Solid-Phase Peptide Capture and Release for Bulk and Single-Molecule Proteomics

Cecil J Howard,[a] Brendan M. Floyd, [b] Angela M. Bardo,[b] Jagannath Swaminathan,[b] Edward M. Marcotte,*[b] and Eric V. Anslyn*[a]

[a] Department of Chemistry, University of Texas at Austin, Austin, TX 78712

[b] Department of Molecular Biosciences, University of Texas at Austin, Austin, TX 78712

Corresponding author: anslyn@austin.utexas.edu

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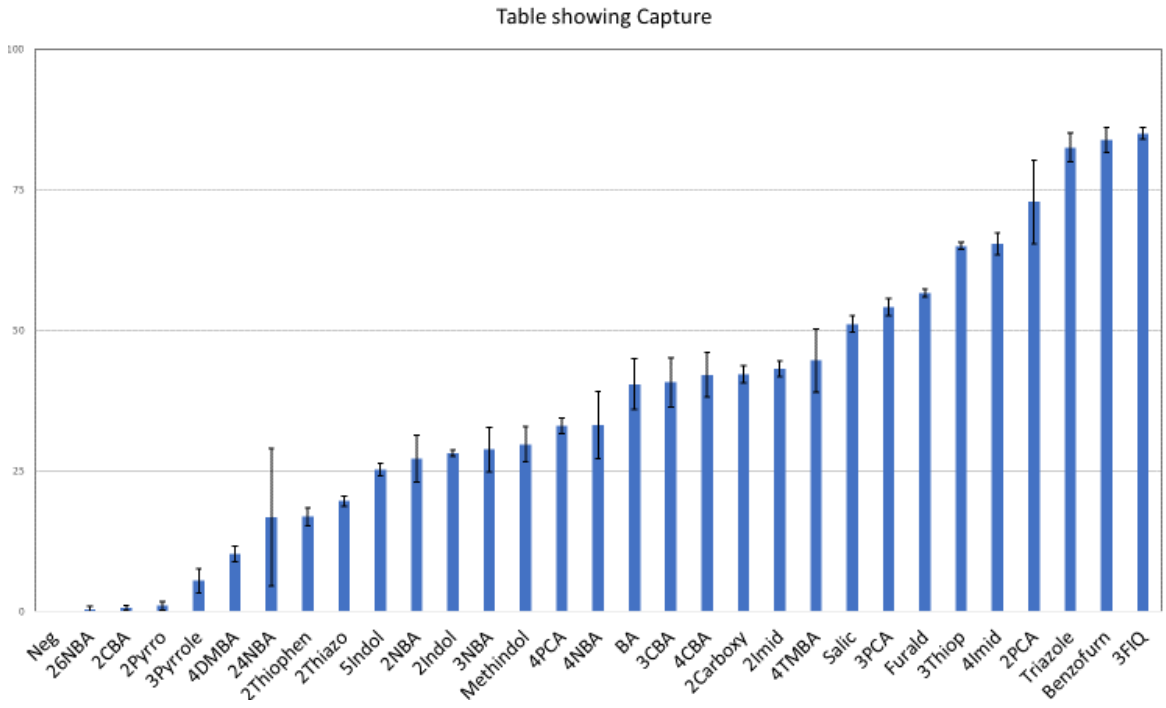


