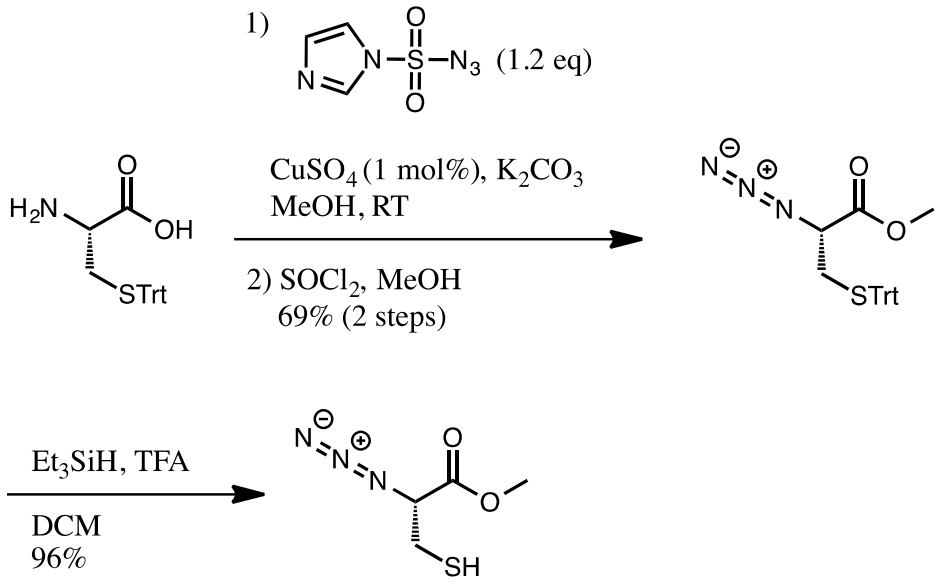
New Methods for the Site-Selective Placement of Peptides on a Microelectrode Array: Probing VEGF – v107 Binding as Proof of Concept

Matthew D. Graaf,[a] Bernadette V. Marquez,[b] Nai-Hua Yeh,[a] Suzanne E. Lapi,[a,b]* and Kevin D. Moeller[a]*

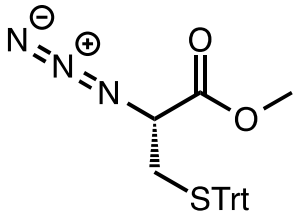
[a]Department of Chemistry, Washington University in St. Louis, St. Louis, MO, USA 63130
[b]Department of Radiology, Washington University in St. Louis, St. Louis, MO, USA 63110

SUPPORTING INFORMATION

Synthesis of azido-cysteine-methylester:



N_3-Cys(Trt)-OMe

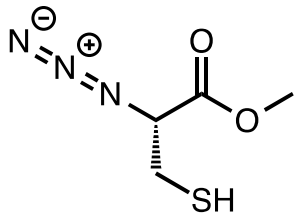


In a 25 mL, flame-dried round bottom flask, 726 mg (2.0 mmol) H-Cys(Trt)-OH, 5 mg (0.020 mmol) $CuSO_4$, and 552 mg (4 mmol) K_2CO_3 were dissolved in 10 mL anhydrous methanol. The imidazole-

sulfonyl-azide (HCL) diazotransfer agent (0.5 g, 2.4 mmol) was slowly added to the solution and allowed to stir overnight while open to the atmosphere. Upon completion, the methanol was removed and the crude mixture was suspended in H_2O before being acidified with concentrated HCl. The acidified mixture was extracted with ethyl acetate. All organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*.

