Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information:

Development of a chemical probe for identifying protein targets of α-oxoaldehydes

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General

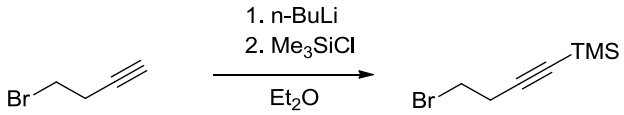
NMR spectra were acquired on a Varian AS 400 spectrometer, running at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm relative to residual solvent signals ($CHCl_3$, 7.26 ppm; D_2O, 4.79 ppm) for ^{1}H NMR spectra and relative to the central solvent resonance ($CDCl_3$, 77.00 ppm) for ^{13}C NMR spectra. The following abbreviations are used to indicate the multiplicity in ^{1}H NMR spectra: s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; dt, double triplet; quin, quintet; m, multiplet; bs, broad signal. ^{13}C NMR spectra were acquired in broadband decoupled mode. Mass spectra of synthetic products and intermediates were recorded on a micromass LCT spectrometer using electrospray (ES+) ionization. pH was measured with a Metrohm 744 pH-meter. Analytical thin layer chromatography (TLC) was performed using pre-coated aluminium-backed plates (Merck Kieselgel 60 F254) and visualized by ultraviolet irradiation or $KMnO_4$ dip. Commercially available reagents and solvents for flash chromatography were used without further purification. Et_2O was dried over activated 4Å molecular sieves overnight. Dry THF was acquired from an MBraun SPS-800 solvent purification system and used immediately. Glassware was dried by warming with a heat gun under high vacuum, then flushed with N_2.

Water for biological assays was purified using a Milli-Q system (Millipore). Proteins and peptides for mass spectrometry analysis were handled in 1.5 mL Protein LoBind tubes (Eppendorf). Phosphate buffer saline (PBS) was Mg^{2+} and Ca^{2+} free and adjusted to pH = 7.4.

SDS loading buffer consisted of 1% SDS, 10% glycerol, 10 mM Tris-HCl, pH 6.8, 1 mM ethylene diamine tetraacetic acid (EDTA), 80 mM dithiothreitol (DTT) and 0.1 mg/mL bromophenol blue.

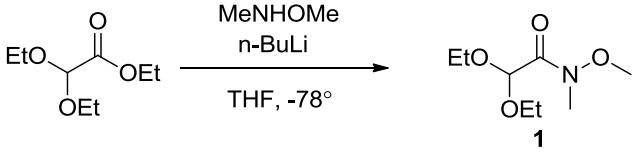
Synthesis

(4-Bromobut-1-yn-1-yl)trimethylsilane



n-Butyllithium (1.6 M in hexanes, 11.28 mL, 18 mmol) was added dropwise to a solution of 1-bromobut-4-yne (1.42 mL, 2,00 g, 15 mmol) in 50 mL Et_2O at -78 °C under N_2 atmosphere and stirred for 1 h. Chlorotrimethylsilane (3.78 mL, 3.27 g, 30 mmol) was added and the solution was allowed to warm to rt and stirred overnight. The reaction mixture was poured into 120 mL 2 M HCl. The organic phase was isolated and the aqueous phase was extracted 3 times with 50 mL Et_2O each. The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo* to afford the product as a clear oil (2.94 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41 (t, *J* = 7.6 Hz, 2H), 2.75 (t, *J* = 7.6 Hz, 2H), 0.14 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 103.4, 87.2, 29.3, 24.5, 0.14 (3C).

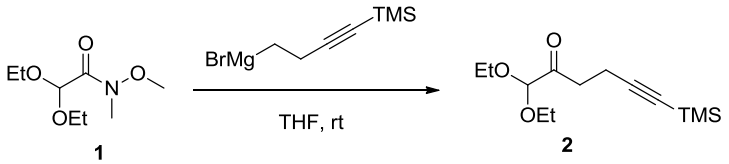
2,2-Diethoxy-N-methoxy-N-methylacetamide, **1**



1

N,O-Dimethylhydroxylamine (0.51 g, 8.4 mmol) was dissolved in 30 mL THF under N_2 atmosphere and cooled to -78 °C. *n*-Butyllithium (1.6 M in hexanes, 10.5 mL, 16.8 mmol) was added dropwise and the solution was allowed to warm to rt and stirred for 10 min before cooling to -78 °C again. Ethyl diethoxyacetate (1.0 mL, 0.99 g, 5.6 mmol) was added and the solution was stirred at this temperature for 2 h before quenching with 40 mL of a saturated aqueous solution of NH_4Cl. The reaction mixture was extracted 3 times with 50 mL Et_2O each. The extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography in 10% Et_2O in CH_2Cl_2, affording the product as a clear oil (0.59 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (s, 1H), 3.72 (s, 3H), 3.70 (q, *J* = 7.2 Hz, 4H), 3.19 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 94.6, 61.5 (2C), 61.2, 31.7, 14.7 (2C). HRMS: Calculated for $[C_8H_{17}NO_4Na]^+$: 214.1050 found: 214.1052.

1,1-Diethoxy-6-(trimethylsilyl)hex-5-yn-2-one, **2**

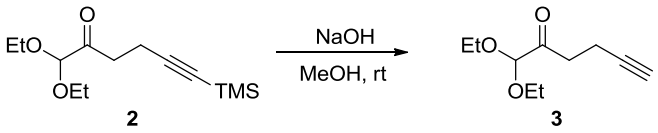


1 **2**

Magnesium flakes (54 mg, 2.2 mmol) were suspended in 10 mL THF at rt under N_2 atmosphere. (4-Bromobut-1-yn-1-yl)trimethylsilane (0.45 g, 2.2 mmol) was dissolved in 10 mL THF and 1 mL of the solution was added to the magnesium suspension. The Grignard reaction was initiated by heating the flask slightly with a heat gun until reflux.

The rest of the solution was then added dropwise while stirring. The solution was then stirred at reflux for 30 min until all magnesium had disappeared. The resulting solution of (4-(trimethylsilyl)but-3-yn-1-yl)magnesium bromide was then added slowly to a solution of **1** (0.21 g, 1.1 mmol) in 20 mL THF and stirred at rt for 1 h before quenching with 40 mL of a saturated aqueous solution of NH_4Cl. The reaction mixture was diluted with 50 mL Et_2O, washed once with brine and dried over $MgSO_4$ before concentrating *in vacuo*. The residue was purified by column chromatography in 10% EtOAc in pentane, affording the product as a clear oil (0.16 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.55 (s, 1H), 3.71-3.64 (m, 2H), 3.58-3.50 (m, 2H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.46 (t, *J* = 7.6 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 6H), 0.11 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.2, 105.9, 102.6, 84.9, 63.5 (2C), 36.3, 15.3 (2C), 13.9, 0.2 (3C). HRMS: Calculated for $[C_{13}H_{24}O_3SiNa]^+$: 279.1387 found: 279.1388.

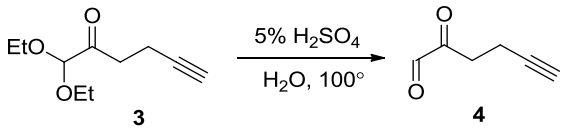
1,1-Diethoxyhex-5-yn-2-one, **3**



2 **3**

Compound **2** (200 mg, 0.8 mmol) was stirred in 15 mL MeOH with K_2CO_3 (160 mg, 1.16 mmol) at rt. The reaction was monitored by TLC in 15% EtOAc in pentane and full conversion was observed in 2 h. 30 mL H_2O was added and the mixture was extracted 3 times with CH_2Cl_2. The organic phase was washed once with 20 mL H_2O, then dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography in 15% EtOAc in pentane, affording the product as a clear oil (115 mg, 80%). %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.53 (s, 1H), 3.70-3.62 (m, 2H), 3.56-3.48 (m, 2H), 2.82 (t, *J* = 7.6 Hz, 2H), 2.40 (dt, *J* = 7.6, 2.7 Hz, 2H), 1.90 (t, *J* = 2.6 Hz, 1H), 1.22 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.2, 102.6, 83.9, 68.7, 63.5 (2C), 36.0, 15.3 (2C), 12.5. HRMS: Calculated for $[C_{10}H_{16}O_3Na]^+$: 207.0992 found: 207.0990.

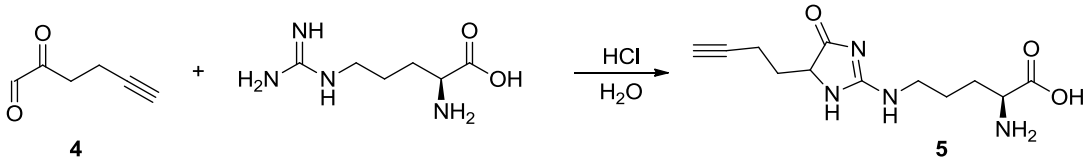
2-Oxohex-5-ynal, AlkMG, **4** - general procedure



3 **4**

Removal of the acetal was carried out immediately before incubation with cells or lysate. 10-100 mg of **3** was suspended in 1 mL 5% aqueous H_2SO_4 in a glass vial and immersed in an oil bath at 100 °C for 30 min with stirring. The pH was then adjusted to 7 with 1 M KOH and this was used as the stock solution.

(2S)-2-Amino-5-((5-(but-3-yn-1-yl)-4-oxo-4,5-dihydro-1H-imidazol-2-yl)amino)pentanoic acid, AlkMG-H1, **5**

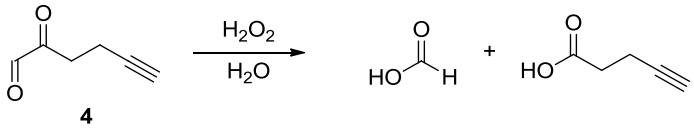


4 **5**

Arginine (40 mg,0.23 mmol) and **3** (125 mg, 0.68 mmol, 3 eq) were suspended in 1 mL 1 M HCl and stirred vigorously in a glass vial at 100 °C for 20 min. The temperature was then lowered to 60 °C and the solution was stirred overnight, changing in appearance to dark brown. The reaction was concentrated *in vacuo*, dissolved in 2 mL H_2O, concentrated again and then dissolved in 2 mL MeOH and concentrated on celite and purified by column chromatography in (0-40% MeOH in EtOAc with 5% AcOH). Product fractions were identified by TLC in 1:1:3 AcOH/H_2O/n-BuOH and subsequent staining with ninhydrin. The pure fractions were combined and concentrated in vacuo, then taken up in 2 mL 1 M HCl and concentrated again to remove AcOH. The residue was stored under high vacuum overnight to remove solvents and yield the product as the hydrochloride salt, appearing as a light brown residue (48 mg, 73%). ^{1}H NMR (400 MHz, D_2O) δ 4.68 (t, *J* = 5.7 Hz, hydroimidazol H conj.), δ 4.63 (m, hydroimidazol H isol.), 3.98 (s, 1H), 3.53 (quin, *J* = 6.2 Hz, hydroimidazolonyl CH_2 conj.), 5.38 (m, hydroimidazolonyl CH_2 isol.), 2.50 (s, 1H), 2.35-2.56 (m, bs, 2H), 2.13-2.34 (m, bs, 2H), 1.95-2.13 (m, bs, 2H) 1.70-1.95 (m, bs, 2H). ^{13}C NMR (100 MHz, D_2O) δ 177.0, 157.2, 119.7, 83.7, 71.9, 59.4, 54.5, 42.5, 29.2, 28.2, 24.5, 14.2. HRMS: Calculated for $[C_{12}H_{19}N_4O_3]^+$: 267.1452 found: 267.1454.

