Supplementary Information

Reversible chemoselective tagging and functionalization of methionine containing peptides

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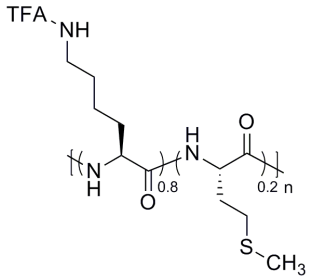
1. Materials and Methods

Unless stated otherwise, reactions were conducted in oven-dried glassware under an atmosphere of nitrogen using anhydrous solvents. Hexanes, THF, and DCM were purified by first purging with dry nitrogen, followed by passage through columns of activated alumina. Deionized water (18 MΩ-cm) was obtaining by passing in-house deionized water through a Millipore Milli-Q Biocel A10 purification unit. All commercially obtained reagents were used as received without further purification unless otherwise stated. Reaction temperatures were controlled using an IKA magnetic temperature modulator, and unless stated otherwise, reactions were performed at room temperature (RT, approximately 20 °C). Thin-layer chromatography (TLC) was conducted with EMD gel 60 F254 precoated plates (0.25 mm) and visualized using a combination of UV, anisaldehyde, and phosphomolybdic acid staining. Selecto silica gel 60 (particle size 0.032–0.063 mm) was used for flash column chromatography. ^{1}H NMR spectra were recorded on Bruker spectrometers (at 500 MHz) and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. ^{13}C NMR spectra were recorded on Bruker spectrometers (at 125 MHz). Data for ^{13}C NMR spectra are reported in terms of chemical shift. High-resolution mass spectrometry (HRMS) was performed on a Micromass Quatro-LC Electrospray spectrometer with a pump rate of 20 μL/min using electrospray ionization (ESI). Matrix assisted laser desorption ionization (MALDI) mass spectrometry was performed on an Applied Biosystems Voyager-DE STR using an α-cyano-4-hydroxycinnamic acid matrix. All Fourier Transform Infrared (FTIR) samples were prepared as thin films on NaCl plates and spectra were recorded on a Perkin Elmer RX1 FTIR spectrometer and are reported in terms of frequency of absorption (cm^{-1}). Tandem gel permeation chromatography/light scattering

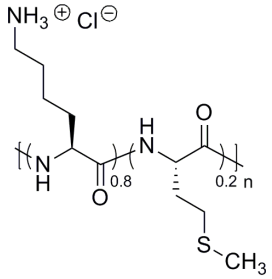
(GPC/LS) was performed on a SSI Accuflow Series III liquid chromatograph pump equipped with a Wyatt DAWN EOS light scattering (LS) and Optilab rEX refractive index (RI) detectors. Separations were achieved using 10^5, 10^4, and 10^3Å Phenomenex Phenogel 5 μm columns using 0.10 M LiBr in DMF as the eluent at 60 °C. All GPC/LS samples were prepared at concentrations of 5 mg/mL.

2. Experimental Procedures



General Procedure for Preparation of Poly[(N_ε-trifluoroacetyl-L-lysine)$_{0.8}$-*stat*-(L-methionine)$_{0.2}$]$_n$

Inside a dinitrogen filled glove box, a solution of N_ε-TFA-L-lysine-N-carboxyanhydride[1] (TFA-Lys NCA), (616 mg, 2.30 mmol, 4 eq) and L-methionine-*N*-carboxyanhydride (Met NCA)[2] (100 mg, 0.573 mmol, 1 eq) in dry THF (0.15 M) was prepared. A solution of $(PMe_3)_4Co$[3] in dry THF (20 mM) was rapidly added via syringe (26.0 mg, 0.0714 mmol, 0.025 eq). After 45 min, the polymerization reaction was complete as determined by FTIR. An aliquot was removed and analyzed by GPC/LS. The polymerization was removed from the drybox and HCl (2 eq per $(PMe_3)_4Co$, 6M in H_2O) was added to the solution, which turned a blue-green color. After 10 min stirring, the copolymer was collected by precipitation into acidic water (pH 3, HCl, > 10x the reaction volume), followed by centrifugation. The white precipitate was washed with two portions of DI water and then lyophilized to give the copolymer as a fluffy white solid (595 mg, 99 % yield). Spectral data was in agreement with previous reports.[1] Polypeptides used in this study were either 32 (M_n= 6,520, M_w/M_n =1.21) or 135 (M_n= 27,730, M_w/M_n =1.21) residues in length as determined by GPC analysis, and both gave similar results.



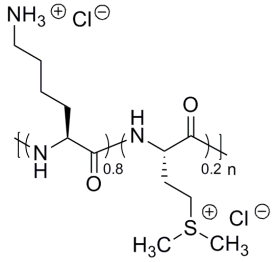
General Procedure for Deprotection of TFA-Lysine, Poly[(L-lysine·HCl)$_{0.8}$-*stat*-(L-methionine)$_{0.2}$]$_n$, KM, 1

Poly[(N_ε-trifluoroacetyl-L-lysine)$_{0.8}$-*stat*-L-methionine)$_{0.2}$]$_n$ was dispersed in methanol:water, 9:1 (20 mg/mL) and K_2CO_3 (2 eq per lysine residue) was added. The reaction was stirred for 8 hours at 50 ^oC and then the methanol was removed by rotory evaporation. The remaining solution was acidified to pH 3 with 6M HCl, and transferred to 2000 MWCO dialysis tubing. The polypeptide was dialyzed at pH 4 (HCl) for 24 hours, followed by DI water for 48 hours with water changes

twice per day. The contents of the dialysis tubing were then lyophilized to dryness to give poly[(L-lysine·HCl)$_{0.8}$-*stat*-(L-methionine)$_{0.2}$]$_n$, **KM** as a white solid. (82% yield) ^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 4.51 (s, 1H), 4.32 (s, 4H), 3.02 (m, 8H), 2.66-2.52 (m, 2H), 2.16-1.97 (m, 5H), 1.88-1.66 (m, 16H), 1.46 (s, 8H).

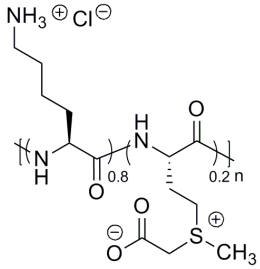
General Procedure for Alkylation of KM

KM was dissolved in 0.2 M formic acid (10 mg/mL) and the alkylating reagent (1.5 eq per methionine residue) was added. The reaction mixture was covered with foil and stirred at room temperature for 48 hours. The reaction was then transferred to 2000 MWCO dialysis tubing and dialyzed against 0.10 M NaCl for 24 hours to exchange all counterions to chloride, followed by dialysis against DI water for 48 hours with water changes twice per day. The contents of the dialysis tubing were then lyophilized to dryness to give the product as a white solid. Note: Reactions performed with 1.0 eq of alkylating reagent completed with reaction times extended to 64 hours.



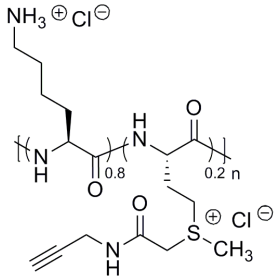
Poly[(L-lysine·HCl)$_{0.8}$-*stat*-(*S*-methyl-L-methionine sulfonium chloride)$_{0.2}$]$_n$, 3a

Polysulfonium **3a** was prepared from **KM** and methyl iodide according to the general procedure for alkylation of **KM**, (88% yield). ^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ ^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 4.56 (s, 1H), 4.28 (s, 4H), 3.39 (s, 2H), 3.04-2.89 (m, 12H), 2.37-2.16 (m, 2H), 1.84-1.60 (m, 16H), 1.45 (s, 8H).



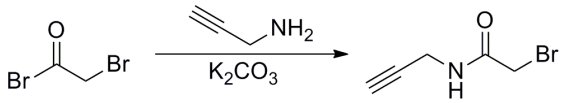
Poly[(L-lysine·HCl)$_{0.8}$-*stat*-(*S*-(carboxymethyl)-L-methionine)$_{0.2}$]$_n$, 3b

Polysulfonium **3b** was prepared from **KM** and bromoacetic acid according to the general procedure for alkylation of **KM**, (85% yield). ^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ ^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 4.56 (s, 1H), 4.26 (s, 4H), 3.46-3.28 (m, 2H), 3.04-2.84 (m, 12H), 2.37-2.14 (m, 2H), 1.85-1.59 (m, 16H), 1.42 (s, 8H).



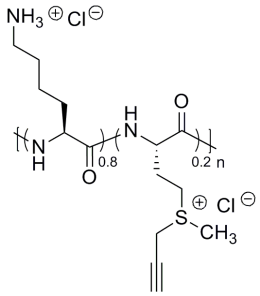
Poly[(L-lysine·HCl)$_{0.8}$-*stat*-(*S*-(*N*-propargyl-acetamido)-L-methionine sulfonium chloride)$_{0.2}$]$_n$, 3c

Polysulfonium **3c** was prepared from **KM** and *N*-propargyl-bromoacetamide **2c** according to the general procedure for alkylation of **KM**, (85% yield). ^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 4.62 (s, 1H), 4.32 (s, 4H), 4.06 (s, 2H), 3.64-3.46 (m, 2H), 3.07-2.97 (m, 12H), 2.69 (s, 1H), 2.44-2.20 (m, 2H), 1.88-1.62 (m, 16H), 1.47 (s, 8H).



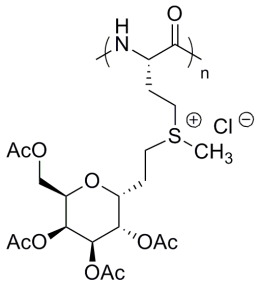
Preparation of *N*-propargyl-bromoacetamide, 2c:

N-propargyl-bromoacetamide was prepared from bromoacetyl bromide according to a modified literature procedure.[4] Propargyl amine (0.166 mL, 2.60 mmol, 1.05 eq) was added dropwise to a solution of K_2CO_3 (0.358 g, 2.60 mmol, 1.05 eq) and bromoacetyl bromide (0.500 g, 2.48 mmol, 1.00 eq) in CH_2Cl_2 (20 mL) at 0 ºC. The resulting solution was allowed to reach RT and stir for 4 hours. The reaction was filtered, the filter cake rinsed with CH_2Cl_2, and the filtrate was evaporated to a brown solid, which was recrystallized from THF and hexanes to give *N*-propargyl-bromoacetamide (0.313 g, 72%). Spectral data were consistent with literature values.



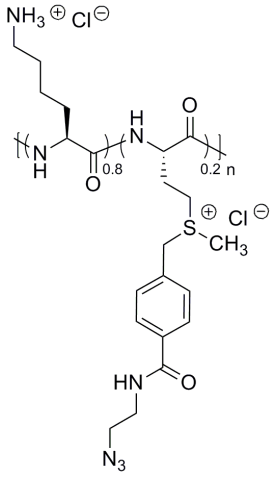
Poly[(L-lysine·HCl)$_{0.8}$-*stat*-(*S*-propargyl-L-methionine sulfonium chloride)$_{0.2}$]$_n$, 3d

Polysulfonium **3d** was prepared from **KM** and propargyl bromide according to the general procedure for alkylation of **KM**, (92% yield). ^{1}H NMR (500 MHz, 2% d-TFA in D_2O, 25 ^{o}C): δ 4.44 (s, 1H), 4.15 (s, 6H), 3.31 (s, 2H), 2.89-2.82 (m, 16H), 2.26-2.15 (m, 3H), 2.08 (s, 1H), 1.70-1.48 (m, 265H), 1.30 (s, 13H).



Poly(*S*-2-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)ethyl-L-methionine sulfonium chloride), 3e

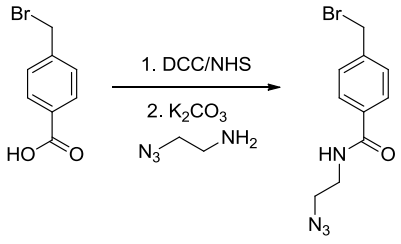
Polysulfonium **3e** was prepared as previously described.[1]



Poly[(L-lysine·HCl)$_{0.8}$-*stat*-(*S*-(4-(*N*-azidoethylcarboxamide)phenylmethyl))-L-methionine sulfonium chloride)$_{0.2}$]$_n$, 3f

Polysulfonium **3f** was prepared from **KM** and α-bromomethyl-(*N*-azidoethyl)-*p*-toluamide **2f** according to the general procedure for alkylation of **KM**, except that α-bromomethyl-(*N*-azidoethyl)-*p*-toluamide was added as a 25 mg/mL solution in ethanol (87% yield).

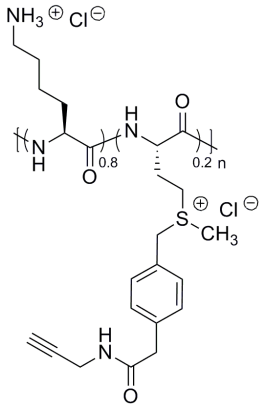
^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 7.91-7.307 (m, 4H), 4.47 (s, 1H), 4.19 (s, 3H), 3.58-3.11 (m, 4H), 2.89 (s, 1H), 2.74 (s, 1H), 2.25-1.96 (m, 2H), 1.78-1.11 (m, 23H).



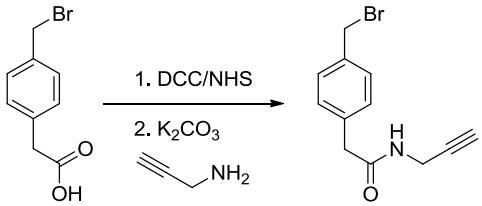
Preparation of α-bromomethyl-(*N*-azidoethyl)-*p*-toluamide, 2f:

The NHS ester of α-bromomethyl-*p*-toluic acid was prepared according to a literature procedure.[5] α-Bromomethyl toluic acid (0.140 g, 0.651 mmol, 1.00 eq) was dissolved in DMF/ethyl acetate 1/1 (5 mL). NHS (0.0787 g, 0.684 mmol, 1.05 eq) and then DCC (0.141 g, 0.684 mmol, 1.05 eq) were added. A white precipitate formed within 10 minutes. The reaction was stirred for 2 hours, filtered, the filter cake was washed with ethyl acetate, and the filtrate was

condensed to a white solid. The crude NHS ester was redissolved in DMF (5 mL) and K_2CO_3 was added (0.105 g, 0.716 mmol, 1.10 eq) followed by 2-azidoethylamine[6] (0.0654 g, 0.716 mmol, 1.10 eq). The reaction was stirred for 4 hours, then diluted with water (200 mL). The product was extracted with 3 portions of ethyl acetate (50 mL), the combined organic layers were washed with water and brine, dried over sodium sulfate, and condensed. The pale yellow solid was chromatographed on silica in 5% methanol in benzene to give α-bromomethyl-(*N*-azidoethyl)-*p*-toluamide, 0.140 g (76%).
^{1}H NMR (500 MHz, $CDCl_3$, 25 ^{o}C): δ 7.74 (d, ^3J (H, H) = 8.0, 2H), 7.43 (d, ^3J (H, H) = 8.0, 2H), 6.70 (s, 1H), 4.49 (s, 2H), 3.61-3.58 (m, 2H), 3.53 (t, ^3J (H, H)= 5.5, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 ^{o}C): δ 167.0, 141.3, 133.9, 129.2, 127.4, 50.7, 39.4, 32.2. HRMS-ESI (m/z) $[M + H]^+$ $C_{10}H_{11}BrN_4O$, calcd: 282.01; found: 282.01.

