

Supporting Information

Thiol-yne radical reaction mediated site-specific protein labeling via genetic incorporation of an alkynyl-L-lysine analogue

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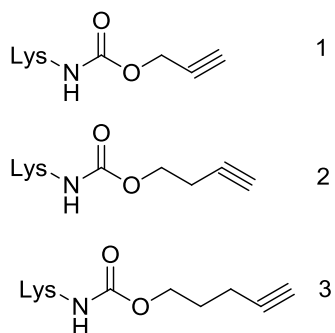
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Experimental details

a. Preparation of compounds 1-3



Compound 1: ¹H NMR (300MHz, D2O): δ(ppm) 1.40-1.57(m,4H), 1.89-1.99(m,2H), 2.87(s,1H), 3.14(m,2H), 4.03(m,1H), 4.64 (m,2H). ¹³C NMR (300MHz, D2O): δ(ppm)=21.0, 27.9, 29.0, 39.5, 52.3, 52.5, 75.2, 78.2, 157.2, 172.0. HRMS: m/z calculated for C₁₀H₁₆N₂O₄. [M+H]⁺:(M/Z)229.11 ; found :229.2.

Compound 2: ¹H NMR (300MHz, D2O): δ(ppm) 1.41-1.56(m,4H), 1.94-1.98(m,2H), 2.38(s,1H), 2.53(m,2H), 3.12(m,2H), 4.06(m,1H), 4.12 (m,2H). ¹³C NMR (300MHz, D2O): δ(ppm) 18.7, 21.4, 28.4, 29.4, 39.9, 52.8, 62.9, 70.6, 81.7, 158.3, 172.1. HRMS: m/z calculated for C₁₁H₁₈N₂O₄. [M+H]⁺:(M/Z):243.13; found :243.2.

Compound 3: ¹H NMR (300MHz, DMSO-D): δ(ppm) 1.34-1.35(m,4H), 1.64-1.76(m,3H), 2.15-2.18(m,2H), 2.76(s,1H), 2.90-2.92(m,2H), 3.76(m,2H), 3.91-3.96 (m,2H), 7.08-7.11(m,1), 8.43(s,2H). ¹³C NMR (300MHz, DMSO-D): δ(ppm) 14.9, 22.1, 28.2, 29.3, 30.1, 40.8, 52.3, 62.7, 72.1, 84.0, 156.6, 171.4. HRMS: m/z calculated for C₁₂H₂₀N₂O₄. [M+H]⁺:(M/Z):257.14; found:257.1

b. Protein expression and purification

Protein sequence:

WT: HdeA-myc-His₆

ADAQKAADNKKPVNSWTCEDFLAVDESFPQPTAVGF~~AE~~ALNNKDKPEDAVLDVQGIAT~~Y~~TPAIVQACTQ
DKQANFKDKVKG~~EW~~DKIKKDMKLGPEQKLISEEDLNSAVDHHHHHH

HdeA protein expression was carried out in *E. coli* DH10B or BL-21DE3 cells co-transformed with plasmids expressing ACPK-RS-tRNA^{Pyl}_{CUA} pair and HdeA with different TAG mutation sites. Cells were grown in LB medium containing ampicilline (50 µg/mL) and chloramphenicol (34 µg/mL) with shaking overnight at 37 °C. After 1:100 dilution in LB medium containing ampicilline (50 µg/mL) and chloramphenicol (34 µg/mL), the culture was grown at 37 °C to an OD₆₀₀~0.5. Compound **1-3** (100 mM for stock solution) was added by 1:100 dilution to a final concentration of 1 mM and incubated with cell culture for 30 min. Protein expression was induced by the addition of arabinose and IPTG to the final concentration of 0.2% and 0.5 mM at 30 °C, respectively. After expression for 12 h, cells were harvested by centrifugation (10000 g, 5 min), and resuspended in lysis buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.0). Bacterial lysate after sonication was loaded onto a Ni-NTA column (Hisrap 5 mL, GE healthcare). The column was washed with 30 mL washing buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.0 with 30 mM imidazole) and then eluted with elution buffer (20 mM Tris-HCl, 300 mM NaCl, pH 7.0 with 250 mM imidazole). Then the eluted protein was concentrated by using centrifugal filters (10000 MW cutoff) and further purified process RP-HPLC. The yield of the protein (as solid powder) was quantified after lyophilization.

