Supporting Information

A Clickable Analogue of Cerulenin as Chemical Probe to Explore Protein Palmitoylation

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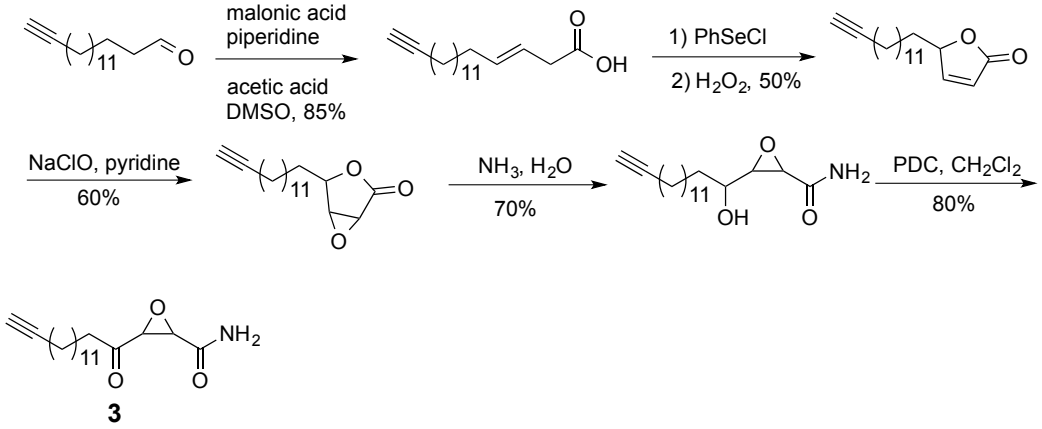
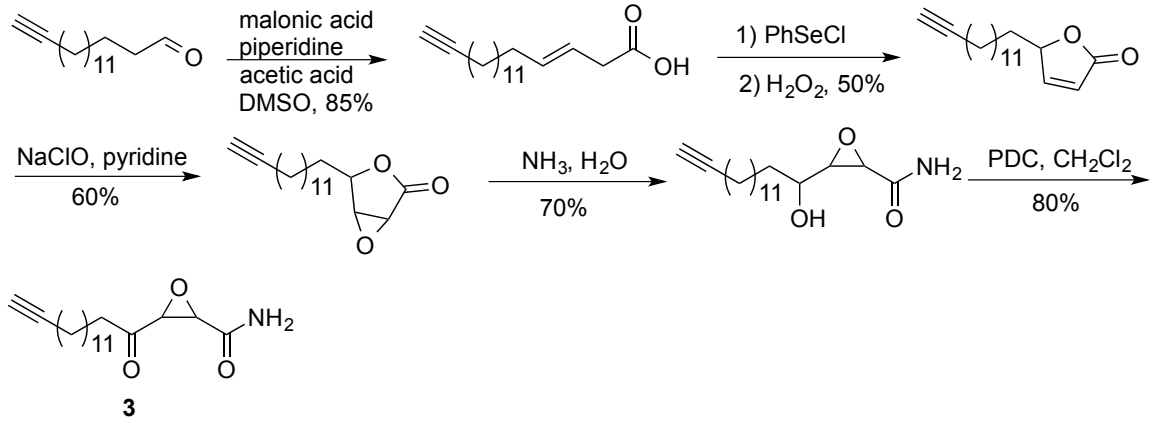
General procedures

All chemicals were purchased from Sigma-aldrich, Alfa Aesar, Fisher Scientific or Acros and were used as received. Azide-biotin was purchased from Click Chemistry Tools. The silica gel used in flash column chromatography was from Aldrich (Cat. 60737, pore size 60 Å, 230-400 mesh). The ^{1}H and ^{13}C NMR spectra were obtained on a Varian 500M spectrometer. Chemical shifts are reported in δ ppm values downfield from tetramethylsilane and *J* values are reported in Hz.



Scheme S1. Synthesis of cerulenin analogue 3.

1. Synthesis of *cis*-2, 3-Epoxy-4-oxooctadec-17-ynamide (3)



(*E*)-octadec-3-en-17-ynoic acid

Piperidine (11.7 μL, 0.118 mmol) and acetic acid (6.8 μL, 0.118 mmol) were stirred for 5 min in 0.5 mL DMSO at room temperature. Afterwards, malonic acid (3.7 g, 35.5 mmol), hexadec-15-ynal (2.8 g,

11.8 mmol) in 10 mL DMSO were added and the reaction mixture was stirred under N_2 atmosphere for 20 min. The solution was stirred at 100 °C for 8 h. The solution was cooled to room temperature and water 20 mL, Et_2O 20 mL were added sequentially. The aqueous phase was extracted with Et_2O, washed with water. The combined organic phases were dried (Na_2SO_4) and evaporated. The crude product was purified by flash chromatography which gave (*E*)-octadec-3-en-17-ynoic acid as white solid (2.94 g, 85% yield). ^{1}H NMR (500 MHz, CDCl3) δ 5.60 – 5.50 (m, 1H), 5.37 (ddd, *J*=15.2, 7.6, 6.3, 1H), 3.62 (dd, *J*=11.9, 6.0, 2H), 2.29 – 2.23 (m, 2H), 2.18 (td, *J*=7.1, 2.6, 2H), 2.05 – 1.97 (m, 2H), 1.93 (dd, *J*=3.0, 2.2, 1H), 1.58 (d, *J*=1.8, 1H), 1.52 (dt, *J*=15.2, 7.2, 2H), 1.44 – 1.21 (m, 20H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.43, 125.63, 84.82, 68.00, 62.02, 35.98, 32.66, 29.61, 29.58, 29.48, 29.45, 29.18, 29.10, 28.75, 28.49, 18.39.

octadec-2-en-17-ynoic acid γ-lactone

PhSeCl solution (78 mg, 0.41 mmol in CH_2Cl_2) was added to a solution of (E)-octadec-3-en-17-ynoic acid (103 mg, 0.37 mmol) and Et_3N (41 mg, 0.41 mmol) in CH_2Cl_2 (10 mL) at –78 °C. The mixture was warmed to –40 °C over 40 minutes and was then concentrated in vacuum. The residue was purified by flash chromatography (50% EtOAc/hexanes) to afford the intermediate product which could be used directly in the next step.