Figure 1: Graphic representation of the aldehydes screened

Aldehydes tested in Main Figure 1. Bars indicate percent peptide capped by PCA. Error bars are the standard deviation based off of three replicates: A) 2,6-dinitrobenzaldehyde B) 2-cyanobenzaldehyde C) Pyrrole-2-carboxaldehyde D) Pyrrole-3-carboxaldehyde E) 4-(dimethylamino)benzaldehyde F) 2,6-dinitrobenzaldehyde G) Thiophene-2-carboxaldehyde H) 2-Thiazolecarboxaldehyde I) Indole-5-carboxaldehyde J) 2-nitrobenzaldehyde K) Indole-2-carboxaldehyde L) 3-nitrobenzaldehyde M) 1-methyl-1H-indole-5-carboxaldehyde N) 4-pyridinylcarboxaldehyde O) 4-nitrobenzaldehyde P) benzaldehyde Q) 3-cyanobenzaldehyde R) 4-cyanobenzaldehyde S) 2-carboxybenzaldehyde T) 2-imidazolecarboxaldehyde U) 4-(trimethylamine)benzaldehyde V) salicylaldehyde W) 3-pyridinecarboxaldehyde X) 5-hydroxymethyl-2-furaldehyde Y) thiophene-3-carboxaldehyde Z) 4-imidazolecarboxaldehyde AA) 2-pyridinylcarboxaldehyde BB) 1H-1,2,3-Triazole-5-carbaldehyde CC) benzofuran-2-carboxaldehyde DD) 3-formylisoquinoline.

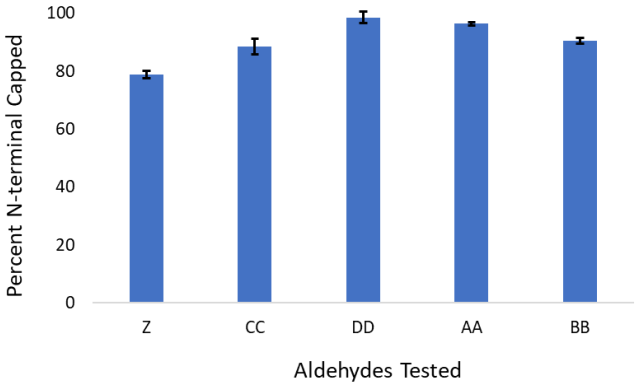


Figure 2. Percent of N-terminal capped product of SGKW peptide (4 eq) with aldehydes (1eq). Labels are taken from Figure 1. Z) 4-imidazolecarboxaldehyde AA) 2-pyridinylcarboxaldehyde BB) 1H-1,2,3-Triazole-5-carbaldehyde CC) benzofuran-2-carboxaldehyde DD) 3-formylisoquinoline.

The five most reactive aldehydes were selected to further test the ability for this reaction to discriminate the N-terminus of a peptide of the amine from an internal Lys residue. For this, we used a H_2N-Ser-Gly-Lys-Trp-COOH peptide that was solubilized at 1 mM in 50 mM sodium phosphate buffer pH 7.5 and incubated with the aldehydes (4 mM final concentration) at 37 °C for six hours. The reactions were analyzed by LC/MS. The only detectable masses were two characteristic diasteromeric peaks that are present when the imidazolinone product is formed. Further, the five aldehydes showed similar imidazolinone formation as in the initial screen, and no product could be detected that corresponded to peptide with both an N-terminal imidazolinone and an imine on the lysine side chain (Figure 2). From the lack of product containing 2 aldehyde attached products, we confirm that these five aldehydes are N-terminal specific.

