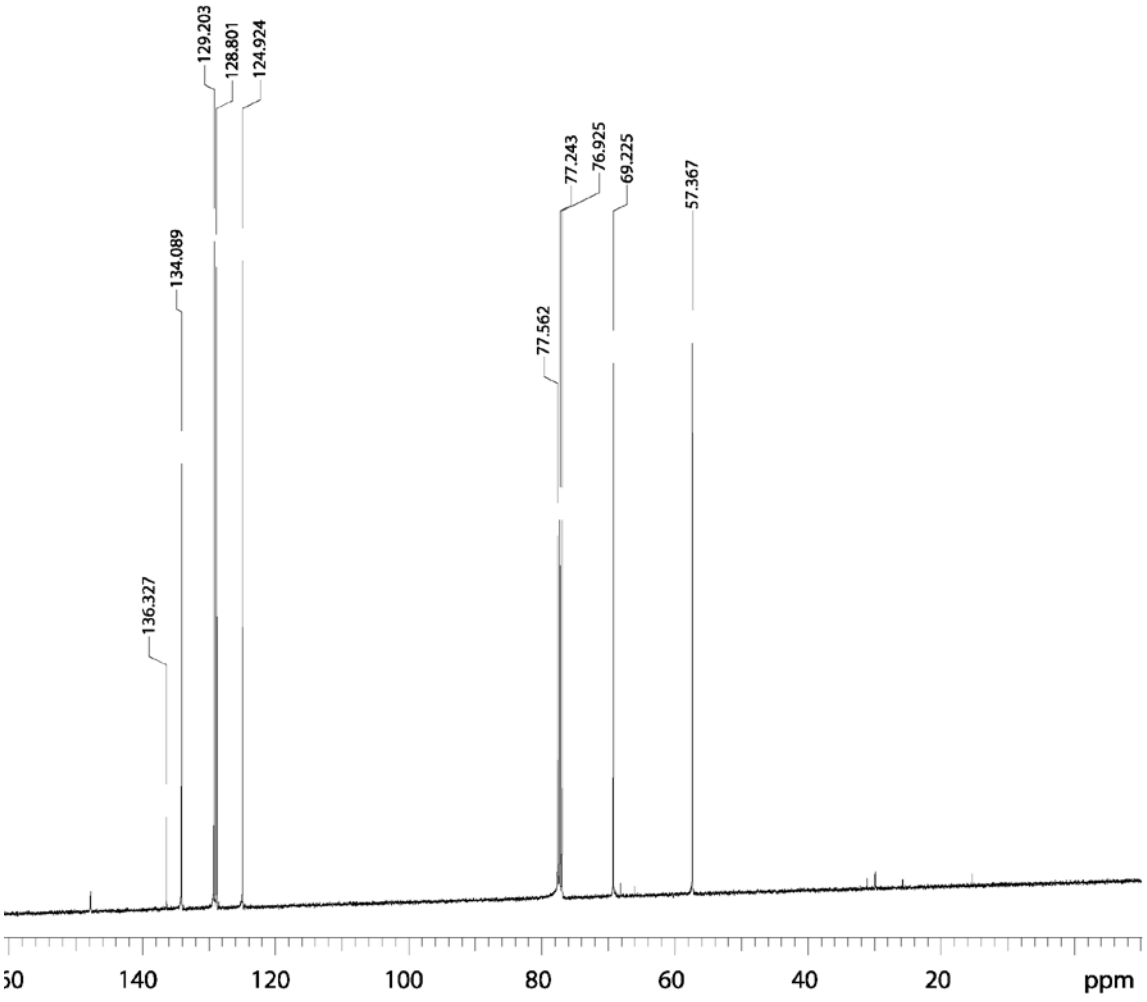
^{1}H NMR: (399.947 MHz, $CDCl_3$) δ=2.64 (d, J = 3.7 Hz, 1H, OH), 3.44 (dd, J = 7.6, 4.9 Hz, 1H, CH_2), 3.74 (dd, J = 3.0, 9.5 Hz, 1H, CH_2), 5.49 (m, 1H, CH), 7.47 (t, J = 7.8 Hz, 1H, CH_{phenyl}), 7.69 (t, J = 8.0 Hz, 1H, CH_{phenyl}), 7.90 (d, J = 7.8 Hz, 1H, CH_{phenyl}), 8.00 (d, J = 8.3 Hz, 1H, CH_{phenyl}).

^{13}C NMR: (100.527 MHz, $CDCl_3$) δ=57.4, 69.2, 125.0, 128.8, 129.2, 134.1, 136.3, 147.9.

HRMS $[M + Na]^+$ $C_8H_8N_4O_3Na$ calc'd = 231.0494, found = 231.0483.



^{1}H NMR of **16**.



^{13}C NMR of **16**.

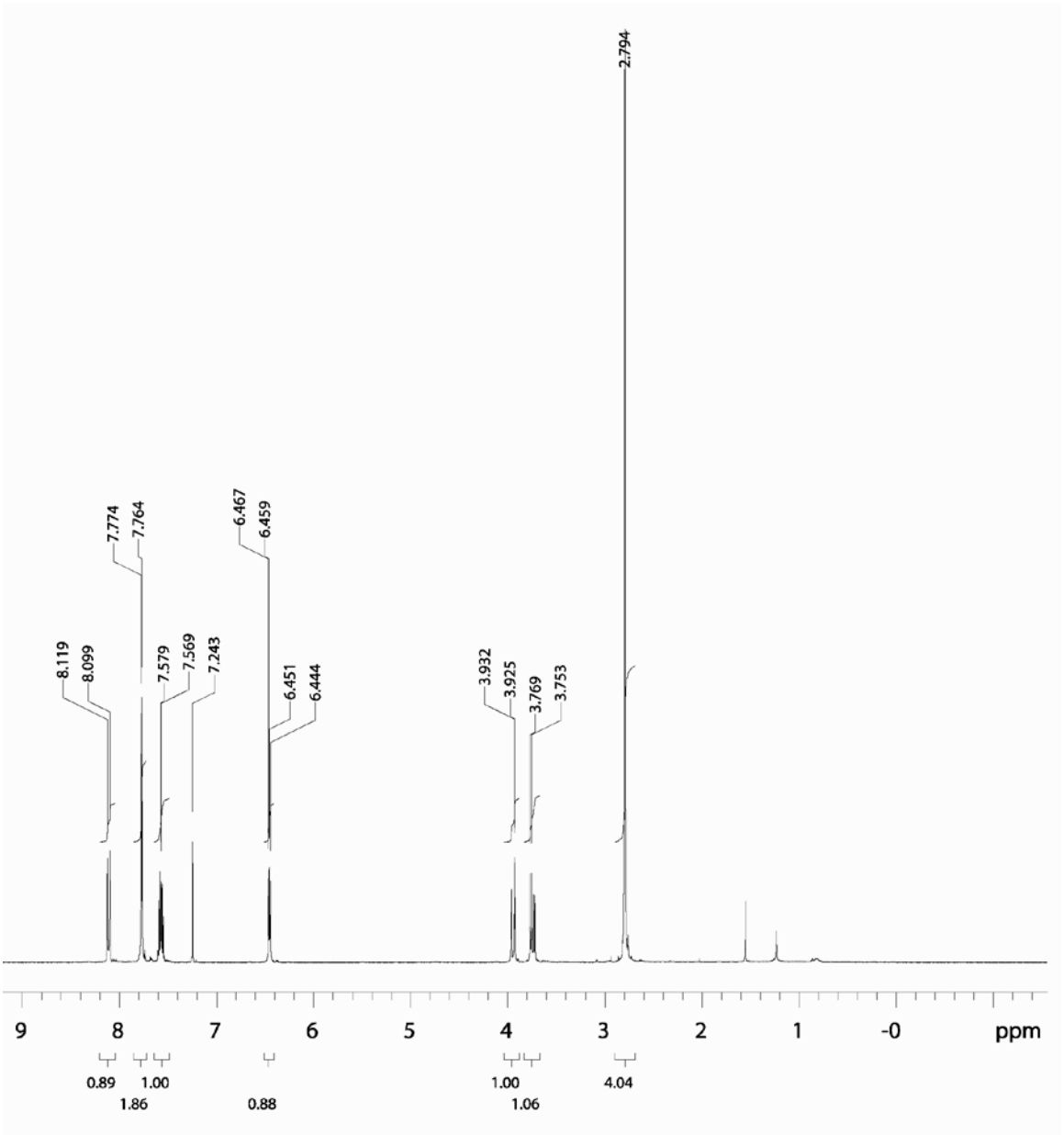
2-Azido-1-(2-nitrophenyl)ethyl (2,5-dioxopyrrolidin-1-yl) carbonate *(17)*:
Into a 10 mL round-bottomed flask was placed a solution of azide derivative **16** (41.6 mg, 0.2 mmol, 1 equiv) in DMF (1.0 mL) and N-hydroxysuccinimidyl carbonate (85.0 mg, 0.33 mmol, 1.65 equiv) was added followed by NEt_3 (83.6 μL, 0.60 mmol, 3 equiv). The reaction was stirred at room temperature for 16 h. At this time, solvent was removed by rotary evaporation and crude product was purified by SiO_2 gel column chromatography (eluted with hexanes/EtOAc (1:1); Rf = 0.45). Fractions were collected and concentrated by rotary evaporation yielding **17** (55.9 mg, 80% yield) as a white solid.

^{1}H NMR: (499.947 MHz, $CDCl_3$) δ=2.80 (s, 4H, CH_2), 3.76 (dd, J = 6.4, 7.1 Hz, 1H, CH_2), 3.93 (dd, J = 3.0, 10.7 Hz, 1H, CH_2), 6.46 (dd, J = 3.0, 3.7 Hz, 1H, CH), 7.57 (m, J = 4.3 Hz, 1H, CH_{phenyl}), 7.77 (d, J = 4.1 Hz, 2H, CH_{phenyl}) 8.11 (d, J = 8.3 Hz, 1H, CH_{phenyl}).