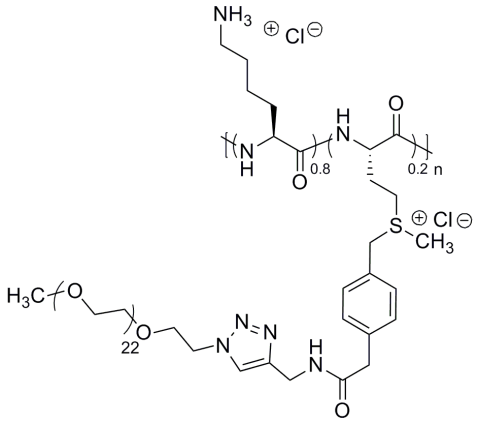


Poly[(L-lysine·HCl)$_{0.8}$-*stat*-(*S*-(4-(*N*-propargyl-acetamido)phenylmethyl)-L-methionine sulfonium chloride)$_{0.2}$]$_n$, 3g
Polysulfonium **3g** was prepared from **KM** and 4-bromomethyl-*N*-propargyl-phenylacetamide **2g** according to the general procedure for alkylation of **KM**, except that 4-bromomethyl-*N*-propargyl-phenylacetamide was added as a 25 mg/mL solution in ethanol, (92% yield). ^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 7.39-7.24 (m, 4H), 4.48 (s, 1H), 4.19 (s, 4.5H), 3.85 (s, 2H), 3.57-3.48 (m, 2H), 3.34-3.13 (m, 2H), 2.90 (m, 9H), 2.73-2.61 (m, 3H), 2.50 (s, 1H), 2.23-2.03 (m, 2H), 1.74-1.48 (m, 18H), 1.34 (s, 9H).

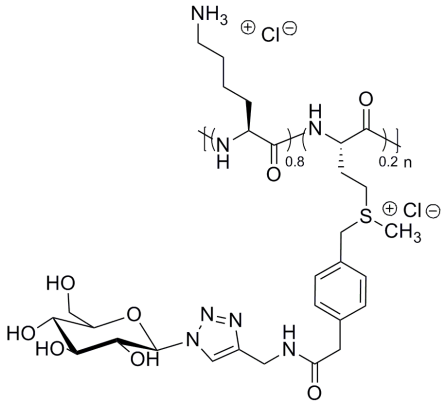


Preparation of 4-bromomethyl-*N*-propargyl-phenylacetamide, 2g:
4-Bromomethyl-phenylacetic acid (0.509 g, 2.22 mmol, 1.00 eq) was dissolved in dry THF (20 mL). NHS (0.258 g, 2.24 mmol, 1.01 eq) and then DCC (0.463 g, 2.24 mmol, 1.01 eq) were added. A white precipitate formed within 10 minutes. The reaction was stirred for 2 hours, filtered, the filter cake was washed with THF, and the filtrate was condensed to a white solid. The crude NHS ester was redissolved in DMF (20 mL) and K_2CO_3 was added (0.307 g, 2.22 mmol, 1.00 eq) followed by propargyl amine (0.149 mL, 2.33 mmol, 1.05 eq). The reaction was stirred for 4 hours, then diluted with water (200 mL). The product was extracted with 3 portions

of ethyl acetate (50 mL), the combined organic layers were washed with water and brine, dried over sodium sulfate, and condensed. The pale yellow solid was chromatographed on silica in 5% methanol in benzene to give 4-bromomethyl-*N*-propargyl-phenylacetamide, 0.496 g (84%).
^{1}H NMR (500 MHz, MeOD, 25 ^{o}C): δ 7.38 (d, ^{3}J (H, H) = 8.2, 2H), 7.28 (d, ^{3}J (H, H) = 8.2, 2H), 4.55 (s, 2H), 3.95 (d, ^{3}J (H, H) = 2.5, 2H), 3.51 (s, 2H), 2.58 (t, ^{3}J (H, H) =2.6, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 ^{o}C): δ 170.0, 137.0, 134.5, 129.8, 129.6, 79.2, 71.6, 43.0, 32.9, 29.3. HRMS-ESI (m/z) $[M + H]^+$ $C_{12}H_{13}BrNO$, calcd for 266.01; found 266.02.



Copper(I)-catalyzed Azide-Alkyne Cycloaddition of azide terminated polyethyleneglycol with sulfonium 3g to give 5a:
Polysulfonium **3g** was dissolved in water (5 mg/mL) and azide terminated polyethyleneglycol (Sigma, MW=1,000), (1.2 eq / alkyne) was added. The solution was degassed by bubbling N_2 through the solution for 20 minutes and then stirred under N_2. Separately, a solution of Cu(I) was prepared by addition of sodium ascorbate (0.5 eq / alkyne) to a degassed solution of $Cu(II)SO_4$ (0.1 eq / alkyne) and pentamethyldiethylenetriamine (0.1 eq / alkyne). The solution turned dark blue. The Cu(I) solution was transferred to the azide/alkyne solution via syringe. The reaction was stirred at room temperature for 48 hours and then transferred to 8000 MWCO dialysis tubing and dialyzed against 0.10 M NaCl for 24 hours, followed by dialysis against DI water for 72 hours with water changes twice per day. The contents of the dialysis tubing were then lyophilized to dryness to give the product, **5a**, as a white solid (95% yield).
^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 7.92 (s, 1H), 7.46-7.18 (m, 4H), 4.27 (s, 4H), 4.00-3.42 (m, 152 H), 3.20 (s, 2H), 2.97 (s, 8H), 2.77 (s, 3H), 2.53 (m, 2H), 2.19 (m, 2H), 1.83-1.59 (m, 16 H), 1.42 (s, 8H).



Copper(I)-catalyzed Azide-Alkyne Cycloaddition of β-D-glucopyranosyl azide with sulfonium 3g to give 5b:

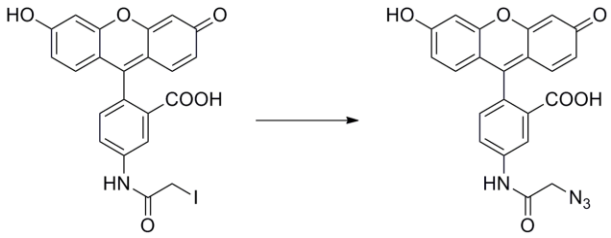
Polysulfonium **3g** was dissolved in water (5 mg/mL) and β-D-glucopyranosyl azide (Carbosynth, 1.2 eq / alkyne) was added. The solution was degassed by bubbling N_2 through the solution for 20 minutes and then stirred under N_2. Separately, a solution of Cu(I) was prepared by addition of sodium ascorbate (0.50 eq / alkyne) to a degassed solution of $Cu(II)SO_4$ (0.10 eq / alkyne) and pentamethyldiethylenetriamine (0.10 eq / alkyne). The solution turned dark blue. The Cu(I) solution was transferred to the azide/alkyne solution via syringe. The reaction was stirred at room temperature for 48 hours and then transferred to 2000 MWCO dialysis tubing and dialyzed against 0.10 M NaCl for 24 hours, followed by dialysis against DI water for 48 hours with water changes twice per day. The contents of the dialysis tubing were then lyophilized to dryness to give the product, **5b**, as a white solid (95% yield).

^{1}H NMR (500 MHz, D_2O, 25 ^{o}C): δ 8.13 (s, 1H), 7.49-7.31 (m, 4H), 5.73 (s, 1H), 4.59 (s, 1H), 4.49 (s, 2H), 4.30 (s, 5.5H), 4.00-3.86 (m, 2H), 3.81-3.58 (m, 7H), 3.41 (s, 1H), 3.29 (s, 1H), 3.01 (s, 11H), 2.84-2.72 (m, 3H), 2.35-2.15 (m, 22H), 1.45 (s, 11H).

Copper(I)-catalyzed Azide-Alkyne Cycloaddition of 5-azidoacetamido-fluorescein with sulfonium 3g to give 5c:

Polysulfonium **3g** was dissolved in water (5 mg/mL) and 5-azidoacetamido-fluorescein **4c**, (0.020 eq / alkyne, 1 eq per **3g** chain) was added. The solution was degassed by bubbling N_2 through the solution for 20 minutes and then stirred under N_2. Separately, a solution of Cu(I) was prepared by addition of sodium ascorbate (0.50 eq / alkyne) to a degassed solution of $Cu(II)SO_4$ (0.10 eq / alkyne) and pentamethyldiethylenetriamine (0.10 eq / alkyne). The solution turned dark blue. The Cu(I) solution was transferred to the azide/alkyne solution via syringe. The reaction was covered with foil and stirred at room temperature for 48 hours and then transferred to 2000 MWCO dialysis tubing and dialyzed against 0.10 M NaCl for 24 hours, followed by dialysis against DI water for 48 hours with water changes twice per day. The contents of the dialysis tubing were then lyophilized to dryness to give the product, **5c**, as a white solid (95% yield).

Preparation of 5-azidoacetamido-fluorescein:



5-iodoacetamido-fluorescein (5 mg, 9.70 μmol) was dissolved in DMSO (1 mL). NaN_3 (1.89 mg, 29.1 μmol, 3.00 eq) was added. The reaction was covered with foil and stirred at 50 ^oC for 8 hours. Water was added (20 mL) and the mixture was extracted with 3 portions of 1/1 ethyl acetate/isopropanol (10 mL each). The combined organic phases were washed with 2 small portions of brine, dried with magnesium sulfate, and condensed to give **4c** as an orange solid (4.1 mg, 99%)
^1H NMR (500 MHz, d-DMSO, 25 ^oC): δ 10.76 (s, 1H), 10.15 (s, 1H), 8.29 (s, 1H), 7.84 (d, ^3J (H, H) = 7.2, 2H), 7.22 (d, ^3J (H, H) = 7.2, 2H), 6.66 (s, 2H), 6.58-6.51 (m, 4H), 4.13 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 ^oC): δ 169.0, 167.5, 159.9, 152.4, 147.7, 140.5, 129.49, 127.5, 127.0, 125.1, 114.2, 113.1, 110.0, 102.6, 55.3.

General Procedure for Dealkylation of Methionine Sulfonium Salts to Regenerate KM:
Alkylated **KM** was dissolved in 0.1 M nucleophile (2-mercaptopyridine, thiourea, mercaptoethanol, or glutathione) in PBS buffer, pH 7.4 and stirred at 37 ^oC. At different time points, an aliquot of each reaction was removed and transferred to 2000 MWCO dialysis tubing. Samples were dialyzed against 0.10 M NaCl for 24 hours to exchange all counterions to chloride, followed by dialysis against DI water for 48 hours with water changes twice per day. Reactions with glutathione were dialyzed against 0.10 M NaCl at pH 3 for 24 hours to disrupt polyelectrolyte complexes between glutathione and the polypeptide, followed by dialysis against DI water for 48 hours with water changes twice per day. The contents of the dialysis tubing were then lyophilized to dryness.

^1H NMRs of the products of dealkylation reactions of polysulfoniums **3a, 3b,** and **3e** were found to be identical to the respective alkylated starting materials, and no regeneration of methionine was observed. Products of dealkylation reactions of polysulfoniums **3c, 3d**, **3f, 3g**, and **5a-c** were found to give products with ^1H NMRs identical to the parent polypeptide **KM**.

A dealkylation reaction was performed using **3g** and 1 eq of 2-mercaptopyridine per sulfonium group (0.02 M in DI water, 37 ^oC). Complete dealkylation was found to occur in 36 hours under these conditions, and to yield the parent polypeptide **KM**.

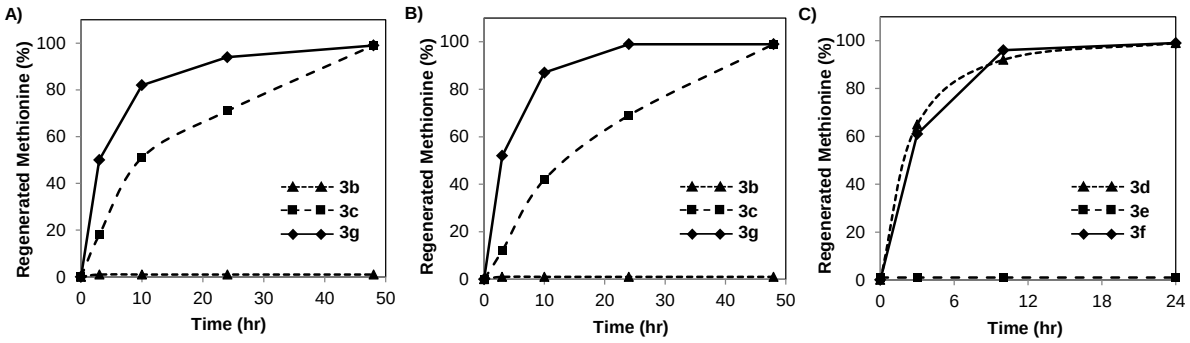


Figure S1: Regeneration of **KM** from polysulfoniums over time using different nucleophiles (37 ^{o}C, PBS buffer). A) 0.1 M 2-mercaptoethanol, B) 0.1 M thiourea, C) 0.1 M 2-mercaptopyridine.

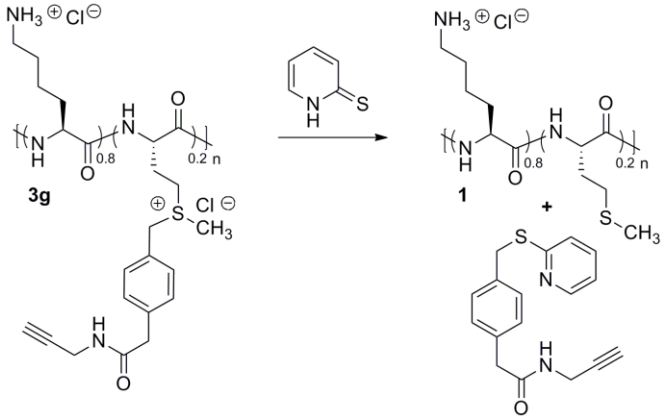
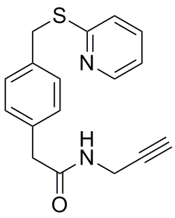


Figure S2: Regeneration of **KM** from **3g** using 0.1 M 2-mercaptopyridine (37 ^{o}C, PBS buffer) and structure of isolated reaction byproduct.



Isolation of byproduct from dealkylation reactions of 3g using mercaptopyridine:
Polysulfonium **3g** was treated with 0.1 M mercaptopyridine in PBS buffer for 24 hours at room temperature. The reaction was extracted with 3 portions of ethyl acetate, and the combined organic extractions were condensed by rotary evaporation. The residue was purified by flash chromatography on silica (5% methanol in benzene) and was found to contain only excess mercaptopyridine and the expected thioether reaction byproduct. This stucture was confirmed by preparation of an authentic sample by reaction of mercaptopyridine (1.5 eq) with 4-bromomethyl-*N*-propargyl-phenylacetamide **2g** (1 eq) and K_2CO_3 (1.5 eq) in DMF for 16 hours. Spectral data were identical to the byproduct isolated from the polypeptide dealkylation reaction.

^{1}H NMR (500 MHz, $CDCl_3$, 25 ^{o}C): δ 8.46 (d, ^{3}J (H, H) = 4.9, 1H), 7.48 (dd, ^{3}J (H, H) = 8.4, 7.0 1H), 7.40 (d, ^{3}J (H, H) = 8.0, 2H), 7.18 (d, ^{3}J (H, H) = 8.2, 2H), 7.16 (s, 1H), 7.00 (dd, ^{3}J (H, H) = 6.9, 5.4 1H), 5.54 (s, 1H), 4.44 (s, 2H), 4.00 (dd, ^{3}J (H, H) = 5.3, 2.5 2H) , 3.56 (s, 2H), 2.17 (t, ^{3}J (H, H) = 2.5, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, 25 ^{o}C): δ 170.5, 158.5, 149.4, 137.6, 136.0, 133.0, 129.7, 129.6, 122.2, 119.7, 79.3, 71.6, 43.2, 33.9, 29.3. HRMS-ESI (m/z) $[M + H]^{+}$ $C_{17}H_{16}N_2OS$, calcd: 296.10; found: 296.10.