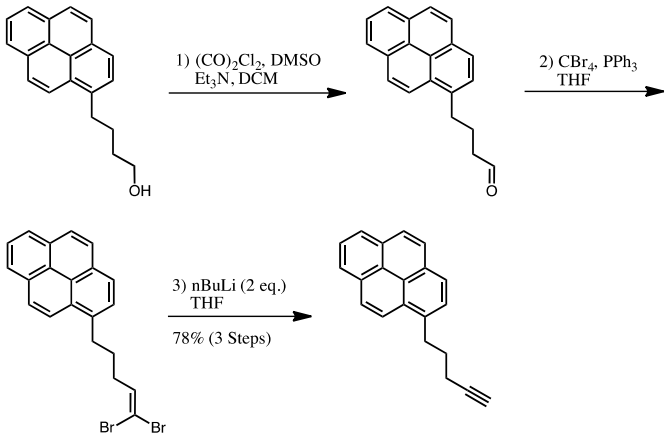
Esterification of the azido-cysteine was performed without further purification. The mixture was dissolved in 5.0 mL methanol and 0.05 mL thionyl chloride was added to the solution, being allowed to stir overnight. The crude mixture was purified via column chromatography using 9:1 hexanes:ethyl ether obtaining the product in a 69% yield (558 mg, MW = 403.5 g/mol). ^{1}H-NMR (300 MHz; $CDCl_3$): δ7.47-7.43 (m, 6H), 7.33-7.21 (m, 9H), 3.71 (s, 3H), 3.22 (dd, *J* = 8.1, 5.9 Hz, 1H), 2.71 (dd, *J* = 13.4, 5.9 Hz, 1H), 2.60-2.53 (dd, *J* = 13.4, 8.1 Hz, 1H); ^{13}C NMR (75 MHz; $CDCl_3$): δ169.3, 144.2, 129.5, 128.1, 126.9, 67.3, 61.3, 52.7, 33.2; IR (KBr) 3467, 3055, 2950, 2505, 2107, 1745, 1593, 1487, 1443, 1206, 740 cm^{-1}; HRESI MS m/z [M+Na$^+$] Found: 426.1244, Calculated: 426.1254

N_3-Cys-OMe



The trityl deprotection of the azido-cysteine was in a flamed-dried 25 mL round bottom flask. 530 mg N_3-Cys(Trt)-OMe (1.31 mmol) was dissolved in 5 mL dichloromethane. 0.5 mL TFA and 0.3 mL Et_3SiH were added to the flask and allowed to stir overnight. Upon completion, the mixture was diluted with DCM and concentrated *in vacuo*. The crude mixture was purified via column chromatography using 9:1 hexanes:ethyl ether. The deprotected product was obtained in a 96% yield (203 mg, MW = 161.18 g/mol). ^{1}H-NMR (300 MHz; $CDCl_3$): δ4.25 (dd, *J* = 8.0, 5.3 Hz, 1H), 3.82-3.81 (s, 3H), 3.21 (dd, *J* = 14.0, 5.3 Hz, 1H), 3.00 (dd, *J* = 14.0, 8.1 Hz, 1H), 1.25-1.23 (s, 1H); ^{13}C NMR (126 MHz; $CDCl_3$): δ 164.1, 55.8, 47.9, 34.8 ppm; IR (KBr) 3365, 2952, 2920, 2493, 2115, 1741, 1435, 1246, 1205, 1004 cm^{-1}; HRESI MS m/z [M+Na$^+$] Found: 184.0172, Calculated: 184.0159

Synthesis of 1-(pent-4-yn-1-yl)pyrene:



In a flame-dried, 50 mL round bottom flask, 0.11 mL oxalyl chloride was dissolved in 3 mL dichloromethane and cooled to -78°C. 0.2 mL dimethylsulfoxide was added to the flask and stirred for two

minutes. 1-pyrenebutanol (300 mg , 1.09 mmol) was dissolved in 2 mL DCM and added slowly to the reaction solution and stirred for 15 minutes. 1 mL triethylamine was added and stirred for 5 minutes before warming to room temperature. The solution was quenched with water and extracted with DCM. The organic layers were dried over $MgSO_4$, filtered and rotavapped. No further purification was performed.

In a flame-dried, 50 mL round bottom flask, 362 mg carbon tetrabromide and 571 mg triphenylphosphine were dissolved in 10 mL DCM. The crude aldehyde from the previous Swern oxidation was dissolved in DCM, slowly added to the reaction solution and stirred for one hour. Upon completion, the reaction was quenched with water and extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. No further purification was performed.

The final elimination/dehalogenation step was performed on the Wittig crude. The dibromoalkene product was dissolved in 10 mL THF. Two equivalents of nBuLi (1.6M solution in hexanes) was slowly added to the flask and stirred for 10 minutes. The reaction was quenched with water and extracted with DCM. The organic layers were dried over $MgSO_4$, filtered and rotavapped. The product was purified via column chromatography using 95:5 hexanes:ethyl ether as the eluent. The pyrene-labeled alkyne was obtained in a 78% yield over the three steps (228 mg, MW = 268.36 g/mol). ^{1}H-NMR (300 MHz; $CDCl_3$): δ8.31 (d, J = 9.3 Hz, 1H), 8.15 (dd, J = 17.2, 7.5 Hz, 4H), 8.06-8.00 (m, 3H), 7.90 (d, J = 7.8 Hz, 1H), 3.49 (t, J = 7.6 Hz, 2H), 2.34 (td, J = 6.9, 2.5 Hz, 2H), 2.15-2.05 (m, 3H) ppm; ^{13}C NMR (75 MHz; $CDCl_3$): δ135.8, 131.4, 130.9, 130.0, 128.7, 127.51, 127.37, 127.33, 126.7, 125.8, 125.01, 124.91, 124.81, 124.75, 123.3, 84.3, 69.1, 32.2, 30.3, 18.3 ppm; IR (KBr) 3509, 3296, 3037, 2939, 2113, 1918, 1648, 1601, 1456, 1181, 637 cm^{-1}; GC-HRMS m/z [M^+] Found: 268.1217, Calculated: 268.1252.