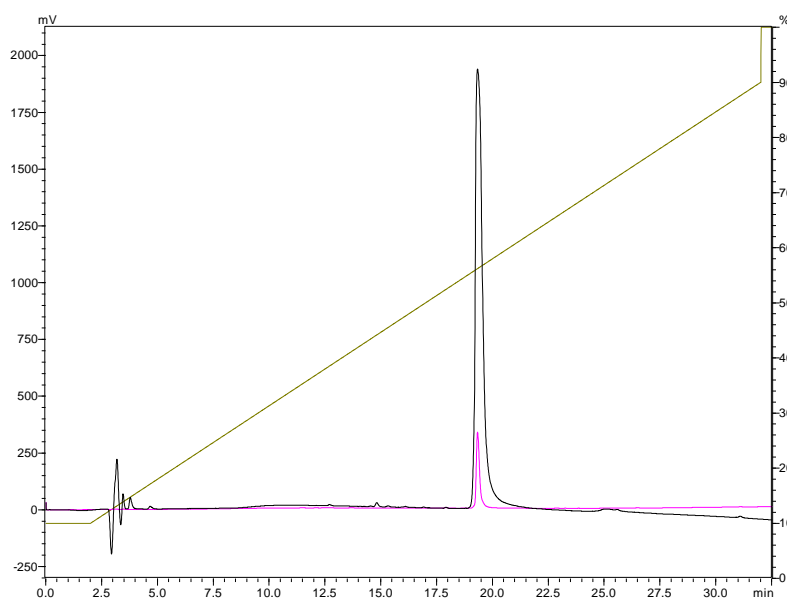
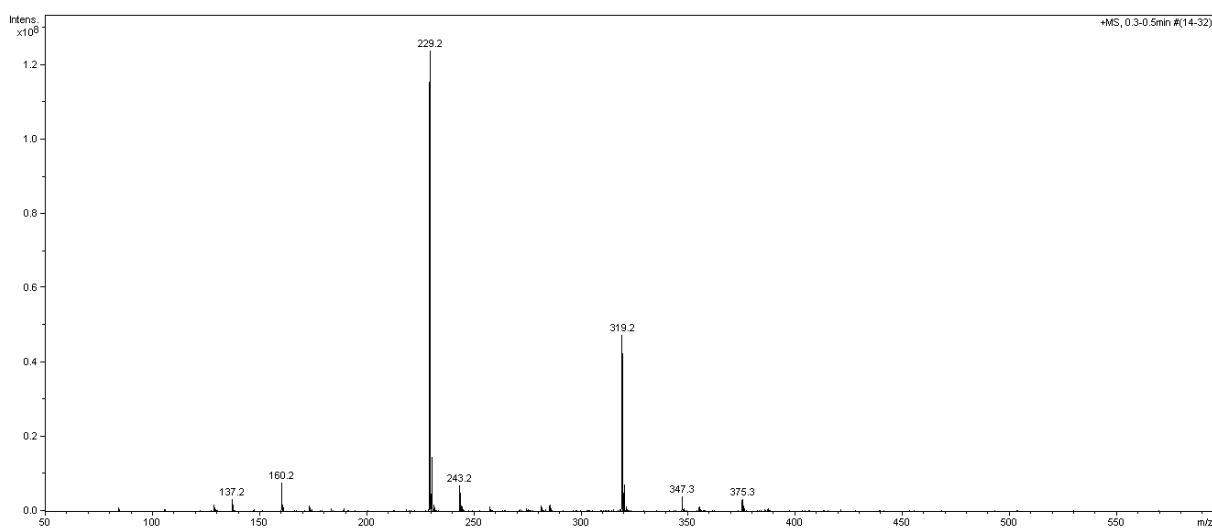