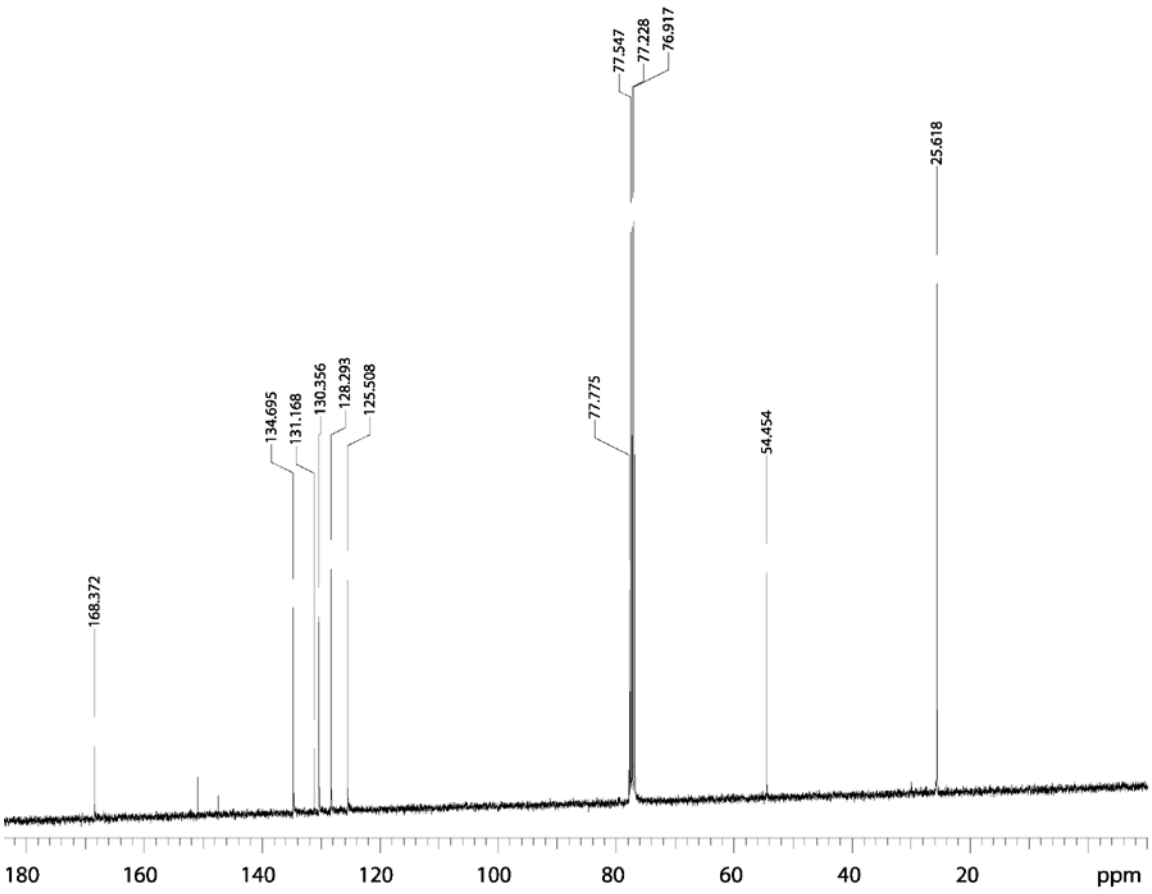
^{13}C NMR: (100.578 MHz, $CDCl_3$) δ=25.6, 54.5, 77.8, 125.5, 128.3, 130.4, 131.2, 134.7, 147.5, 150.9, 168.4.

HRMS $[M+H]^+$ $C_{13}H_{12}N_5O_7$ calc'd = 350.0737, found = 350.0727.

M.P. = 110-115 ^oC.



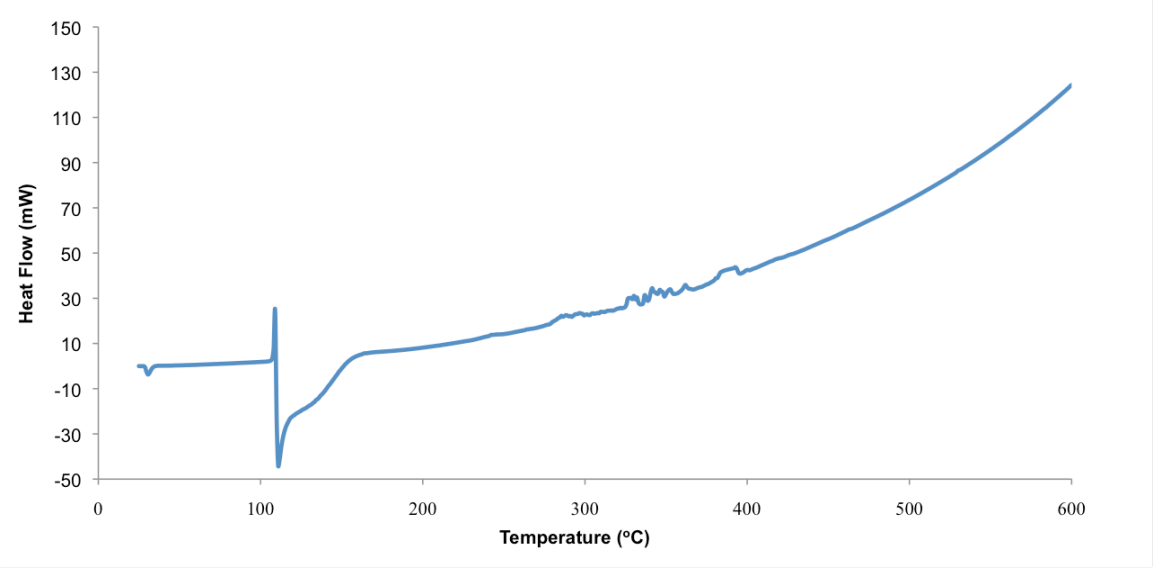
^{1}H NMR of **17**.



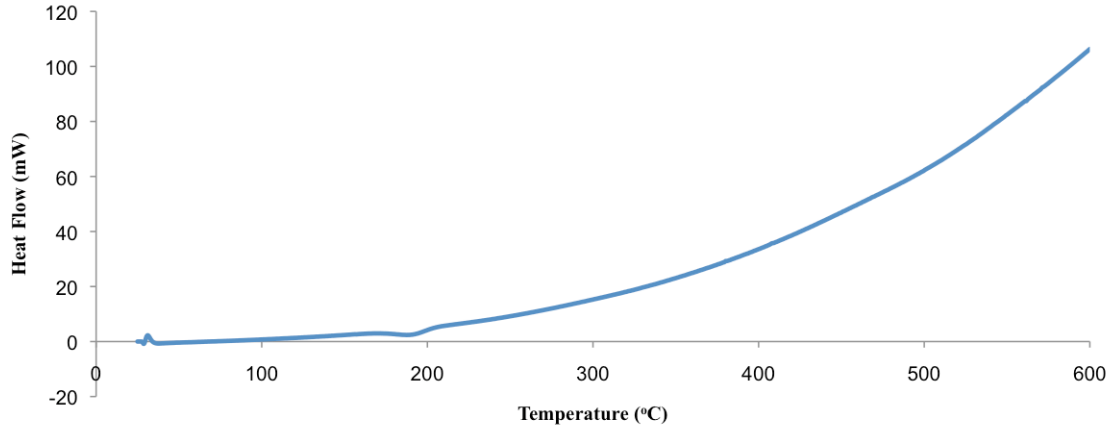
^{13}C NMR of **17**.

Differential Scanning Calorimetry (DSC) of compounds 14-17.

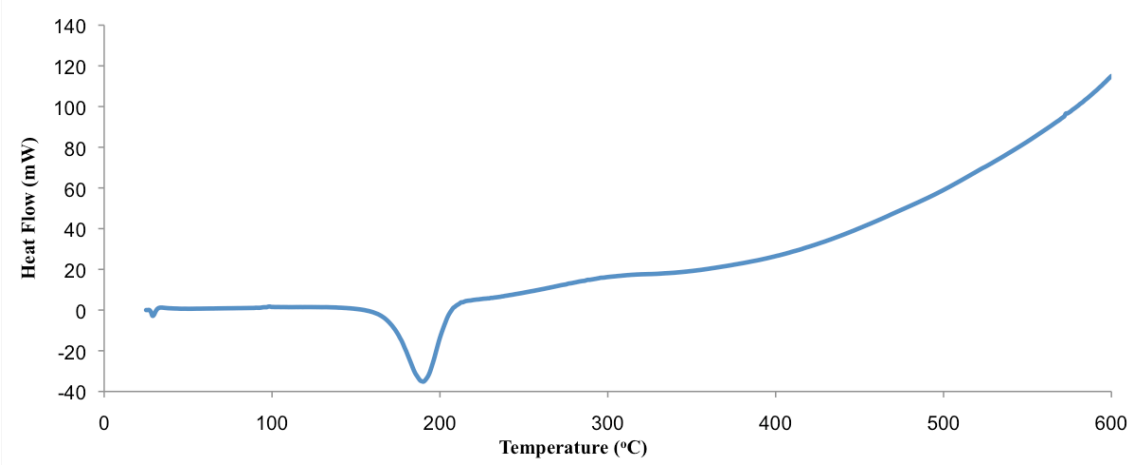
Because of the large ratio of oxygen and nitrogen atoms to carbon atoms, compounds **14-17** were analyzed by DSC to determine whether they qualify as Class 5 explosives. Each compound was tested in comparison to benzoyl peroxide (BPO) as standard reference material.[1] Briefly, if the heat quantities of the decomposition reactions of compounds **14-17** were found to be lower than the heat quantity of decomposition of BPO then the compound was judged not to be a Class 5 explosive.



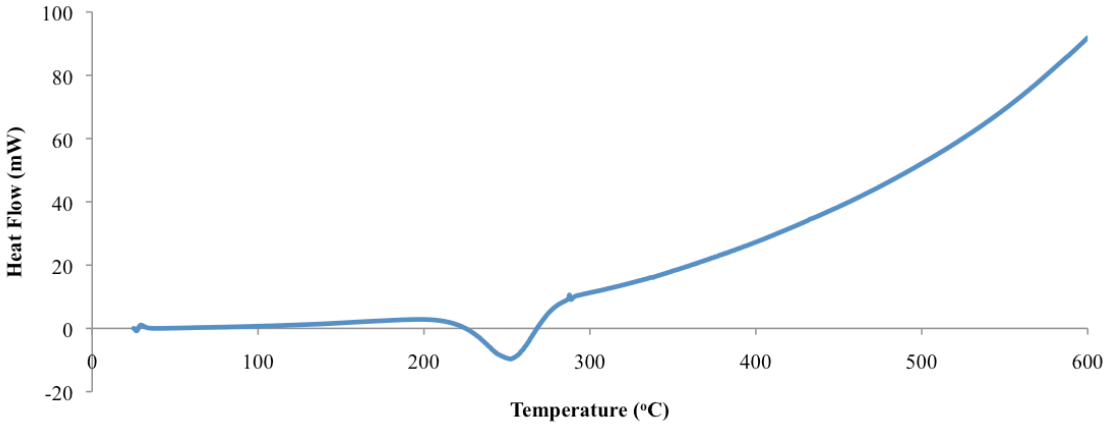
DSC data of standard reference compound BPO. Peak onset = 109 ^{o}C, peak = 110 ^{o}C, exotherm = -2350 J/g.