Solution Phase Coupling Experiment (Text, Scheme 6):

A 100 mL round bottom flask was charged with 5.45 g (30 mmol) cupric acetate, 0.6 g (4 mmol) 4-vinylphenylboronic acid, 0.6 g activated 4 Å molecular sieves, 0.6 mL (4 mmole) triethylamine, and 30 mL anhydrous dichloromethane. After stirring for 1 hour, 0.22 mL (2 mmol) phenylacetylene (dissolved in 10 mL anhydrous dichloromethane) was slowly added to the flask. The solution was then stirred under air at room temperature for 48 hours. The reaction was quenched with ethylenediaminetetraacetic acid solution and then acidified to pH 3 with 1.5 N HCl. The reaction was extracted with ethyl acetate (3x 20mL). The organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude reaction was passed through a chromatography column using hexane eluent to afford a mixture of three known compounds that could not be easily separated but could be readily identified in the mixture by both proton NMR and mass spectrometry. The yield of each product was determined by integration of the H^1-NMR spectroscopy after the addition of 1,3,5-trimethoxybenzene as an internal standard. The reaction afforded 11 % yield of 4-phenylethynyl styrene, 85% yield of 1,4-diphenyldiacetylene, and 22% yield of bis(4-vinylphenyl) ether. The yields of the 4-phenylethynyl styrene and 1,4-diphenyldiacetylene were calculated based on the phenylacetylene starting material that was used as the limiting reagent. The yield of the bis(4-vinylphenyl) ether was based on the amount of vinylboronic acid used since it was derived exclusively from that substrate and water.

Homology Modeling for the Human and Murine VEGF Binding Epitopes:

Data was taken from the Protein Model Portal:
http://www.proteinmodelportal.org/?aid=getModelQualityAnalysis&sid=PMPSID-B54869D2FC55AF3961DCD7C0CCB714CA&queryfromto=0_214&ref_ac=Q00731