3FIQ-SGW Capped Peptide H1 NMR

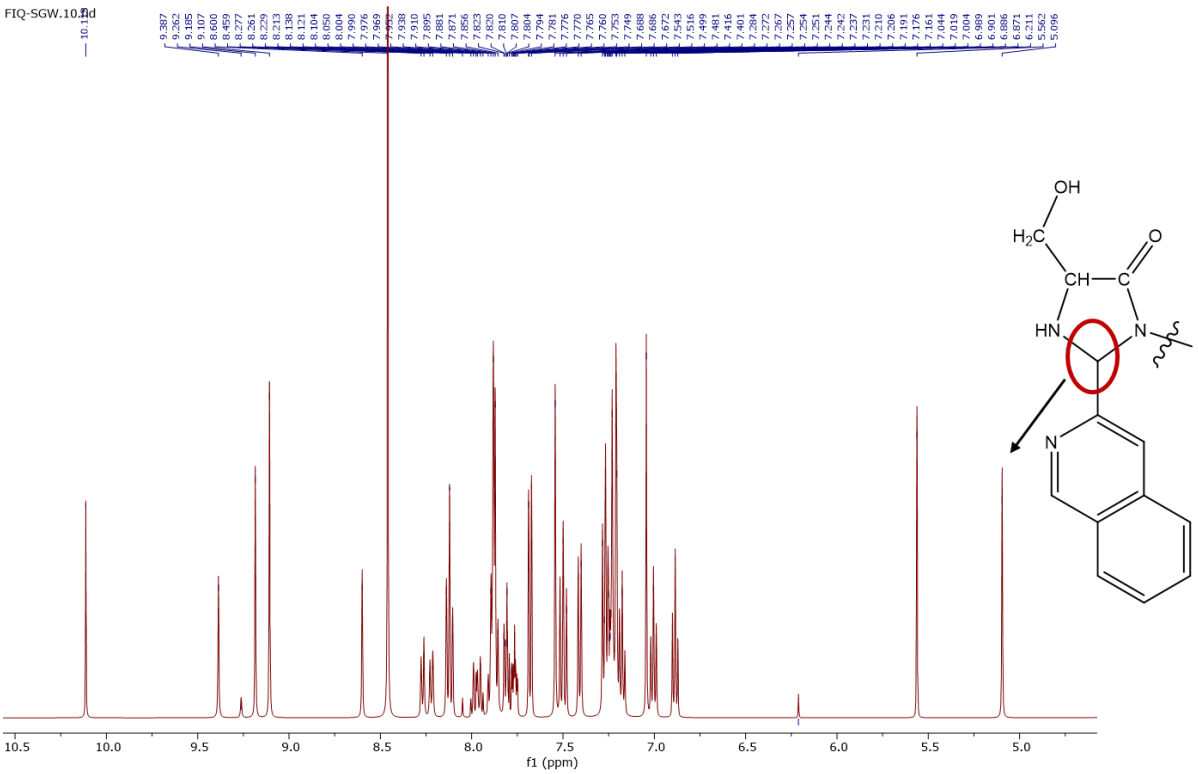


Figure 2: ^{1}H-NMR of 3-formylisoquinoline-SGW peptide.

^{1}H-NMR was taken in D_2O of purified aldehyde labeled Ser-Gly-Trp peptide with the imidazolinone proton labeled. No proton that corresponded to the aldehyde or imine was observed