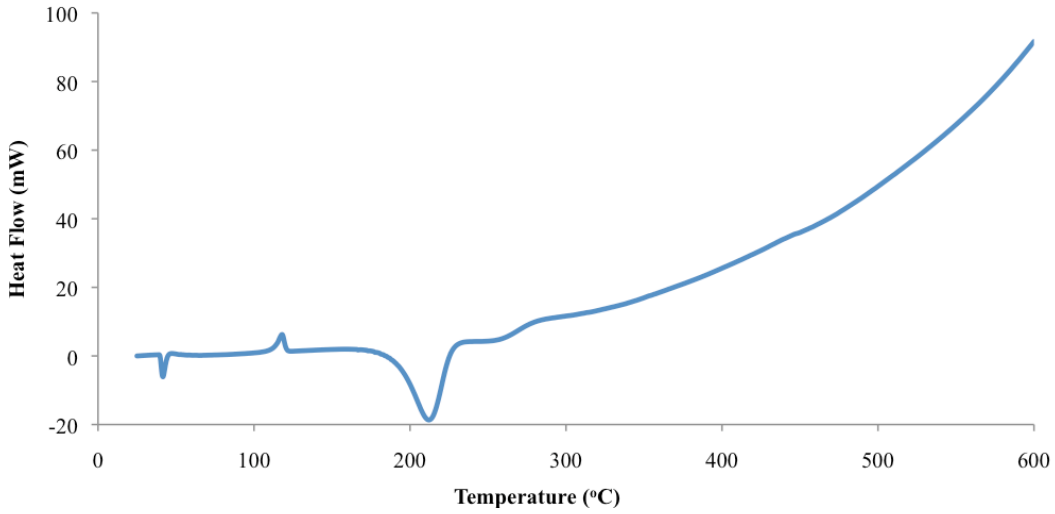
DSC of compound **14**. Peak onset = 170 ^{o}C, peak = 191 ^{o}C, exotherm = -1220 J/g.



DSC data for compound **15**. Peak onset = 170 ^{o}C, peak = 190 ^{o}C, exotherm = -860 J/g.

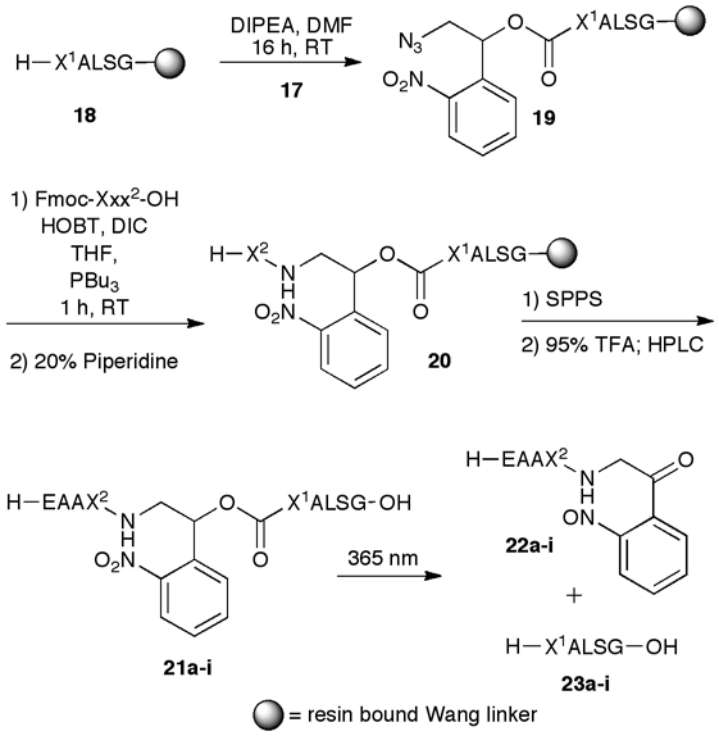


DSC data of compound **16**. Peak onset = 225 ^{o}C, peak = 252 ^{o}C, exotherm = -1595 J/g.



DSC data for compound **17**. Peak onset = 190 °C, peak = 212 °C, exotherm = -1220 J/g.

Each of the compounds tested had heat quantities of decomposition less than that of benzoyl peroxide. However, the baseline of each of the spectra increased dramatically after 250 °C. Therefore, we cannot rule out exotherms at high temperatures that could have been obscured by the baseline. Therefore, care should still be taken before using these compounds at higher temperatures than used in this study and DSC analysis that is accurate at higher temperatures should be employed prior to use at high temperatures.



Synthetic scheme for peptides **21a-i** and photocleavage resulting in peptides **22a-i** and **23a-i**.

General procedure for solid-phase peptide synthesis:
C-terminal peptides (**18**) were synthesized using standard fluorenylmethyloxycarbonyl (Fmoc) based solid phase peptide synthesis (SPPS) techniques using an automated peptide synthesizer (either Aapptec 396 or Rainin PS3 synthesizers). Preloaded resin (either Wang or 2-chlorotrityl; 0.1 mmol) was first swollen in dimethylformamide (DMF) (3 × 5 mL × 10 min). Fmoc-amino acids (0.4 mmol, 4 equiv) were coupled using O-(1H-6-chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HCTU, 165 mg, 0.4 mmol, 4 equiv) as coupling reagent and 0.4 M N-methyl morpholine (NMM) as activating reagent (45 min). Fmoc deprotection was performed with piperidine (3 × 5 mL × 3 min; 20% in DMF). After completion of the coupling of the final amino acid, the Fmoc group was removed to create the free amino group.

Peptide attachment to photocleavable linker *(19)*:
For the synthesis of azidopeptides **19**, pre-swelled resin containing a five-mer peptide (**18**, 0.1 mmol, 1 equiv) was added to a 5 mL round-bottomed flask. To it was added photocleavable linker **17** (70 mg, 0.2 mmol, 2 equiv) dissolved in DMF (3 mL) and the flask was sparged with N_2 for five min. Diisopropylethylamine (DIEA) (87 μL, 0.5 mmol, 5 equiv) was added and the solution was stirred at room temperature with a non-ridged silica coated stir bar for 12-16 h. At this time, the resin was filtered and washed with DMF (3 × 5 mL).

One pot azide reduction and amino acid coupling *(20)*:
Pre-swelled resin containing the azidopeptide **19** (0.1 mmol, 1 equiv) was added to a 5 mL round-bottomed flask. To this flask was added a solution of various Fmoc protected amino acids (Fmoc-Xxx^2-OH, 0.4 mmol, 4 equiv), and hydroxybenzotriazole (HOBt) (54 mg, 0.4 mmol, 4 equiv) in THF (3 mL). Diisopropylcarbodiimide (DIC) (62 μL, 0.4 mmol, 4 equiv) was then added followed by the addition of tri-n-butylphosphine (125 μL, 0.5 mmol, 5 equiv). The reaction was stirred at room temperature with a non-ridged silica coated stir bar for 1 h. At this time, the resin was filtered and washed with DMF (3 × 5 mL).

Note:
The compatibility of the linker with SPPS was first investigated with short peptides with a C-terminal alanine. However, during the reduction step, the C-terminal alanine was epimerized, presumably by the phosphazene base created between tri-n-butylphosphine and azide. This problem could be overcome either by using Gly as the C-terminal residue or by using chlorotrityl resin preloaded with alanine, which did not result in epimerization, presumably due to the steric hindrance of the trityl group on the α hydrogen of alanine.

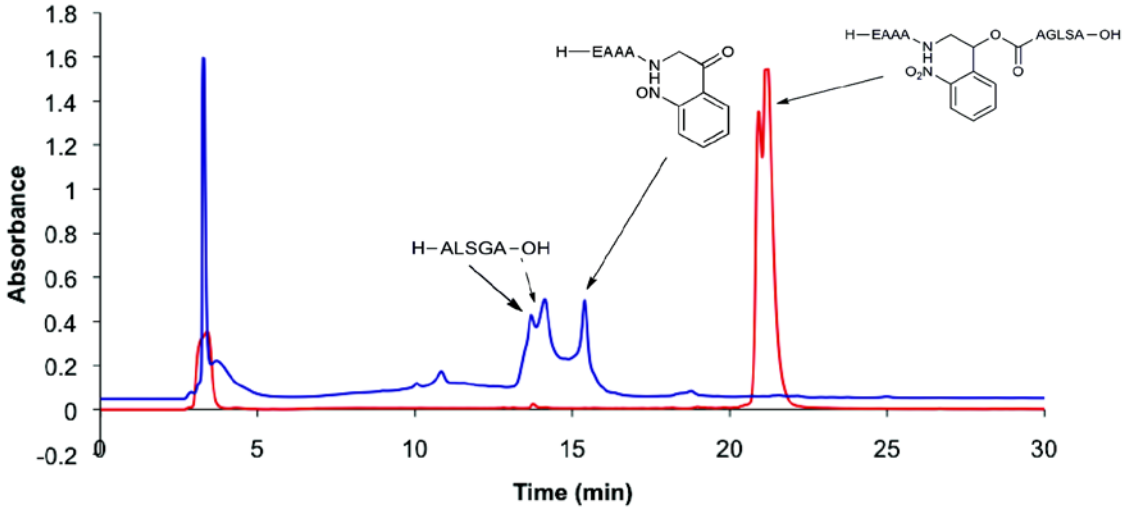


Illustration that after photocleavage of the full peptide with a C-terminal alanine, epimerization of the C-terminal alanine was observed.

Extension of the peptide chain:
The final amino acids were added by standard Fmoc based SPPS techniques as described above starting and ending with an Fmoc deprotection.

General procedure for peptide cleavage from resin and purification:
Peptides prepared on Wang resin were cleaved from the resin by adding a solution of trifluoroacetic acid (TFA) (5 mL), triisopropylsilane (TIPS) (100 μL) and H_2O (100 μL) to the resin (0.1 mmol) and stirring the solution for 2 h at room temperature. The solution was concentrated by purging with a nitrogen stream and peptide was precipitated with cold diethyl ether. The crude peptides were dissolved in an aqueous 0.1% TFA solution, lyophilized, and purified by preparative RP-HPLC on a Waters Delta-Pak^{TM} C18 column (2.5 cm × 10.0 cm) employing a water-acetonitrile solvent system. A linear gradient was used from 2% to 80% solvent B over 45 min (solvent A = 0.1% TFA in H_2O; solvent B = 80% acetonitrile in H_2O, 0.086% TFA). Peptides were monitored by their absorbance at 220 nm. Fractions containing product **21a-i** as analyzed by matrix assisted laser desorption ionization (MALDI) or electrospray ionization (ESI) mass spectrometry were collected, lyophilized, and weighed to determine yield.
Note:
The linker contains a stereogenic center and was prepared in racemic form, resulting in two diastereomers of peptides **21a-i** (as seen in Figure 1).

H—EAAA—Linker—TALSG—OH (21a). RP-HPLC; R_t = 20.2-21.4 min. LRMS (ESI): calcd $C_{41}H_{63}N_{11}O_{18}$, $[M+H]^+$ 998.4, observed 998.1.
H—EAAA—Linker—VALSG—OH (21b). RP-HPLC; Rt = 23.0-24.0 min. LRMS (ESI): calcd $C_{42}H_{65}N_{11}O_{17}$, $[M+H]^+$ 996.4, observed 996.1.
H—EAAA—Linker—KALSG—OH (21c). RP-HPLC; Rt = 19.2-20.6 min. LRMS (MALDI): calcd $C_{43}H_{68}N_{12}O_{17}$, $[M+H]^+$ 1025.5, observed 1027.3.
H—EAAG—Linker—GALSG—OH (21d). RP-HPLC; Rt = 19.7-21.0 min. LRMS

(ESI): calcd $C_{38}H_{57}N_{11}O_{17}$, $[M+H]^+$ 940.4, observed 940.1.
H—EAAL—Linker—LALSG—OH (21e). RP-HPLC; Rt = 28.0-29.2 min. LRMS (MALDI): calcd $C_{46}H_{73}N_{11}O_{17}$, $[M+H]^+$ 1052.5, observed 1052.9.
H—EAAY—Linker—YALSG—OH (21f). RP-HPLC; Rt = 23.6-25.0 min. LRMS (MALDI): calcd $C_{52}H_{69}N_{11}O_{19}$, $[M+H]^+$ 1152.5, observed 1152.9.
H—EAAP—Linker—PALSG—OH (21g). RP-HPLC; Rt = 22.0-23.9 min. LRMS (MALDI): calcd $C_{44}H_{65}N_{11}O_{17}$, $[M+H]^+$ 1020.5, observed 1021.2.
H—EAAW—Linker—WALSG—OH (21h). RP-HPLC; Rt = 29.9-31.2 min. LRMS (MALDI): calcd $C_{56}H_{71}N_{13}O_{17}$, $[M+H]^+$ 1198.5, observed 1199.4.
H—EAAE—Linker—EALSG—OH (21i). RP-HPLC; Rt = 19.0-20.2 min. LRMS (MALDI): calcd $C_{44}H_{65}N_{11}O_{21}$, $[M+H]^+$ 1084.4, observed 1085.7.