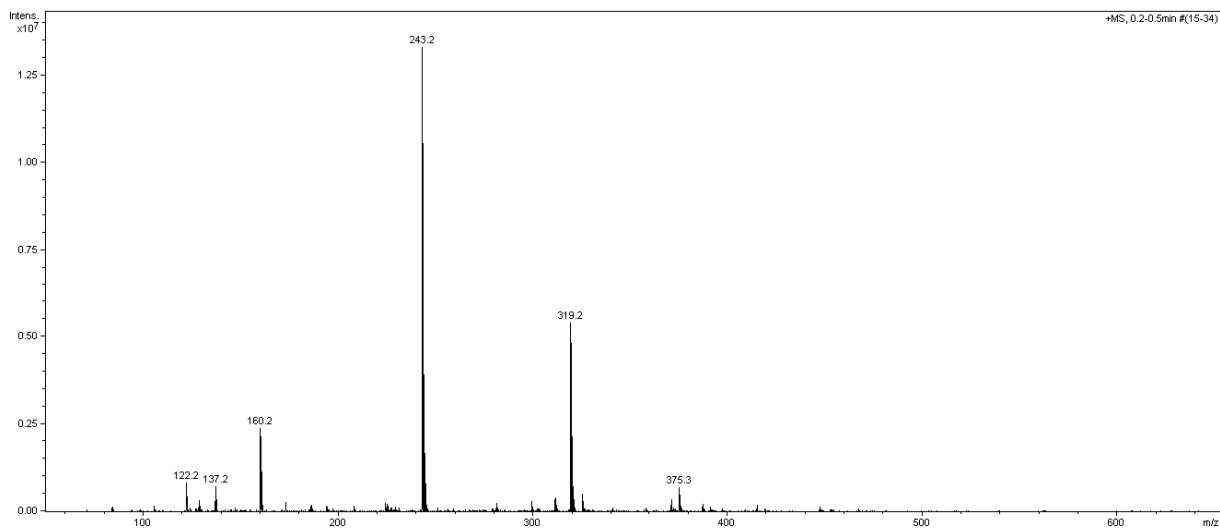
Figure S1: HPLC analysis the purified HdeA-V58-3

c. ESI MS of peptides or proteins

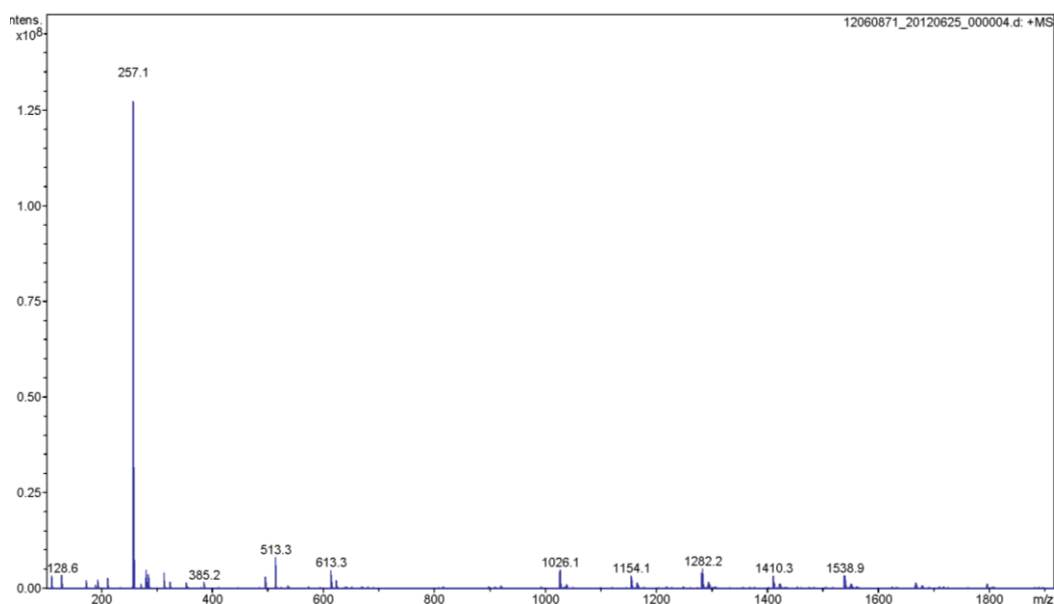
Compound **1**: m/z calculated: 229.11; Found: 229.2 Da.