To a solution of the above product in CH_2Cl_2 (10 mL) at 0 °C was added H_2O_2 (35% in H_2O, 200 µL). After stirring for 5 min, another 200 µL of H_2O_2 solution was added. The reaction mixture was stirred for another 40 minutes and then concentrated in vacuum. The residue was purified by flash chromatography (50% EtOAc/hexanes) to afford product, octadec-2-en-17-ynoic acid γ-lactone (51 mg, 50% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (dd, *J*=5.7, 1.5, 1H), 6.10 (dd, *J*=5.7, 2.0, 1H), 5.03 (ddt, *J*=7.3, 5.3, 1.7, 1H), 2.17 (td, *J*=7.1, 2.6, 2H), 1.93 (t, *J*=2.7, 1H), 1.80 – 1.71 (m, 1H), 1.70 – 1.61 (m, 1H), 1.57 – 1.47 (m, 2H), 1.47 – 1.20 (m, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.11, 156.24, 121.51, 84.79, 83.40, 68.01, 33.18, 29.53, 29.45, 29.44, 29.35, 29.28, 29.08, 28.73, 28.47, 24.95, 18.38.

***cis*-2, 3-Epoxyoctadec-17-ynoic acid γ-lactone**

To the octadec-2-en-17-ynoic acid γ-lactone (500 mg, 1.81 mmol) in pyridine (25 mL) at 0 °C was added sodium hypochlorite solution (5%, 13.5 mL, 9 mmol). The mixture was stirred at 0 °C for 1 h and allowed to reach room temperature over an additional hour. The mixture was poured into CH_2Cl_2-water (30-10 mL) and $NaHCO_3$ solution added (1M, 10 mL). The bicarbonate extract was washed with ethyl acetate, acidified to pH 1, and extracted with ethyl acetate (20 mL). The acid extracts were combined, dried, concentrated, and purified, affording analytically pure epoxy lactone (317 mg, 60% yield). ^{1}H NMR (500 MHz, CDCl3) δ ^{13}C NMR (126 MHz, $CDCl_3$) δ 4.60 – 4.53 (m, 2H), 3.96 (d, *J*=2.5, 2H), 3.77 (dd, *J*=2.5, 0.6, 2H), 2.17 (td, *J*=7.1, 2.6, 4H), 1.93 (t, *J*=2.7, 2H), 1.74 – 1.61 (m, 5H), 1.58 – 1.46 (m, 7H), 1.46 – 1.18 (m, 40H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.33, 84.79, 79.81, 68.02, 58.01, 49.79, 32.09, 29.52, 29.46, 29.43, 29.31, 29.22, 29.08, 28.74, 28.48, 24.20, 18.39.

***cis*-2, 3-Epoxy-4-hydroxyoctadec-17-ynamide**

The epoxy lactone (38 mg, 0.13 mmol) in methanol (2 mL) was treated with ammonium hydroxide (15 M, 0.03 mL) at 0 °C. After being stirred at 0 °C for 25 min, the mixture was diluted with CH_2Cl_2 (20 mL) and 0.5 N HCl (5 mL), and the organic phase was dried, concentrated, and purified, affording the hydroxyl amide (28 mg, 70% yield). ^{1}H NMR (500 MHz, CDCl3) 6.27 (d, *J*=20.0, 2H), 3.54 (d, *J*=4.6, 1H), 3.45 (dd, *J*=7.9, 5.0, 1H), 3.13 (dd, *J*=8.1, 4.6, 1H), 2.18 (td, *J*=7.1, 2.6, 2H), 1.94 (t, *J*=2.7, 1H), 1.67 (ddd, *J*=9.5, 6.6, 4.5, 2H), 1.52 (dt, *J*=14.7, 7.0, 3H), 1.45 – 1.20 (m, 18H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.41, 84.81, 68.87, 68.04, 60.12, 54.28, 34.92, 29.61, 29.60, 29.57, 29.53, 29.48, 29.10, 28.75, 28.48, 24.88, 18.39.

***cis*-2, 3-Epoxy-4-oxooctadec-17-ynamide**

The hydroxy amide (13 mg, 0.042 mmol) in CH_2Cl_2 (3 mL) was added at room temperature to a stirred solution of the pyridine dichromate anhydride complex (20 mg) in CH_2Cl_2 (5 mL). After 15 min the mixture was diluted with ether (5 mL), filtered through Celite, washed with water and then dilute acid, and the organic phase was dried, concentrated, and purified, affording **3** (10.3 mg, 80%). ^{1}H NMR (500 MHz, CDCl3) 6.32 (s, 1H), 5.61 (s, 1H), 3.87 (d, *J*=5.3, 1H), 3.72 (d, *J*=5.3, 1H), 2.65 – 2.49 (m, 2H), 2.17 (td, *J*=7.1, 2.6, 2H), 1.93 (t, *J*=2.7, 1H), 1.70 – 1.56 (m, 2H), 1.55 – 1.46 (m, 2H), 1.43 – 1.15 (m, 17H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.65, 167.45, 84.81, 68.03, 58.33, 55.32, 41.13, 29.52, 29.51, 29.45, 29.35, 29.24, 29.08, 29.02, 28.74, 28.48, 23.08, 18.39.

2. Cell culture, transfection and labeling

HEK293A cells (ATCC) were grown in DMEM, supplemented with 10% fetal bovine serum (FBS), 100U/mL penicillin and 100μg/mL streptomycin. Cells were transfected with DHHC-PATs expression vectors using Lipofectamine 2000 (Invitrogen) and further cultured 40-45 h for protein expression.

To label the proteome, cells were cultured with DMEM, supplemented with 1% fatty acid free bovine serum albumin (BSA) for 1 h before labeling. Cells were then treated with probes at concentrations described in the text for indicated time period.

For direct labeling experiments, cell lysates and heat inactivation were prepared as described previously[1].

3. Cell lysate preparation, immunoprecipitation and click reaction

After removal of the growth medium, cells were washed once with cold PBS, collected and centrifuged at 1200g for 5 min. To lyse cells, cells were re-suspend in 0.150 mL lysis buffer [50 mM TEA-HCl (pH=7.4), 150 mM NaCl, 1% Triton X-100, 0.1% SDS and 1 mM PMSF, 1x Roche complete mini protease inhibitor cocktail]. The mixture was shaken gently for 15 minutes on ice, and

then centrifuged at 15,000 g for 15 minutes to pellet the cell debris. The resulting supernatant was collected to a new tube and protein concentration was analyzed by the BCA assay.