The structure of murine VEGF-A (UniprotKB identifier Q00731) was compared with that of human VEGF-A from the NMR studies with VEGF-v107 complex conducted by Pan et. al (PDB 1KAT). Amino acid sequences of both proteins were aligned and the sequence from 37-134 were compared. Model 1 indicates murine VEGF and 1katW indicates human VEGF. It is important to note that the v107 peptide binding epitope lies within the 37-134 amino acid sequence.

```
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1katW          -----------------------------------HEVVKFMDVYQRSYCHPIETLVDIFQEYP

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1katW          PKKD------------------------------------------------------------

querysequence  KARQLELNERTCRCDKPRR
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1katW          -------------------
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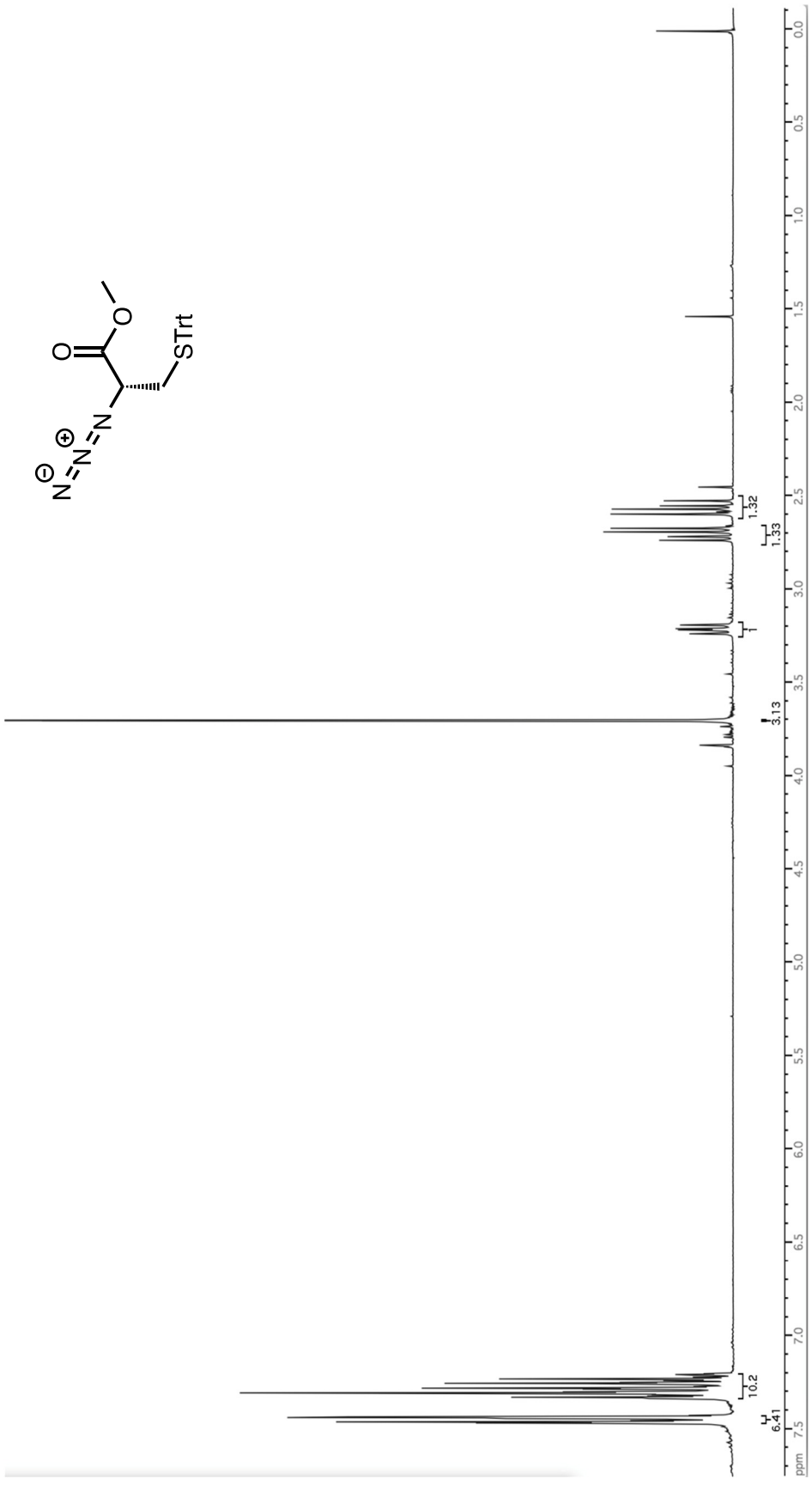
Superposition of Human and Murine VEGF Structures

Human VEGF is depicted in green and murine VEGF in black, showing homology from amino acids 37 – 134. The arrow points to VEGF Lys-48, an important amino acid for v107 peptide binding.

Results of Superposition