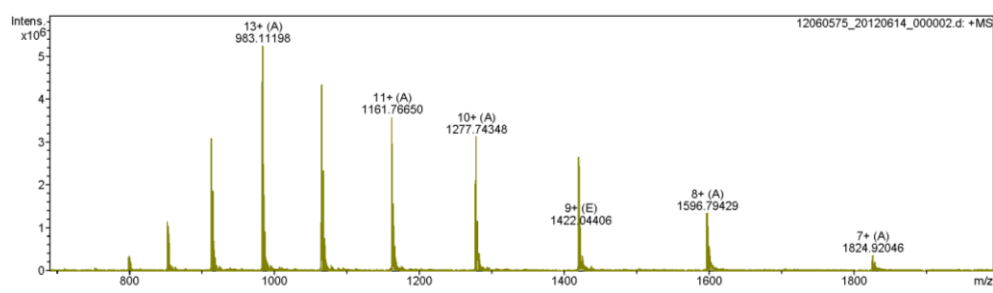
Compound **2**: m/z calculated: 243.13; Found: 243.2 Da.



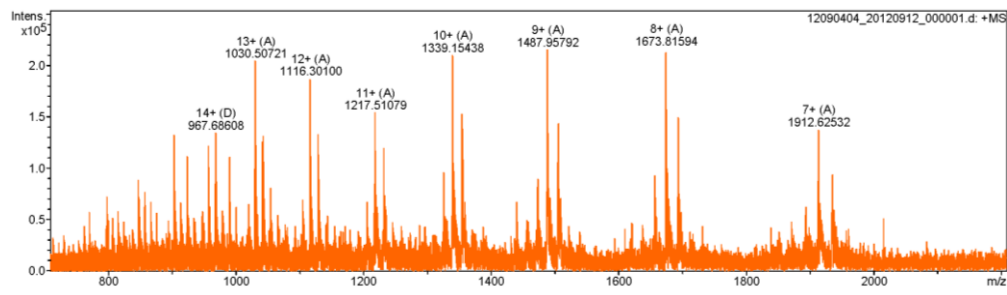
Compound **3**: m/z calculated: 257.14; Found: 257.1 Da.



HdeA-V58-3 (m/z calculated: 12768.5, Found: 12767.8 Da)