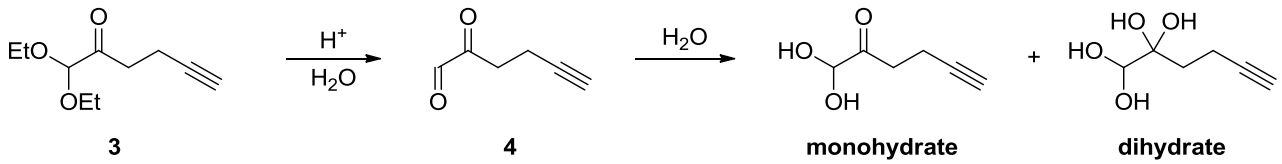
The signals designated "conj." and "isol." are from the isomers of MG-H1 with conjugated and isolated double bonds respectively. By comparing the integrals, we observed that the latter form makes up ~25% of the product. This corresponds to what Hellwig *et al.* report in their synthesis of MG-H1.[1]

Friedemann titration



A solution of **4** (100 mg, 0.54 mmol) was prepared as described above and pH of the solution was adjusted to 7.0 with 1 M KOH. The pH was measured throughout the experiment with a pH-meter. Hydrogen peroxide (35% in H_2O, 0.1 mL, 1.09 mmol) was added and the pH of the solution fell to ~5. The solution was then titrated with 1.08 mL 1 M KOH until the pH was stable at 8.3 and then with 0.02 mL 1.2 M HCl until the pH was stable at 7.0 again. This corresponded to 1.06 mmol of acid formed during oxidation. As 2 equivalents of acid are formed from each mol of **4**, this suggests that 0.53 mmol was effectively deprotected, giving a near quantitative yield of 98%.

Deprotection of the probe for characterization of 4



10 mg Dowex 50WX8 (H+ form) resin was washed once in D_2O, then suspended in 1 mL D_2O in a glass vial. 20 mg of compound **3** (0.11 mmol) was added and the vial was immersed in a 100 °C hot oil bath stirred vigorously for 30 min, where TLC (10% EtOAc in pentane) indicated full conversion of the starting material. The solution was allowed to cool

[1] M. Hellwig, S. Geissler, R. Matthes, A. Peto, C. Silow, M. Brandsch, and T. Henle, *ChemBioChem*, 2011, **12**, 1270–1279.

to rt, filtered and analyzed by NMR. The spectrum shows that compound **4** exists as a 1:03 mixture of the monohydrate and dihydrate forms in aqueous solutions. The aldehyde is not observed at all. This corresponds with what is observed for methylglyoxal in the literature.[2]

Monohydrate: ^{1}H NMR (400 MHz, D_2O) δ 5.37 (s, 1 H), 3.04 (t, *J* = 6.8 Hz, 2 H), 2.57 (dt, *J* = 6.8 Hz, 1.9 Hz, 2H), 2.42 (t, *J* = 2.0 Hz, 1 H). ^{13}C NMR (100 MHz, D_2O) δ 211.1, 92.1, 86.7, 72.0, 38.8, 14.5.

Dihydrate: ^{1}H NMR (400 MHz, D_2O) δ 4.95 (s, 1 H), 2.44 (m, 1 H), 2.03 (t, *J* = 7.7 Hz, 2H), 1.32 (t, *J* = 7.5 Hz, 2 H). ^{13}C NMR (100 MHz, D_2O) δ 98.1, 93.5, 88.0, 71.7, 36.9, 14.3.

[2] I. Nemet, D. Vikić-Topi and L. Varga-Defterdarović, *Bioorg. Chem.*, 2004, **32**, 560–570.

Biological Evaluation

Human serum albumin binding site identification and comparison to methylglyoxal

Stock solutions of **4** and methylglyoxal were prepared. Methylglyoxal was prepared from methylglyoxal 1,1-dimethyl acetal following the same procedure as for **4**. Aliquots of each solution were added to Eppendorf tubes with 1 mL of a human serum albumin solution (10 µM, 0.67 mg/mL) in PBS to afford final concentrations of 500 µM of each glyoxal. The solutions were incubated for 24 h at 37 °C. After incubation, the protein was concentrated on a 0.5 mL "10 kDa" Amicon ultracentrifugal filter unit (Millipore) and the concentration was subsequently adjusted to 1 mg/mL with 10 mM ammonium bicarbonate buffer with 1 mM $CaCl_2$ before incubating overnight with trypsin (1 µg/100 µg protein). The tryptic digests were then purified on Pierce C-18 spin columns, 8 mg C-18 resin (Thermo Scientific) according to manufacturer's instructions and vacuum-dried before subjecting them to mass spectrometry as described below.

Mass spectrometry

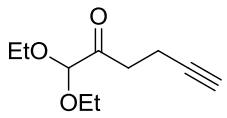
The tryptic digests of albumin were redissolved in 5% acetonitrile (AcN) with 0.4% AcOH. Aliquots were subjected to analysis by nano liquid chromatography (Easy nLC; Proxeon) coupled to mass spectrometry (LTQ-Orbitrap; Thermo Fisher Scientific) through a nano-electrospray source (Proxeon). The reverse phase separation column, 10 cm long and 75 µm in inner diameter (G&T Septech), was packed with 3.5 µm Kromasil C18 particles (Eka Chemicals). The peptides were separated in a 100-min gradient of AcN in 0.4% AcOH, starting with 5% and ending with 36% AcN. MS detection was full scan (m/z 400–2000) with Orbitrap detection at resolution R = 60,000 (at m/z 400) followed by up to four data-dependent MS/MS scans with LTQ detection of the collision-induced dissociation (CID) fragments of the most intense parent ions. The data was then analyzed using Proteome Discoverer software from MS supplier (Thermo Scientific) with the Mascot algorithm (Matrix Science) using 5 parts per million as mass tolerance threshold. The Swiss-Prot Human database was used and 2 missed trypsin cleavages were accepted in the analysis. All reported peptides have $p<0.05$.

Example mass spectra are shown in supporting figures 6-8 and identified peptides are shown on p. 15.

Cell assay - Lysates

HEK 293 cells were grown to 90% confluency in Dulbecco's modified eagle medium (Invitrogen) in T150 flasks before they were harvested using a cell scraper in PBS. Cells were pelleted by centrifugation (5 min, 500 g, rt) in a 15 mL BD Falcon tube (Fisher Scientific) and resuspended in 500 µL PBS, before homogenization with a probe sonicator (Branson Sonifier 250, 3x10 strokes at 30% power) on ice. Protein levels were measured by Bradford assay (Bio-Rad) and subsequently adjusted to 1 mg/mL with PBS. The cell lysate was divided into aliquots of 1 mL and a freshly prepared stock solution of **4** was added to afford a concentration of 0 (control), 0.1, 0.5, 1 or 5 mM. Lysates were incubated for 1 h at 37 °C, before precipitation by addition of 5 mL cold acetone and incubation for 2 h at -20 °C. Precipitated protein was pelleted by centrifugation (10 min, 6000 g, 4 °C) and the supernatant was removed. The pellets were suspended in 500 µL 1% SDS in PBS and resolubilized by probe sonication (3x10 strokes at 30% power).

Control experiment with AlkMG 4, compound 3, hex-5-ynal and hex-5-yn-2-one



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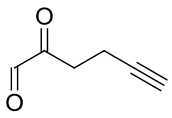

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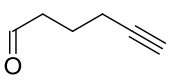
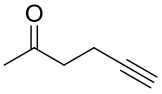


hex-5-ynal



hex-5-yn-2-one

Cells were treated as described above. To each sample was added 1 mM of either compound **3**, AlkMG **4**, hex-5-yn-2-one or hex-5-ynal from a 0.1 M stock solution in H_2O. Compound **3**, hex-5-yn-2-one and hex-5-ynal required the addition of 2-5% DMSO to be dissolved. Samples were incubated for 1 h at 37 °C before precipitation as described above. Hex-5-ynal and hex-5-yn-2-one were synthesized according to literature procedures.[3]

Cell assay – Live cells

HEK-293 cells were grown to ~80% confluency in a T150 flask in Dulbecco's modified eagle medium (Invitrogen) with 10% foetal calf serum before harvesting with a cell scraper in PBS. Cells were centrifuged (500 g, 5 min, rt), resuspended in 2 mL medium and transferred in aliquots of 400 µL into 5 Eppendorf tubes. To each tube was added 0 (control), 0.1, 0.5, 1 or 5 mM of compound **4** from a 0.1 M stock solution.[4] The tubes were incubated at 37 °C for 2 h and the cells were then centrifuged (500 g, 5 min, rt) and washed with 500 µL PBS. This was repeated twice to remove extracellular proteins and excess probe. The cells were then suspended in 200 µL PBS and lysed by probe sonication on ice (3x10 strokes at 30% power). An aliquot was taken out for Bradford analysis, showing protein levels to be ~2 mg/mL. Proteins were precipitated immediately afterwards by the addition of 800 µL cold acetone. Samples were incubated overnight at -20 °C and pelleted by centrifugation (6000 g, 10 min, 4 °C). The protein pellets were dissolved in 200 µL 1% SDS in PBS by probe sonication (3x10 strokes at 30% power).