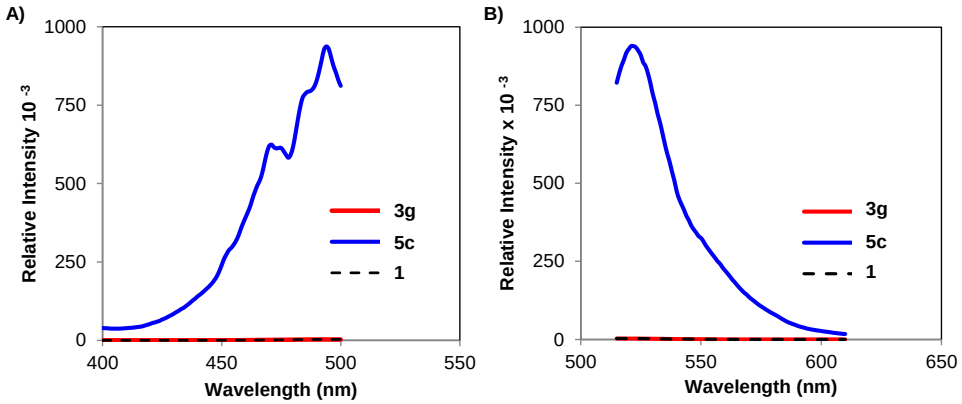
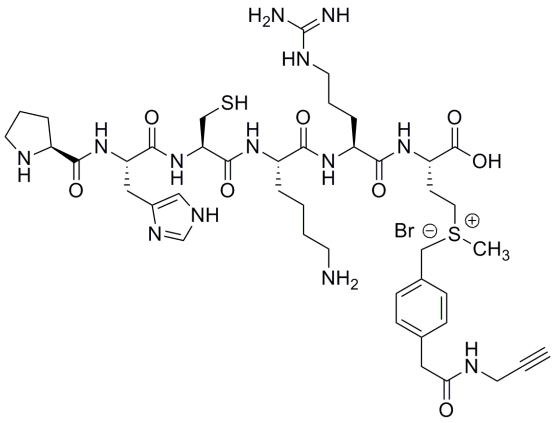


Figure S3: Fluorescence spectra of polypeptide **3g**: initially (**3g**), after copper catalyzed attachment of azidofluorescein **4c** (**5c**), and after dealkylation using mercaptopyridine to give parent polypeptide (**1**). A) absorption spectra. B) emission spectra.



Alkylation of PHCKRM Peptide at pH 2.4:

PHCKRM was purchased from Bachem. PHCKRM (2.0 mg, 2.6 µmol, 1.0 eq) was dissolved in 0.2 M formic acid (0.5 mL) and 4-bromomethyl-*N*-propargyl-phenylacetamide **2g** (0.76 mg, 2.85 µmol, 1.5 eq) was added as a 25 mg/mL solution in ethanol. The reaction was stirred for 48 hours and then extracted with 3 portions of ethyl acetate. The remaining aqueous solution was lyophilized to dryness to give 2.27 mg of **6** (92% yield), which was directly analyzed by mass spectrometry and ^{1}H NMR. (see Fig S4, S5)

Alkylation of PHCKRM at pH 8.3:

PHCKRM (1.0 mg, 1.3 µmol, 1.0 eq) was dissolved in carbonate buffer (0.25 mL) pH 8.3 and 4-bromomethyl-*N*-propargyl-phenylacetamide **2g** (0.068 mg, 2.6 µmol, 2.0 eq) was added as a 25 mg/mL solution in ethanol. The reaction was stirred for 48 hours and then extracted with 3 portions of ethyl acetate. A sample of the remaining aqueous solution was analyzed by mass spectrometry and the remainder was lyophilized to yield a white solid. (see Fig S6)

Dealkylation of Alkylated PHCKRM (6) using mercaptopyridine:
Alkylated PHCKRM **6** (2.3 mg, 2.4 µmol, 1.0 eq) was dissolved in DI water and 2-mercaptopyridine (0.78 mg, 7.1 µmol, 3 eq) was added. The solution was stirred for 24 hours at room temperature and then extracted with 5 portions of ethyl acetate. The remaining aqueous solution was lyophilized to dryness and then directly analyzed by mass spectrometry and ^{1}H NMR (see Fig S4, S5, S7). ^{1}H NMR was found to be identical to the original peptide PHCKRM.

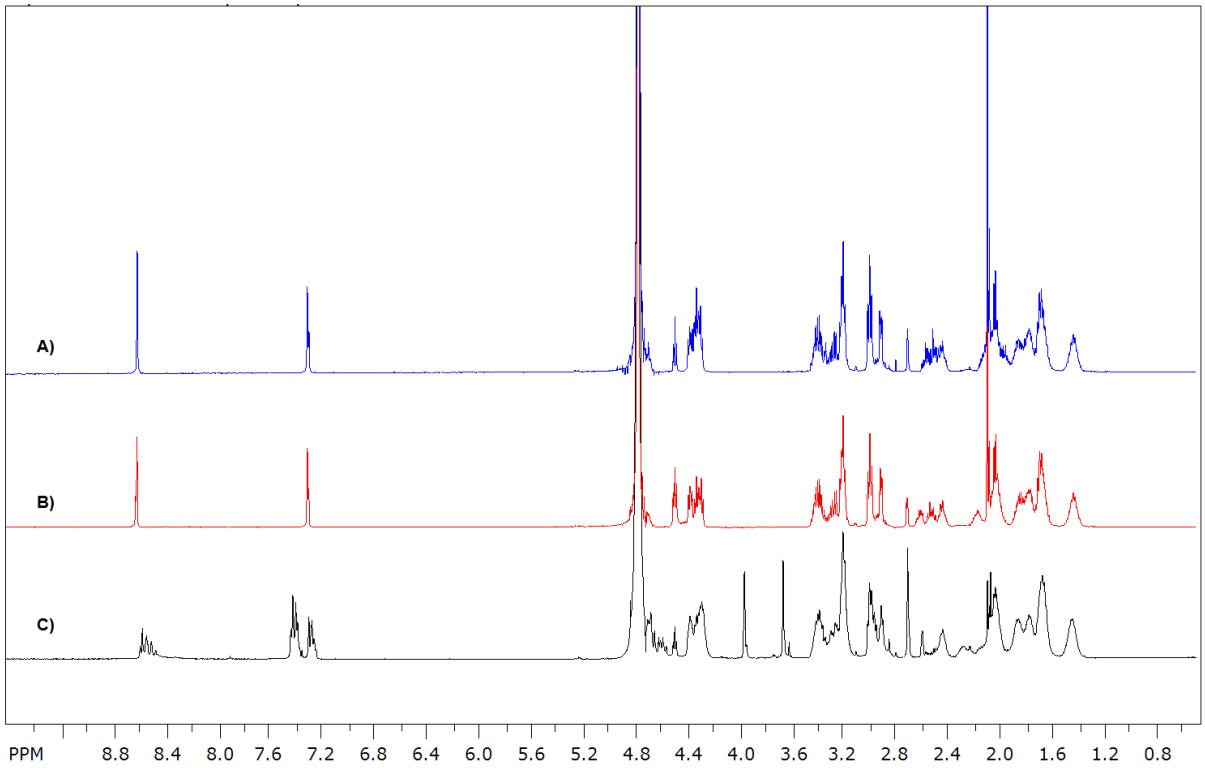


Figure S4: ^{1}H NMR spectra (all are 2 mg/mL in D_2O) of A) PHCKRM; B) PHCKRM regenerated from **6** after treatment with PyS; and C) alkylated PHCKRM, **6**, which is a mixture of diasteromers due to sulfonium chirality. See spectral data section for individual spectra.

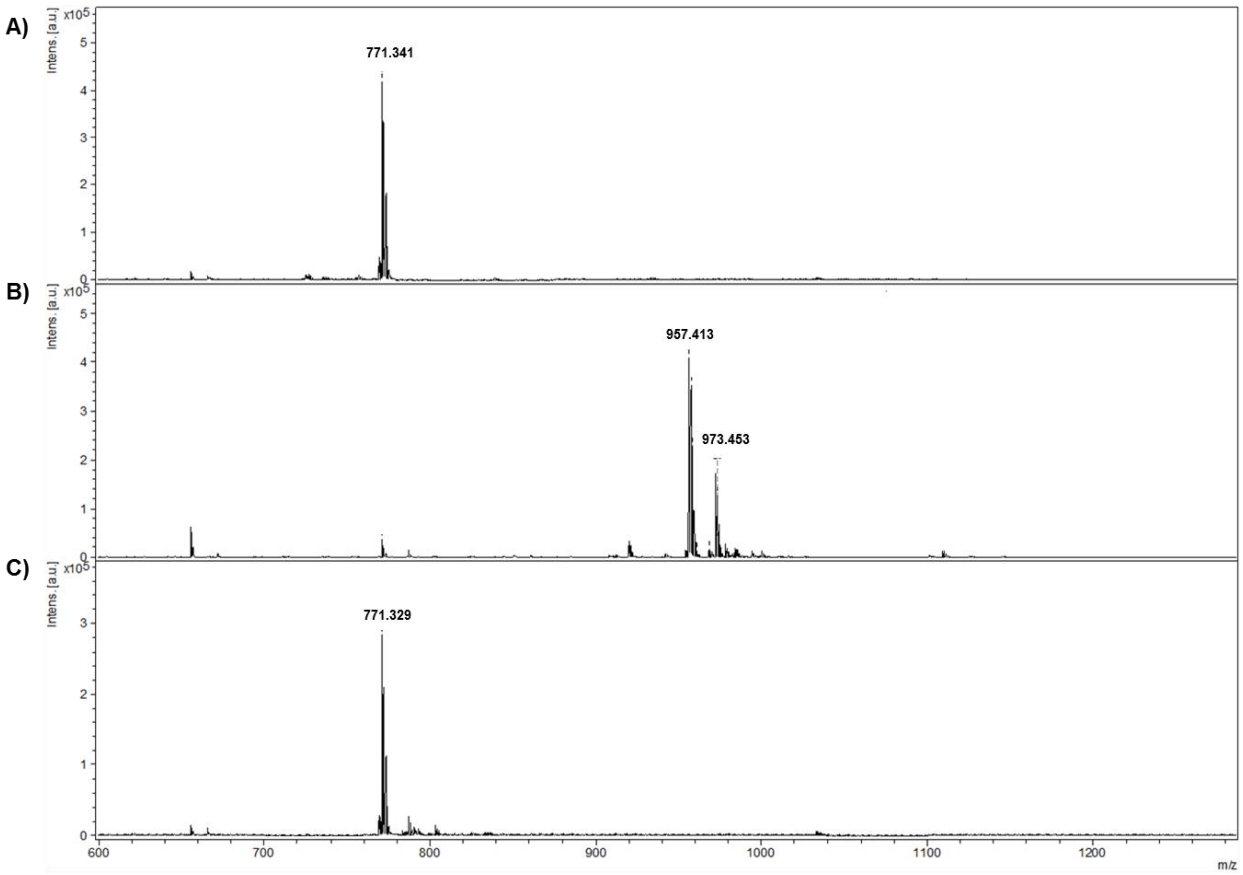


Figure S5: Expanded MALDI-MS spectra of A) PHCRKM, B) PHCKRM alkylated with **2g** to give **6**, and C) **6** after treatment with PyS to regenerate PHCRKM. Negligible multiply alkylated products were observed. The presence of the additional peak in B) at *m/z* 973 is indiciative of oxidation (addition of a single oxygen, Δ*m/z* 16) of alkylated **6** during MALDI laser ionization. The oxidation is not at the Met residue since this is alkylated (*m/z* 973, not *m/z* 786 expected for Met sulfoxide of PHCKRM), and is likely due to oxidation at cysteine or histadine. The MALDI and ESI-MS spectra of dealkylated PHCKRM, as well as the ^{1}H NMR of **6** also show no evidence of oxidation, indicating that the oxidation seen in B) occurs only during MALDI MS analysis.

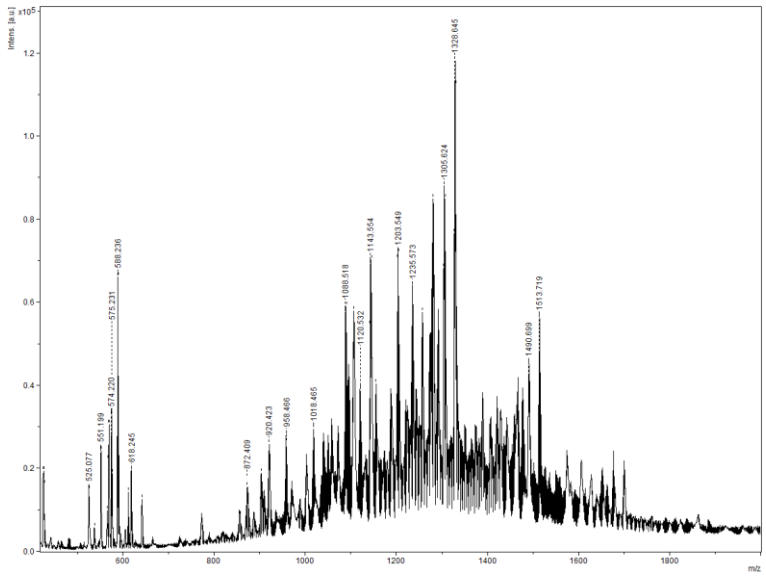


Figure S6: MALDI-MS spectrum of PHCKRM alkylated with **2g** at pH 8.3. Multiply alkylated products are observed.

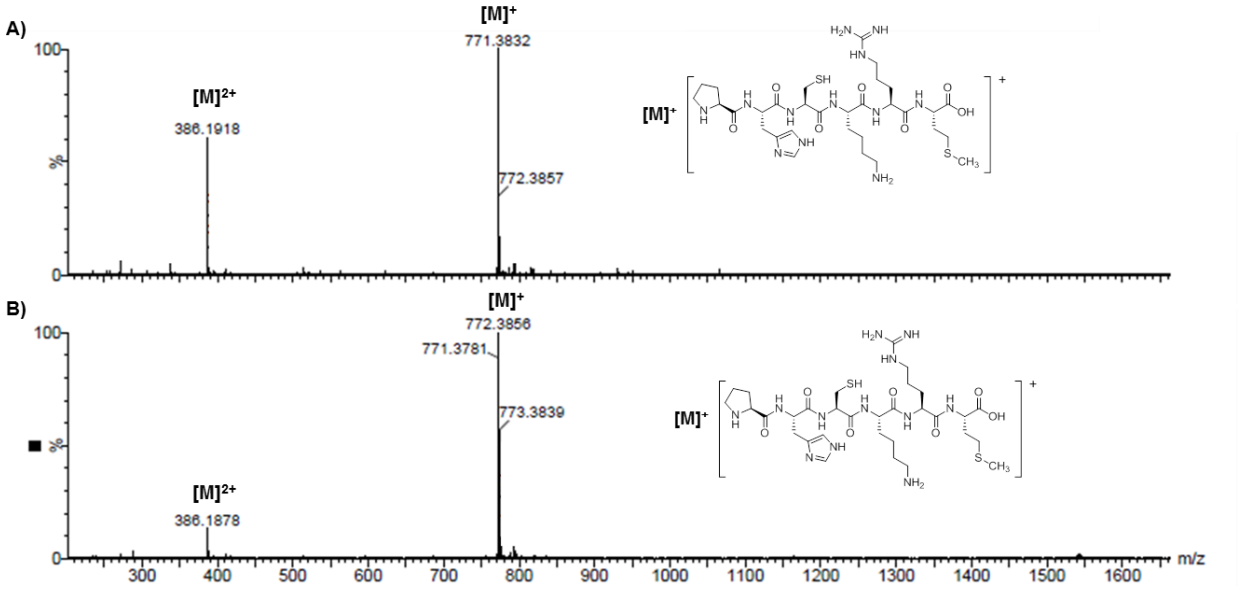


Figure S7: ESI-MS detection of HPLC samples of A) PHCKRM; and B) PHCKRM regenerated after treatment of **6** with PyS. Positive ionization.