Protein lysate (400 µg) were incubated with 1µg anti-HA antibody for 2 h at 4 °C with rocking and were subsequently immunoprecipitated with 30 µL prewashed Pierce Protein A/G Magnetic Beads (Product# 88803) with rocking for 1 h at 4 °C. The beads were washed three times with PBST and then two times with purified water. Proteins captured on beads were subjected to the click reaction in a 100 uL PBST for at RT 1 h.

100µg cell lysate or immunoprecipitated proteins was reacted with 10µl freshly premixed click reaction reactants for 1 hour at room temperature (RT) as shown in the table below.

Reagent	**Stock concentration**	**Final concentration**	**Volume**
tag-azide	10 mM in DMSO	100 µM	1 µL
TCEP	50 mM in deionized water	1 mM	2 µL
TBTA	2.0 mM in DMSO/t-butanol (1/4)	100 µM	5 µL
$CuSO_4$	50 mM in deionized water	1 mM	2 µL

Tag-azide: biotin-azide
TCEP: tris(2-carboxyethyl)phosphine hydrochloride
TBTA: tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methy-l]amine

Reactions were stopped by the addition of 10µL of 6x SDS-sample buffer (50mM Tris-HCl (PH=6.8), 6% SDS, 48% Glycerol, 0.03% Bromphenol Blue, 30mM EDTA, 9% MeSH). The protein samples were then heated for 3 min at 95 °C.

For the hydroxylamine treatment, hydroxylamine (2.5% final concentration) was added to the sample before the addition of loading buffer. ~10 µg protein was loaded per lane (4-20% mini-protein TGX precast gels) and separated by SDS-PAGE.

4. Western blotting

Proteins were transferred onto a PVDF membrane. For immunoblotting of biotin-labeled proteins, the membrane was blocked in 5% BSA TBST solution for 1h at room temperature or overnight at 4 °C. The membrane was then washed 3 times with PBS, incubated with Streptavidin-horseradish peroxidase (HRP) (1 mg/mL, with 1:10,000 dilution in PBST) for 1 h, and developed with ECL Western blotting detection reagents after washing with TBST (3 times, 10 min each washing).

For immunoblotting of HA-tagged proteins, the membrane was blocked in 5% nonfat dried milk in TBST solution for 1h at room temperature or overnight at 4 °C, incubated with anti-HA primary

antibody (diluted 1:10,000 in 5% nonfat dried milk TBST solution) at RT for 1h or overnight at 4 °C, washed with TBST (3 times, 10 min each washing), and incubated with sheep anti-mouse antibody conjugated to HRP (1:10,000 dilution in 5% nonfat dried milk TBST solution) for 1h at RT. At last, the membrane was developed with ECL Western blotting detection reagents after washed with TBST (3 x 10 min).

5. Streptavidin beads pull-down and Mass spectrometry studies

After click chemistry, proteins were precipitated with MeOH at -80 °C overnight, centrifuged at 3500 g and washed twice with chilled MeOH. Protein was air dried and dissolved with 4% SDS buffer (50 mM Tris-HCl, pH 7.4,150 mM NaCl, 10 mM EDTA, 4% SDS) by sonication. The sample was diluted with 1 volume of 1% Brij97 buffer (50 mM Tris-HCl, pH 7.4,150 mM NaCl, 10 mM EDTA, 1% Brij97). Streptavidin-agarose beads were pre-washed 3 times with PBS, and then added to the samples. The resulted mixture was rotated at room temperature for 1.5 h, centrifuged at 2000 g for 2 min, and then washed with 3X 0.2 SDS in PBS and 1x 250 mM ammonium bicarbonate. After removing supernatant, the captured proteins were incubated with 500 μL of 8M urea, 50 μL of 500 mM TCEP and 50 μL of 400 mM iodoacetamide for 40 min in dark and then washed twice with 250 mM ammonium bicarbonate. The samples were directly digested on beads and supernatants were collected for LC-MS/MS analysis as described previously[1].

For endogenous palmitoylated protein detection, cells were labeled using 15-hexadecynoic acid and proteins were pulled down using Strepavidin beads. After washing, the beads were incubated in elution buffer (6 M Urea, 2 % SDS, 30 mM Biotin) for 30 min to elute captured proteins. The eluent samples were boiled for 3 min after addition of loading buffer. Proteins were separated by SDS-PAGE and detected by western blot.

References:

(1) Zheng, B.; DeRan, M.; Li, X.; Liao, X.; Fukata, M.; Wu, X. 2-Bromopalmitate analogues as activity-based probes to explore palmitoyl acyltransferases. *J. Am. Chem. Soc.* **2013**, *135*, 7082-7085.

Supplementary Table 1: The list of identified proteins from the mass spectrometry studies using Probe **3,** with or without the competition of 2-BP (100μM) or cerulenin (10μM). Total spectra counts were listed for each protein. Proteins highlighted in yellow are the targets which could be competed off by cerulenin, but not 2-BP. Proteins highlighted in green are the targets which could be competed off by 2-BP, but not cerulenin.

Symbol	Protein Name	DMSO	Probe 3	Probe 3 + 2BP	Probe 3 + Cerulenin
RTN4	Reticulon-4	4	164	74	78
VDAC2	Voltage-dependent anion-selective channel protein 2	11	140	30	40
FABP5	Fatty acid-binding protein, epidermal	4	103	1	9
VDAC3	Voltage-dependent anion-selective channel protein 3	3	101	48	49
HMOX2	Heme oxygenase	1	100	43	45
ATP2A2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	6	94	30	54
CYB5B	Cytochrome b5 type B	3	63	17	18
ATP2A1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	5	61	29	38
HM13	Minor histocompatibility antigen H13	0	55	21	16
TXNRD1	Thioredoxin reductase 1, cytoplasmic	4	54	40	17
SEC63	Translocation protein SEC63 homolog	0	38	4	5
TXNDC5	Thioredoxin domain-containing protein 5	6	38	20	11
TRAPPC3	Trafficking protein particle complex subunit 3	0	36	2	5
ENDOD1	Endonuclease domain-containing 1 protein	0	35	5	6
XPO1	Exportin-1	3	35	38	22
MTAP	S-methyl-5'-thioadenosine phosphorylase	0	34	11	16
HCCS	Cytochrome c-type heme lyase	0	33	8	7
APOL2	Apolipoprotein L2	0	32	7	9
TMX1	Thioredoxin-related transmembrane protein 1	0	31	18	17
MLANA	Melanoma antigen recognized by T-cells 1	3	28	4	7
BACH1	Transcription regulator protein BACH1	0	27	2	4
SNX1	Sorting nexin-1	0	27	18	12
CD81	CD81 antigen	4	25	5	5
LPCAT2	Lysophosphatidylcholine acyltransferase 2	0	25	2	4
COMT	Catechol O-methyltransferase	2	24	16	8
GPR143	G-protein coupled receptor 143	1	23	19	15
NPC1	Niemann-Pick C1 protein	0	23	10	15
HEATR3	HEAT repeat-containing protein 3	0	21	6	1
TMX3	Protein disulfide-isomerase TMX3	0	21	4	3