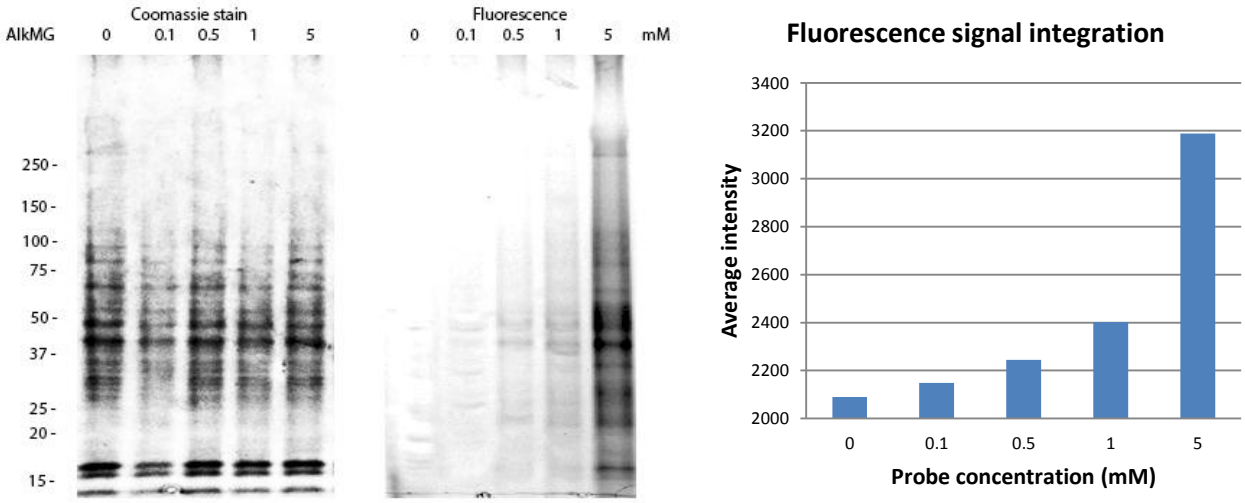
Rhodamine conjugation

50 µL of a 2 mg/mL solution of labeled proteins in 1% SDS in PBS were treated with a "click-chemistry cocktail", consisting of 1.2 µL 10 mM rhodamine-azide in EtOH, 1.2 µL 50 mM TCEP, 3.4 µL 1.7 mM TBTA in 1:4 DMSO/tBuOH and 1.2 µL 50 mM $CuSO_4$,[5] followed by vortexing and incubation for 1 h at rt, vortexing again after 30 min. Proteins precipitated partially during the incubation. Proteins were precipitated by adding 200 µL acetone and incubating for 1 h at -20 °C, then pelleted by ultracentrifugation (6000 g, 10 min, 4 °C). The supernatant was discarded and pellets were redissolved in 50 µL SDS loading buffer, vortexed, incubated for 15 min at 70 °C, vortexed again and centrifuged (10.000 g, 2 min, rt) immediately before loading. 10 µL (20 µg) were subjected to SDS-PAGE on a Criterion TGX "Any kD" precast gel (Bio-Rad) in a Tris-glycine running buffer (0.025M Tris/0.192M glycine/0.1%

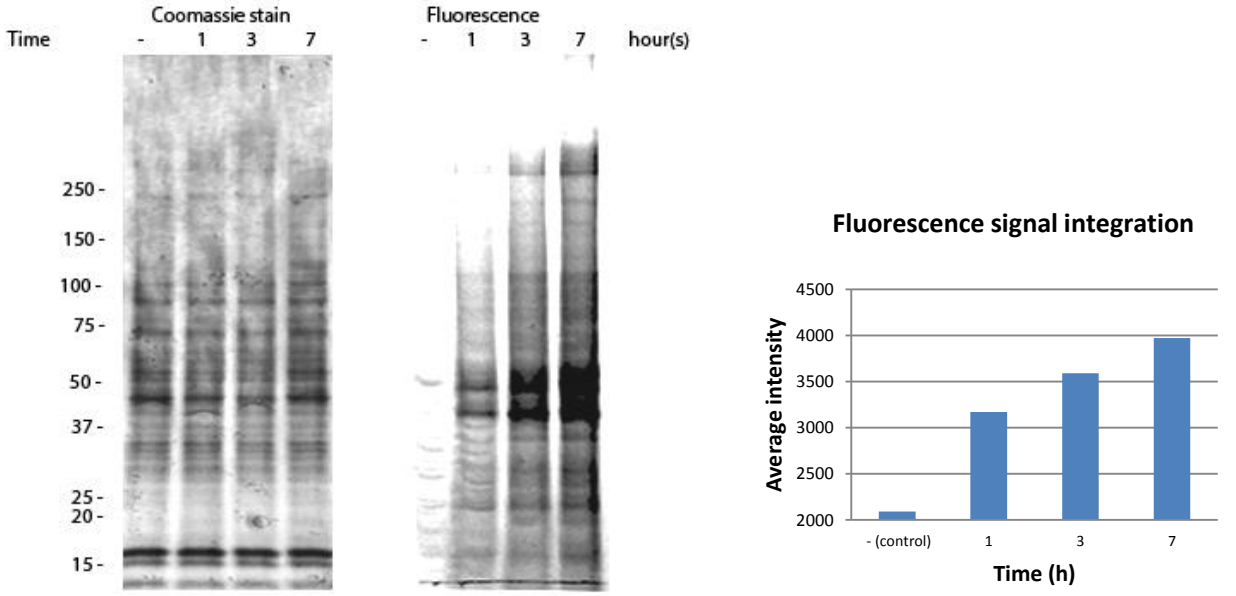
[3] *(a)* Hex-5-ynal: J. W. Amoroso, L. S. Borketey, G. Prasad and N. A. Schnarr, *Org. Lett.*, 2010, **12**, 2330–2333; *(b)* Hex-5-yn-2-one: B. M. Trost and M. T. Rudd, *J. Am. Chem. Soc.*, 2005, **127**, 4763–4776.
[4] The stock solution for this experiment was made using Dowex ion-exchange resin as described on p. 4
[5] A. E. Speers and B. F. Cravatt, in *Current Protocols in Chemical Biology*, eds. A. P. Arkin, L. Mahal, F. Romesberg, K. Shah, C. Shamu, and C. Thomas, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2009.

SDS, pH 8.5) at 300 V. The gel was washed once in deionized water and analyzed using an ImageQuant LAS 4000 biomolecular imager (GE Healthcare) in green fluorescence mode. The total amount of protein on the gel was visualized after in-gel fluorescence by Coomassie staining followed by destaining in destain solution (40% MeOH/10% AcOH in H_2O) for 1 h, to verify that each lane was equally loaded.

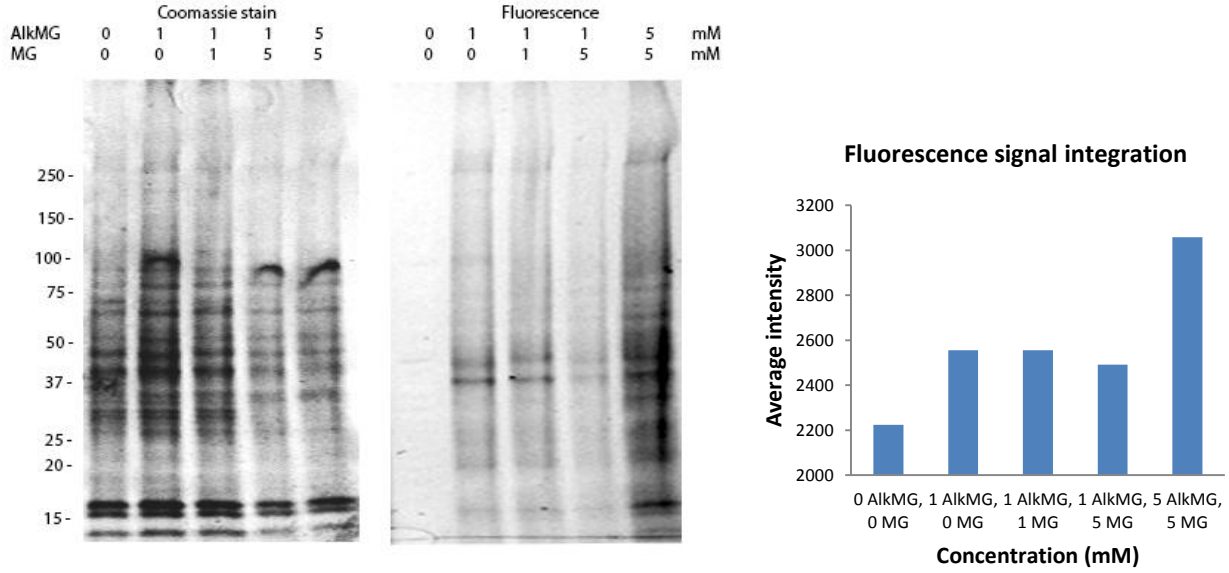
Gel scans – Fluorescence and Coomassie brilliant blue stain



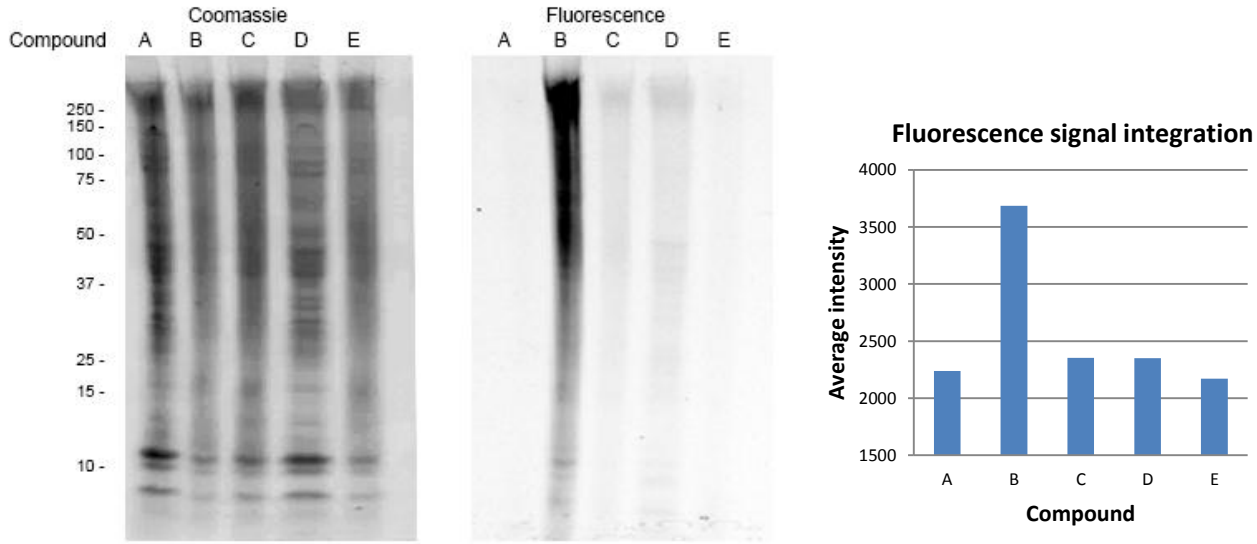
Supporting figure 1 - HEK-293 lysates were incubated with various levels of the probe (AlkMG), then treated with rhodamine-azide and analyzed for fluorescence, before staining with Coomassie brilliant blue. The fluorescence signal was integrated for each sample using ImageQuant TL 1D gel analysis software (GE Healthcare).