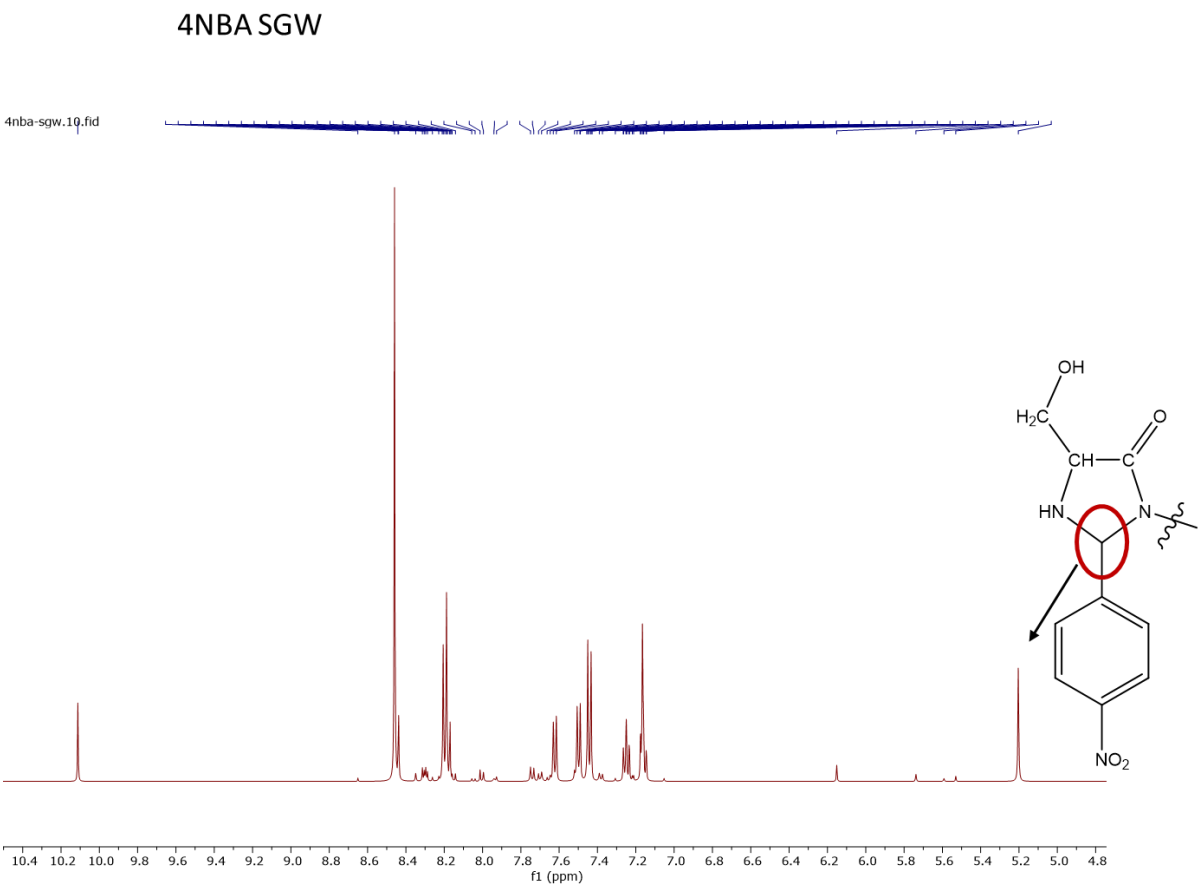


Figure 3: ^{1}H-NMR of 4-nitrobenzaldehyde-SGW peptides.

^{1}H-NMR was taken in D_2O of purified aldehyde labeled Ser-Gly-Trp peptide with the imidazolinone proton labeled. No proton that corresponded to the aldehyde or imine was observed