Reactivity of Cysteine, Histidine, and Lysine with Benzyl Bromide Under Acidic Conditions

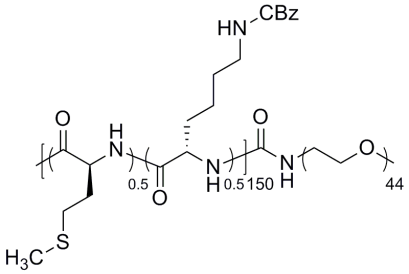
As control experiments, the reactivity of *N*-α-CBz-cysteine, *N*-α-CBz-histidine, or *N*-α-CBz lysine with an alkylating reagent was studied.

Cysteine: *N*-α-CBz-cysteine (50.0 mg, 0.196 mmol, 1.00 eq) was dissolved in 1:1 THF:0.2M aqueous formic acid (2 mL), ~pH 2.4. Benzyl bromide (67.0 mg, 0.392 mmol, 2.00 eq) was added and the reaction was covered with foil and stirred for 48 hours at room temperature. The reaction was diluted with water (30 mL), made basic with 2M NaOH, and extracted with diethyl ether (3 x 15 mL). The combined diethyl ether extracts were dried over magnesium sulfate and condensed to a clear oil. The aqueous phase was made acidic with concentrated HCl and extracted with EtOAc (3 x 15 mL). The EtOAC extracts were pooled, washed with brine, dried over magnesium sulfate, and condensed to a white solid. ^{1}H NMR of the diethyl ether extract was found to contain only benzyl bromide, and the EtOAc extract contained only *N*-α-CBz-cysteine. No alkylation occured at pH 2.4.

Histidine: *N*-α-CBz-histidine (55.5 mg, 0.192 mmol, 1.00 eq) was dissolved 1:1 THF:0.2 M aqueous formic acid (2 mL), pH 2.4. Benzyl bromide (65.7 mg, 0.384 mmol, 2.00 eq) was added and the reaction was covered with foil and stirred for 48 hours at room temperature. The reaction was extracted with diethyl ether (3 x 15 mL). The combined diethyl ether extracts were dried over magnesium sulfate and condensed to a clear oil. The aqueous phase was lyophilized to dryness. ^{1}H NMR of the diethyl ether extract was found to contain only benzyl bromide, and ^{1}H NMR of the aqueous portion contained only *N*-α-CBz-histidine. No alkylation occured at pH 2.4.

Lysine: *N*-α-CBz-lysine (0.0500 mg, 0.178 mmol, 1.00 eq) was treated with benzyl bromide (61.0 mg, 0.357 mmol, 2.00 eq) as previously described for *N*-α-CBz-histidine. ^{1}H NMR of the diethyl ether extract was found to contain only benzyl bromide, and ^{1}H NMR of the aqueous portion contained only *N*-α-CBz-lysine. No alkylation occured at pH 2.4.

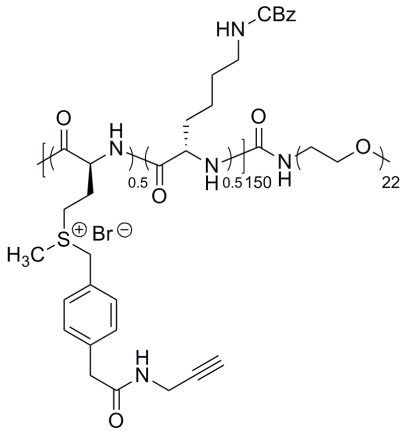
Experiments to Check for Chain Cleavage Resulting from Alkylation or Dealkylation Reactions:



Poly[(N_ε-carbobenzyloxy-L-lysine)$_{0.5}$-*stat*-(L-methionine)$_{0.5}$]$_{150}$-*block*-poly(ethylene glycol)$_{22}$, 8

Inside a dinitrogen filled glove box, a solution of N_ε-carbobenzyloxy-L-lysine-N-carboxyanhydride[7] (Cbz-Lys NCA), (25 mg, 0.082 mmol, 1 eq) and Met NCA (14 mg, 0.082 mmol, 1.0 eq) in dry THF (0.15 M) was prepared. A solution of $(PMe_3)_4Co$ in dry THF (20 mM) was rapidly added via syringe (1.5 mg, 4.1 µmol, 0.025 eq). After 45 min, the polymerization reaction was complete as determined by FTIR. An aliquot was removed and analyzed by GPC/LS. A solution of α-methoxy-ω-isocyanoethyl-poly(ethylene glycol)[1] (PEG-NCO, MW= 2,000) in THF (12 mg, 0.012 mmol, 3.0 eq per $(PMe_3)_4Co$) was added to the polymerization reaction. The reaction immediately turned from pale orange to green and was stirred overnight at

room temperature. The reaction was then removed from the glove box and HCl (6 M in H_2O, 2.0 eq per $(PMe_3)_4Co$) was added to the solution, which turned a blue-green color. After 10 min stirring, the PEG-endcapped copolypeptide was collected by precipitation into water (pH 3, HCl, > 10x the reaction volume), followed by centrifugation. The white solids were washed with 3 portions of DI water to remove all unconjugated PEG-NCO, collected by centrifugation, and lyophilized to give 44 mg of a white solid (99% yield). Since it has been shown that end-capping is quantitative for $(PMe_3)_4Co$ initiated NCA polymerizations when excess isocyanate is used[8], integrations of copolypeptide resonances versus the polyethylene glycol resonance at δ 3.64 could be used to obtain copolypeptide lengths. M_n = 29,490, M_w/M_n = 1.14, DP = 150. ^{1}H NMR (500 MHz, $CDCl_3$ with 1% *d*-TFA, 25 ^{o}C): δ 8.16 (br s, 2H), 7.31 (s, 5H), 5.11 (s, 2H), 4.19 (s, 1H), 3.95 (s, 1H), 3.75 (s, 1.18), 3.14 (s, 2H), 2.74-2.47 (m, 2H), 2.31-1.77 (m, 7 H), 1.60-1.29 (br m, 4H). (see Figure S8).



Poly[(N_ε-carbobenzyloxy-L-lysine)$_{0.5}$-*stat*-(S-(4-(*N*-propargyl-acetamido)phenylmethyl)-L-methionine sulfonium bromide)$_{0.5}$]$_{150}$-*block*-poly(ethylene glycol)$_{22}$, 9

8 (44.1 mg, 1.45 μmol) was dissolved in DMF (3 mL). 4-Bromomethyl-*N*-propargyl-phenylacetamide **2g** (28.9 mg, 0.109 mmol, 2.00 eq per methionine residue) was added. The reaction mixture was covered with foil and stirred at room temperature for 48 hours. Diethyl ether was added (20 mL) and the white precipitate was collected by centrifugation. The precipitate was washed with 2 portions of dichloromethane and then dried under high vacuum to give 77.4 mg (99%). A solution of the alkylated copolypeptide was prepared in 0.1M LiBr in DMF (5 mg/mL) and analyzed by GPC/LS. ^{1}H NMR showed no change in the ratio of PEG to polypeptide. M_n = 53,750; M_w/M_n =1.18. (see Figure S8).
^{1}H NMR (500 MHz, d-TFA, 25 ^{o}C): δ 7.59-7.35 (m, 4H), 7.26 (s, 5H), 6.78 (br s, 1H), 5.15 (s, 2H), 4.80 (s, 1H), 4.65 (s, 2H), 4.52 (s, 1H), 4.15-3.98 (m, 3H), 3.86 (s, 4.02), 3.71 (s, 2H), 3.19 (s, 2H), 3.00 (s, 2H), 2.83-2.55 (m, 2H), 2.25-2.0 (m, 4H), 1.99-1.68 (m, 4H), 1.67-1.30 (m, 4H).

Dealkylation of Sulfonium Groups in 9

9 (77.4 mg, 1.45 μmol) was dissolved in DMF (3 mL). 2-Mercaptopyridine (12.0 mg, 0.109 mmol, 2.00 eq per methionine residue) was added. The reaction mixture was stirred at room temperature for 24 hours. Diethyl ether was added (20 mL) and the white precipitate was collected by centrifugation. The precipitate was washed with 2 more portions of diethyl ether and then dried under high vacuum to give the product 43.9 mg (98%). A solution of the

copolypeptide was prepared in 0.1M LiBr in DMF (5 mg/mL) and analyzed by GPC/LS. GPC/LS and ^{1}H NMR spectra were identical to the parent copolypeptide **8**. (see Figure S8).

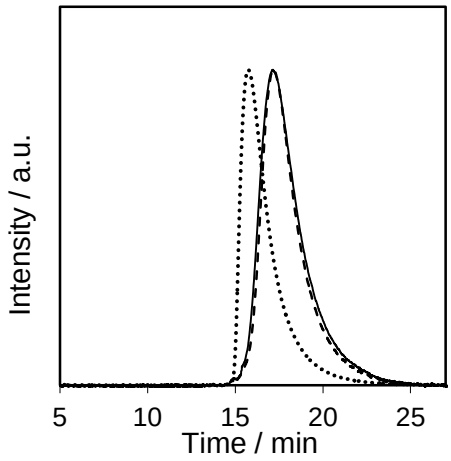


Figure S8: GPC chromatograms (normalized LS intensity versus elution time in arbitrary units (au)) of copolypeptides after initial copolymerization of Met and CBz-Lys NCAs and endcapping with PEG-NCO to give **8** (——), alkylation with **2g** to give polysulfonium **9** (·····), and after dealkylation of the sulfonium groups using mercaptopyridine to regenerate the parent **8** (- - - -).

Reactivity of 2-Mercaptopyridine with Disulfide Bonds

2-Mercaptopyridine (66.7 mg, 0.600 mmol, 3.00 eq) was added to a solution of cystine (48.0 mg, 0.200 mmol, 1.00 eq) in DI water (5 mL). The solution was stirred for 24 hours at 37 ^{o}C. Water was added (3 mL) and the aqueous solution was extracted with EtOAc (3 x 5 mL). The aqueous phase was lyophilized to dryness and analyzed by ^{1}H NMR and ^{13}C NMR. Spectral data was identical to that of an authentic sample of cystine, no disulfide reduction was observed.

Stability of Polysulfoniums:

PBS Buffer:

3c and **3g** were dissolved in PBS buffer (10 mg/mL) and were maintained at room temperature for 2 weeks. Samples were then transferred to 2000 MWCO dialysis tubing, dialyzed against DI water for 16 hours, then lyophilized to dryness. ^{1}H NMR spectra were identical to spectra of the parent copolypeptides. Homopolymers of (*S*-methyl-L-methionine sulfonium chloride) and (*S*-carboxymethyl-L-methionine sulfonium chloride) were previously shown to be stable in water, DMF, PBS buffer, or DMEM cell culture media for >1 week at room temperature.[1]

Storage as Dry Solids:

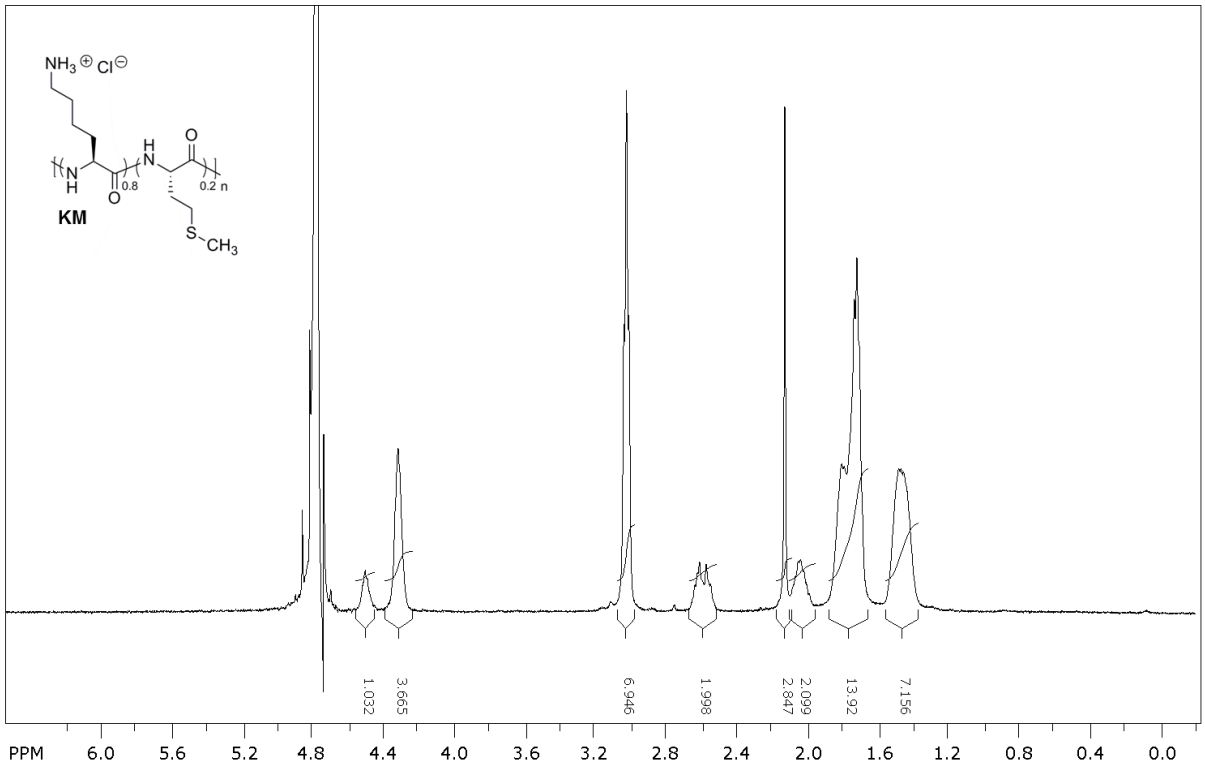
All polysulfoniums described were found to be stable for > 6 months when stored as dry powders at room temperature, with the exception of **3d**. After *ca*. 4 weeks of storage as a dry solid copolypeptide **3d** becomes very difficult to redissolve in previously good solvents (water, TFA).