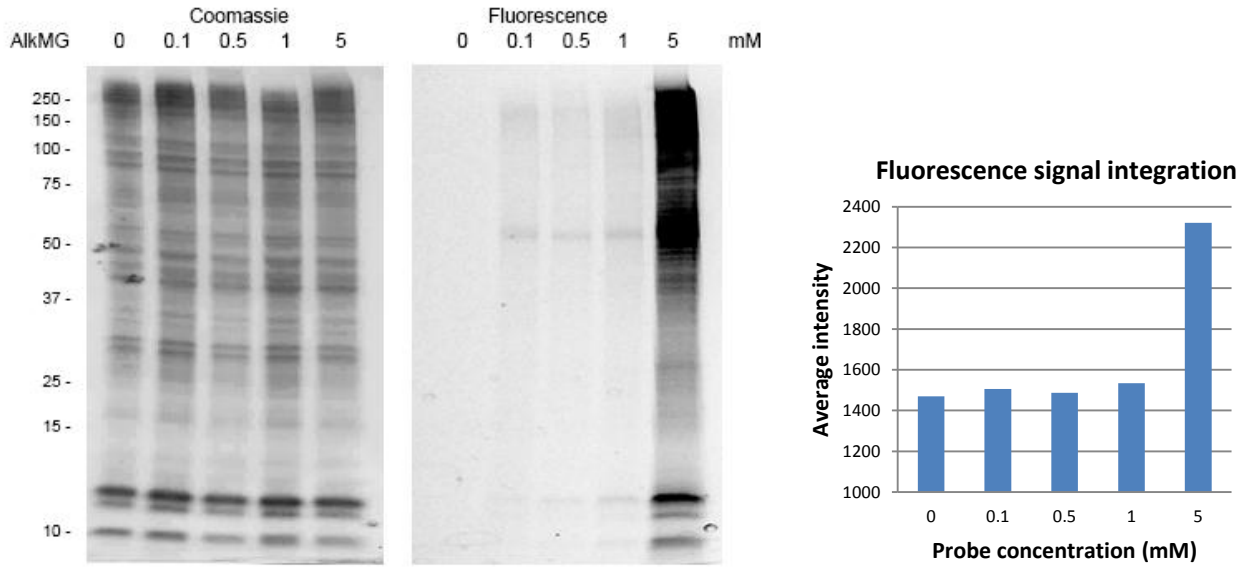
Supporting figure 2 - HEK293 lysates were incubated with AlkMG (1 mM) for 1, 3 or 7 hours, then treated with rhodamine-azide and analyzed for fluorescence, before staining with Coomassie brilliant blue. "-" designates a negative control (lysate with no probe). The fluorescence signal was integrated for each sample using ImageQuant TL 1D gel analysis software (GE Healthcare).



Supporting figure 3 – HEK293 lysates were incubated with methylglyoxal (MG) for 30 min, then with AlkMG for 1 h, then treated with rhodamine-azide and analyzed for fluorescence, before staining with Coomassie brilliant blue. "-" designates a negative control.



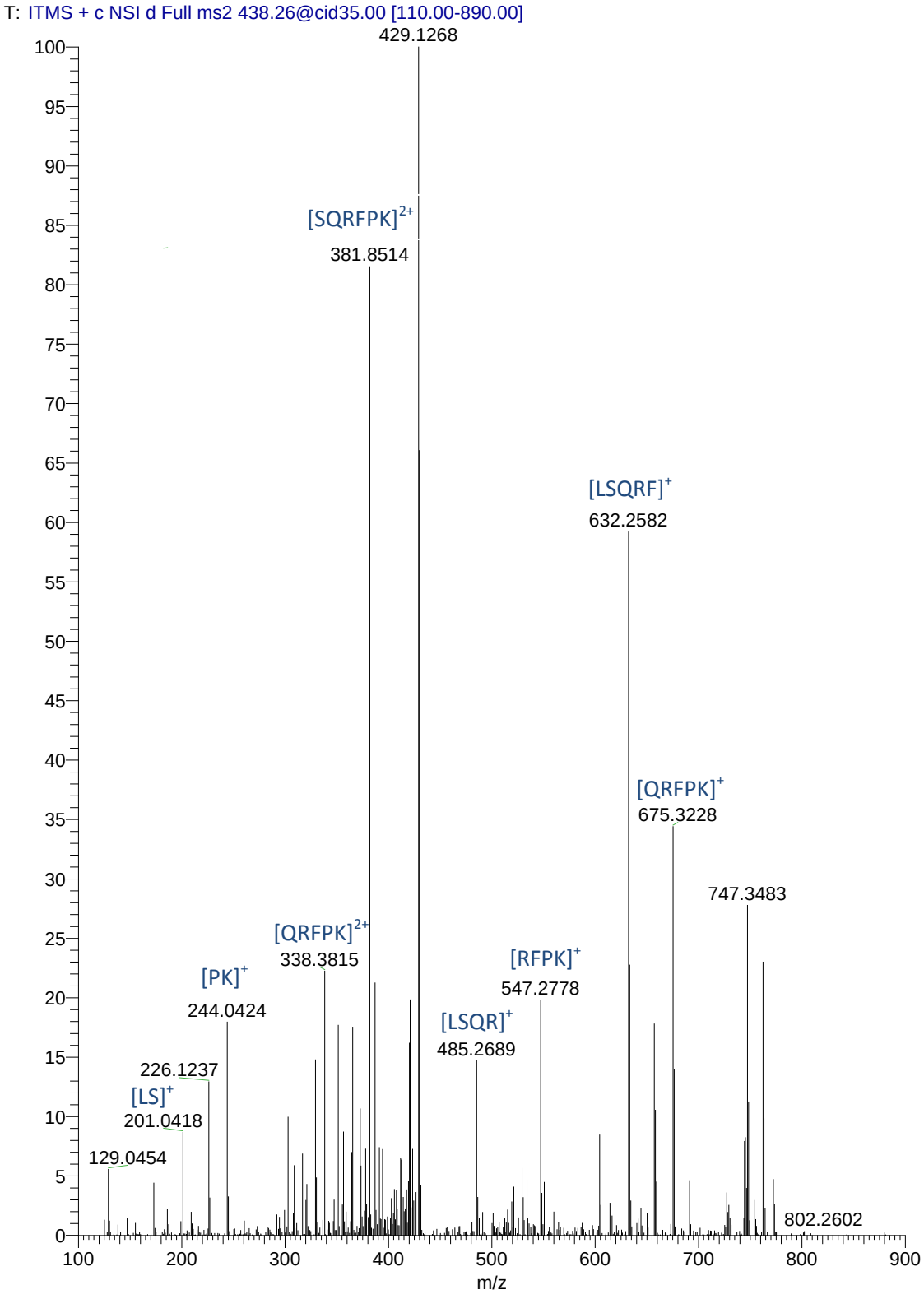
Supporting figure 4 – HEK293 lysates were incubated with 1 mM of the compounds **A** (no treatment, control), **B** (AlkMG, **4**), **C** (1,1-diethoxyhex-5-yn-2-one, **3**), **D** (hex-5-ynal) and **E** (hex-5-yn-2-one) for 1 h at 37 °C, then treated with rhodamine-azide and analyzed for fluorescence, before staining with Coomassie brilliant blue.



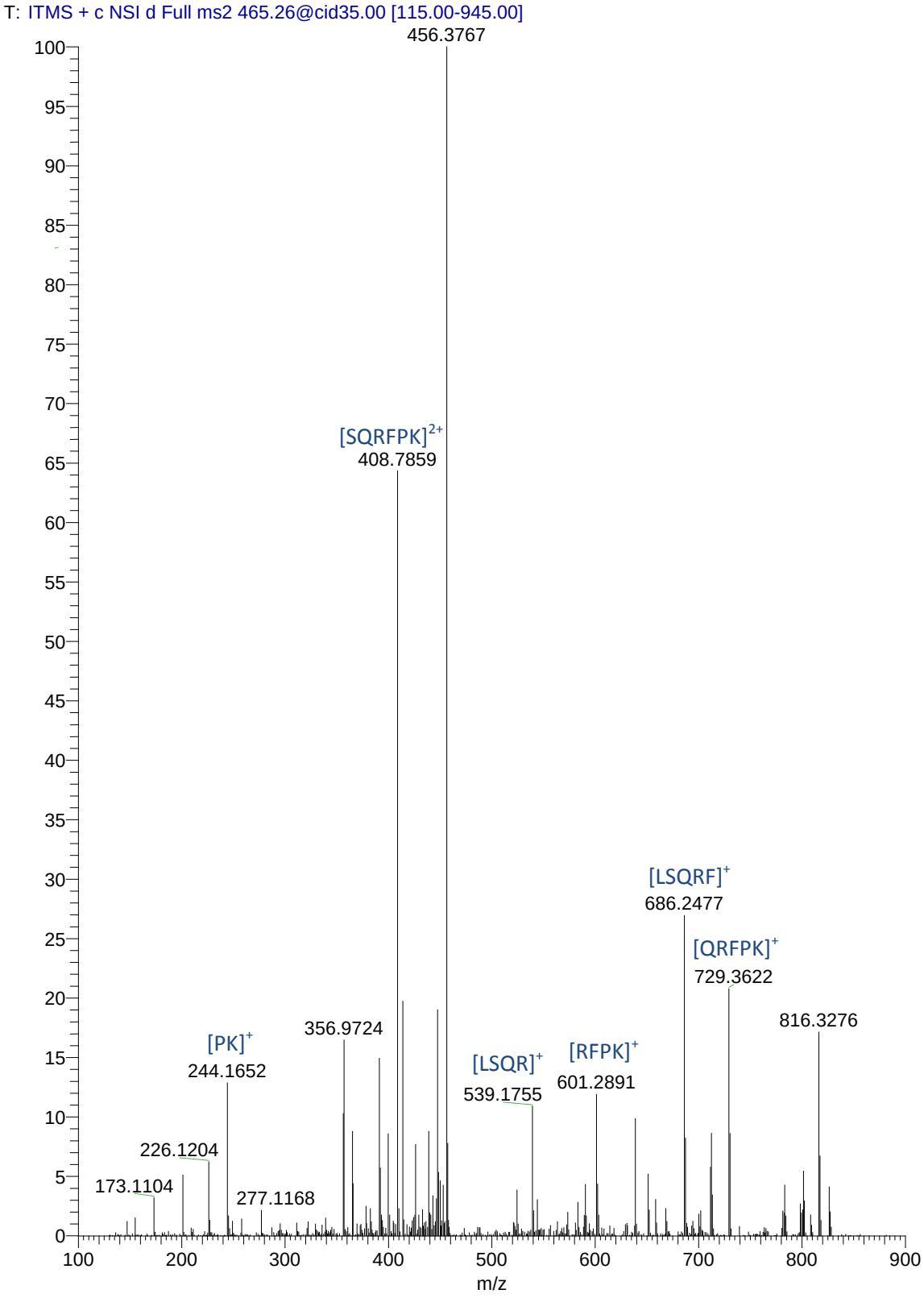
Supporting figure 5 – HEK293 cells were incubated with AlkMG for 2 h, then treated with rhodamine-azide and analyzed for fluorescence, before staining with Coomassie brilliant blue.