Root mean square deviations (RMSD, in Å) based on pairwise superpositions (CA atoms) onto the model/structure with the highest sequence coverage are listed below.

RMSD of	total RMSD	RMSD of final subset
human VEGF (green) to murine VEGF (black)	**1.4 Å** // 98 residues	**0.9 Å** // 95/98 residues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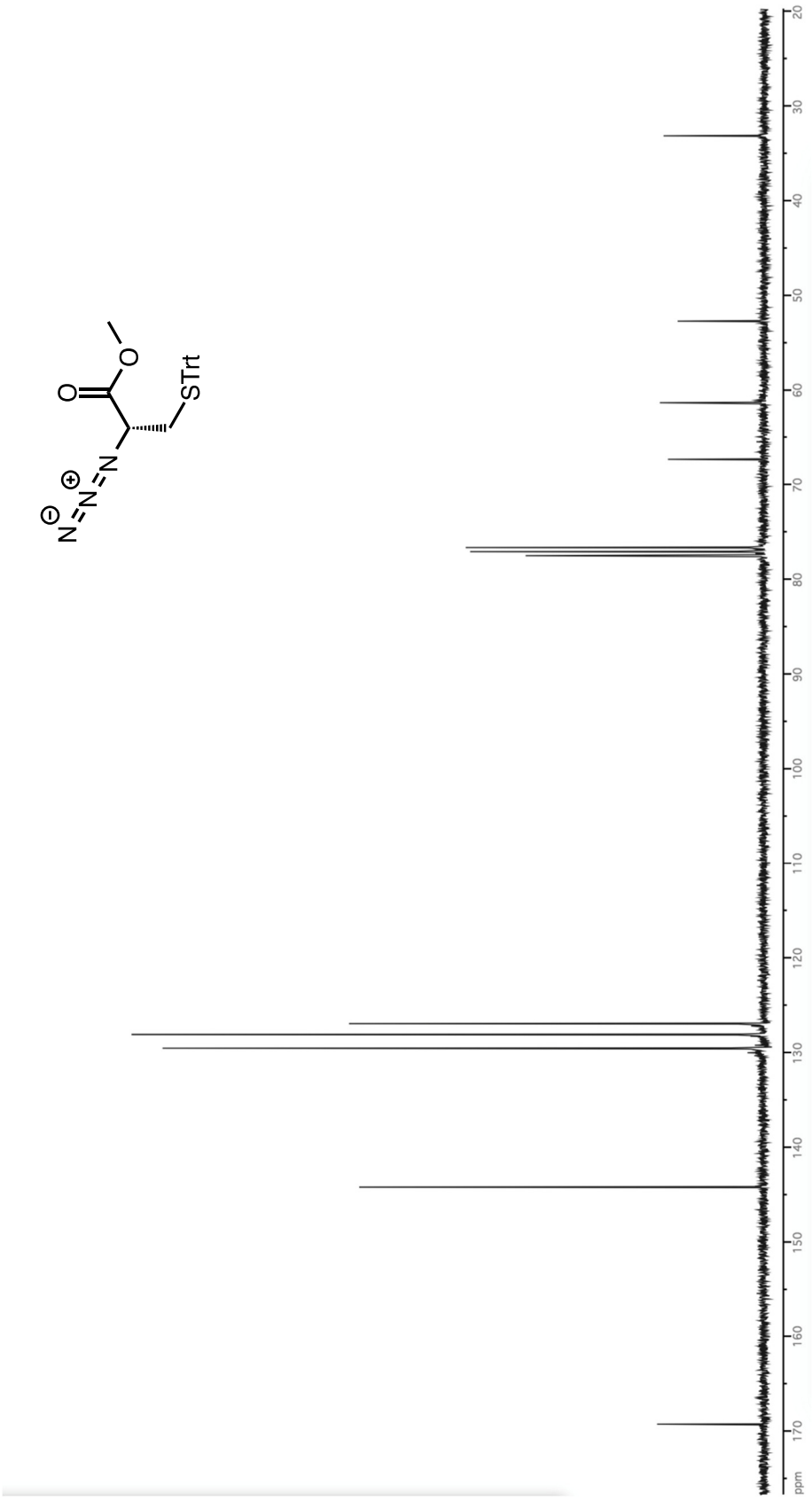