General procedure for photocleavage of the polypeptide backbone:
A solution of peptide **21a-i** (4-20 mg), dissolved in 0.1% TFA (1.0-2.0 mL) was placed into a 5 mL round-bottomed flask. The solution was irradiated at 365 nm for 1-2 h (until starting material had disappeared as shown by MALDI-MS). The crude material was then purified by preparative RP-HPLC on a C18 column employing a water-acetonitrile solvent system. Peptides were monitored by their absorbance at 220 nm. Fractions containing product **23a-i** (as analyzed by ESI-MS) were collected, lyophilized, and weighed to determine the yield.

H—TALSG—OH (23a). Analytical RP-HPLC; R_t = 10.8-12.0 min. LRMS (ESI): calcd $C_{18}H_{33}N_5O_8$, $[M+H]^+$ 448.2, observed 448.0.
H—VALSG—OH (23b). Analytical RP-HPLC; Rt = 13.2-14.4 min. LRMS (ESI): calcd $C_{19}H_{35}N_5O_7$, $[M+H]^+$ 446.3, observed 446.1.
H—KALSG—OH (23c). Analytical RP-HPLC; Rt = 10.1-11.0 min. LRMS (ESI): calcd $C_{20}H_{38}N_6O_7$, $[M+H]^+$ 475.3, observed 475.1.
H—GALSG—OH (23d). Analytical RP-HPLC Rt = 11.2-12.2 min. LRMS (ESI): calcd $C_{16}H_{29}N_5O_7$, $[M+H]^+$ 404.2, observed 404.0.
H—LALSG—OH (23e). Analytical RP-HPLC Rt = 16.2-17.1 min. LRMS (ESI): calcd $C_{20}H_{37}N_5O_7$, $[M+H]^+$ 460.3, observed 460.1.
H—YALSG—OH (23f). Analytical RP-HPLC Rt = 14.7-15.8 min. LRMS (ESI): calcd $C_{23}H_{35}N_5O_8$, $[M+H]^+$ 510.3, observed 510.1.
H—PALSG—OH (23g). Analytical RP-HPLC Rt = 11.9-13.0 min. LRMS (ESI): calcd $C_{19}H_{33}N_5O_7$, $[M+H]^+$ 444.2, observed 444.0.
H—WALSG—OH (23h). Analytical RP-HPLC Rt = 19.4-20.4 min. LRMS (ESI): calcd $C_{25}H_{36}N_6O_7$, $[M+H]^+$ 533.3, observed 533.1.
H—EALSG—OH (23i). Analytical RP-HPLC Rt = 11.5-12.5 min. LRMS (ESI): calcd $C_{19}H_{33}N_5O_9$, $[M+H]^+$ 476.3, observed 476.1.

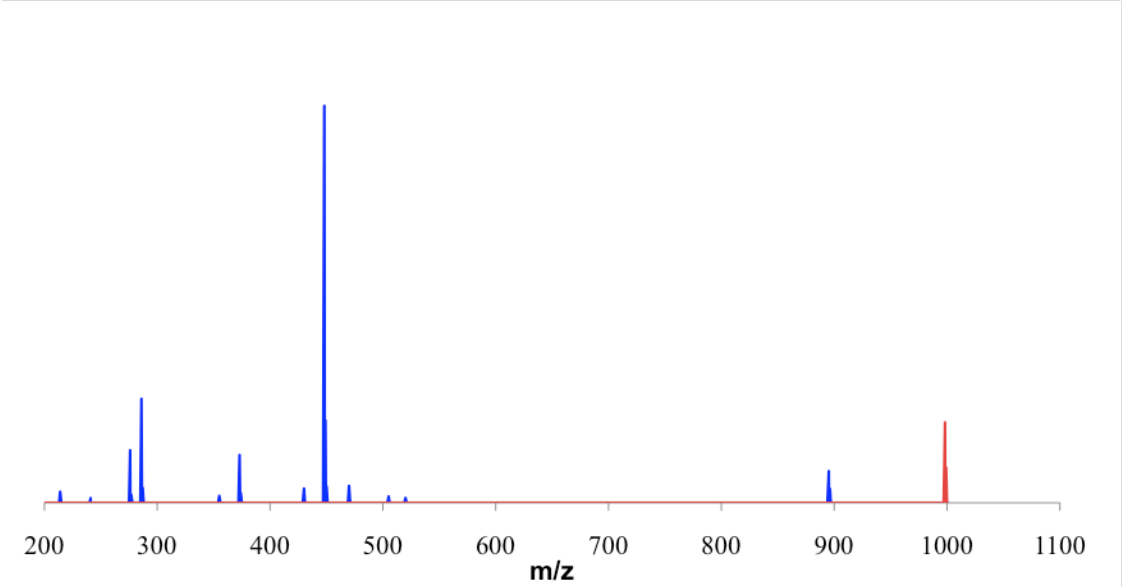


Figure S1. ESI-MS of **21a** before (red) and after (blue) photochemical reaction. $[M+H]^+_{before}$ = 998.1, $[M+H]^+_{after}$ = 448.2.

Representative HPLC traces of peptides 21a-i before (red) and after (blue) the photocleavage reaction: (Grace Vydac® C18 column (4.6 x 250 mm); a linear gradient was used from 2% to 80% solvent B over 45 min (solvent A = 0.1% TFA in H_2O; solvent B = 80% acetonitrile in H_2O, 0.086% TFA))

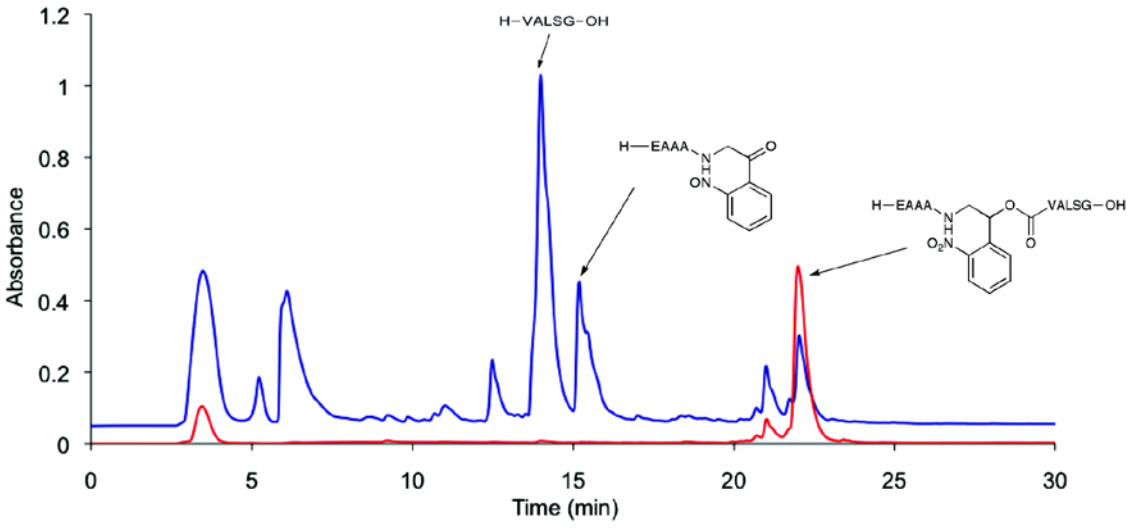


Figure S2. Photocleavage of **21b** to generate **22b** and **23b.**

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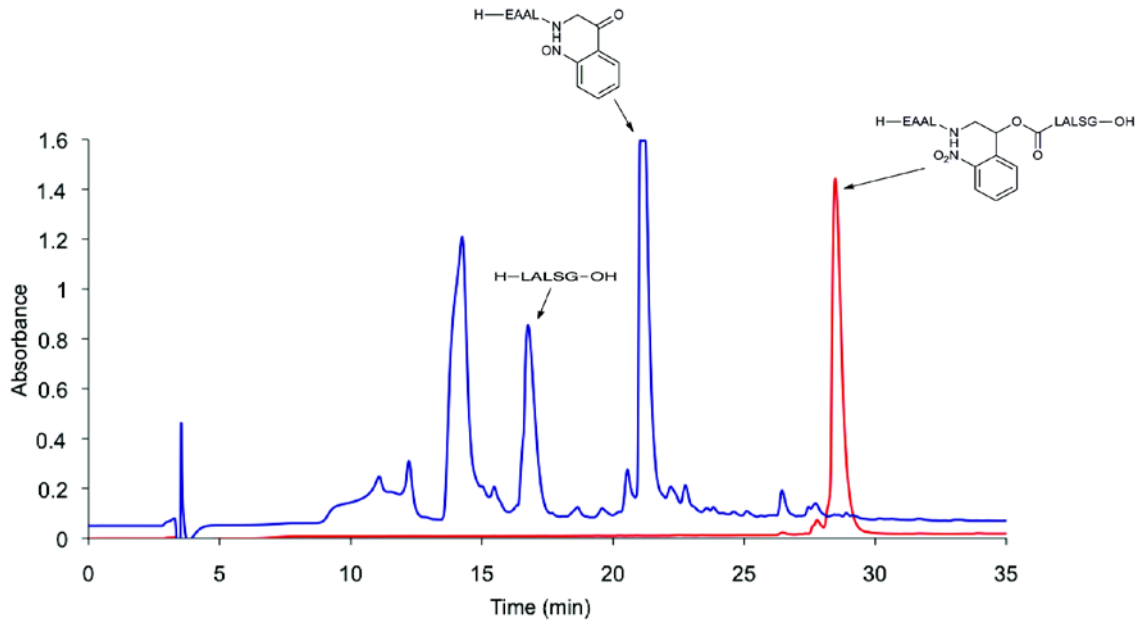


Figure S3. Photocleavage of **21e** to produce **22e** and **23e**.