Mass spectrometric data – Supporting figures 6-8 (following pages)

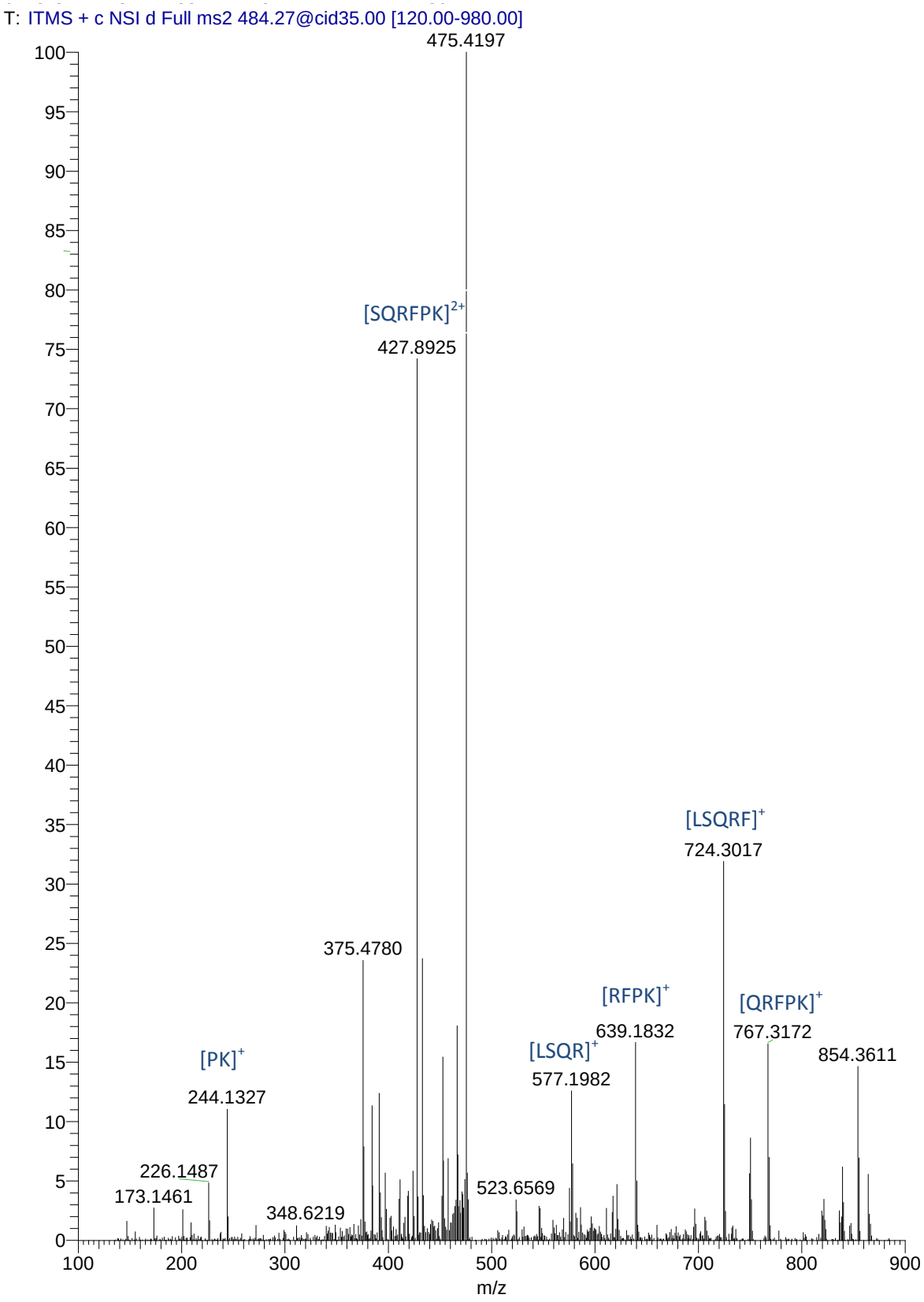
Examples of mass spectra from CID fragmentation of the albumin peptide LSQRFPK from the HSA binding site experiment described earlier. Spectra show the changes in mass of the peptide fragments with and without modification at position R (arginine).



Supporting figure 6 - Example of mass spectrum for peptide: LSQRFPK – no modifications (control)



Supporting figure 7 - Example of mass spectrum for peptide: LSQR(MG)FPK – Methylglyoxal, 100 µM, 1 h (+54.01 Da)



Supporting figure 8 - Example of mass spectrum for peptide: LSQR(AlkMG)FPK – AlkMG, 100 µM, 1 h (+92.03 Da)

Modified peptides detected: AlkMG

Sites of modification detected in the HSA binding site experiment described earlier.

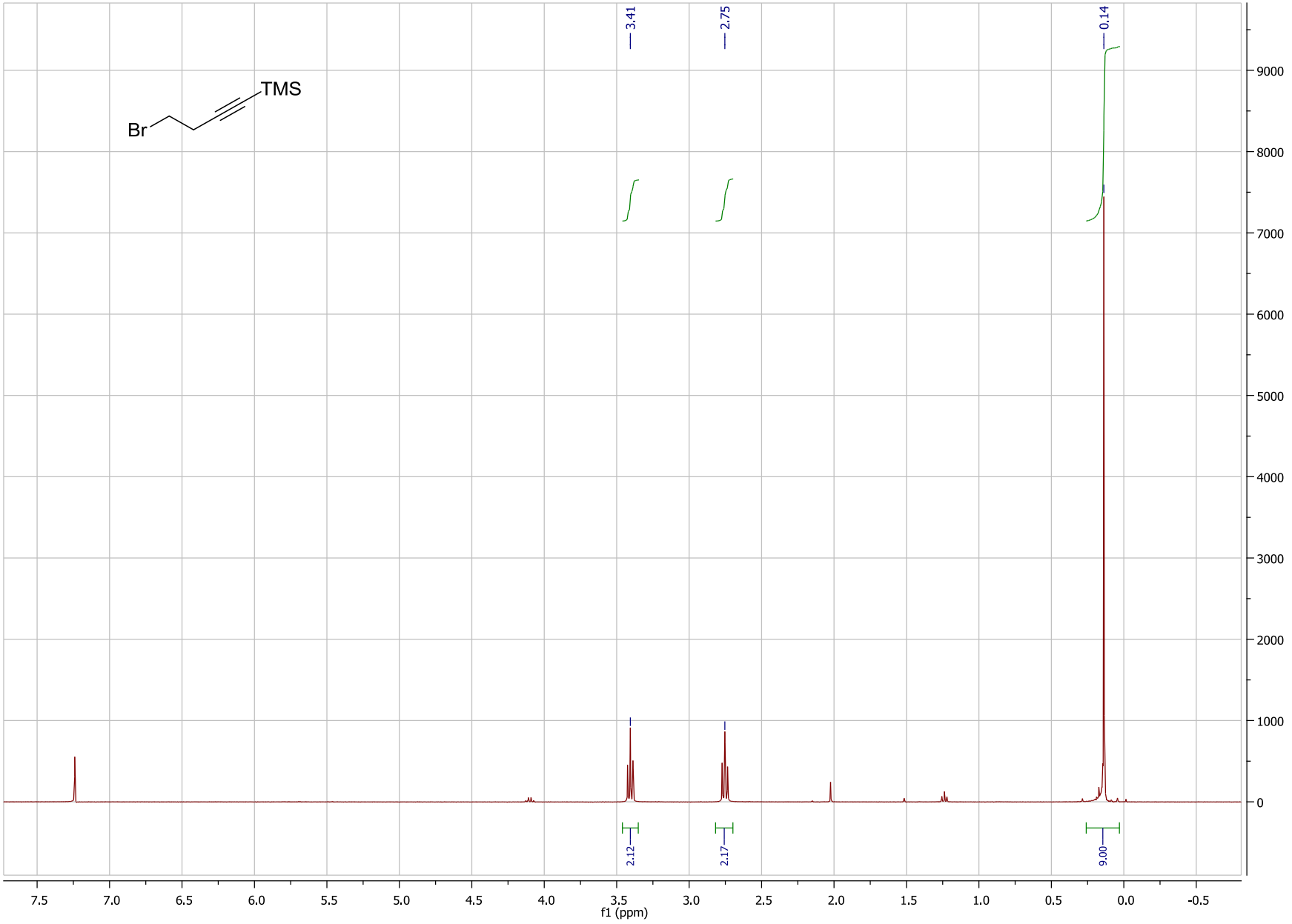
Modified Sequence	Mass	Protein error probability (PEP)
AWAVAR*LSQR	1250.6873	1.1E-16
LSQR*FPK	968.54326	1.1E-16
SEVAHR*FK [a]	1066.5549	8.0E-03
FGER*AFK	947.4854	1.1E-03
FQNALLVR*YTK	1445.802	1.3E-08
KVPQVSTPTLVEVSR*NLGK	2146.2219	5.2E-26
LCTVATLR*ETYGEM(ox)ADCCAK	2459.0699	2.2E-52
LDELR*DEGKASSAK	1613.8239	3.4E-06
LVR*PEVDVMCTAFHDNEETFLKK	2873.407	4.1E-03
RHPDYSVVLLLR*LAK	1875.1072	1.1E-09
R*HPYFYAPELLFFAK	1993.036	5.5E-05
R*MPCAEDYLSVVLNQLCVLHEK	2768.3557	1.7E-31
R*PCFSALEVDETYVPK	2004.9725	2.9E-11
TPVSDR*VTK [a]	1095.5913	8.7E-04
VHTECCHGDLLECADDR*ADLAK	2680.1658	7.2E-22