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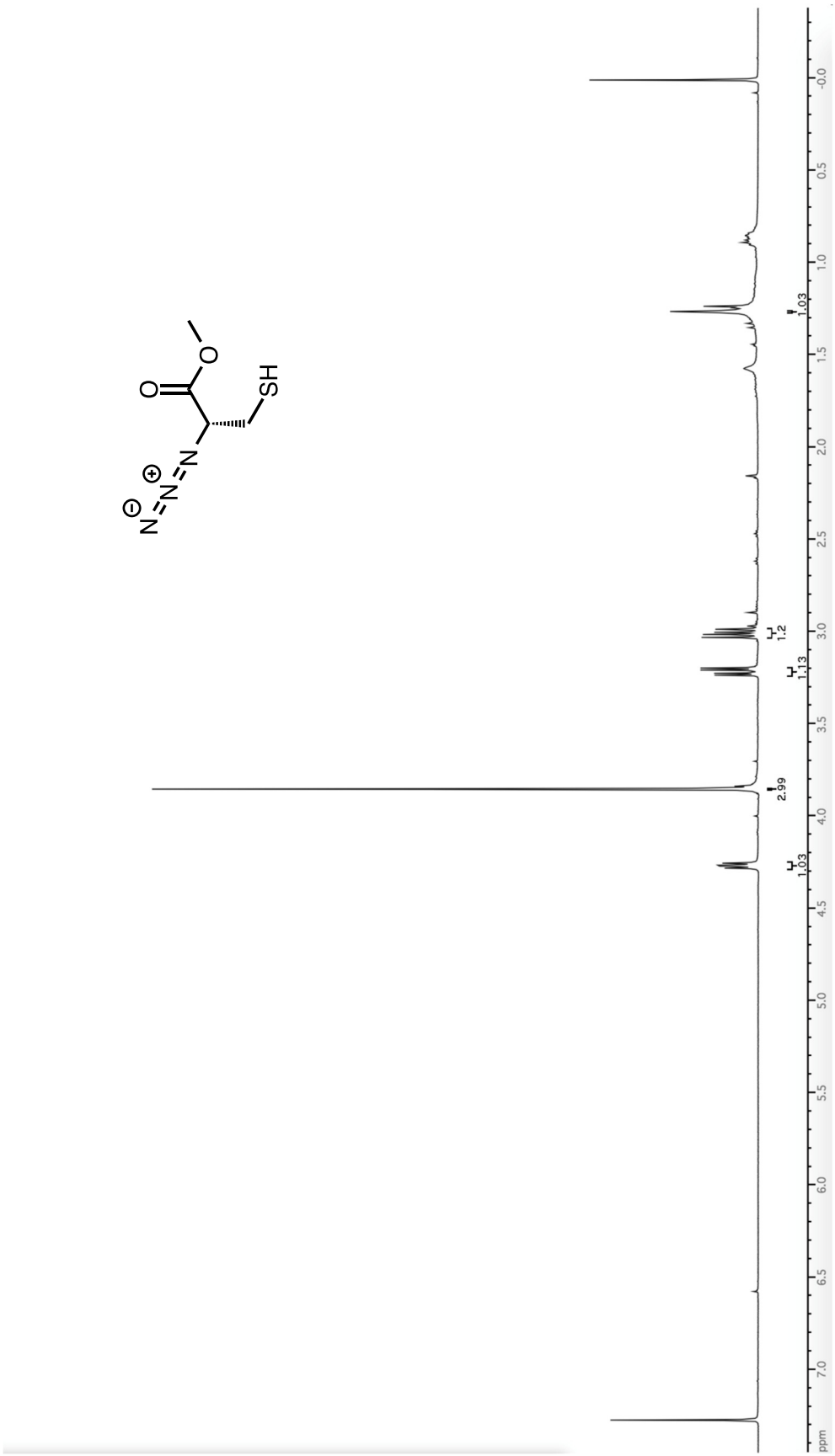
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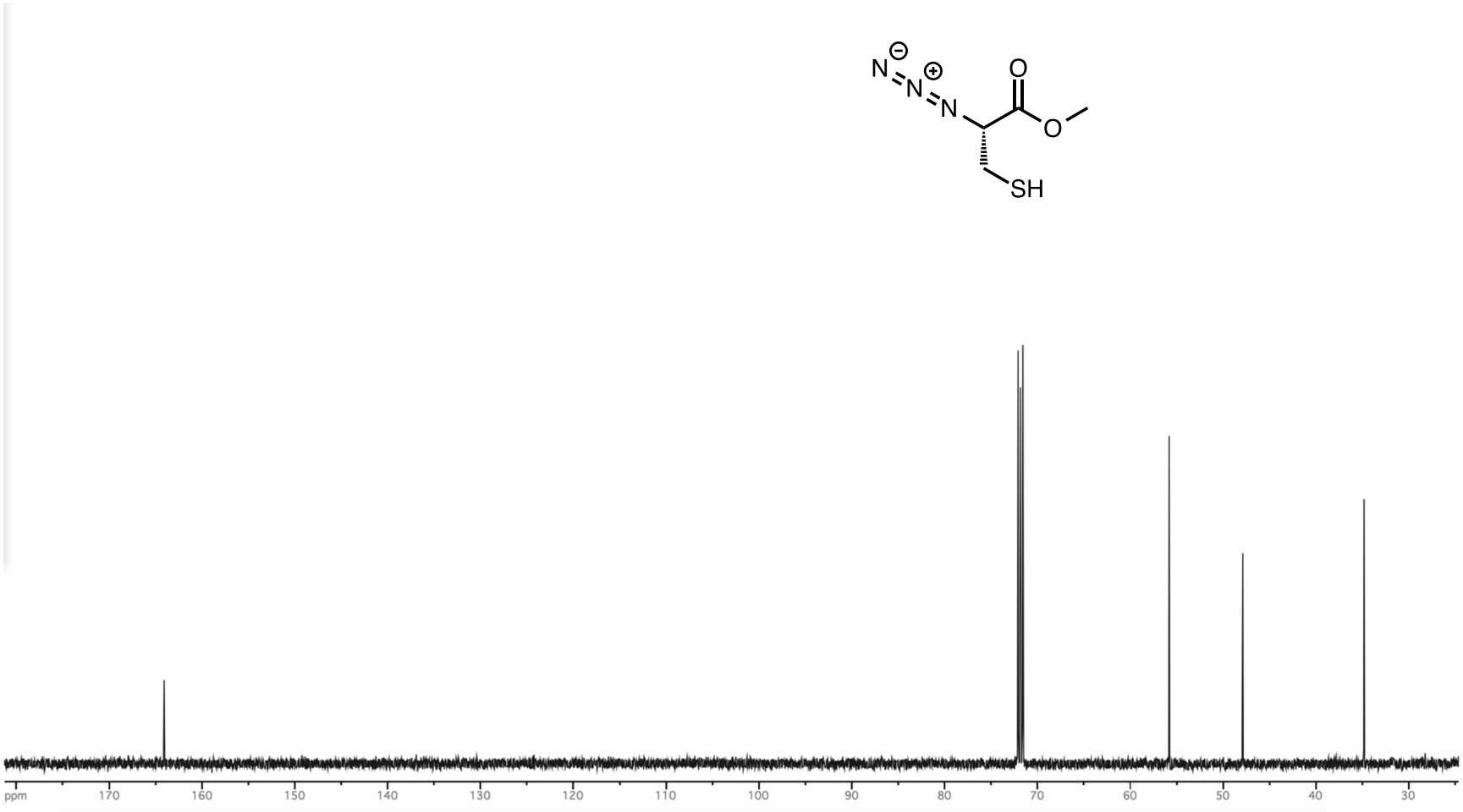
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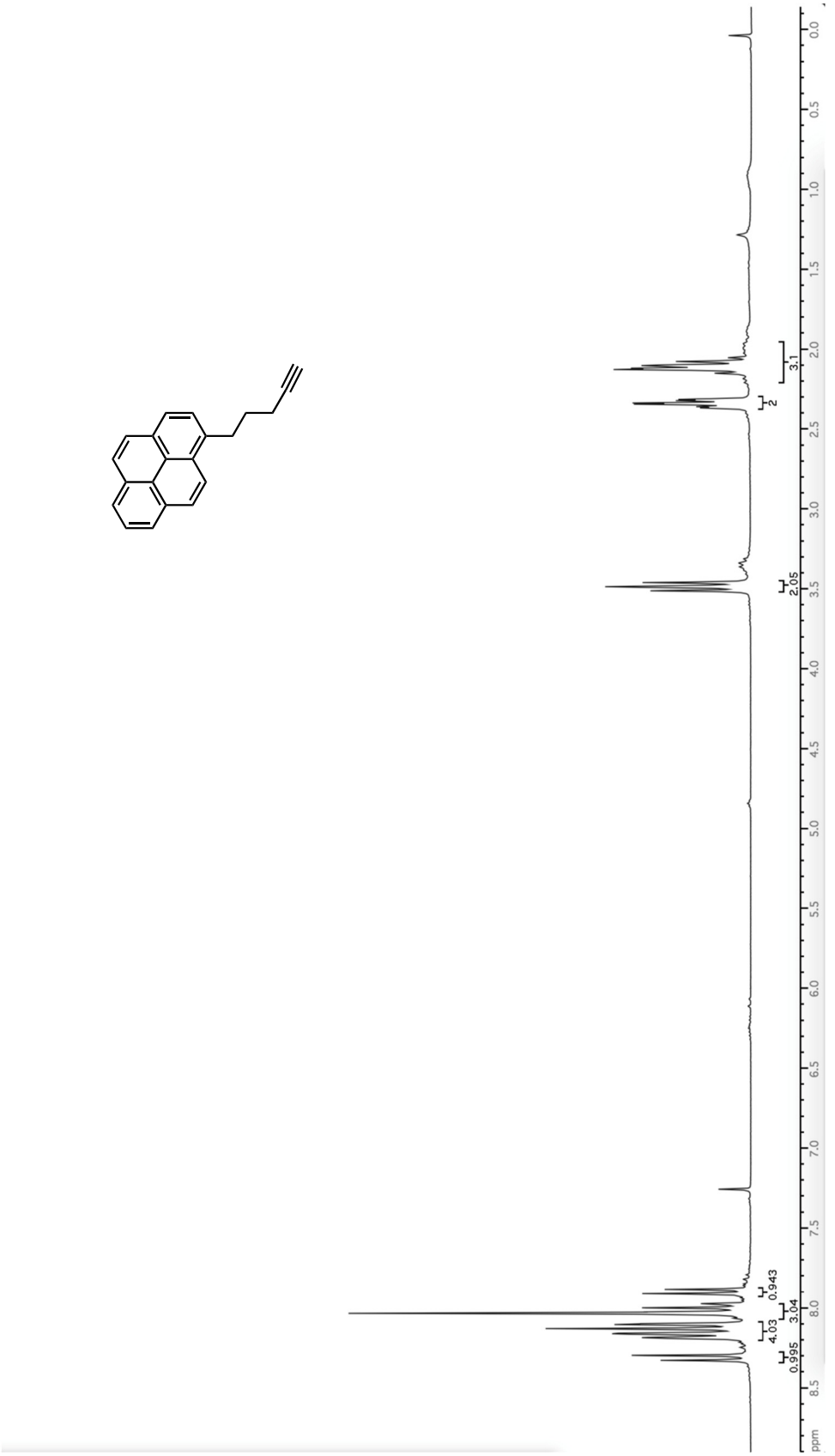
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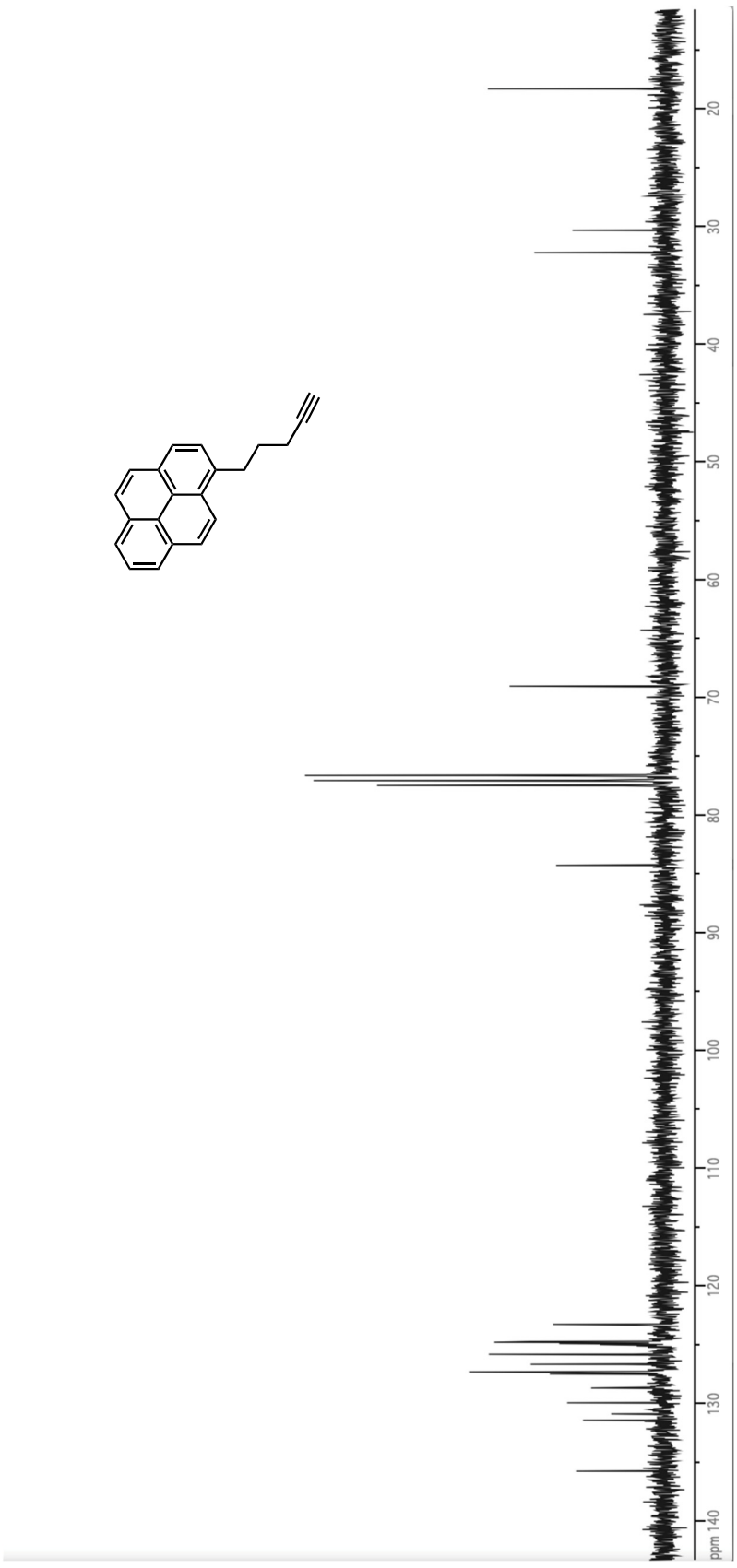
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