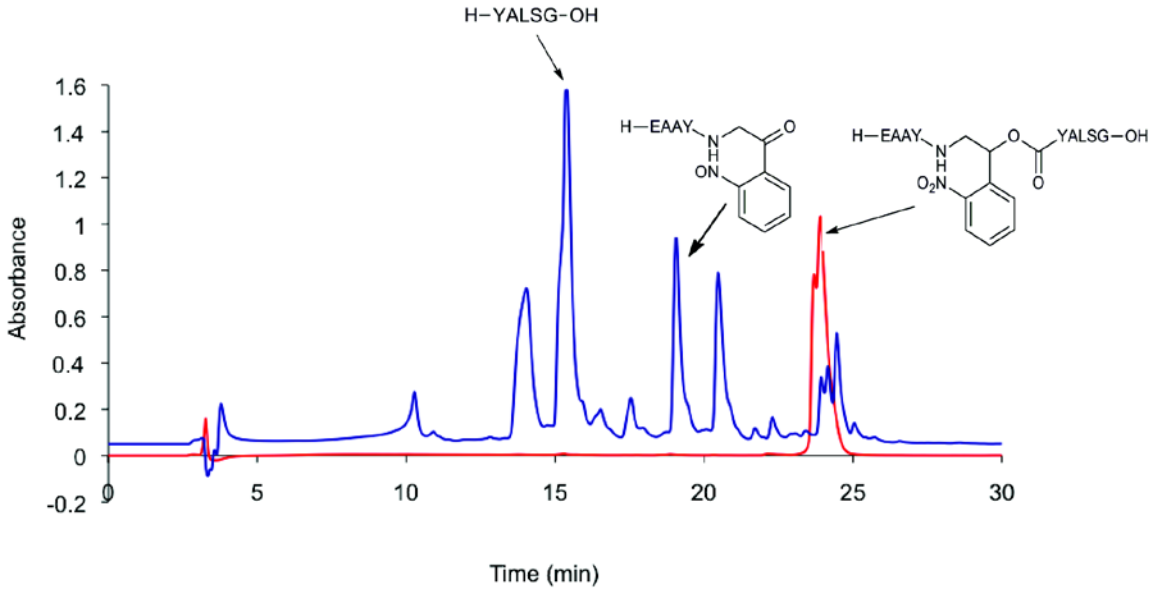


Figure S4. Photocleavage of **21f** to furnish **22f** and **23f**.

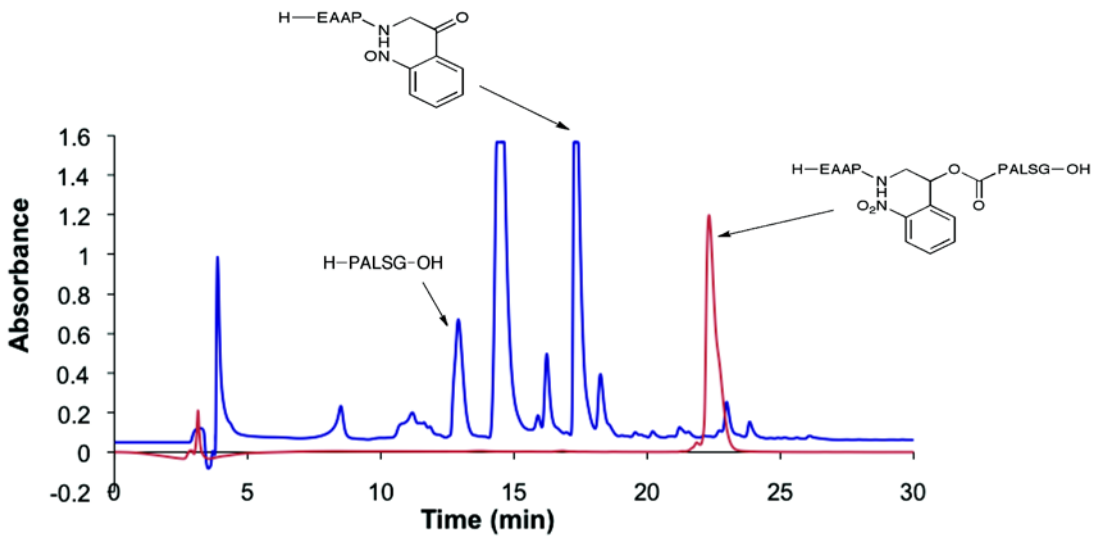


Figure S5. Photocleavage of **21g** to produce **22g** and **23g**.

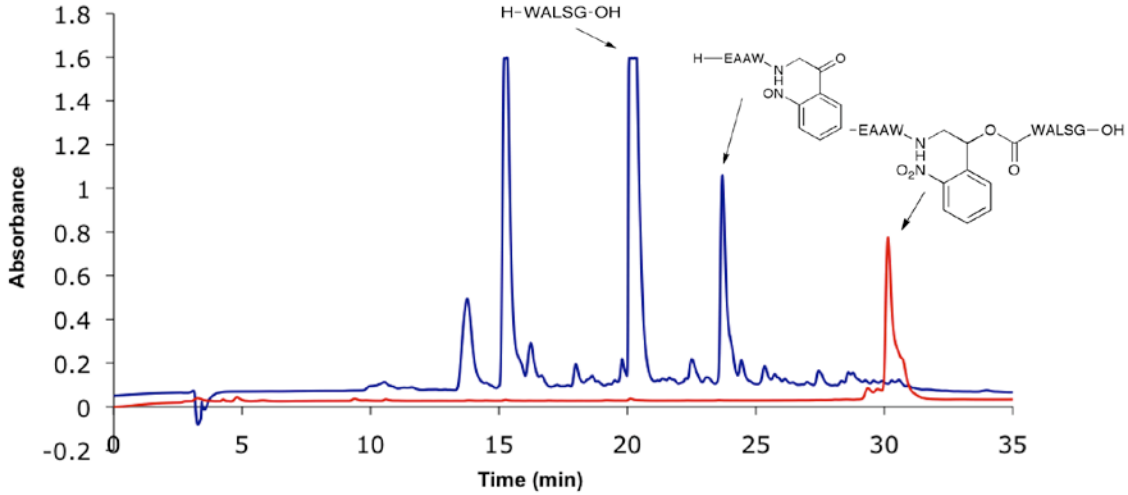


Figure S6. Photocleavage of **21h** to generate **22h** and **23h**.

Representative analytical (C18) HPLC traces of peptides 23 to show purity after HPLC purification (peak from 3-4 min is a salt peak):

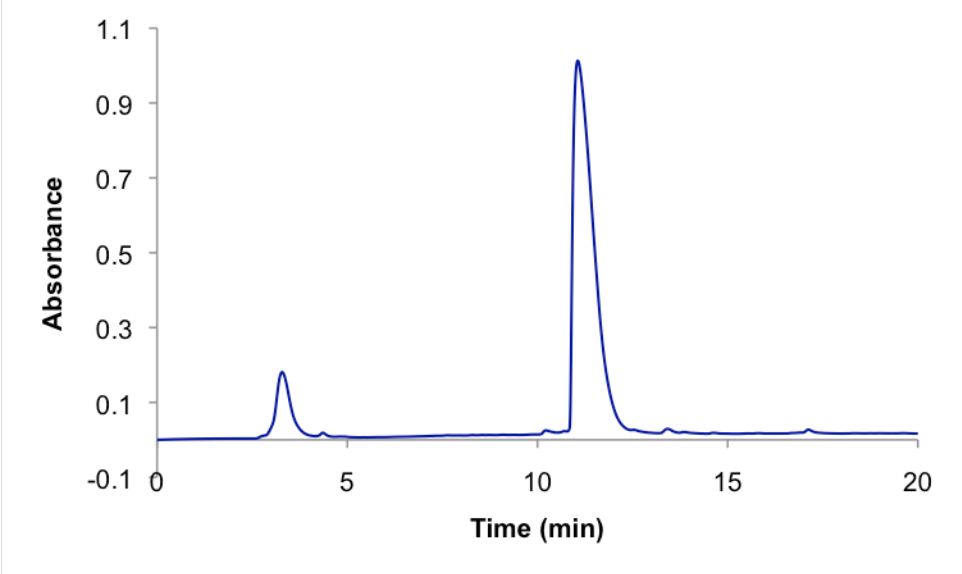


Figure S7. HPLC trace of **23a** after HPLC purification.

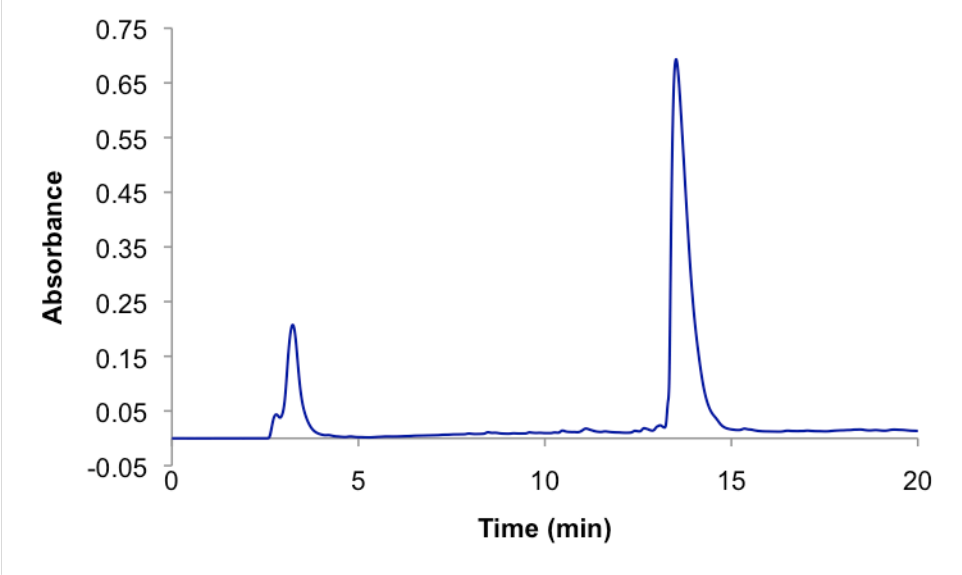


Figure S8. HPLC trace of **23b** after HPLC purification.

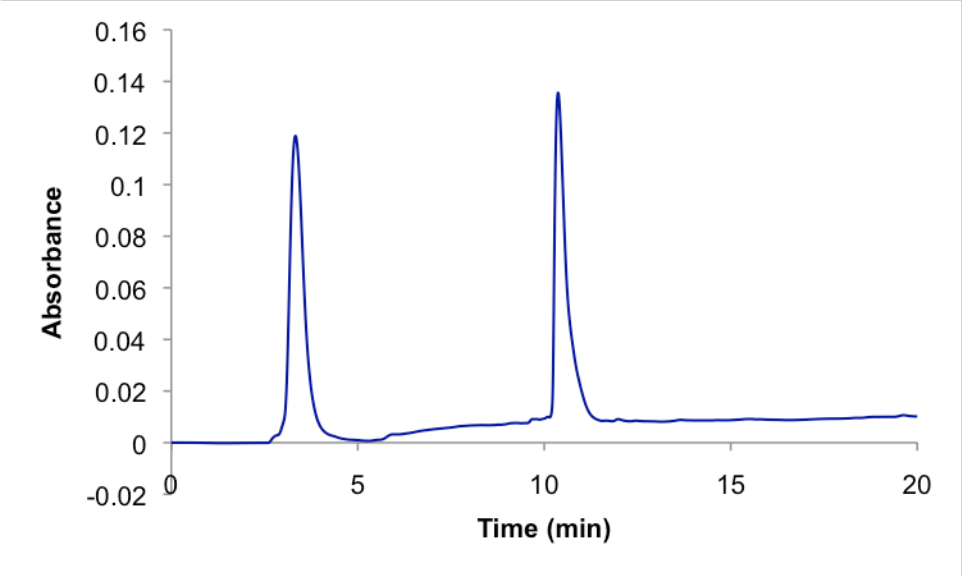


Figure S9. HPLC trace of **23c** after HPLC purification.