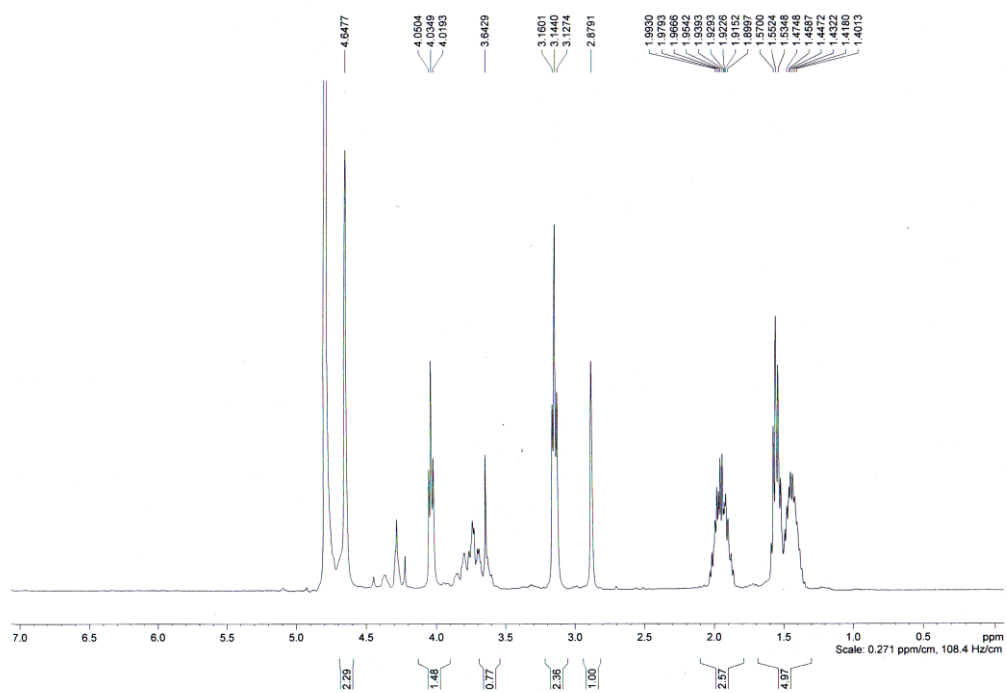


HdeA-V58-3 ligate with N,N'-Bis(dansyl) Cystamine (m/z calculated: 13384.8, Found: 13383.7 Da)