PCA SGW

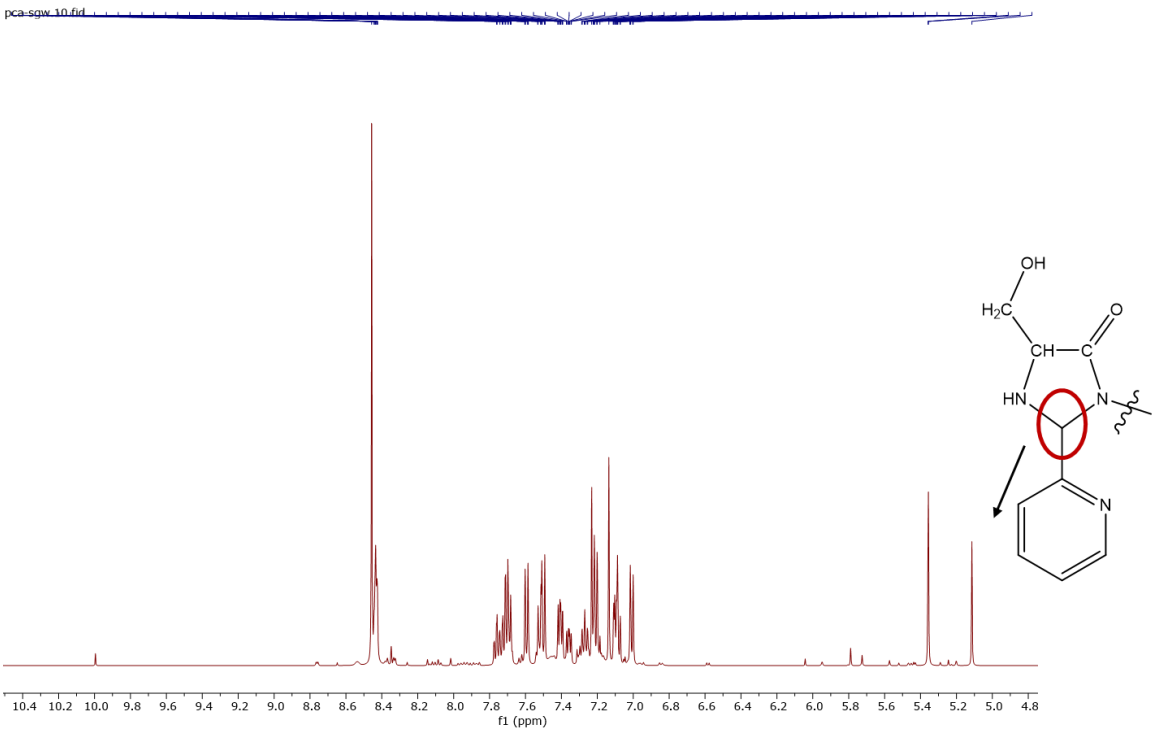


Figure 4: ^{1}H-NMR of pyridinylcarboxaldehyde-SGW peptide.

^{1}H-NMR was taken in D_2O of purified aldehyde labeled Ser-Gly-Trp peptide with the imidazolinone proton labeled. No proton that corresponded to the aldehyde or imine was observed

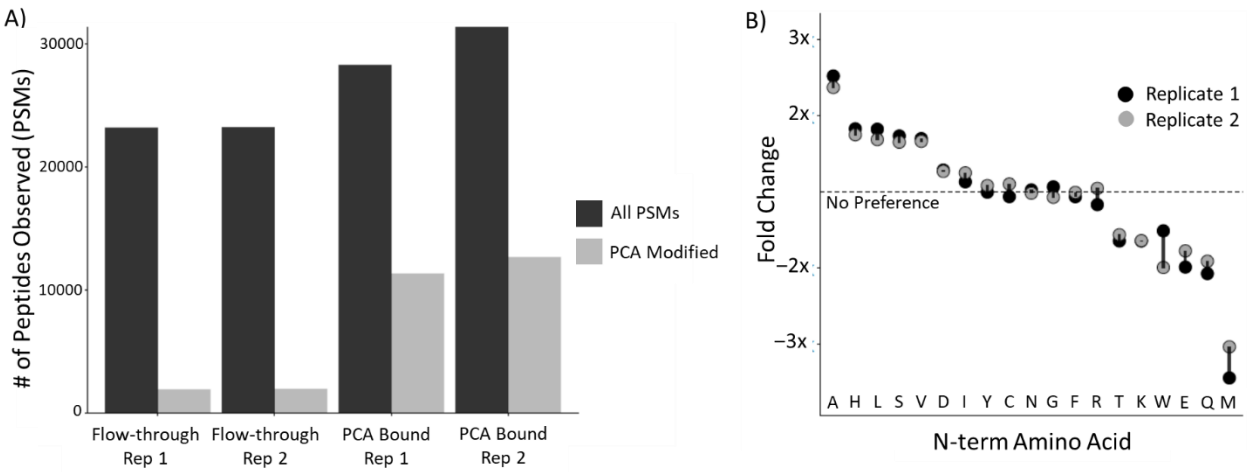


Figure 4. A) The number of unique proteins identified with high confidence (FDR < 1 %) in two replicate HEK293T cell lysate proteomics experiments. B) The normalized bias of the PCA capture reagent against the N-termini of the peptides bound to the resin. A total of 133,793 tandem mass spectra were collected and assigned, corresponding to 39,581 (from the bound sample) and 25,049 (from the flowthrough) in replicate 1, and 43,986 (from bound) and 25,177 (from flowthrough) in replicate 2.