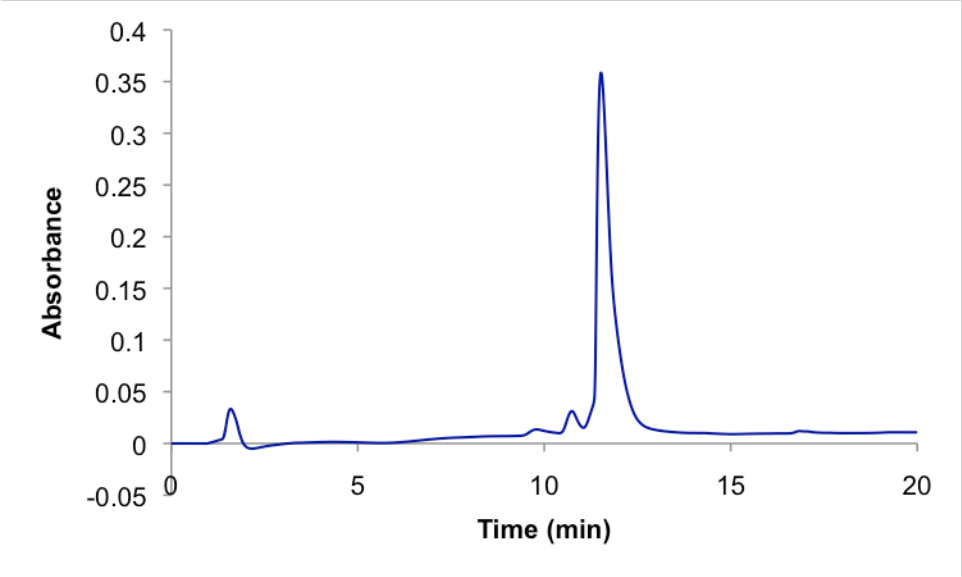


Figure S10. HPLC trace of **23d** after HPLC purification.

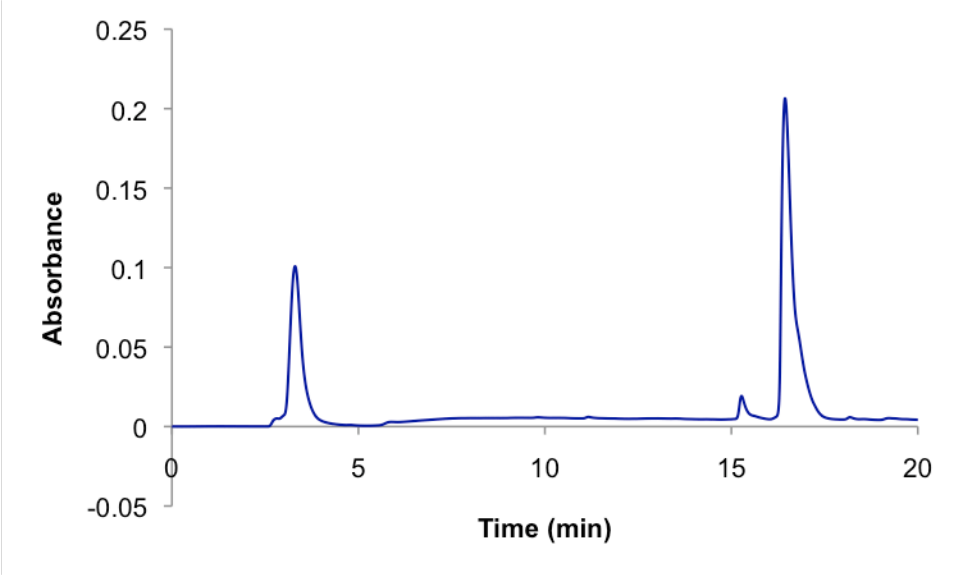


Figure S12. HPLC trace of **23e** after HPLC purification.

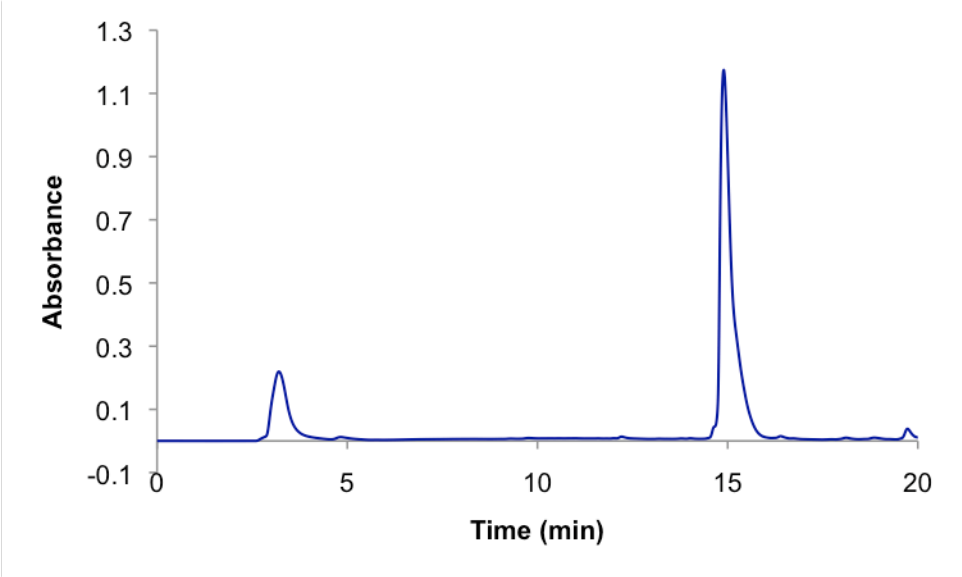


Figure S14. HPLC trace of **23f** after HPLC purification.

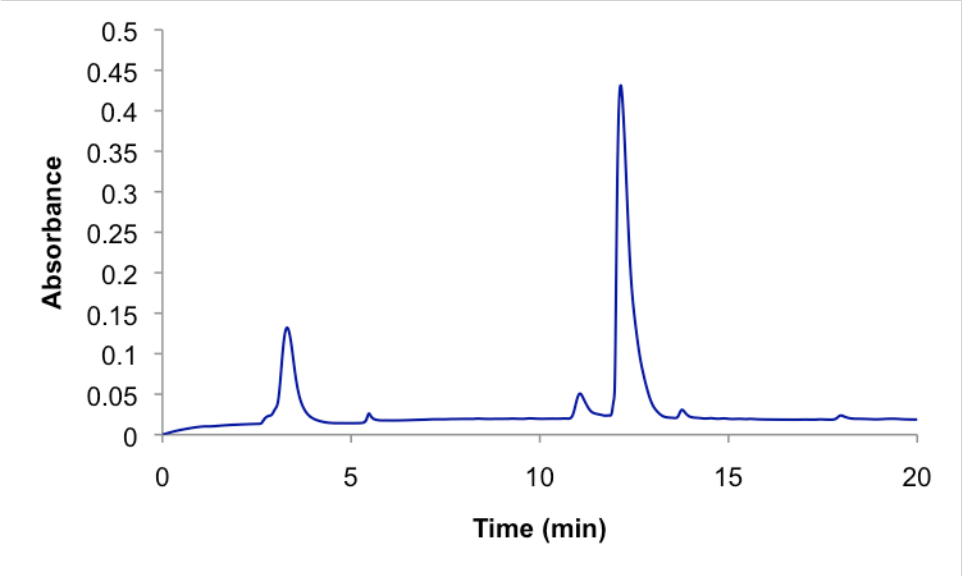


Figure S15. HPLC trace of **23g** after HPLC purification.

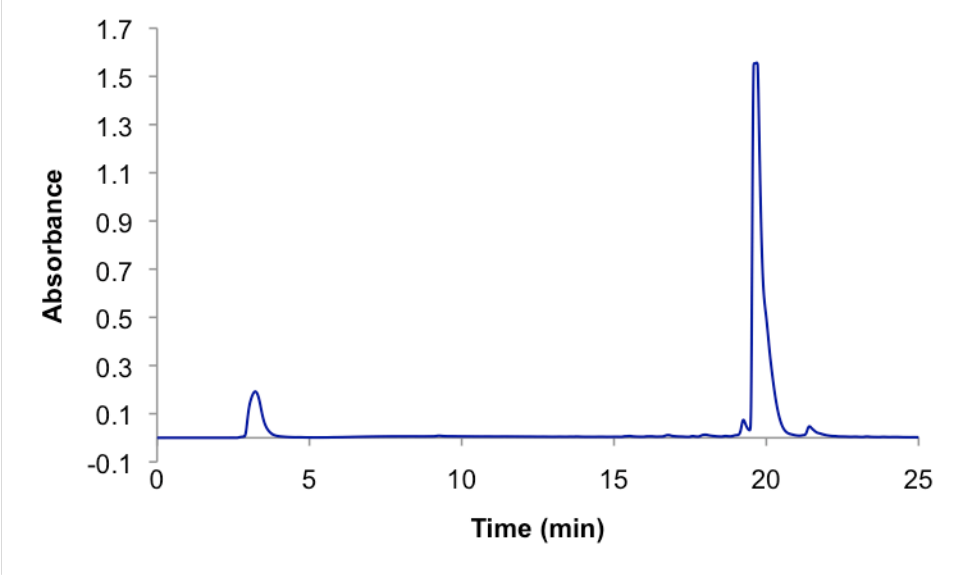


Figure S16. HPLC trace of **23h** after HPLC purification.