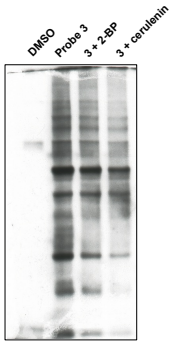
TXNDC12	Thioredoxin domain-containing protein 12	4	20	7	0
GGT7	Gamma-glutamyltransferase 7	0	19	4	7
ZDHHC20	Probable palmitoyltransferase ZDHHC20	0	19	4	7
PCYOX1	Prenylcysteine oxidase 1	0	18	5	3
SCAMP3	Secretory carrier-associated membrane protein 3	0	18	11	10
TMX4	Thioredoxin-related transmembrane protein 4	0	18	10	2
CISD2	CDGSH iron-sulfur domain-containing protein 2	0	17	4	7
FKBP8	Peptidyl-prolyl cis-trans isomerase FKBP8	1	17	8	10
TRPV2	Transient receptor potential cation channel subfamily V member 2	0	17	9	9
AP1AR	AP-1 complex-associated regulatory protein	0	16	2	0
C16orf58	UPF0420 protein C16orf58	0	16	4	10
SNAP23	Synaptosomal-associated protein 23	0	16	7	6
TTYH2	Protein tweety homolog 2	0	16	9	9
CISD1	CDGSH iron-sulfur domain-containing protein 1	0	15	6	6
DAGLB	Sn1-specific diacylglycerol lipase beta	0	15	7	5
NCEH1	Neutral cholesterol ester hydrolase 1	0	15	5	27
TM9SF4	Transmembrane 9 superfamily member 4	0	15	7	6
TMEM245	Transmembrane protein 245	0	15	6	6
XPOT	Exportin-T	0	15	6	5
CDKAL1	Threonylcarbamoyladenosine tRNA methylthiotransferase	0	14	6	9
RTN3	sapiens	0	14	4	5
SLC24A5	Sodium/potassium/calcium exchanger 5	0	14	3	3
TMED1	Transmembrane emp24 domain-containing protein 1	0	14	2	4
VEZT	Vezatin	0	14	5	2
ERO1L	ERO1-like protein alpha	0	13	5	9
GLTP	Glycolipid transfer protein	0	13	1	0
PLIN3	Perilipin-3	0	13	1	2
SLC44A1	Choline transporter-like protein 1	0	13	3	1
ALG6	Dolichyl pyrophosphate Man9GlcNAc2 alpha-1,3-glucosyltransferase	0	12	1	0
BABAM1	BRISC and BRCA1-A complex member 1	0	12	6	3
HMGCS1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	0	12	9	5
MIEN1	Migration and invasion enhancer 1	0	12	0	0
PTGR2	Prostaglandin reductase 2	0	12	5	2

RFTN1	Raftlin	0	12	6	2
SLC7A1	High affinity cationic amino acid transporter 1	0	12	5	9
SNX11	Sorting nexin-11	0	12	3	7
TSPAN14	Tetraspanin-14	0	12	0	3
ANXA11	Annexin A11	0	11	5	4
PGRMC1	Membrane-associated progesterone receptor component 1	2	11	1	1
SLC15A4	Solute carrier family 15 member 4	0	11	3	5
SPRY4	Protein sprouty homolog 4	0	11	3	3
TM9SF3	Transmembrane 9 superfamily member 3	0	11	1	1
UBR7	Putative E3 ubiquitin-protein ligase UBR7	0	11	0	0
PLSCR1	Phospholipid scramblase 1	0	10	5	4
PPM1G	Protein phosphatase 1G	2	10	0	2
LPCAT1	Lysophosphatidylcholine acyltransferase 1	0	9	1	2
RABAC1	Prenylated Rab acceptor protein 1	0	9	4	3
SLC19A2	Thiamine transporter 1	0	9	6	6
SORT1	Sortilin	0	9	6	4
ST6GALNAC3	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3	0	9	0	0
VKORC1L1	Vitamin K epoxide reductase complex subunit 1-like protein 1	0	9	6	2
BCCIP	BRCA2 and CDKN1A-interacting protein	0	8	1	4
CD276	CD276 antigen	0	8	5	2
DFNA5	Non-syndromic hearing impairment protein 5	0	8	7	2
MT-ND5	NADH-ubiquinone oxidoreductase chain 5	0	8	1	1
RABGGTB	Geranylgeranyl transferase type-2 subunit beta	0	8	0	1
TM9SF2	Transmembrane 9 superfamily member 2	1	8	2	1
TMEM222	Transmembrane protein 222	0	8	0	5
TOMM20	Mitochondrial import receptor subunit TOM20 homolog	0	8	4	3
TRMT112	tRNA methyltransferase 112 homolog	0	8	11	3
TSPAN4	Tetraspanin-4	0	8	6	0
C18orf32	UPF0729 protein C18orf32	0	7	0	1
MCOLN1	Mucolipin-1	0	7	6	4
PSEN2	Presenilin-2	0	7	0	2
SLC30A1	Zinc transporter 1	0	7	0	2
SLC7A5	Large neutral amino acids transporter small subunit 1	0	7	3	2
STX8	Syntaxin-8	0	7	2	3