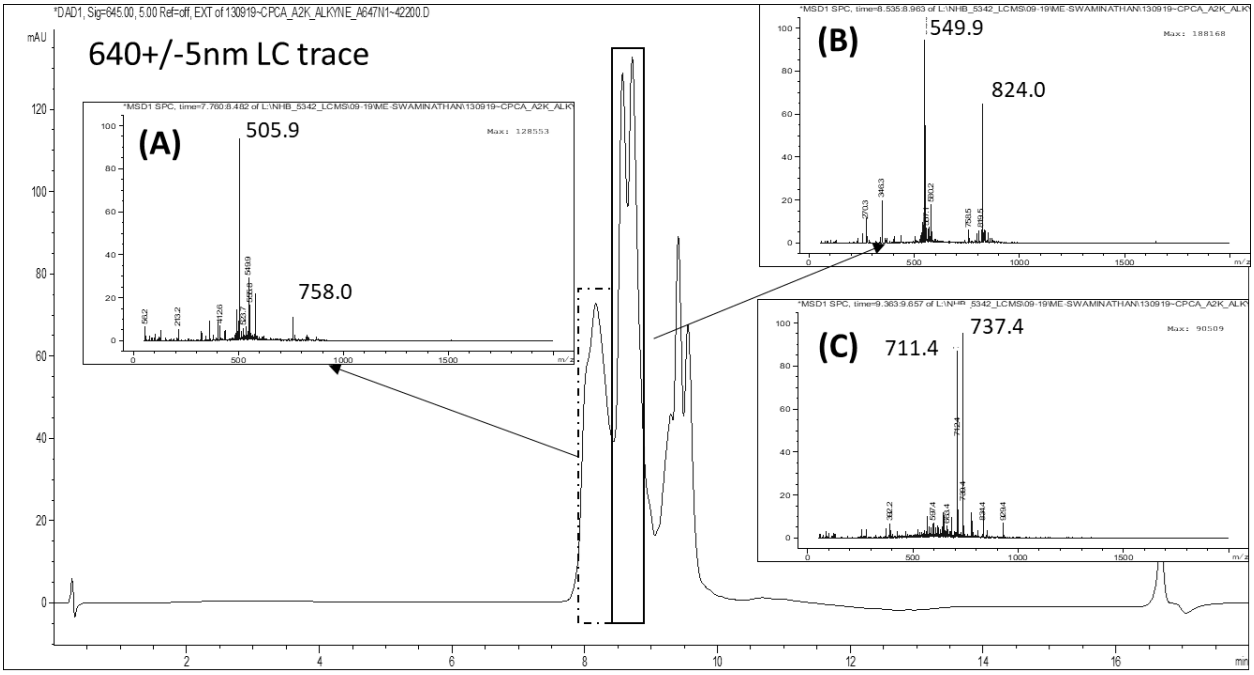
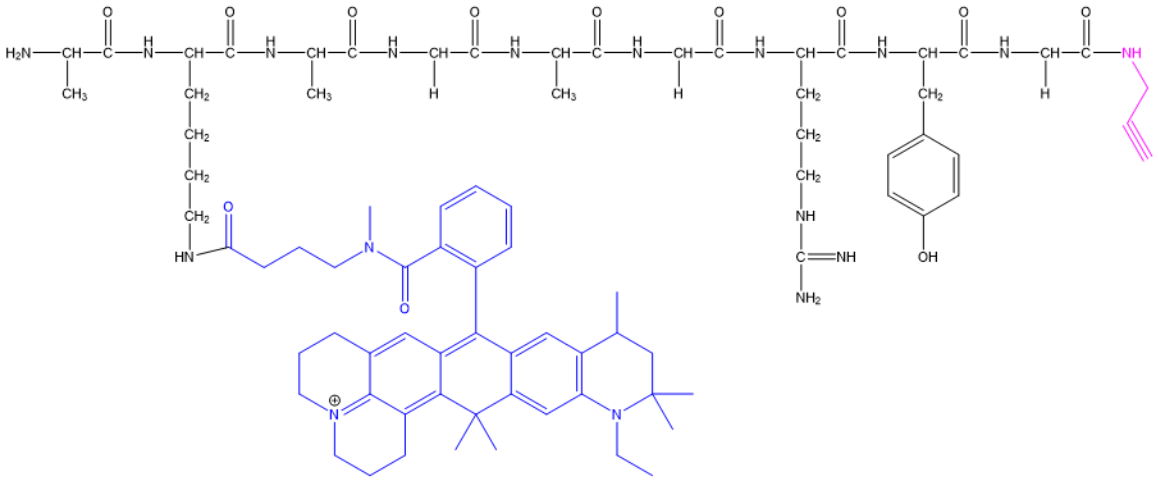


Figure 5: Multiple derivatizations on resin-captured peptides was performed and verified.