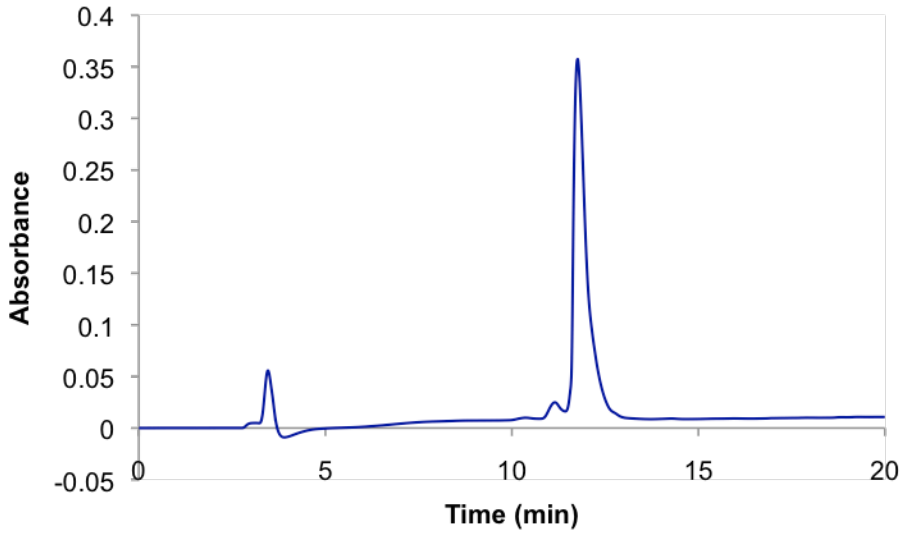
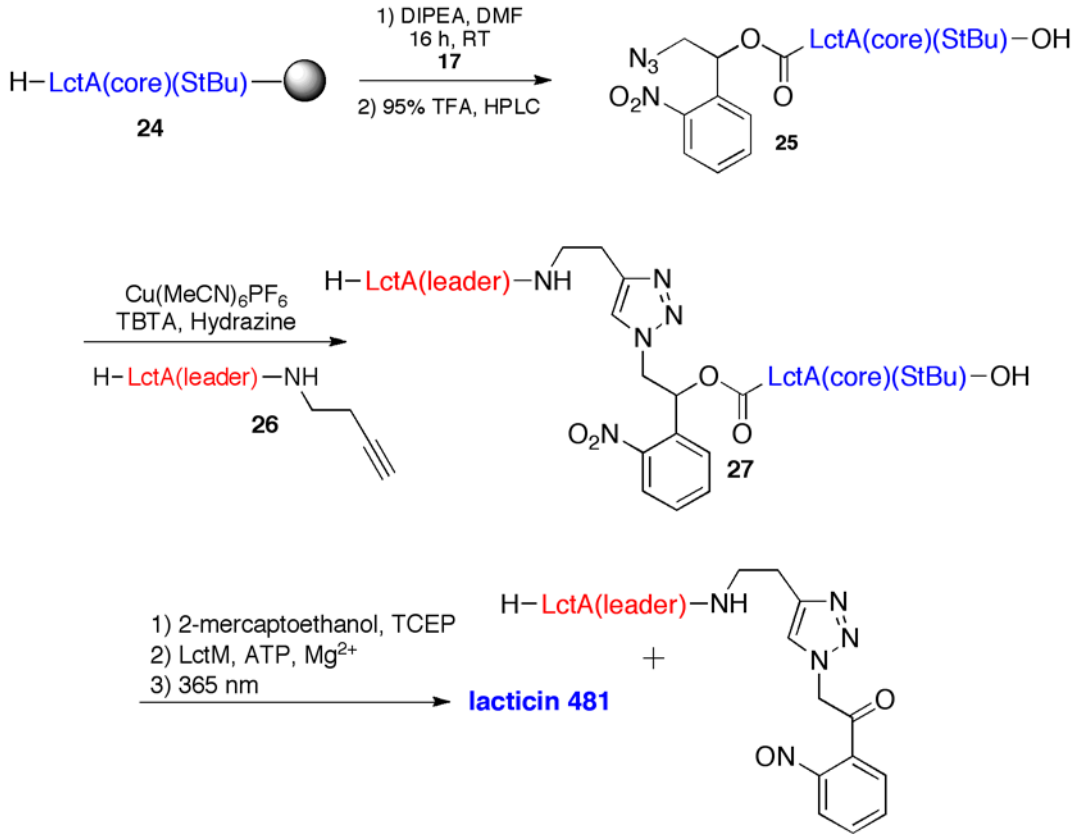


Figure S17. HPLC trace of **23i** after HPLC purification.



Scheme S1. Copper catalyzed azide/alkyne click reaction between LctA(leader) and LctA(core) followed by photolysis to afford lacticin 481.

LctA(leader) = H-KEQNSFNLLQEVTESELDLILGG[a]-OH

LctA(core) = H-KGGSGVIHTISHECR[b]MNSWQH[b]VFTCCS-OH

LctA(core)(StBu) = H-KGGSGVIHTISHECR[b]MNSWQH[b]VFTCCS-OH
StBu Stbu StBu

[a] A mutant of wild type LctA(leader) was used containing the mutation A24/G.
[b] A mutant of wild type LctA(core) was used containing the mutations N39R/F45H. Previous studies have shown this to be a more soluble substrate without significant decrease in the biological activity. See reference 2.

Synthesis of linker modified LctA(core)(StBu) (25)
The core peptide of LctA with its Cys residues protected as S^{t}Bu disulfides, LctA(core)(StBu), was synthesized as described above using a Rainin PS3 peptide synthesizer (0.1 mmol; see also reference 4). StBu protected Fmoc-cysteine residues were coupled manually using (HOBt) (54 mg, 0.4 mmol, 4 equiv) as coupling reagent and DIC (62 μL, 0.4 mmol, 4 equiv) as activating reagent (45 min). The terminal Fmoc group was removed at the end of the synthesis.

For the synthesis of azido peptide LctA(core)(StBu) (**25**), pre-swelled resin containing peptide **24** (0.1 mmol, 1 equiv) was added to a 10 mL round-bottomed flask. To it was added photocleavable linker **17** (70 mg, 0.2 mmol, 2 equiv) dissolved in DMF (5 mL). DIEA (87 μL, 0.5 mmol, 5 equiv) was added and the solution was stirred at room temperature with a non-ridged silica coated stirbar for 12-16 h. At this time, the resin was filtered, washed with DMF (3 × 5 mL), and pipetted into a 20 mL scintillation vial with DMF. The peptide was cleaved from resin by adding a solution of TFA (5 mL), thioanisole (200 μL), H_2O (200 μL) and TIPS (100 μL) to the resin and stirring for 2 h at room temperature. The solution was concentrated by purging with a nitrogen stream and the peptide was precipitated with cold diethyl ether. The crude peptide was dissolved in 0.1% aqueous TFA, lyophilized, and purified by preparative RP-HPLC on a C18 column (solvent A = 0.1% TFA in H_2O; solvent B = 80% acetonitrile in H_2O, 0.086% TFA). Fractions containing product were collected and lyophilized yielding **25** (42.5 mg, 12%, 96% yield per step based on resin loading).
N_3—Linker—LctA(core)(StBu)—OH (25). RP-HPLC; Gradient, 10-48% solvent B in 25 min, 48-68% solvent B in 15 min, R_t = 39.4-41.4 min. LRMS (MALDI): calcd $C_{147}H_{225}N_{44}O_{42}S_7[M+H]^+$ 3503.5, observed 3504.0.

Coupling of C-terminal alkyne to LctA(leader) (26):
LctA(leader) peptide was prepared on 2-chlorotrityl chloride resin (0.1 mmol) on a CEM Liberty microwave peptide synthesizer. Preloaded 2- chlorotrityl chloride glycine resin (0.1 mmol) was first swollen in a 1:1 DMF:DCM mixture (10 mL). Fmoc-amino acids (0.2 M in DMF, 2.5 mL) were coupled using HCTU (0.5 M in DMF, 1 mL) as coupling reagent and DIEA (2.0 M in 1-methyl-2-pyrrolidinone, 0.5 mL) as activating reagent with microwave irradiation (3 min, 20 W, 75 ^oC). Fmoc deprotection was performed with

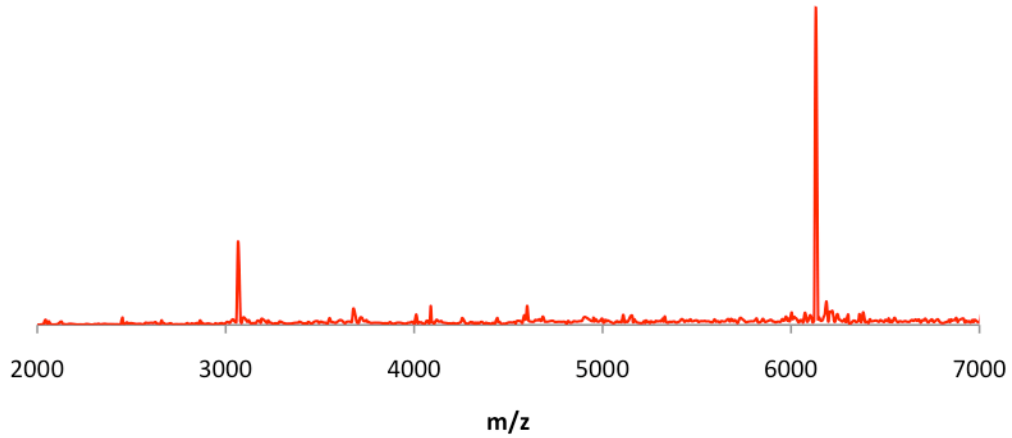
Supplementary Material (ESI) for Chemical Communications

piperidine (20% in DMF) with microwave irradiation (3 min, 30 W, 75 ^{o}C). After completion of the coupling of the final amino acid, the Fmoc group was not removed.

Peptide cleavage from resin was achieved by stirring the resin in a mixture of methylene chloride (8 mL), acetic acid (1 mL), and trifluoroethanol (1 mL) at room temperature for 1 h. (Note: peptide cleavage from 2-chlorotrityl chloride resin in 10% acetic acid does not cleave the amino acid protecting groups). At this time, the solid resin was removed by filtration, and the resulting solution was concentrated by rotary evaporation, and the AcOH was removed in an azeotrope with hexanes (3 × 8 mL). The peptide was then dissolved in THF (10 mL) and DMF (10 mL).
In a separate 50 mL round-bottomed flask was added but-3-yn-1-amine hydrochloride (0.5 mmol, 5 equiv) and N,N-diisopropylethylamine (0.5 mmol, 5 equiv) in THF (5 mL). To this solution was added a premixed solution of hydroxybenzotriazole (0.5 mmol, 5 equiv) and N,N-diisopropylcarbodiimide (0.5 mmol, 5 equiv) in 5 mL of THF and 5 mL of DMF. This mixture was then added to the flask containing the purified leader peptide and the reaction mixture was stirred for 12 h at room temperature. At this time, the solution was concentrated by rotary evaporation and a 20% piperidine/DMF solution (15 mL) was added to remove the terminal Fmoc group. The solution was stirred for 30 min followed by the removal of the piperidine and DMF under reduced pressure. The protecting groups were then removed from the amino acid side chains by adding a solution of TFA (7 mL), EDT (100 μL), TIPS (100 μL) and H_2O (200 μL) and stirring the solution for 2 h at room temperature. The solution was concentrated by purging with a nitrogen stream and was precipitated with cold diethyl ether. The peptide was redissolved in a 1:1 solution of MeCN and H_2O, lyophilized, and purified by preparative RP-HPLC on a Phenomenex C18 column (1.0 cm × 25.0 cm) employing a water-acetonitrile solvent system. The gradient used was from 2-80% solvent B in 45 min (solvent A = 0.1% TFA in H_2O; solvent B = 80% acetonitrile in H_2O, 0.086% TFA). Peptides were monitored by their absorbance at 220 nm. Fractions containing product as analyzed by MALDI-TOF MS were collected and lyophilized yielding product as a white powder.
H—LctA(Leader)—Alkyne (26). Preparative RP-HPLC; R_t = 38.6-39.4 min. LRMS (MALDI): calcd $C_{116}H_{187}N_{29}O_{40,}$ $[M+H]^+$ 2627.4, observed 2623.2