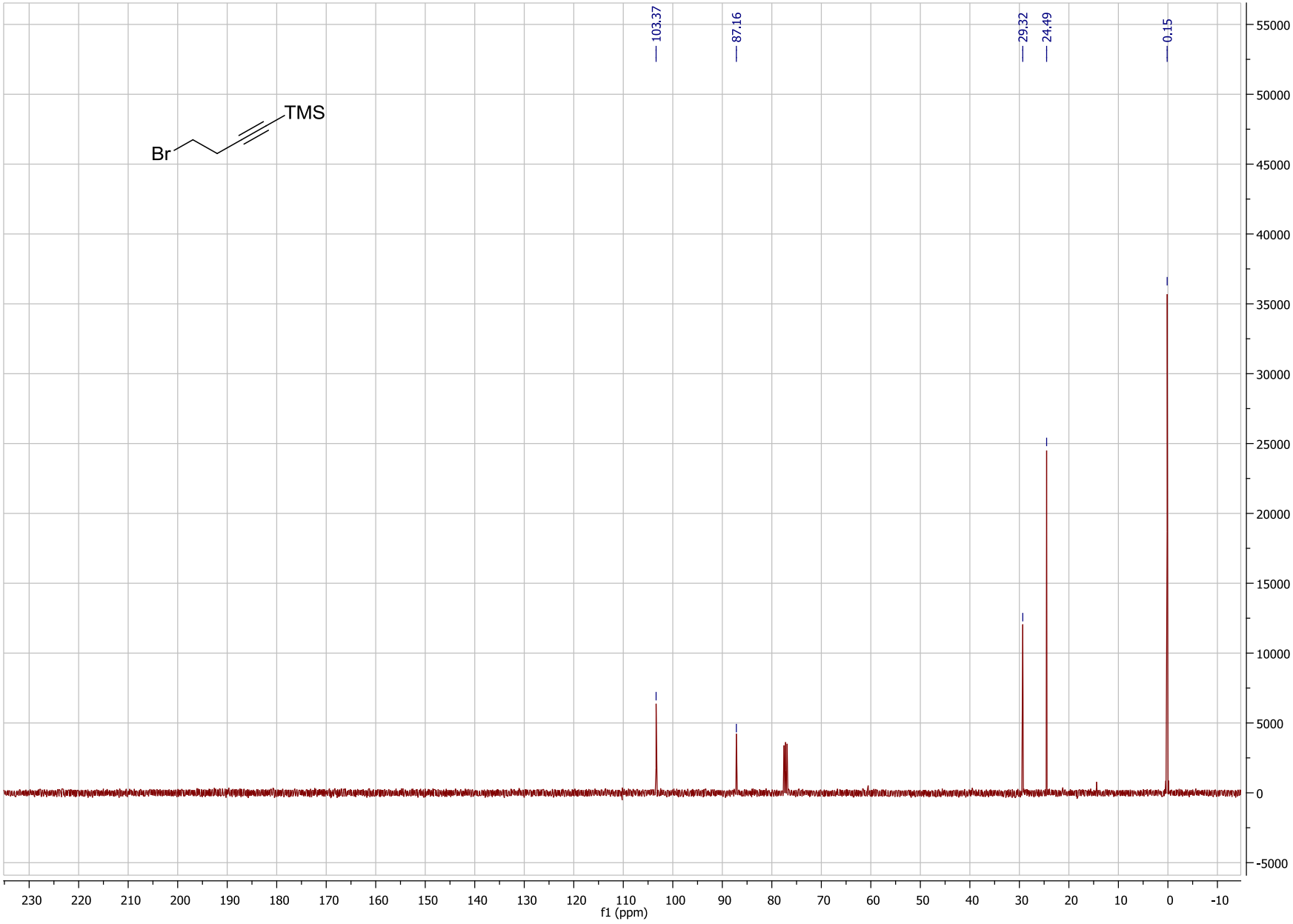
[a] Not identified in the methylglyoxal samples

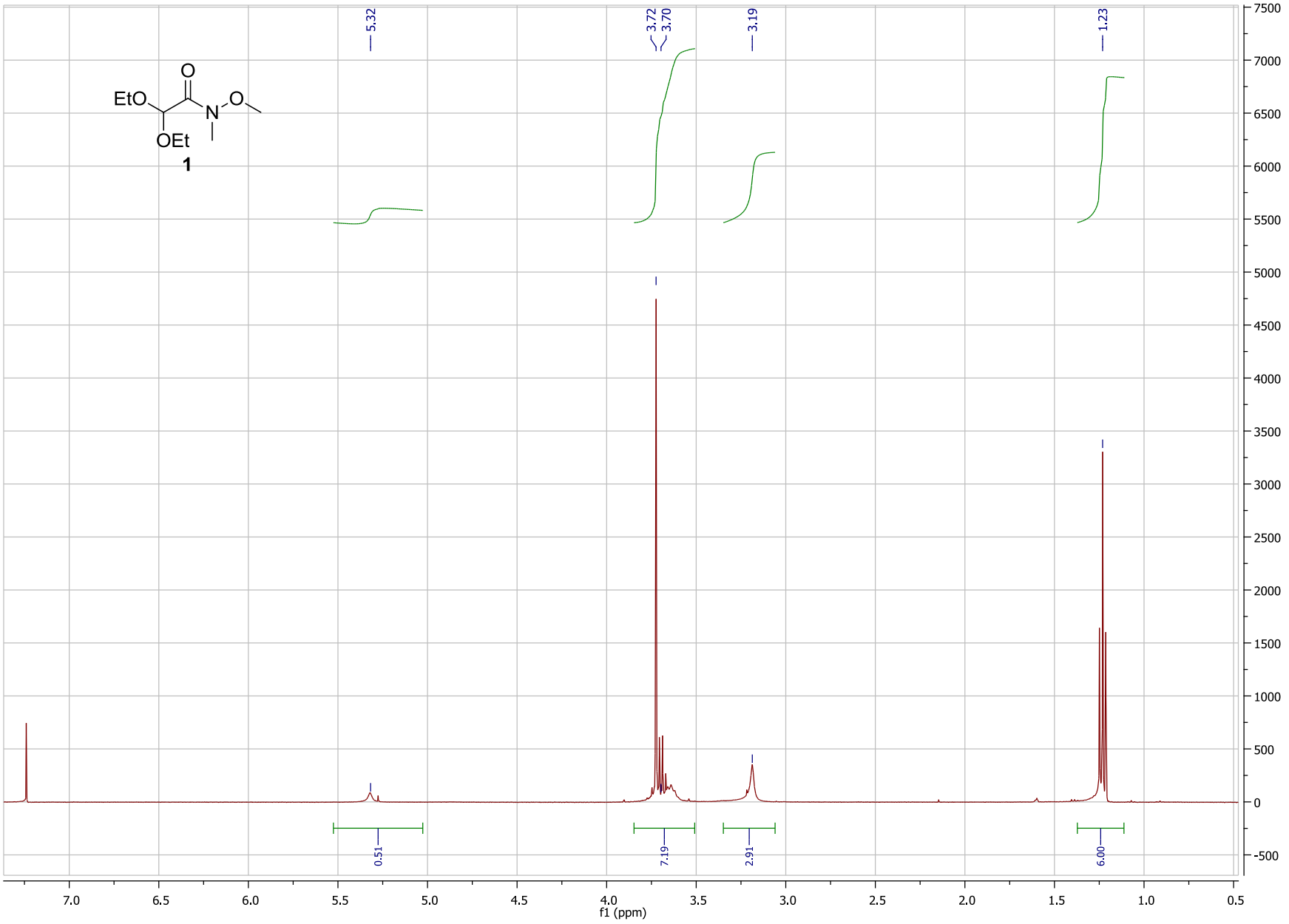
Modified peptides detected: Methylglyoxal

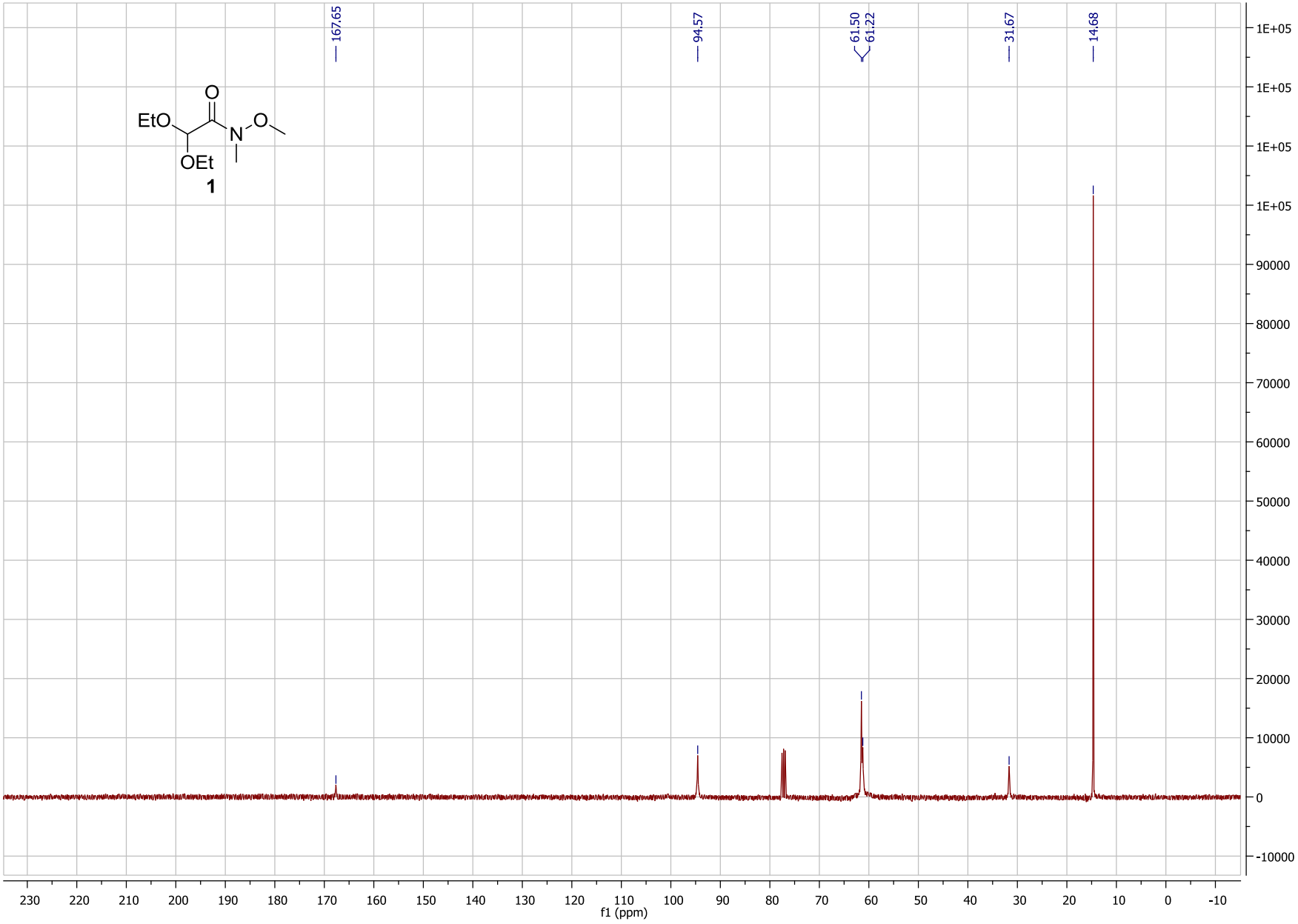
Modified Sequence	Mass	Protein error probability (PEP)
AWAVAR*LSQR	1212.67164	3.1E-21
LSQR*FPK	930.5276	3.1E-21
LVR*PEVDVM(ox)CTAFHDNEETFLKK	2852.39355	1.0E-04
FGER*AFK	909.46976	1.1E-03
FQNALLVR*YTK	1408.79361	4.5E-05
KVPQVSTPTLVEVSR*NLGK	2108.20629	2.0E-17
LCTVATLR*ETYGEMADCCAK	2405.05929	9.9E-33
LDELR*DEGK	1129.56042	8.7E-04
RHPDYSVVLLLR*LAK	1837.0916	9.7E-08
R*HPYFYAPELLFFAK	1955.02032	3.9E-03
R*M(ox)PCAEDYLSVVLNQLCVLHEK	2747.34228	1.5E-14
R*PCFSALEVDETYVPK	1966.95678	9.2E-04
VHTECCHGDLLECADDR*ADLAK	2643.1574	6.0E-40