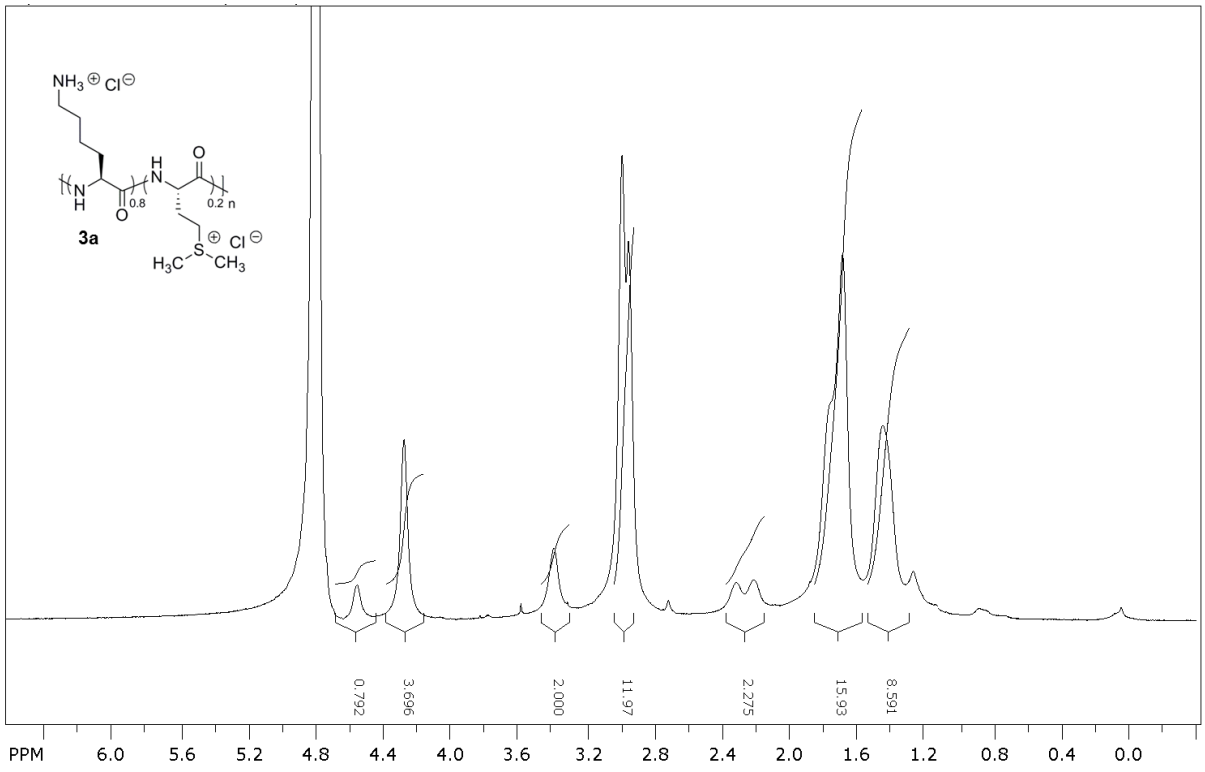
Base:

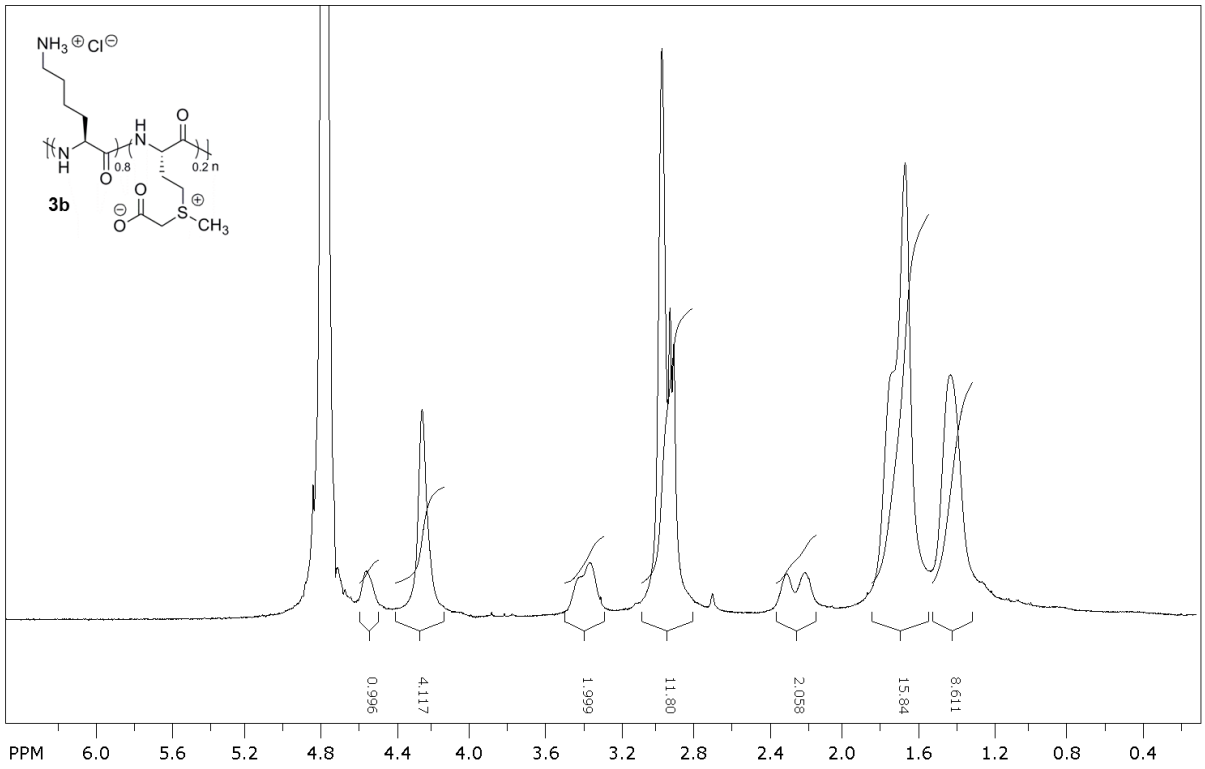
Water was made basic to pH 10.1 by addition of drops of 0.5 M NaOH. **3c, 3d,** and **3g** were each dissolved in pH 10.1 water at a concentration of 1 mg/mL. The solutions were allowed to stand

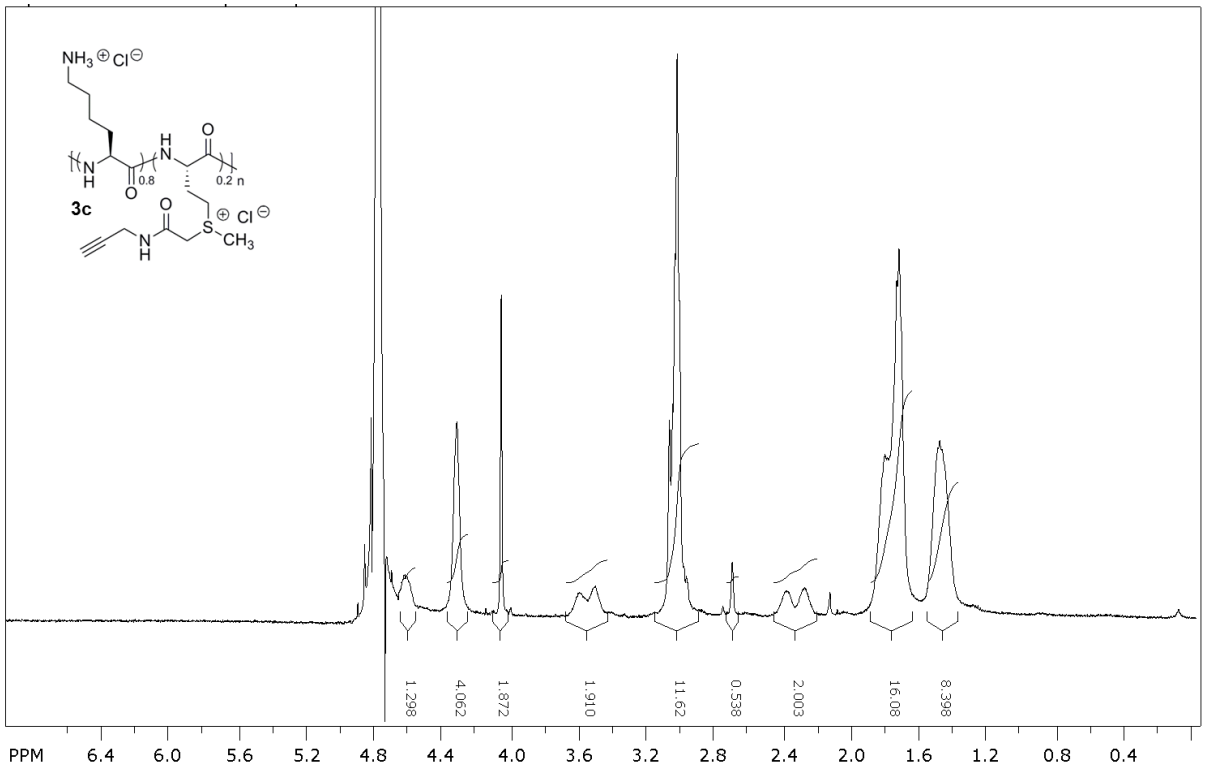
at room temperature for 10 hours and then transferred to 2000 MWCO dialysis tubing. The solutions were then dialyzed against DI water for 16 hours, and then the contents of the dialysis tubing were lyophilized to dryness. ^{1}H NMR spectra of **3c,** and **3g** before and after treatment with base were identical. ^{1}H NMR spectra of **3d** showed complete dealkylation to give the parent **KM**. Treatment of **3d** with aqueous bicarbonate at pH 9.8 also resulted in complete dealkylation to give the parent **KM**. Homopolymers of (*S*-methyl-L-methionine sulfonium chloride) and (*S*-carboxymethyl-L-methionine sulfonium chloride) were previously shown to be stable in water at pH 10 (NaOH) for >3 hours.[1]

Spectral Data:





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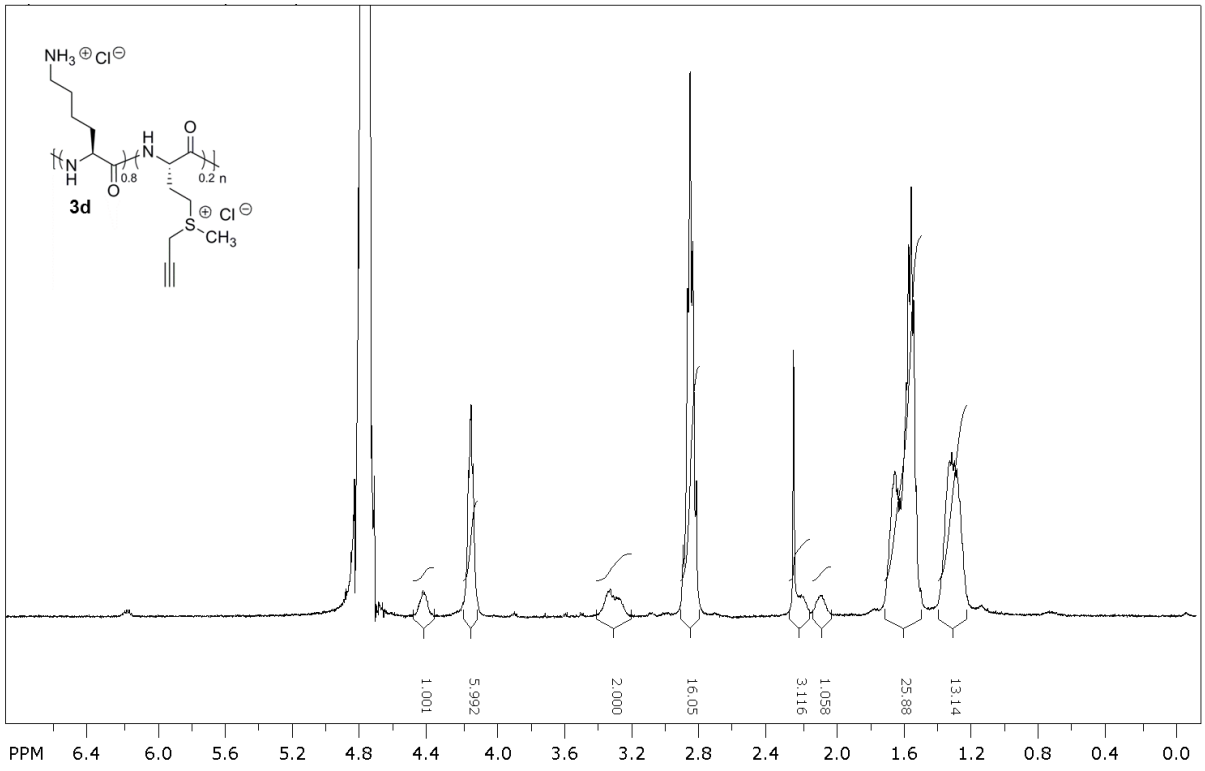
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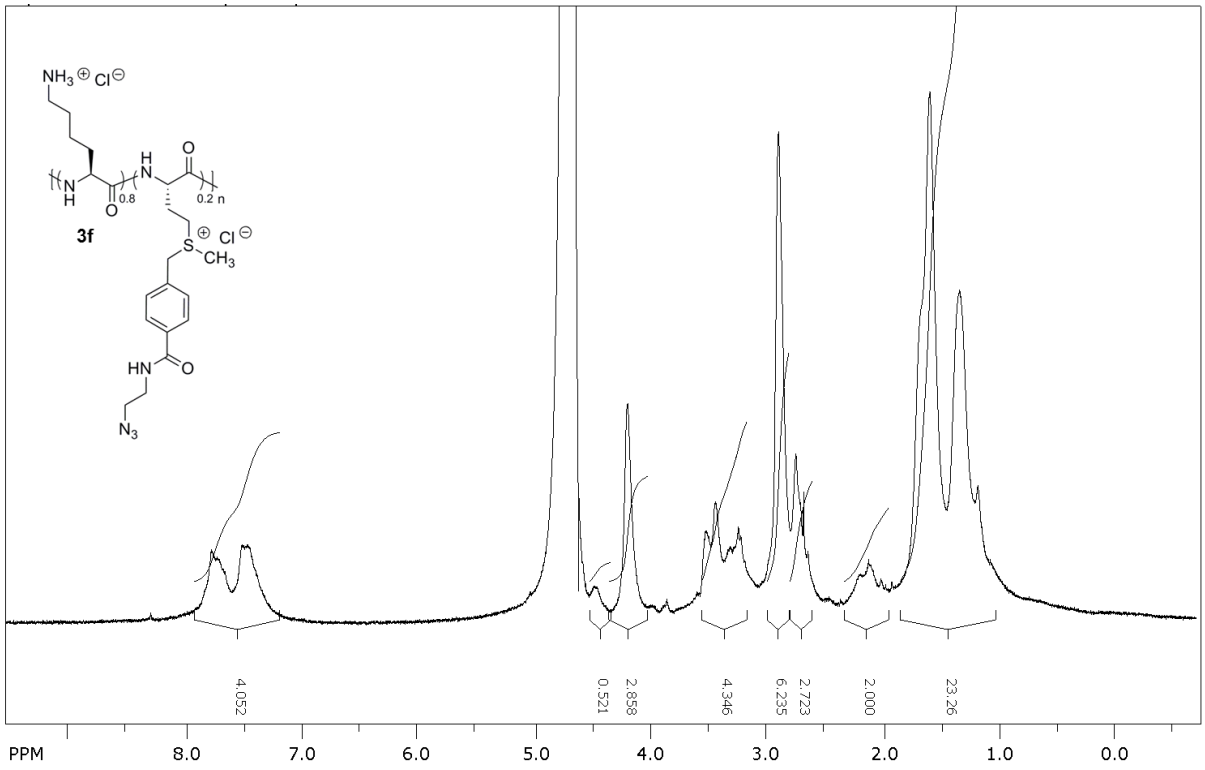
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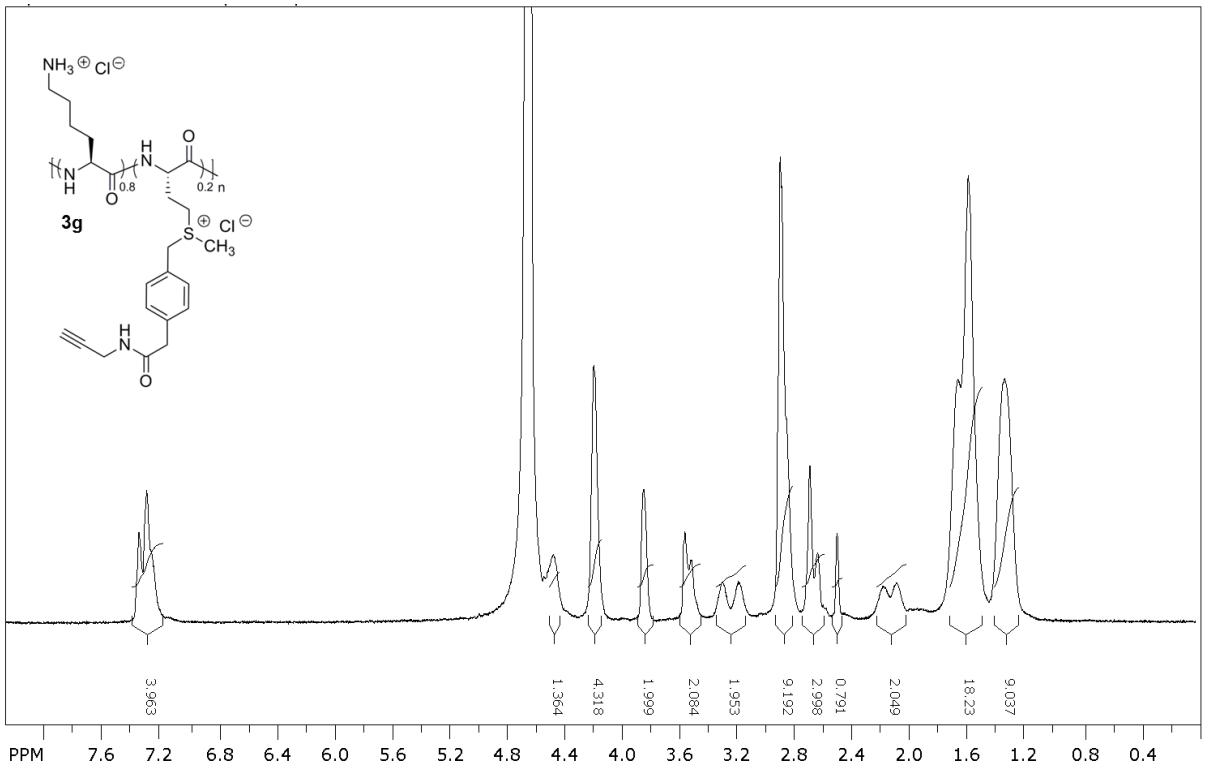
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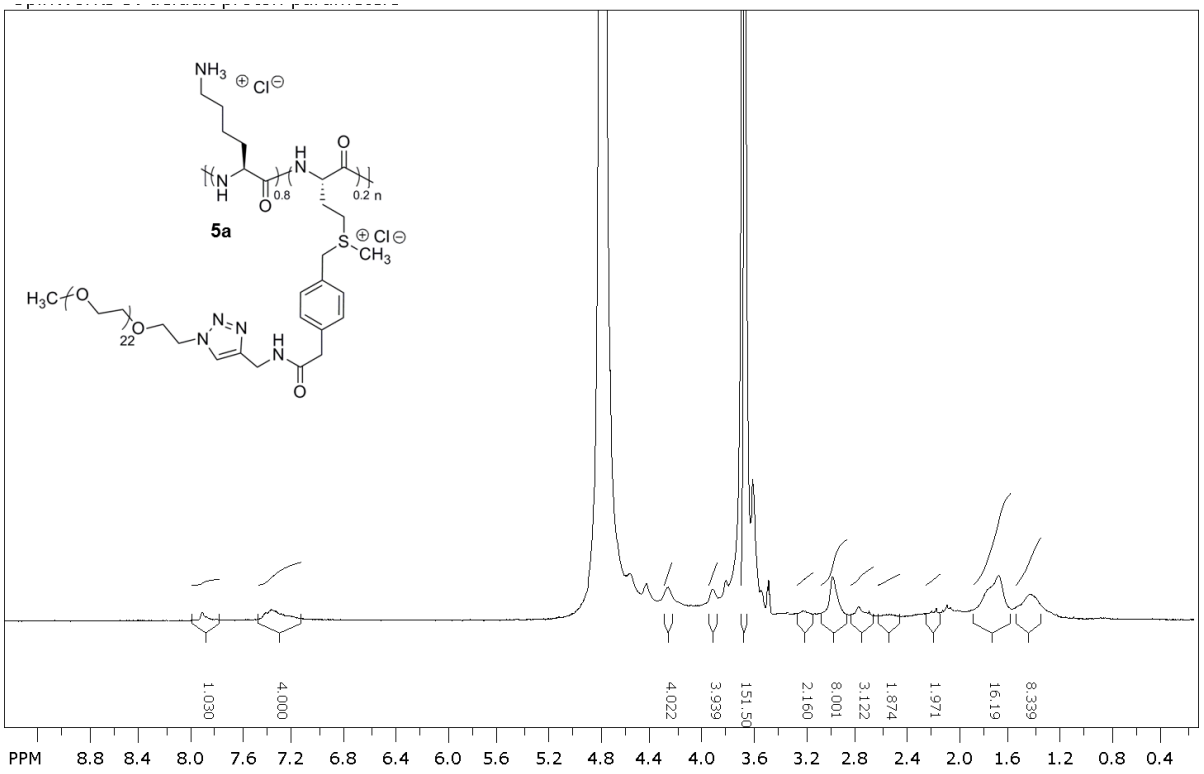
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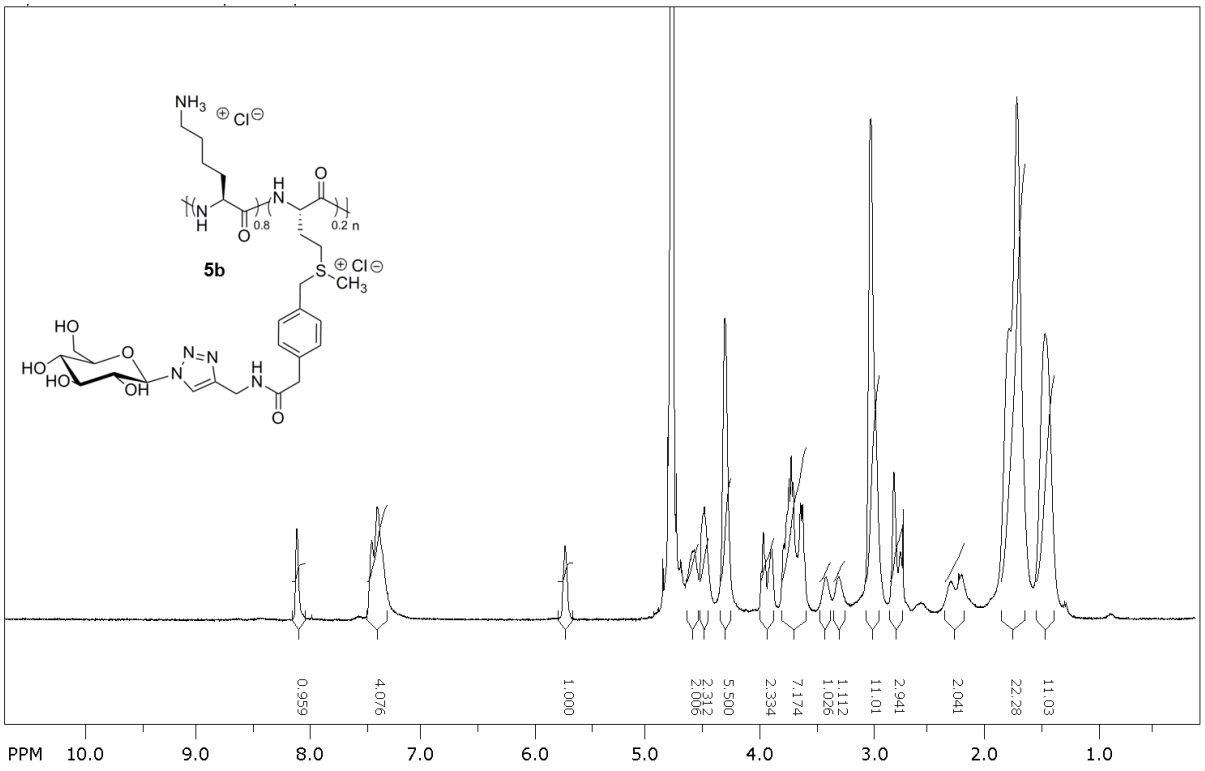
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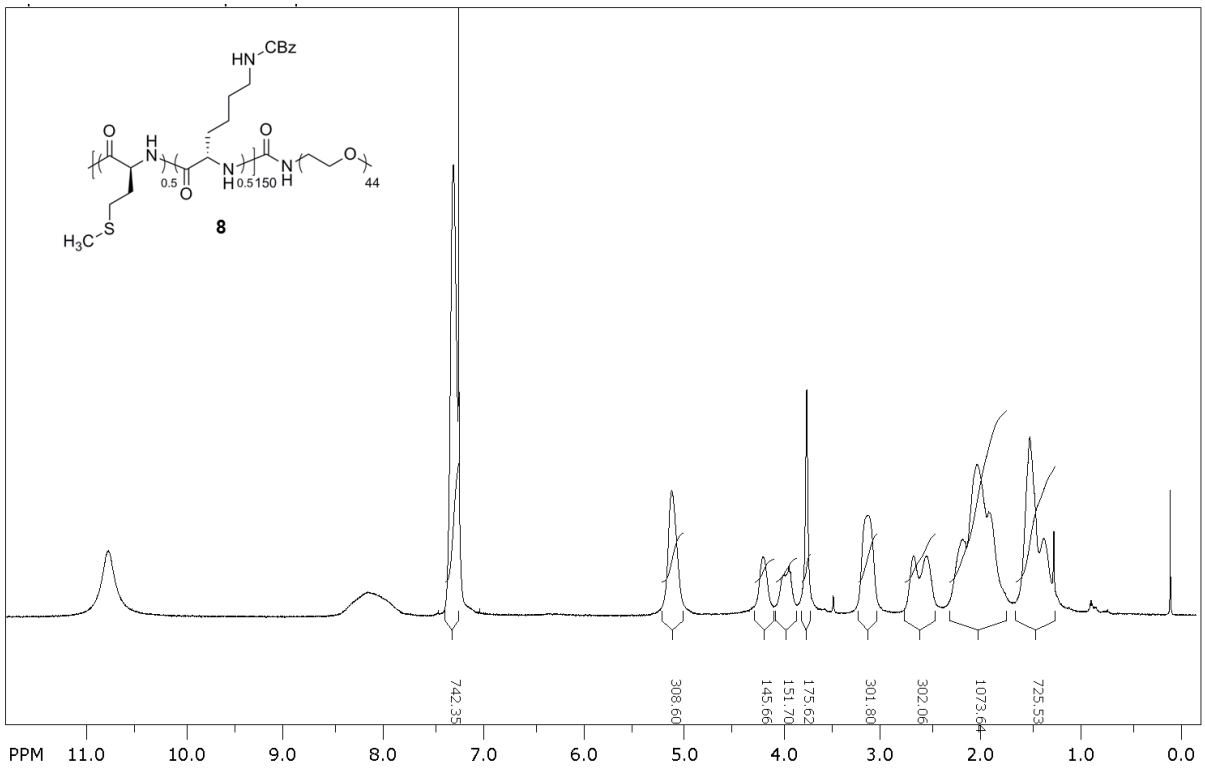
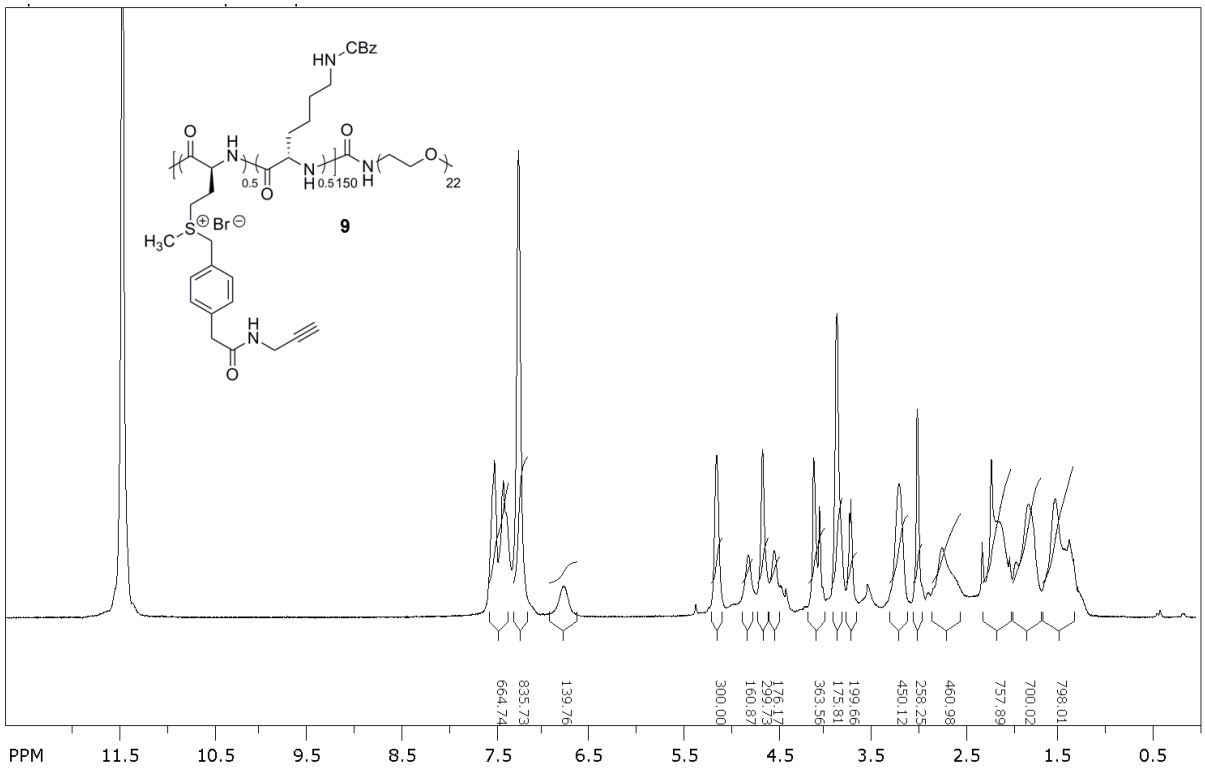
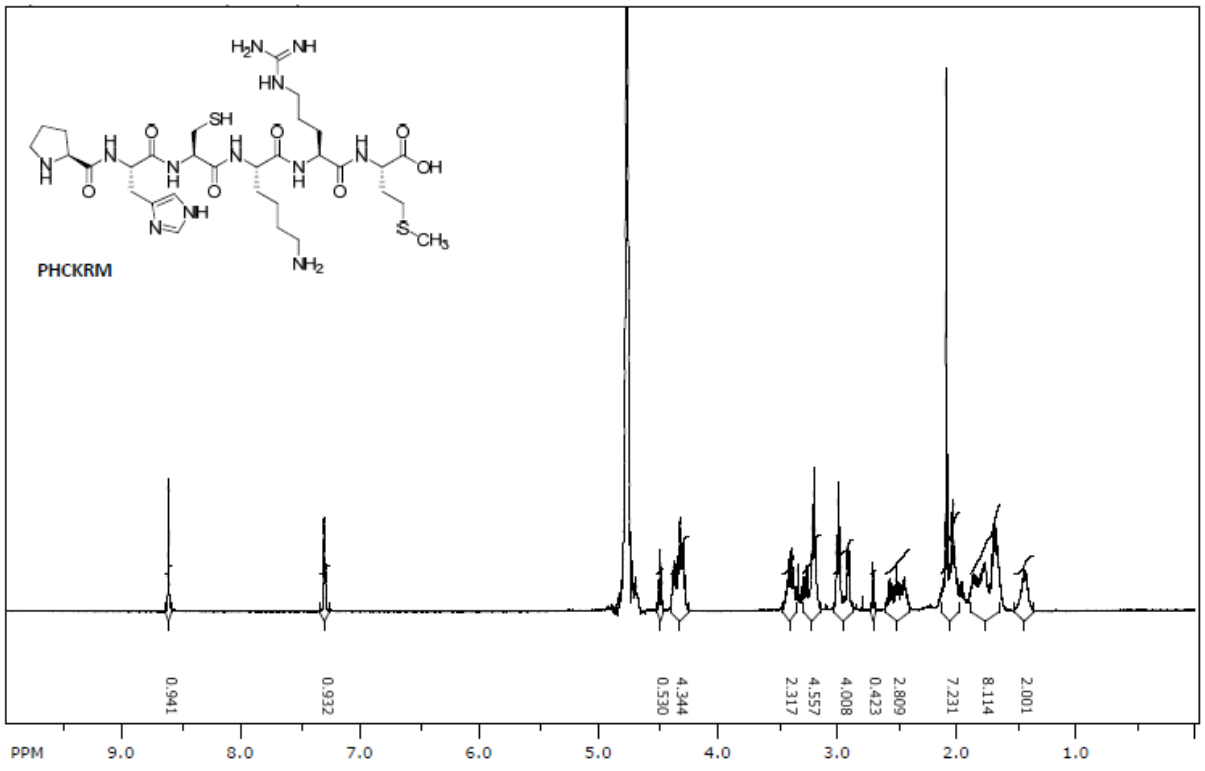
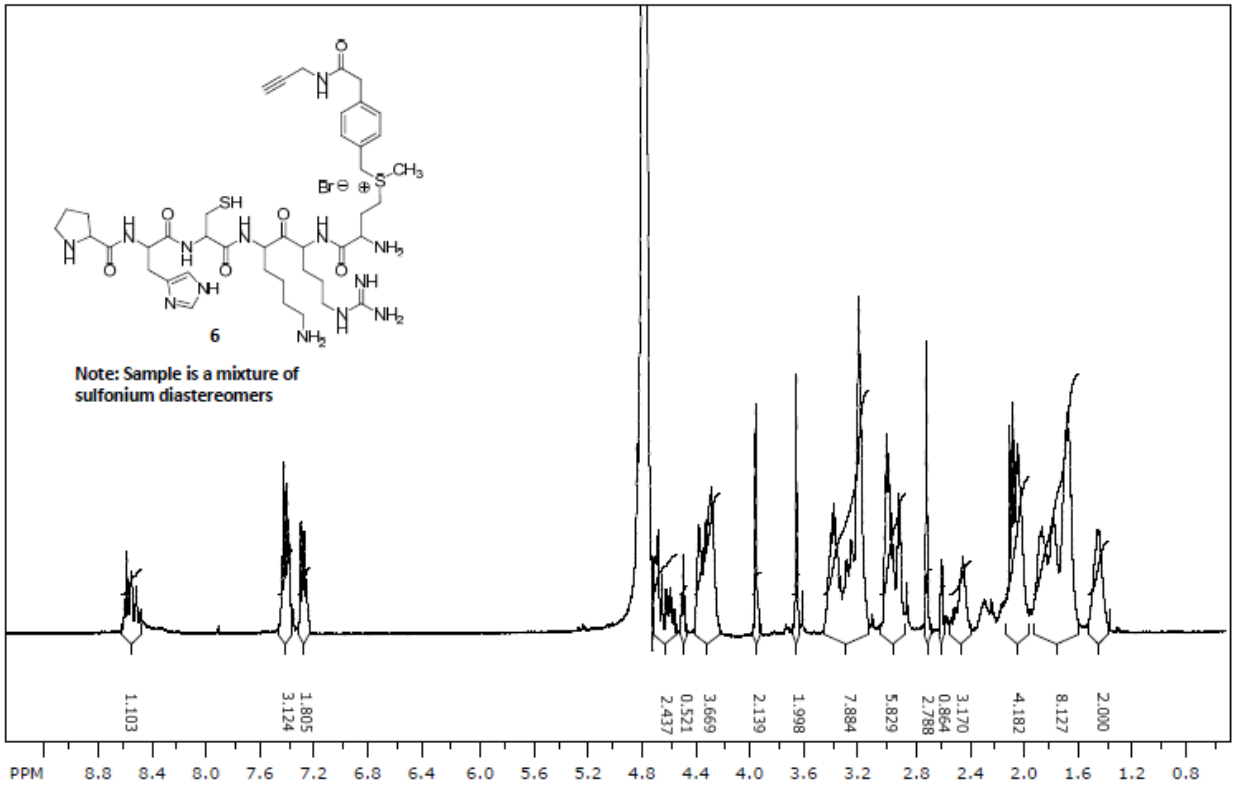
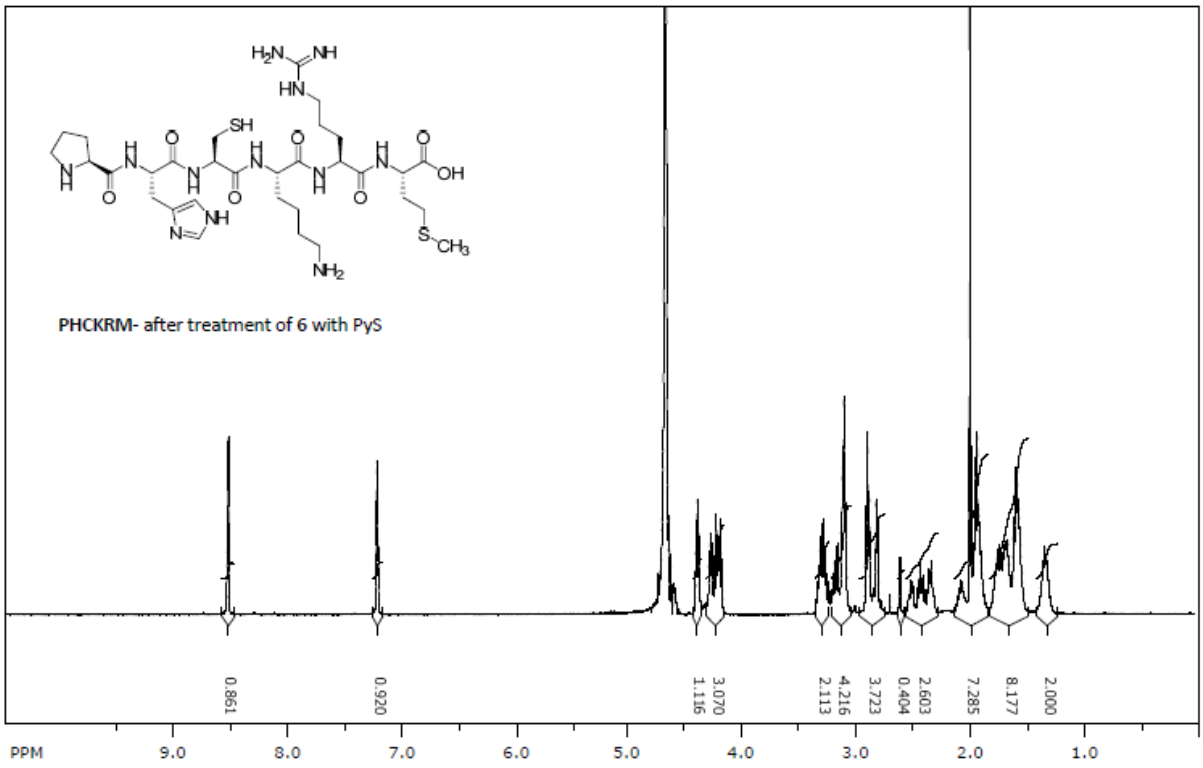
11.03
PPM 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0