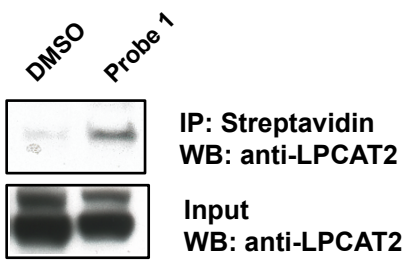
SURF4	Surfeit locus protein 4	0	7	3	2
VAMP2	Vesicle-associated membrane protein 2	0	7	3	4
FAM108B1	Abhydrolase domain-containing protein FAM108B1	0	6	1	2
PLP1	Myelin proteolipid protein	0	6	3	2
SELT	Selenoprotein T	0	6	3	4
SNX6	Sorting nexin-6	0	6	2	1
TBC1D15	TBC1 domain family member 15	0	6	1	3
TMEM192	Transmembrane protein 192	0	6	0	2
TTYH3	Protein tweety homolog 3	0	6	7	0
VIMP	Selenoprotein S	0	6	3	3
AGPAT9	Glycerol-3-phosphate acyltransferase 3	0	5	1	5
CRKL	Crk-like protein	0	5	4	2
NCDN	Neurochondrin	0	5	2	0
NDUFB11	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial	0	5	1	1
PML	Protein PML	0	5	2	5
RER1	Protein RER1	0	5	2	2
SEC62	Translocation protein SEC62	0	5	2	2
SLC5A6	Sodium-dependent multivitamin transporter	0	5	2	3
SPRYD7	SPRY domain-containing protein 7	0	5	4	2
TMEM55B	Transmembrane protein 55B	0	5	0	2
VKORC1	Vitamin K epoxide reductase complex subunit 1	0	5	0	0
CEND1	Cell cycle exit and neuronal differentiation protein 1	0	4	1	0
COX4I1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	0	4	0	0
CTAG1A	Cancer/testis antigen 1	0	4	1	5
DCTN2	Dynactin subunit 2	0	4	0	5
EPT1	Ethanolaminephosphotransferase 1	0	4	0	0
FAM118B	Protein FAM118B	0	4	0	0
SCAMP2	Secretory carrier-associated membrane protein 2	0	4	1	2
SLC39A14	Zinc transporter ZIP14	0	4	2	1
TMEM63A	Transmembrane protein 63A	0	4	3	0
ALG11	GDP-Man:Man(3)GlcNAc(2)-PP-Dol alpha-1,2-mannosyltransferase	0	3	0	2

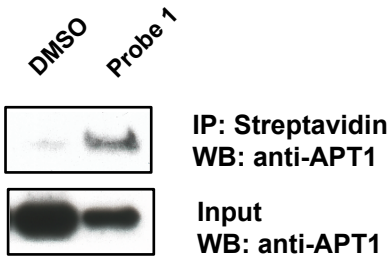
C9orf91	Transmembrane protein C9orf91	0	3	1	2
CEPT1	Choline/ethanolaminephosphotransferase 1	0	3	0	0
LNPEP	Leucyl-cystinyl aminopeptidase	0	3	1	0
PLP2	Proteolipid protein 2	0	3	0	0
PLS1	Plastin-1	0	3	1	2
PQLC3	PQ-loop repeat-containing protein 3	0	3	0	1
SDHD	Succinate dehydrogenase [ubiquinone] cytochrome b small subunit, mitochondrial	0	3	0	0
SLC22A18	Solute carrier family 22 member 18	0	3	1	1
TMEM164	Transmembrane protein 164	0	3	0	0
TPCN2	Two pore calcium channel protein 2	0	3	0	1
VAMP4	Vesicle-associated membrane protein 4	0	3	0	2
ALG3	Dol-P-Man:Man(5)GlcNAc(2)-PP-Dol alpha-1,3-mannosyltransferase	0	2	0	0
DPY19L1	Protein dpy-19 homolog 1	0	2	3	0
GLTPD1	Glycolipid transfer protein domain-containing protein 1	0	2	0	0
LYPLA1	Acyl-protein thioesterase 1	0	2	0	2
MBOAT7	Lysophospholipid acyltransferase 7	0	2	1	1
OR1M1	Olfactory receptor 1M1	0	2	0	0
RCE1	CAAX prenyl protease 2	0	2	0	2
RFT1	Protein RFT1 homolog	0	2	0	0
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	0	2	1	1
SLC2A13	Proton myo-inositol cotransporter	0	2	0	0
SLC39A10	Zinc transporter ZIP10	0	2	0	0
SLC43A3	Solute carrier family 43 member 3	0	2	1	0
ST3GAL4	CMP-N-acetylneuraminate-beta-galactosamide -alpha-2,3-sialyltransferase 4	0	2	0	1
TBC1D9B	TBC1 domain family member 9B	0	2	0	0
TMBIM1	Protein lifeguard 3	0	2	1	0
XRCC4	DNA repair protein XRCC4	0	2	0	0

Supplementary Figure S1. Protein labeling of Probe **3** (5μM) in 501Mel cells with or without palmitoylation inhibitors 2-BP (100 μM) or cerulenin (10μM). The cell lysate is subjected to Click chemistry with biotin-azide, the labeled protein is detected by streptavidin blot.