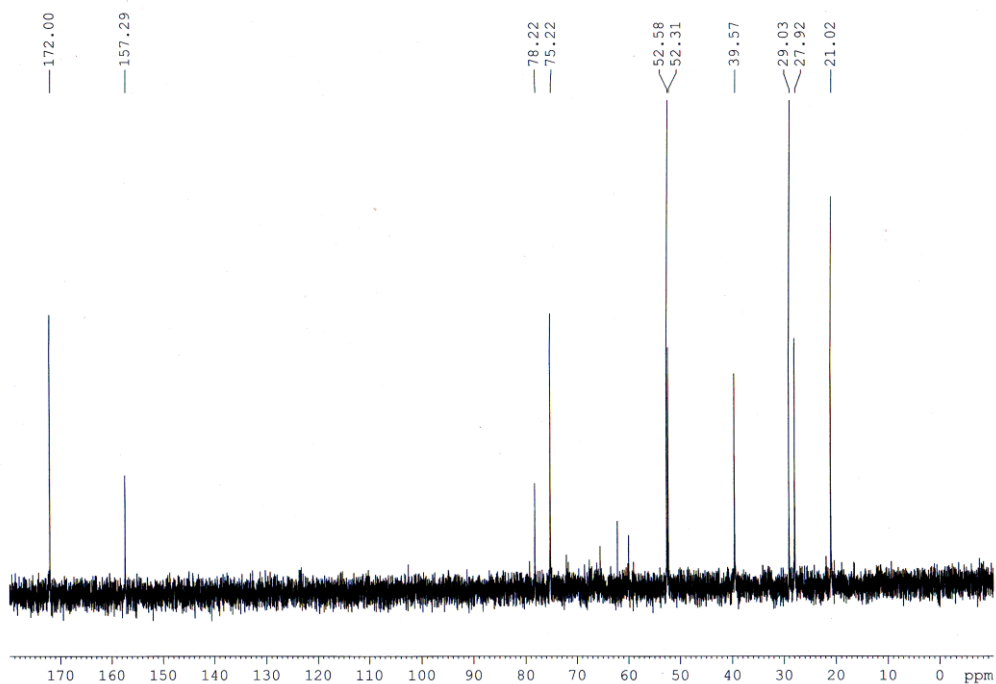


d. ^1H NMR and ^{13}C NMR

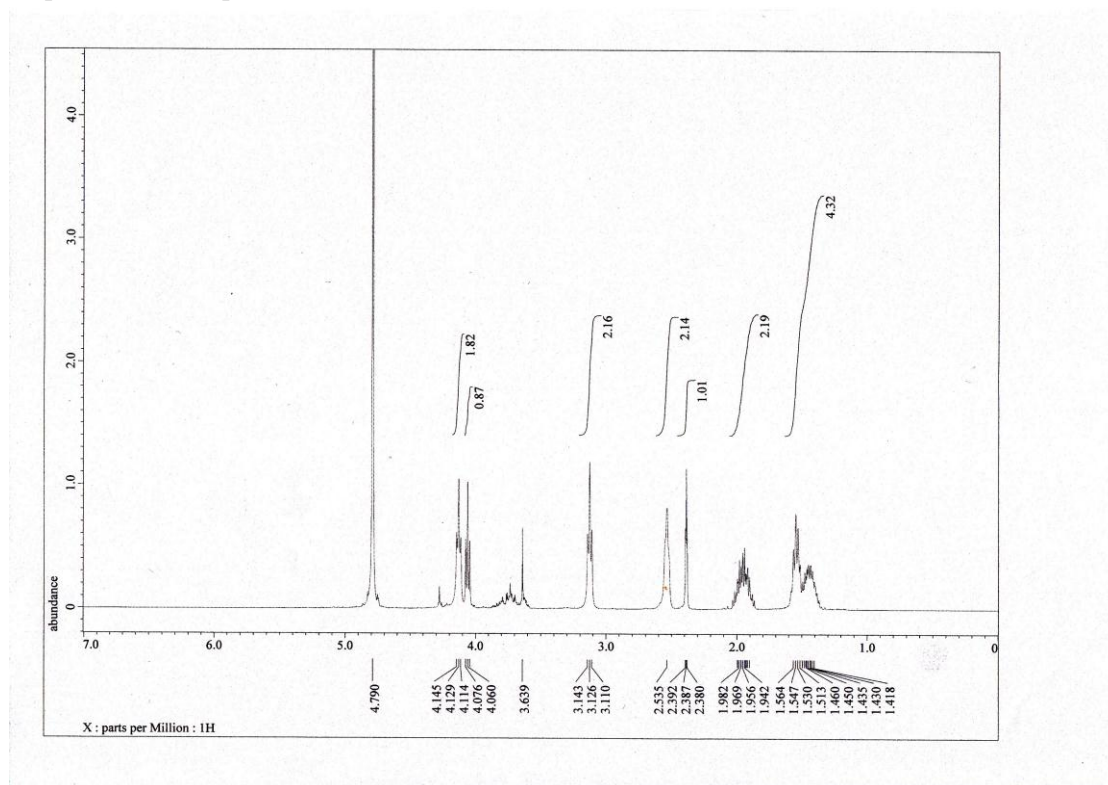
^1H NMR Spectrum of compound **1**



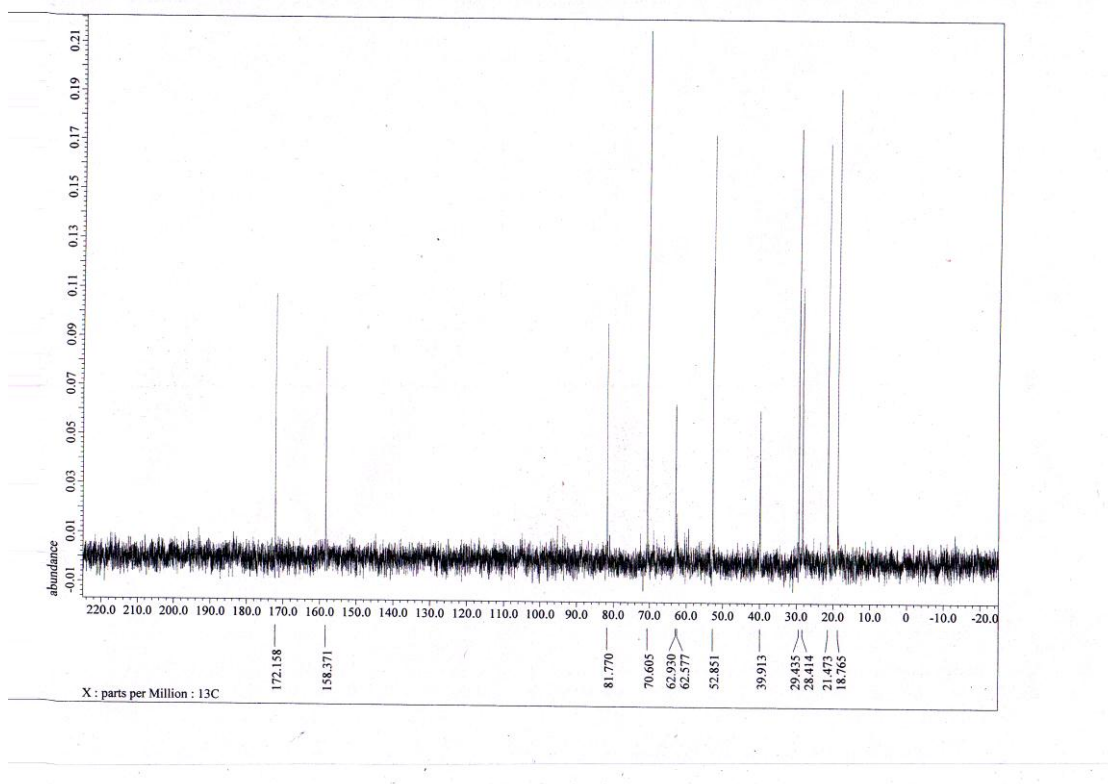