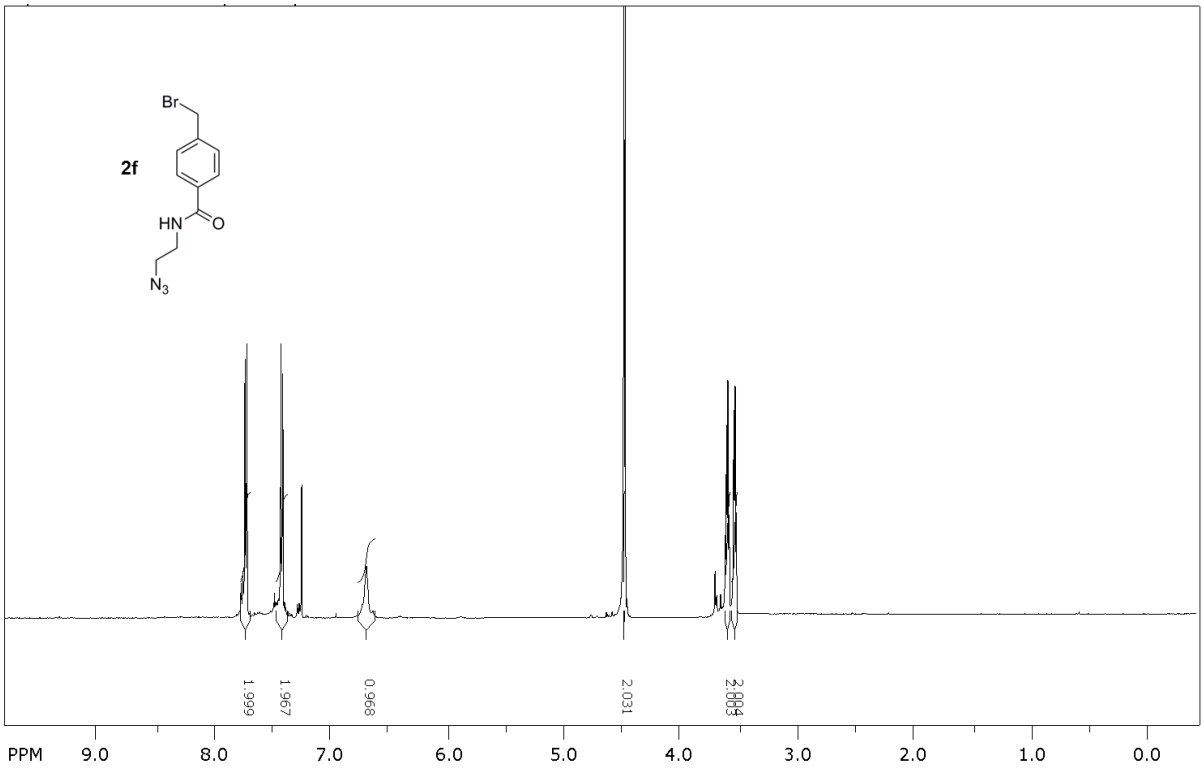

HN
CBz
O
H
N
0.5
O
N
H
0.5
150
O
N
H
O
44
H3C
S
8
742.35
308.60
145.66
151.70
175.62
301.80
302.06
1073.64
725.53
PPM
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

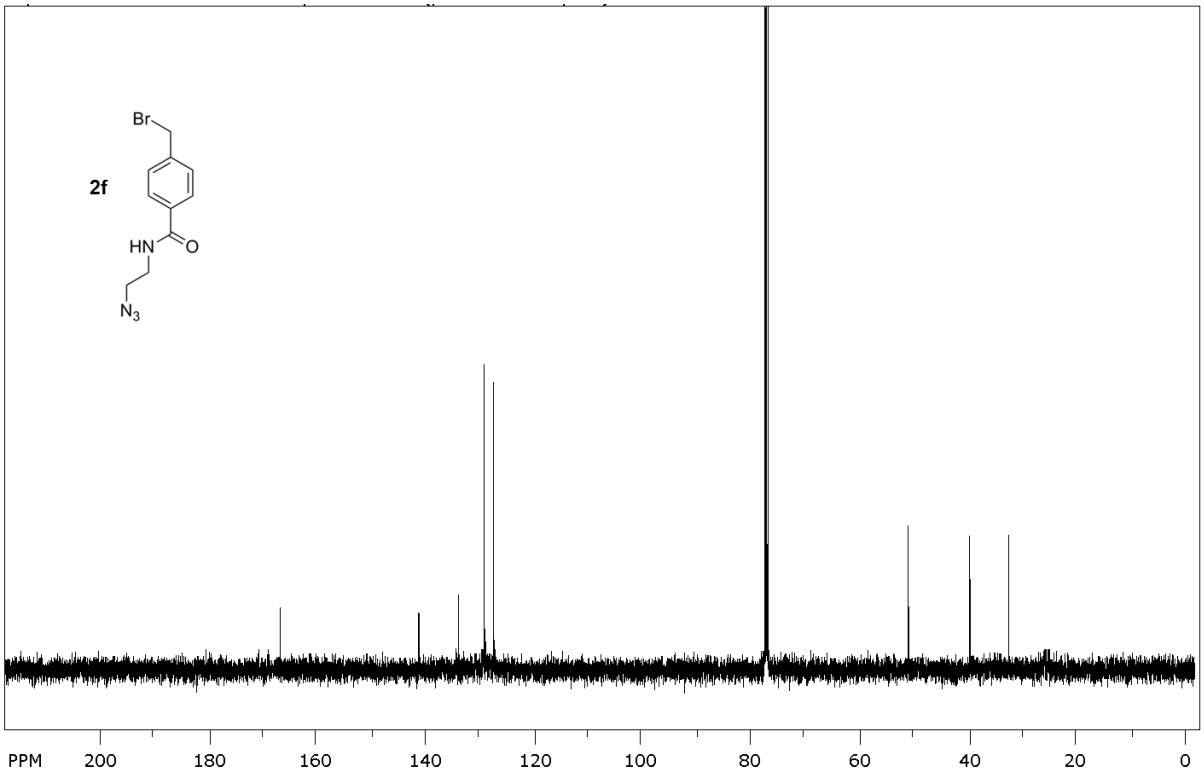

HN
CBz
O
H
N
0.5
O
N
H
0.5
150
O
N
H
O
22
H3C
S
Br
9
H
N
O
664.74
835.73
139.76
300.00
176.17
299.73
160.87
363.56
175.81
199.66
450.12
258.25
460.98
757.89
700.02
798.01
PPM
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5

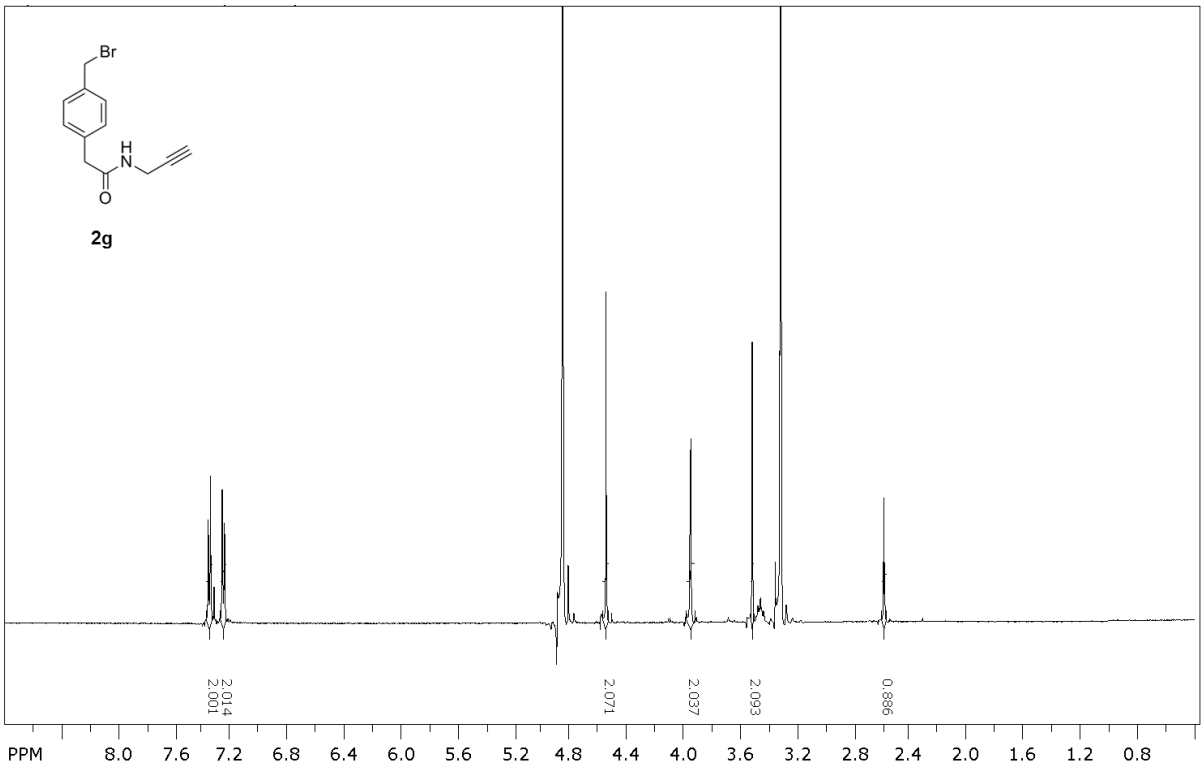

PHCKRM
0.941
0.932
0.530
4.344
2.317
4.557
4.008
0.423
2.809
7.231
8.114
2.001
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