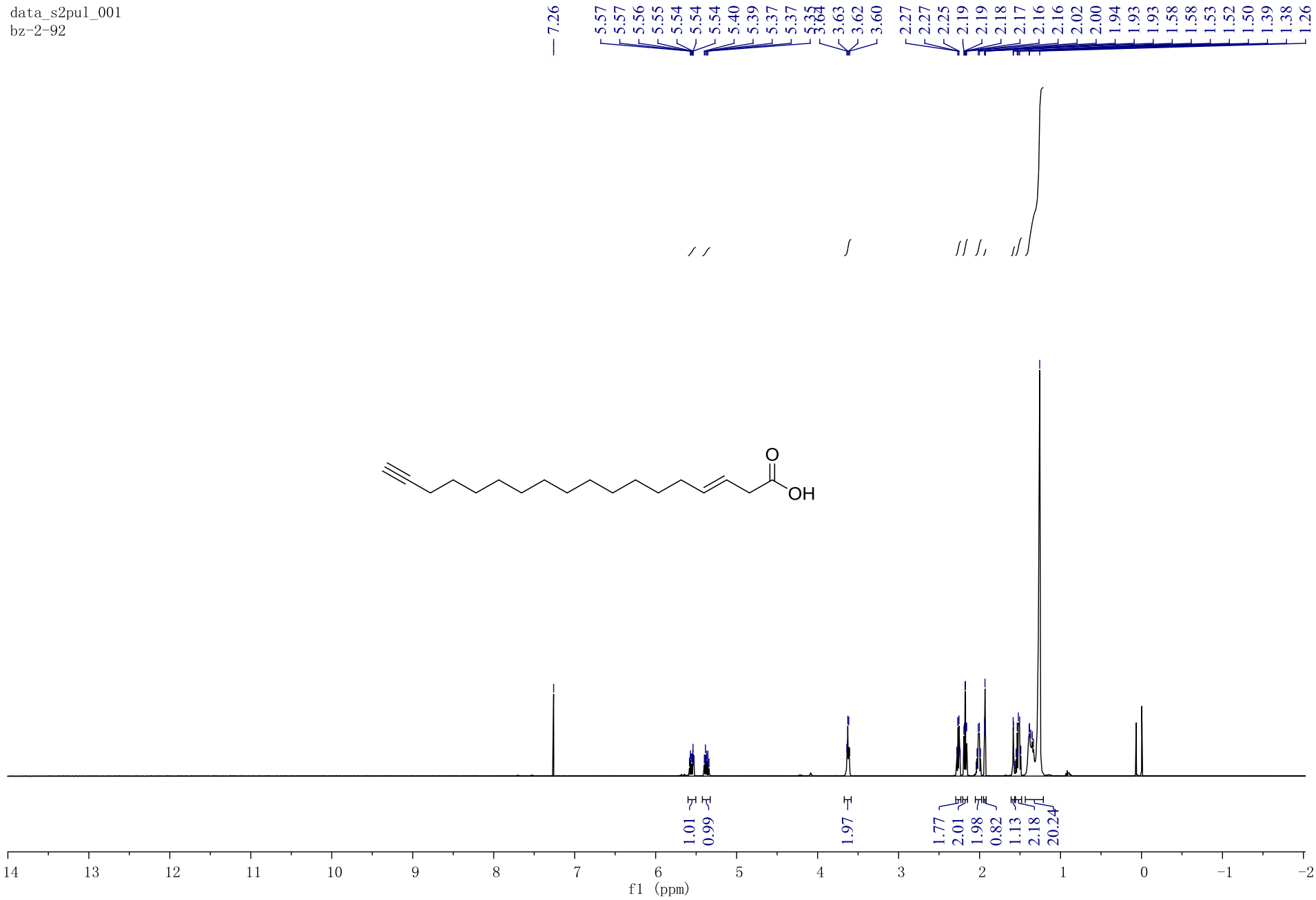
Supplementaty Figure S2. Validating LPCAT2 and LYPLA1/APT1 are palmitoylated proteins. 501Mel cells are labeled by palmitoylation reporter 15-hexadecynoic acid (probe **1**). The cell lysate is subjected to Click chemistry with biotin-azide, and immunoprecipitated with streptavidin beads. The bounded proteins are then eluted, and palmitoylated endogenous LPCAT2 and LYPLA1/APT1 are detected by western blotting.