The resin immobilized peptide's (sequence H_2N-AKAGAGRYG-OH) (1) C-terminal carboxylate was labeled with propargylamine and (2) amine side chain of lysine was labeled with Atto647N fluorophore. The 16 min gradient LC-MS analysis (see methods) indicated that >70% of the products observed with 640 nm LC trace (shown above) corresponds to the multiply labeled peptide. Insert A and B corresponds to the peptide with Atto647N dye and the alkyne label. While inset A corresponds to peptide without the N-terminal PCA adduct (Exp. Mass: $[M+H]^+$ 1514.8 m/z, $[M+2H]^{+2}$ 757.9 m/z), inset B is the PCA capped peptide (Exp. Mass: $[M+H]^+$ 1646.9 m/z, $[M+2H]^{+2}$ 823.9 m/z). Inset C indicates side products observed in the reaction

A)



B)

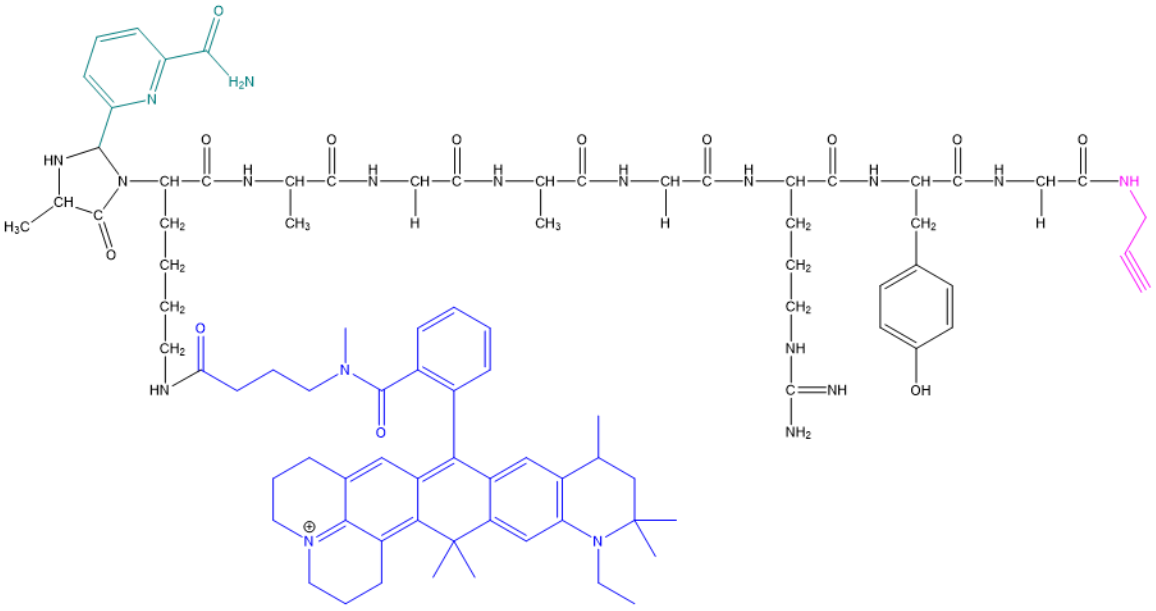


Figure 6: Chemical structures of products from captured peptide labeling.

Schematics of the peptides found in the LC/MS analysis that correspond to the molecular weights seen in Figure 5 A) The product in Figure 5 that is derivatized on the C-terminus and Lys but does not have an N-terminal PCA adduct. Exp. Mass: 1514.8 m/z B) The product in Figure 5 that has both modifications as well as an N-terminal adduct. Exp. Mass: 1646.9 m/z