Preparation of triazole-linked substrate (27).
Peptides **25** (2.0 mg, 0.76 μmol, 1 equiv) and **26** (1.5 mg, 0.76 μmol, 1 equiv) were placed in a 3 mL pear shaped flask equipped with a septum and the flask was sparged with N_2 for 3 min. The peptides were then dissolved in a degassed solution of 1:1 Tris buffer (5 mM, pH 7.0), and dioxane (1 mL) and DMSO (100 μL). In a separate 5 mL round-bottomed flask was placed TBTA (0.1 mg). This flask was sparged with N_2 for 3 min followed by the addition of 500 μL of a 1:1 mixture of Tris (5 mM, pH 7.0) and dioxane. To this solution was added tetrakis(MeCN)copper(I)hexafluorophosphate (40 μL of a 1.25 mg/mL aqueous solution). After brief stirring, an anhydrous hydrazine solution (20 μL of a 1% hydrazine solution in 1:1 Tris (5 mM, pH 7.0): dioxane) was added to the cupric solution followed by the addition of the peptide solution. The solution turned cloudy either the addition of hydrazine and the peptide solution. The solution was stirred under N_2 for 1.5 h and analyzed by MALDI-TOF MS after diluting with 50% MeCN. The lyophilized crude peptides were purified by preparative RP-HPLC on a

Phenomenex C18 column employing a water-acetonitrile solvent system. A linear gradient was used from 2% to 80% solvent B over 45 min (solvent A = 0.1% TFA in H_2O; solvent B = 80% acetonitrile in H_2O, 0.086% TFA). Fractions containing product (as analyzed by ESI-MS) were lyophilized yielding **27** (1.6 mg, 46%) as a white powder.
Triazole-linked LctA(leader)-LctA(core)(StBu) (27). Rt = 34.4-35.4 min LRMS (ESI): calcd $C_{262}H_{415}N_{71}O_{79}S_{07}$ $[M+H]^+$ 6131.85, observed 6131.6.



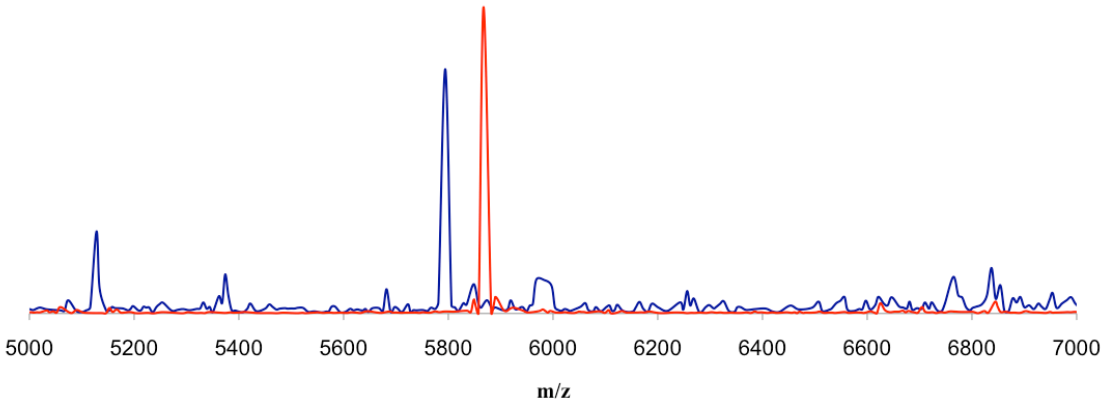
ESI-MS of cycloaddition product **27**. $[M+H]^+$ = 6131.6 m/z, $[M+2H]^{2+}$ = 3065.9 m/z.

Deprotection of StBu protected thiazole-linked substrate.
HPLC purified triazole linked LctA substrates (≈ 3.0 mg) were dissolved in 40% MeCN, 60% Tris (37.5 mM, pH 8.3) (1.3 mL) and added to a 10 mL microwave flask. To this solution was added β-mercaptoethanol (150 μL) and tris-(2-carboxyethyl) phosphine (6 mg). The reaction was heated to 60 ^oC for 30 min using a CEM Discover microwave reactor (100 W, 250 psi). The crude product was acidified (pH 1-2) with 1 M HCl and the lyophilized crude product was purified by preparative RP-HPLC on a C18 column employing a water-acetonitrile solvent system. The gradient used was from 2% to 80% solvent B in 45 min (solvent A = 0.1% TFA in H_2O; solvent B = 80% acetonitrile in H_2O, 0.086% TFA). Peptides were monitored by their absorbance at 220 nm. Fractions containing product (as analyzed by ESI-MS) were collected and lyophilized yielding deprotected product as a white powder.
Triazole-linked LctA(Leader)-LctA(Core) Rt = 35.0-36.5 min. LRMS (ESI): calcd $C_{254}H_{391}N_{70}O_{82}S_4$ $[M+H]^+$ 5867.75, observed 5867.3.

General Procedure for LctM assays.
His-LctM was overexpressed and purified as previously reported.[3] To a 1.6 mL eppendorf tube was added Tris HCl (100 μL, 500 mM, pH 7.5), $MgCl_2$ (100 μL, 100 mM), ATP (40 μL, 50 mM), BSA (12.5 μL, 2 mg/mL), and sterile deionized H_2O (665.9 μL). Triazole-linked LctA(Leader)-LctA(Core) substrate (40 μL, 500 μM in H_2O) was then added followed by LctM (41.5 μL, 34.1 μM) and the solution was incubated for 12 h. At this time the solution was acidified to pH (1-2) with 5% TFA and the crude product

was purified by analytical RP-HPLC on a C4 column employing a water-acetonitrile solvent system. The gradient used was from 2% to 80% solvent B in 45 min. Fractions were analyzed by MALDI-TOF MS.

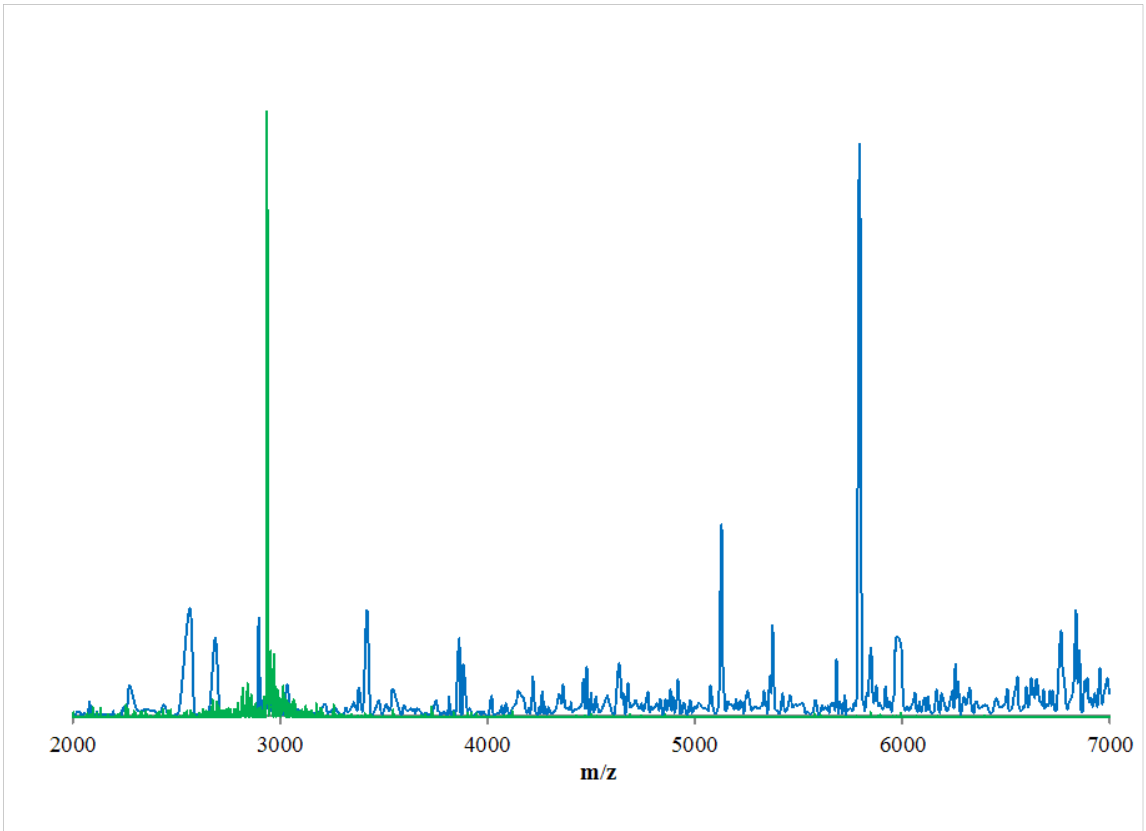
LctM modified Triazole-linked LctA(Leader)-LctA(Core) Rt = 34.4-35.2 min. LRMS (ESI): calcd $C_{254}H_{383}N_{70}O_{78}S_4$ $[M+H]^+$ 5795.67 g/mol, observed 5795.0 g/mol.



ESI-MS before (red) and after (blue) LctM assay. M = 5867.3 m/z, M – 4 H_2O = 5795.0 m/z.

Photolysis of LctM-modified, triazole-linked LctA(Leader)-LctA(Core)
Purified LctM modified peptide (<0.1 mg) in HPLC solvent was irradiated at 365 nm for 20 min. The product was analyzed by MALDI-TOF MS and ESI-MS.

Lacticin 481. LRMS (MALDI): calcd $C_{126}H_{186}N_{40}O_{34}S_4$ $[M+H]^+$ 2934.3, observed 2936.1



ESI-MS and MALDI-TOF-MS of LctM modified-triazole linked LctA(leader)-LctA(core) before (blue) and after (green) the photocleavage reaction. $[M+H]^+_{before}$ = 5795.0 m/z, $[M+H]^+_{after}$ = 2936.1 m/z.

References for Supporting Information.

1. Shimadzu and A. News, *www.shimadzu.com.br/analitica/aplicacoes/analisadores/ta/t97.pdf*.
2. Y. O. You and W. A. van der Donk, *Biochemistry*, 2007, **46**, 5991-6000.
3. L. Xie, L. M. Miller, C. Chatterjee, O. Averin, N. L. Kelleher and W. A. van der Donk, *Science*, 2004, **303**, 679-681.
4. *(a)* M. R. Levengood, C. C. Kerwood, C. Chatterjee, W. A. van der Donk, *ChemBioChem*, 2009, **10**, 911; *(b)* M. R. Levengood, P. J. Knerr, T. J. Oman, W. A. van der Donk, *J. Am. Chem. Soc.*, 2009, **131**, 12024; *(c)* M. R. Levengood, W. A. van der Donk, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3025 (*d*) M. R. Levengood, G. C. Patton and W. A. van der Donk, *J. Am. Chem. Soc.*, 2007, **129**, 10314.