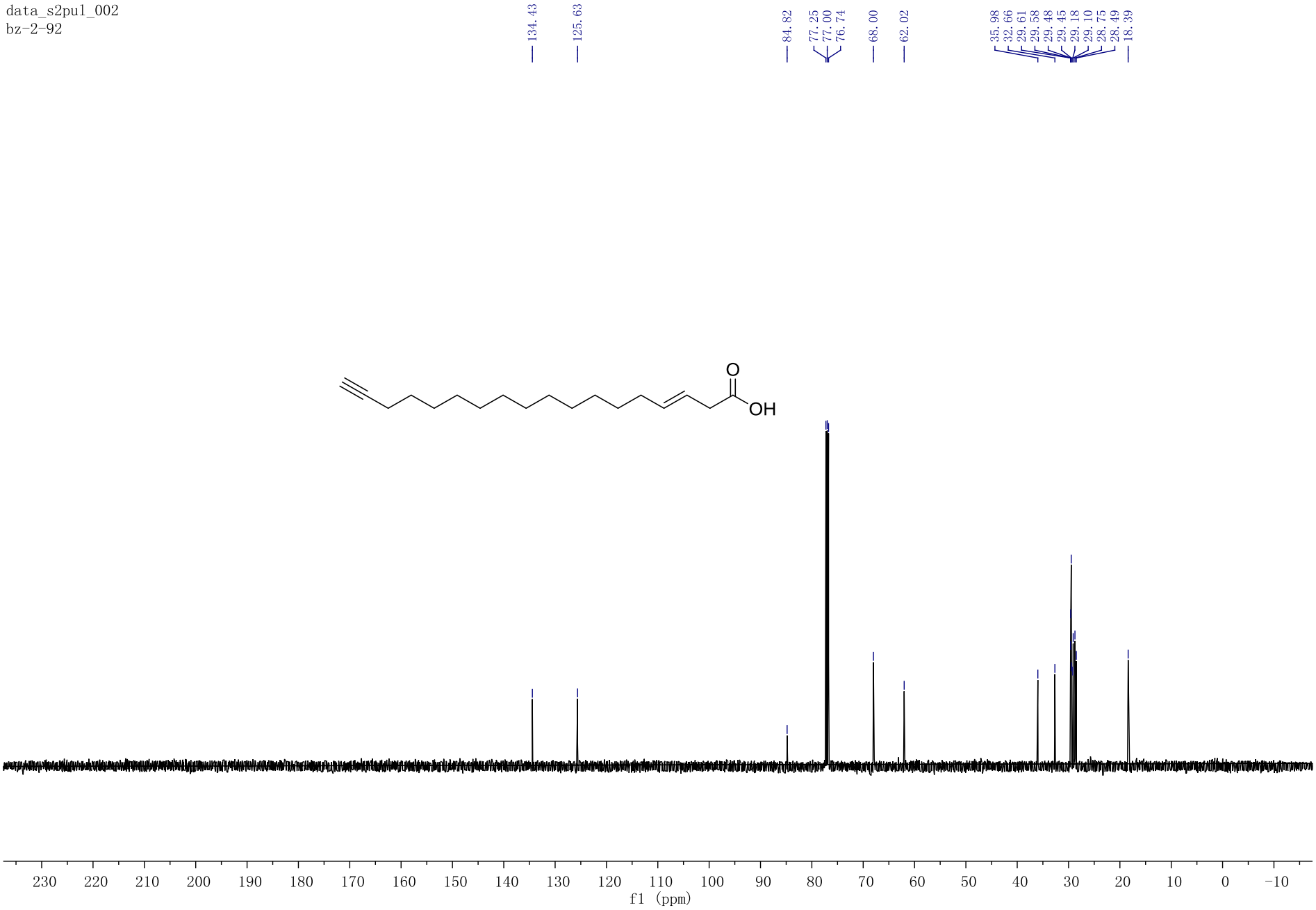


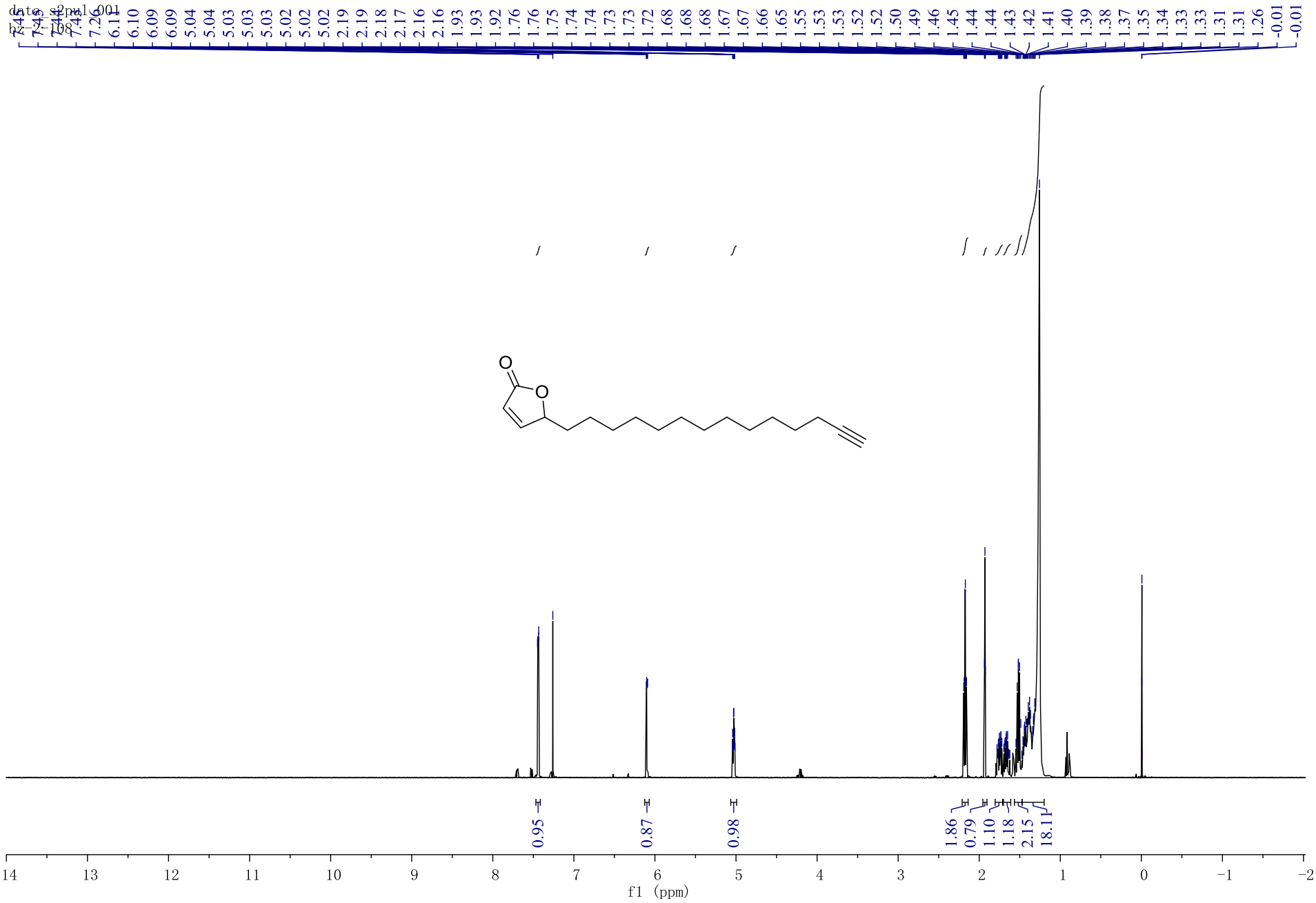
Appendix: NMR spectra of the compounds

data_s2pul_001
bz-2-92



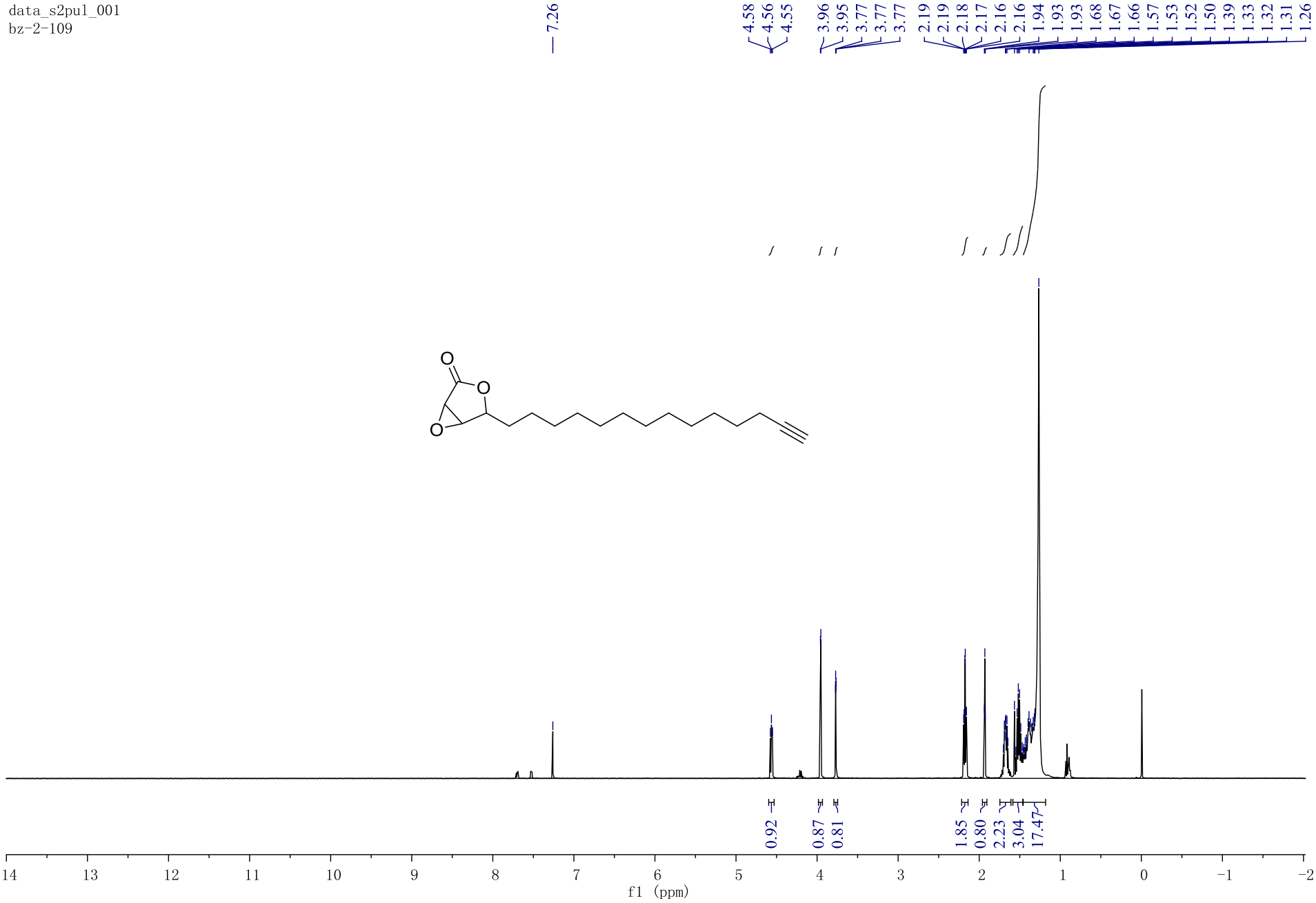
data_s2pul_002
bz-2-92




data s2pul 001
7.45
7.45
7.44
7.44
7.43
7.26
6.11
6.10
6.09
6.09
5.04
5.04
5.03
5.03
5.03
5.02
5.02
5.02
2.19
2.19
2.18
2.17
2.16
2.16
1.93
1.93
1.92
1.76
1.76
1.75
1.74
1.74
1.73
1.73
1.72
1.68
1.68
1.68
1.67
1.67
1.66
1.65
1.55
1.53
1.53
1.52
1.52
1.50
1.49
1.46