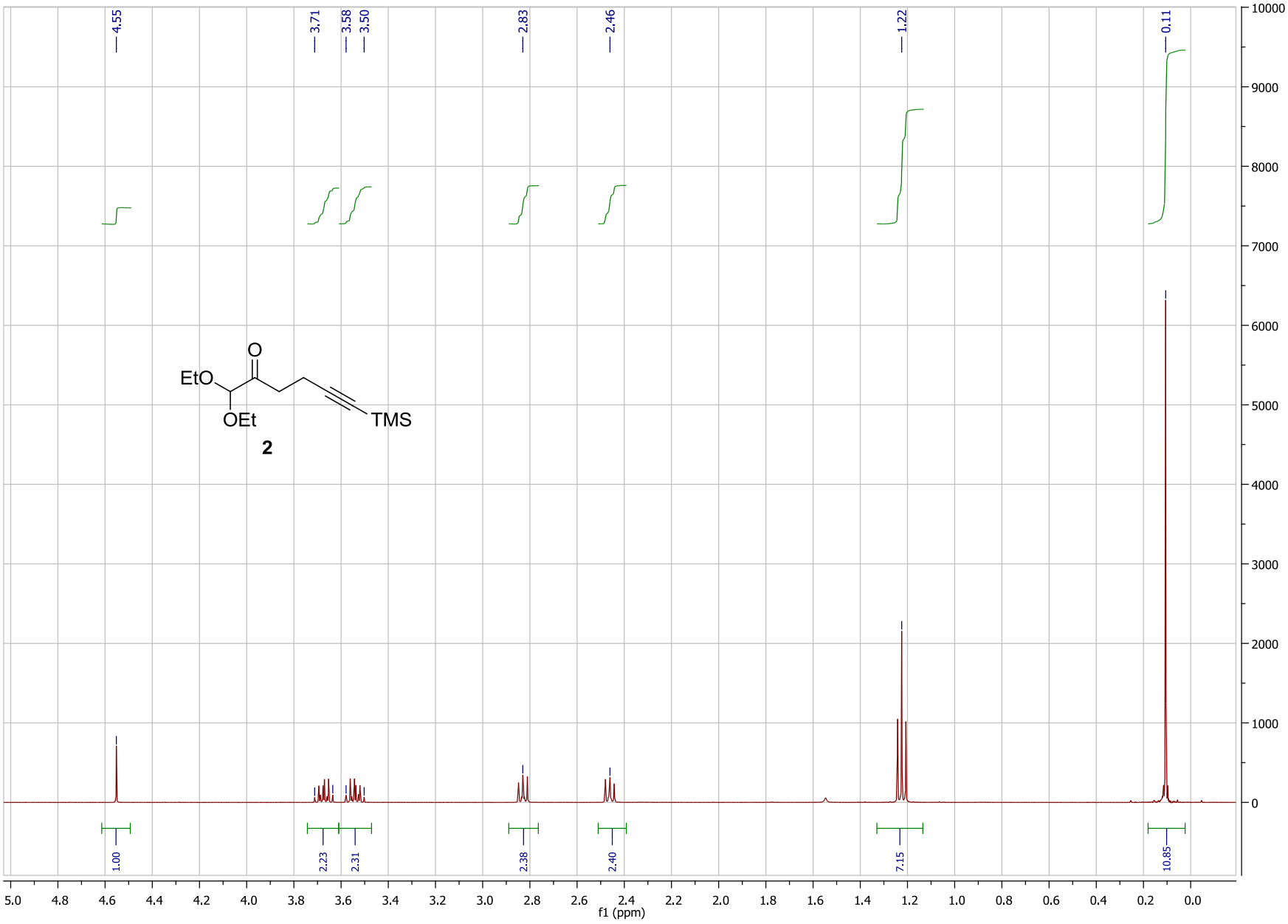
NMR Spectra

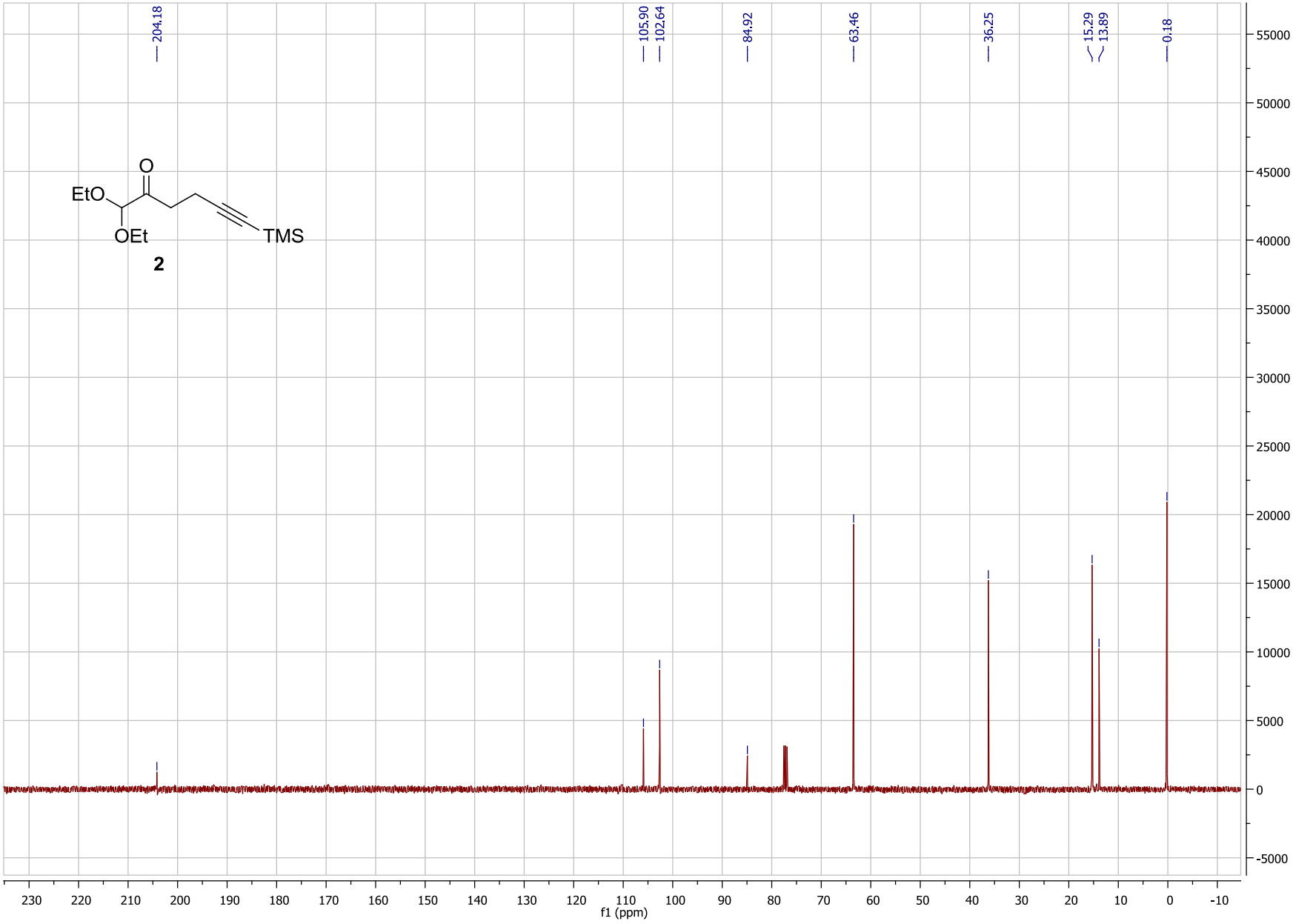


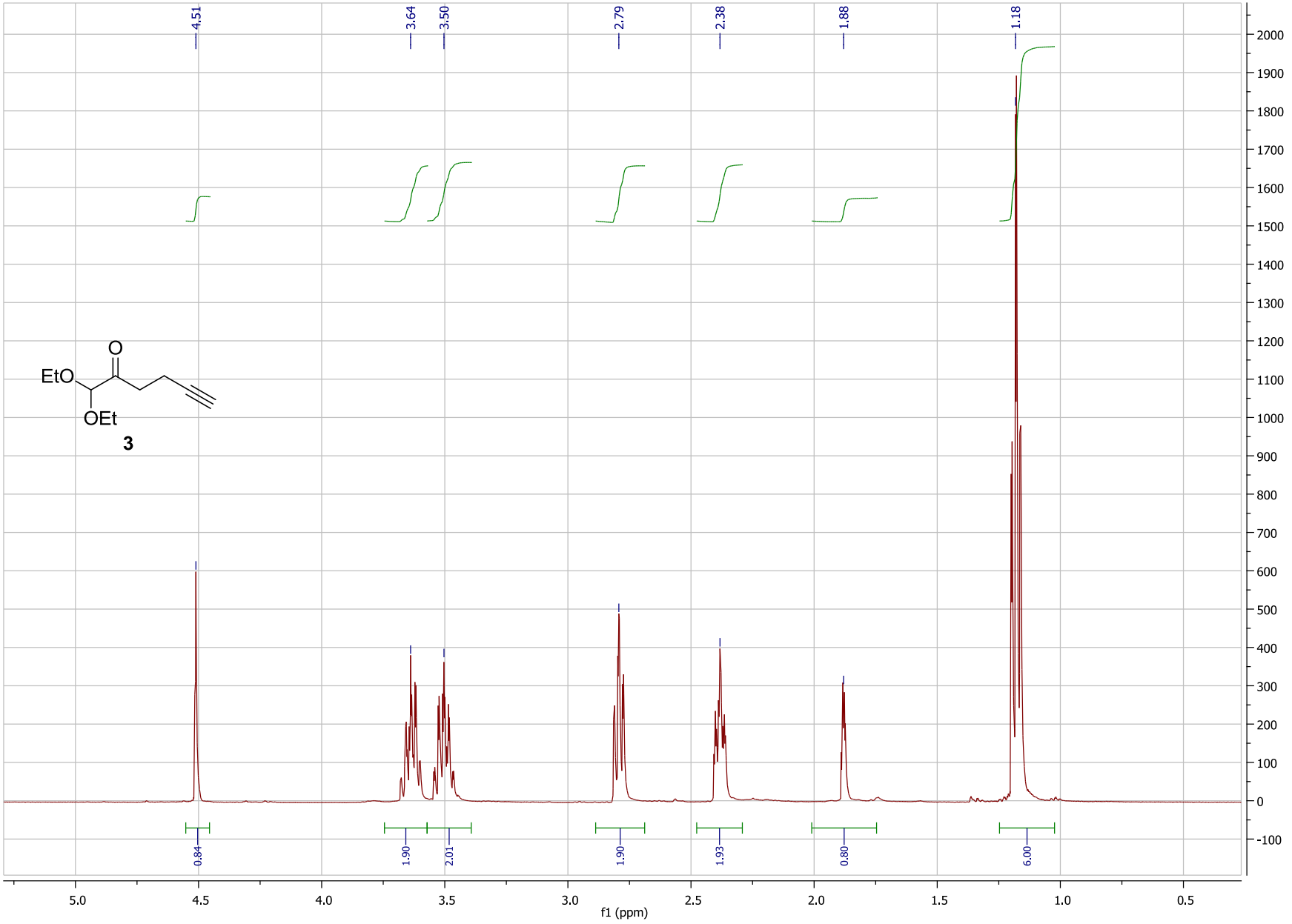