^{13}C NMR Spectrum of compound **1**



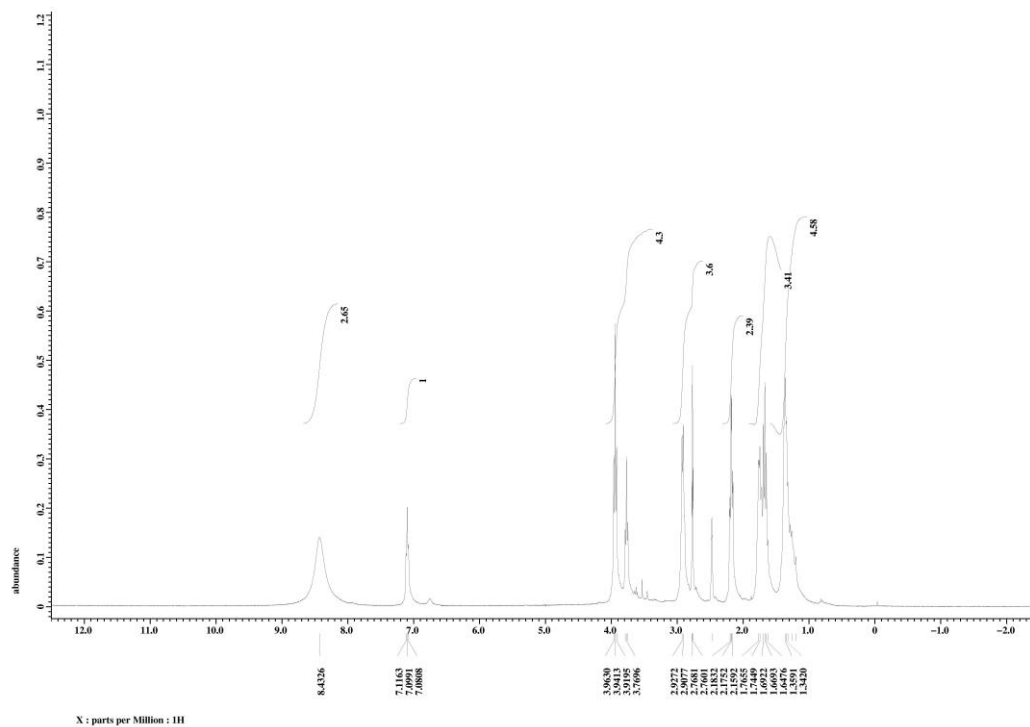
¹H NMR Spectrum of compound 2



¹³C NMR Spectrum of compound 2



^1H NMR Spectrum of compound **3**



^{13}C NMR Spectrum of compound **3**