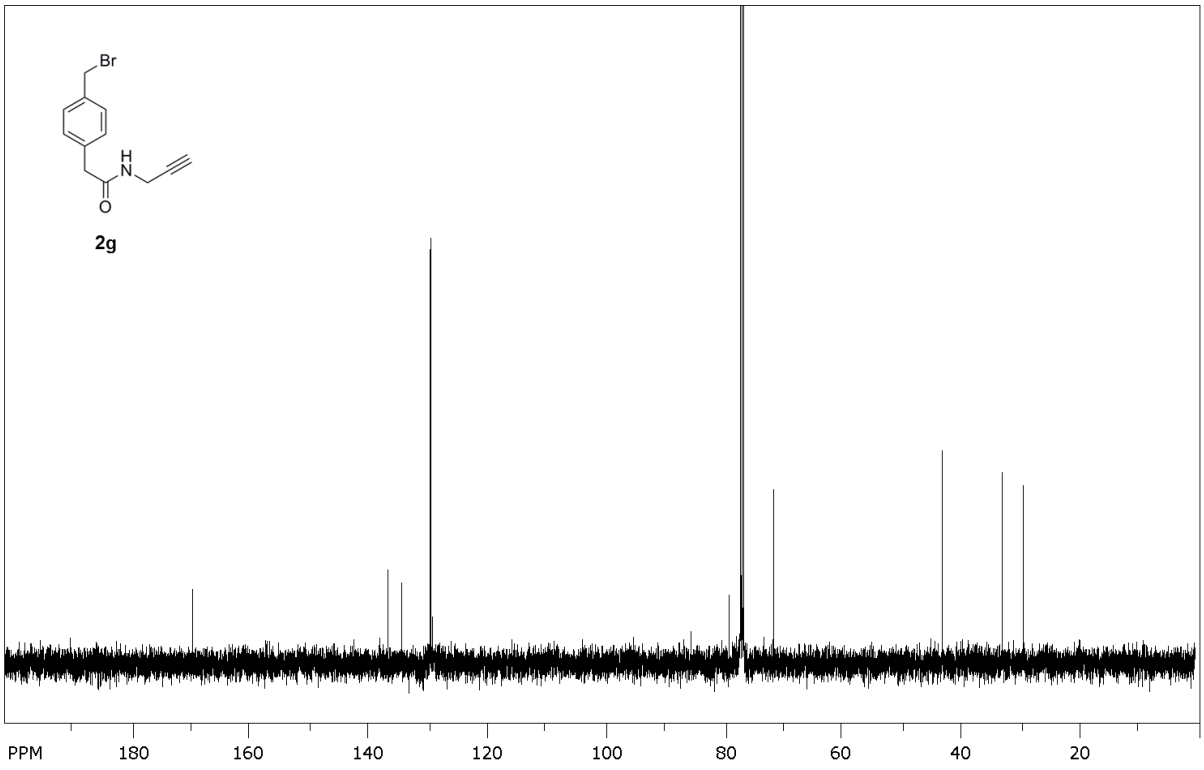

6
Note: Sample is a mixture of
sulfonium diastereomers
1.103
3.124
1.805
2.437
0.521
3.669
2.139
1.998
7.884
5.829
2.788
0.864
3.170
4.182
8.127
2.000
PPM
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8

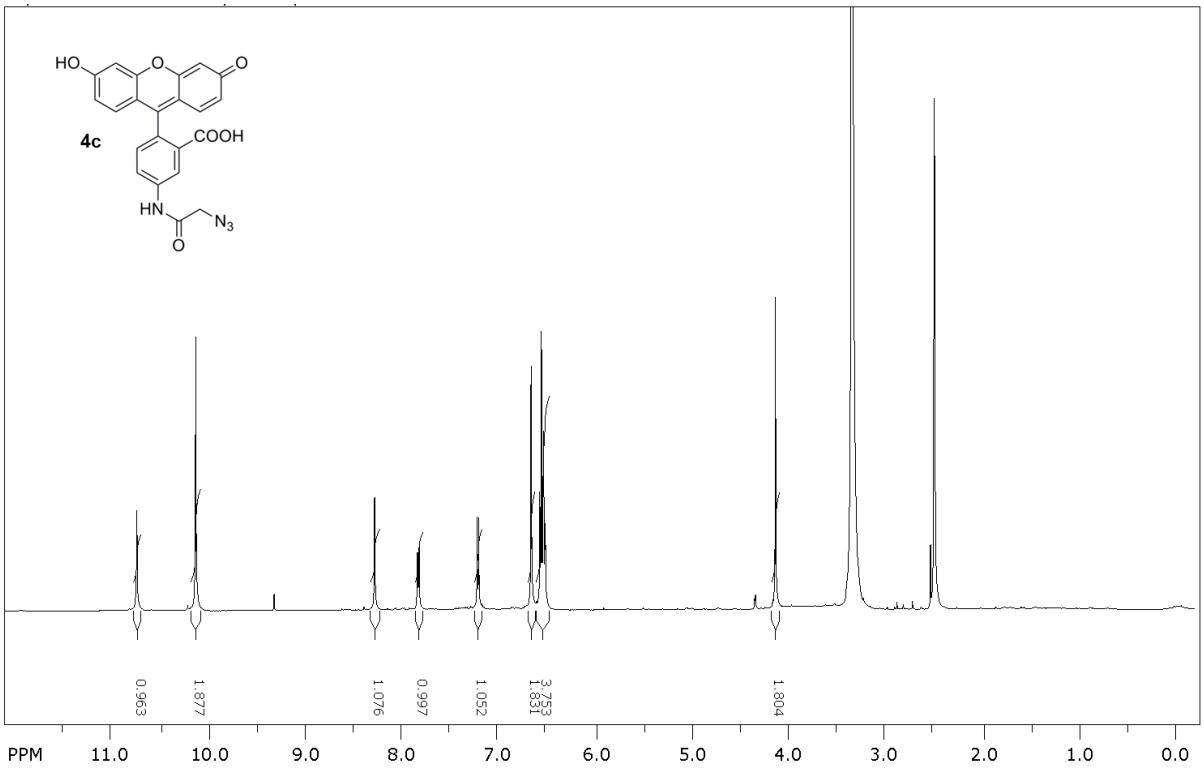

PHCKRM- after treatment of 6 with PyS
0.861
0.920
1.116
3.070
2.113
4.216
3.723
0.404
2.603
7.285
8.177
2.000
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

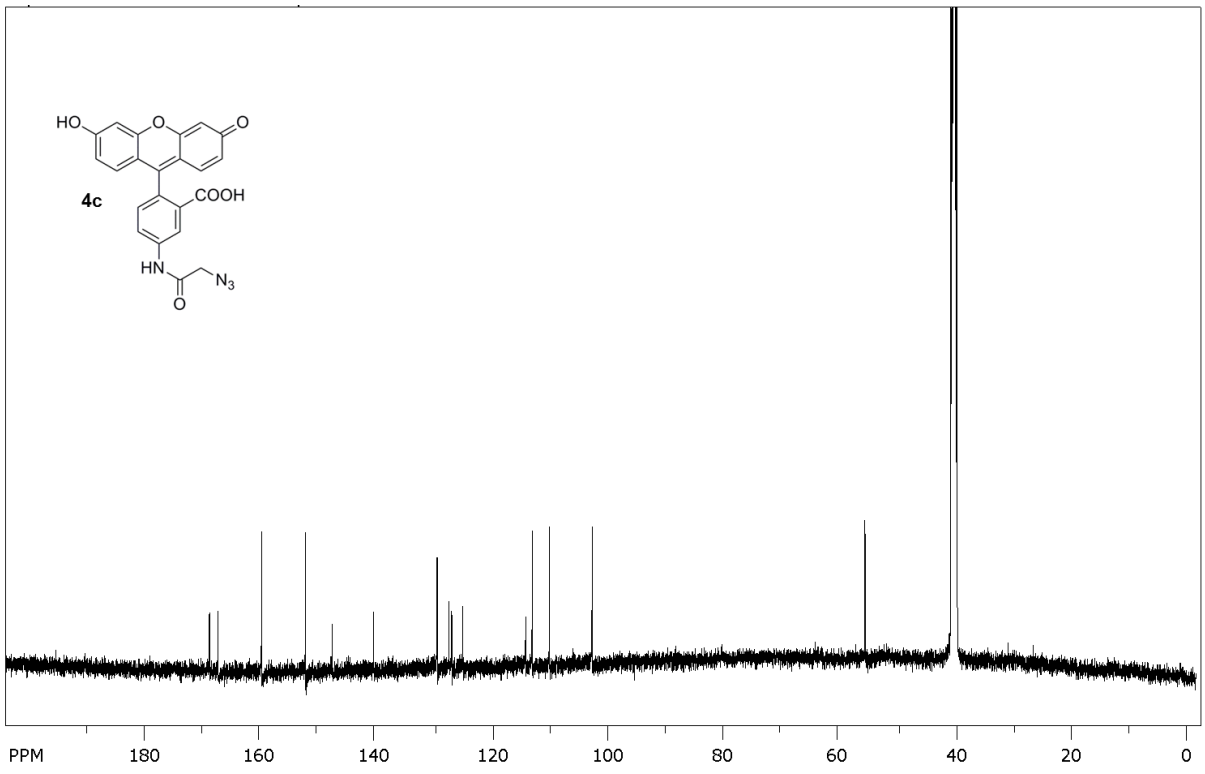

2f
1.999
1.967
0.968
2.031
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0

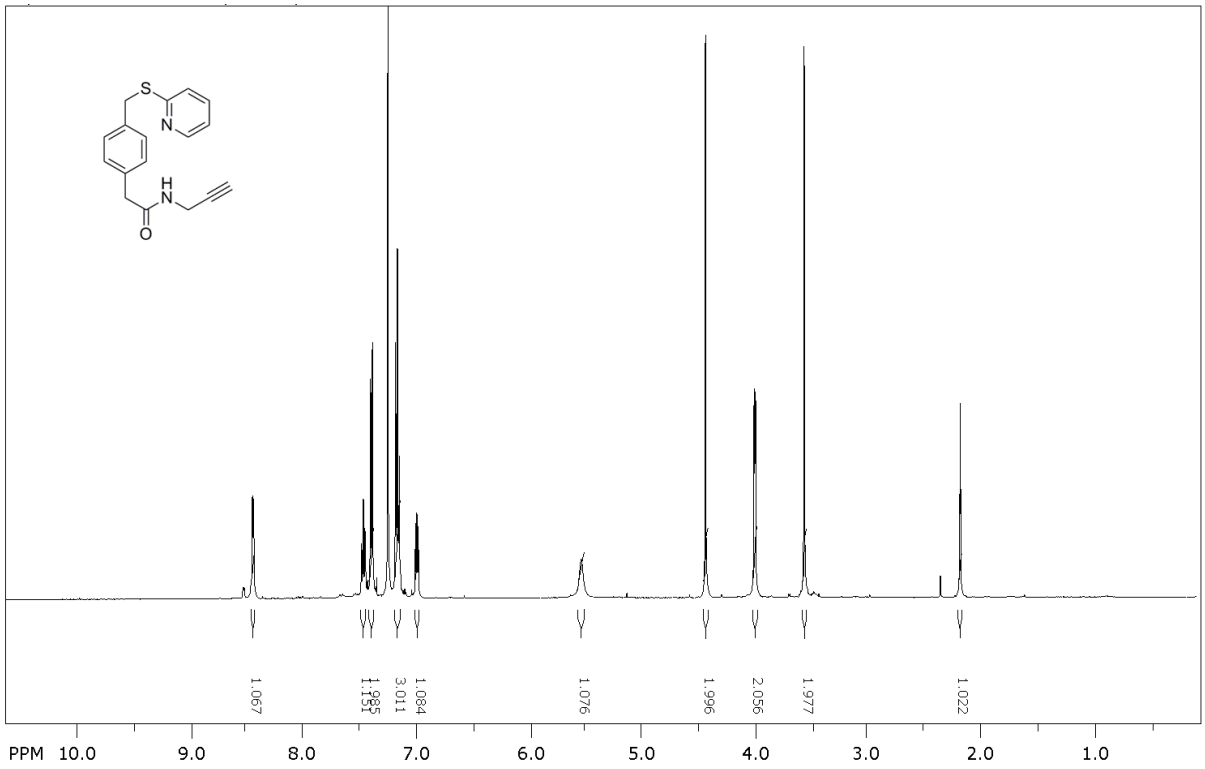

Br
2f
HN
O
N3
PPM
200
180
160
140
120
100
80
60
40
20
0

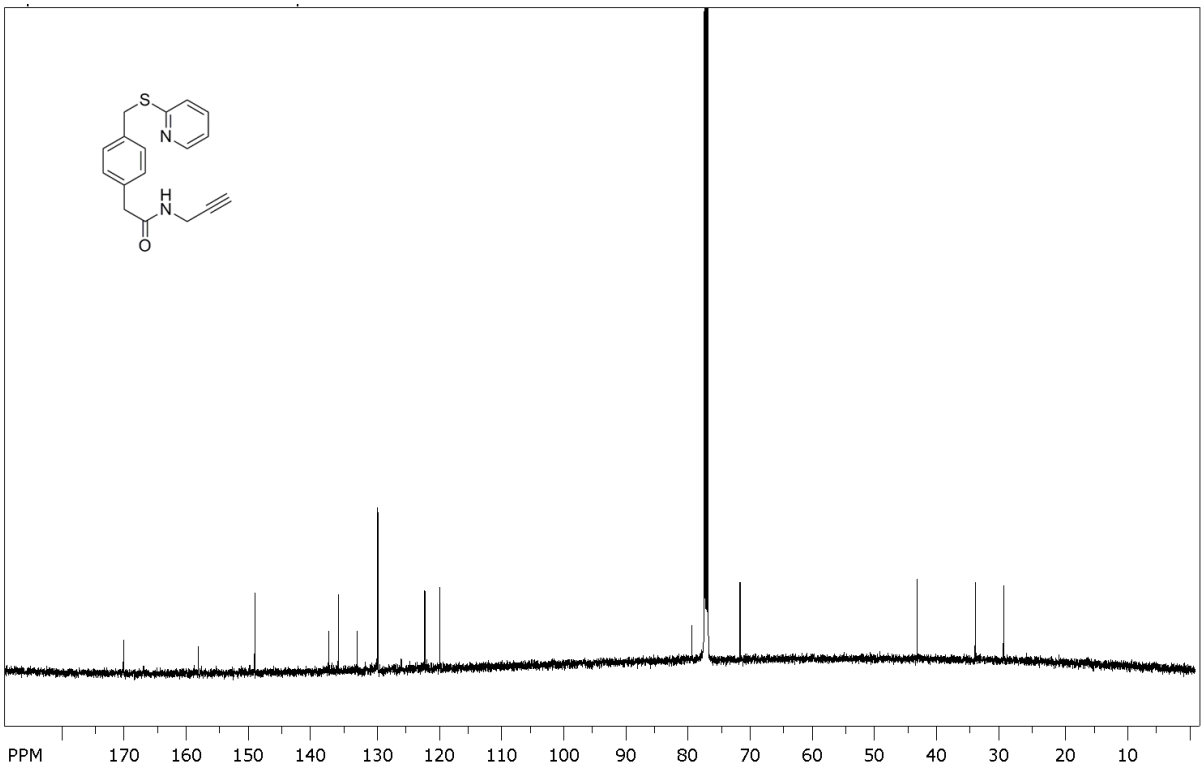

Br
H
N
O
2g
2.014
2.001
2.071
2.037
2.093
0.886
PPM
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8


Br
H
N
O
2g
PPM
180
160
140
120
100
80
60
40
20


HO
O
O
COOH
4c
HN
N3
O
0.963
1.877
1.076
0.997
1.052
3.753
1.831
1.804
PPM
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


HO
O
O
COOH
4c
HN
O
N3
PPM
180
160
140
120
100
80
60
40
20
0


S
N
H
N
O
1.067
1.151
1.985
3.011
1.084
1.076
1.996
2.056
1.977
1.022
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0



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