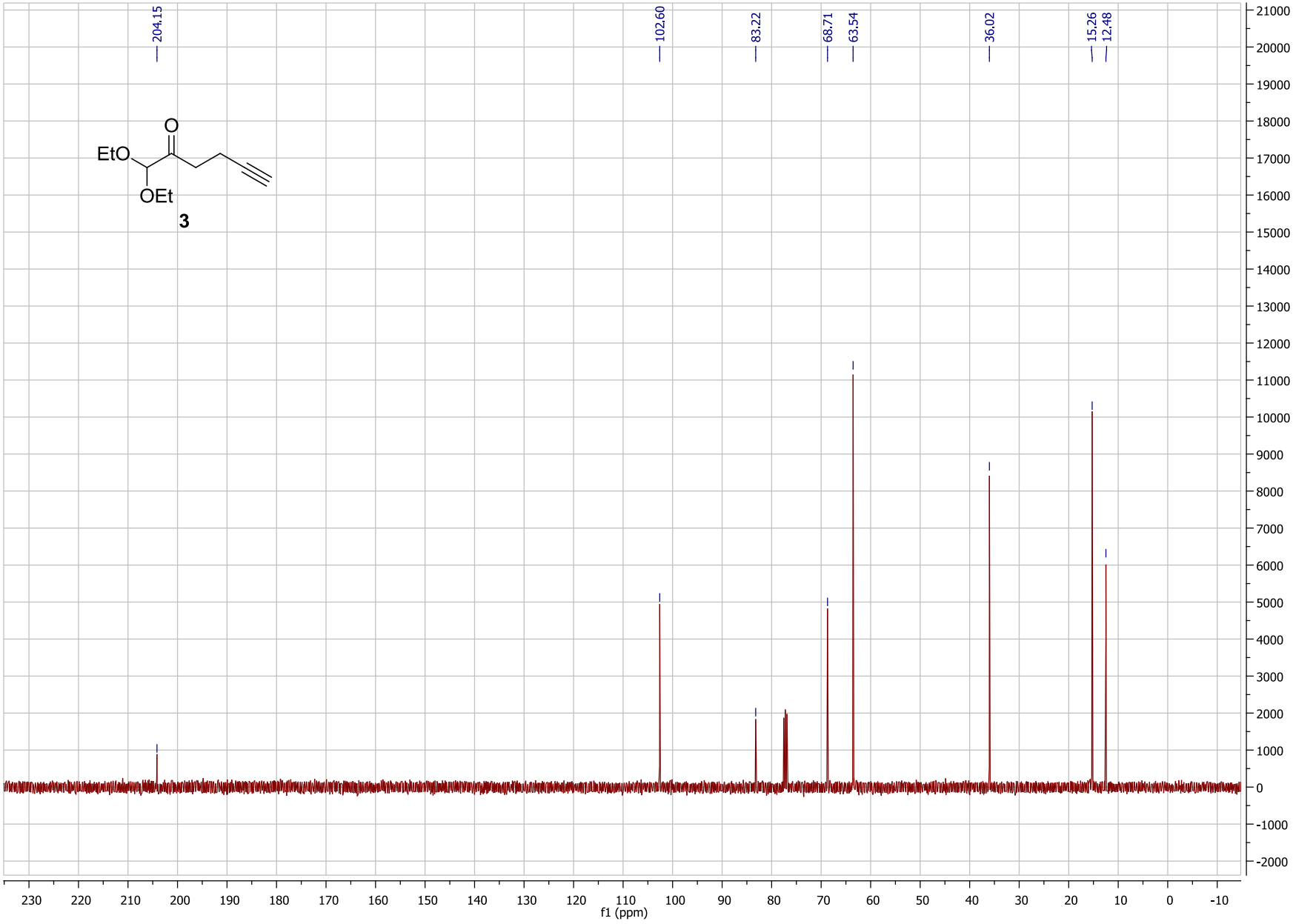


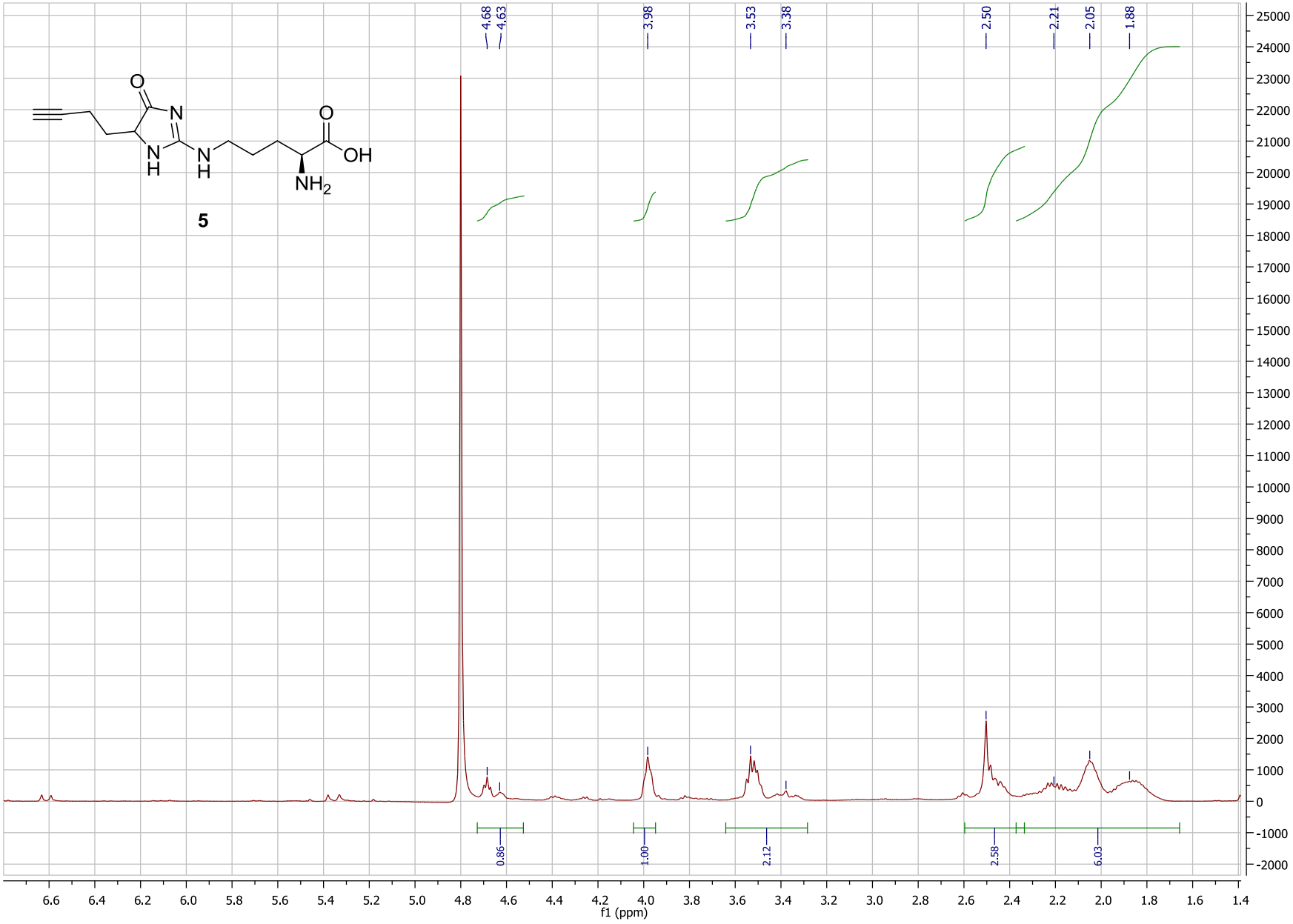












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