1.45
1.44
1.44
1.43
1.42
1.41
1.40
1.39
1.38
1.37
1.35
1.34
1.33
1.33
1.31
1.31
1.26
-0.01
-0.01
O
O
0.95
0.87
0.98
1.86
0.79
1.10
1.18
2.15
18.11
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
f1 (ppm)

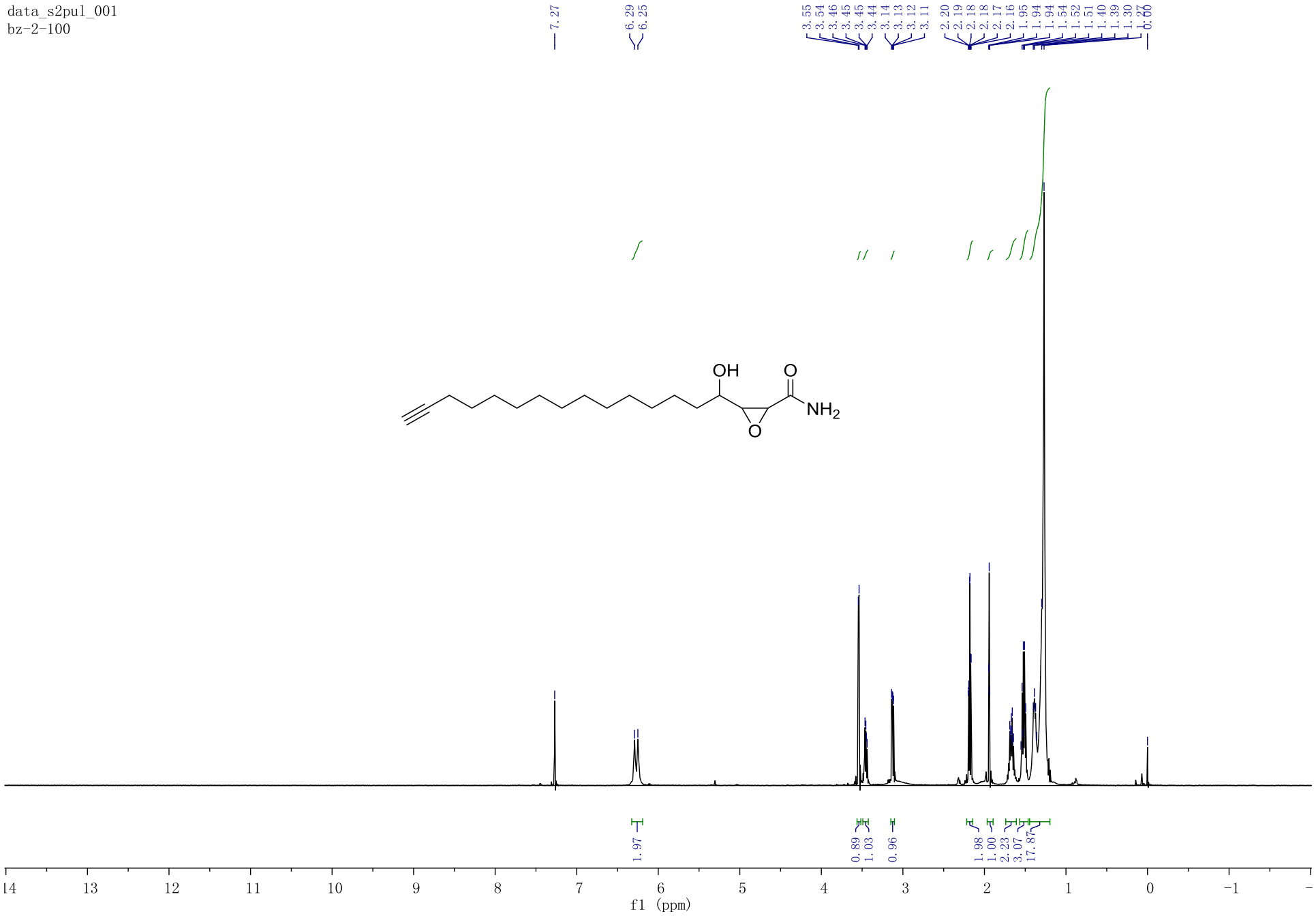
data_s2pul_002
bz-2-108
173.11
156.24
121.51
84.79
83.40
77.25
77.00
76.75
68.01
33.18
29.53
29.45
29.44
29.35
29.28
29.08
28.73
28.47
24.95
18.38
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
O
O

data_s2pul_001
bz-2-109

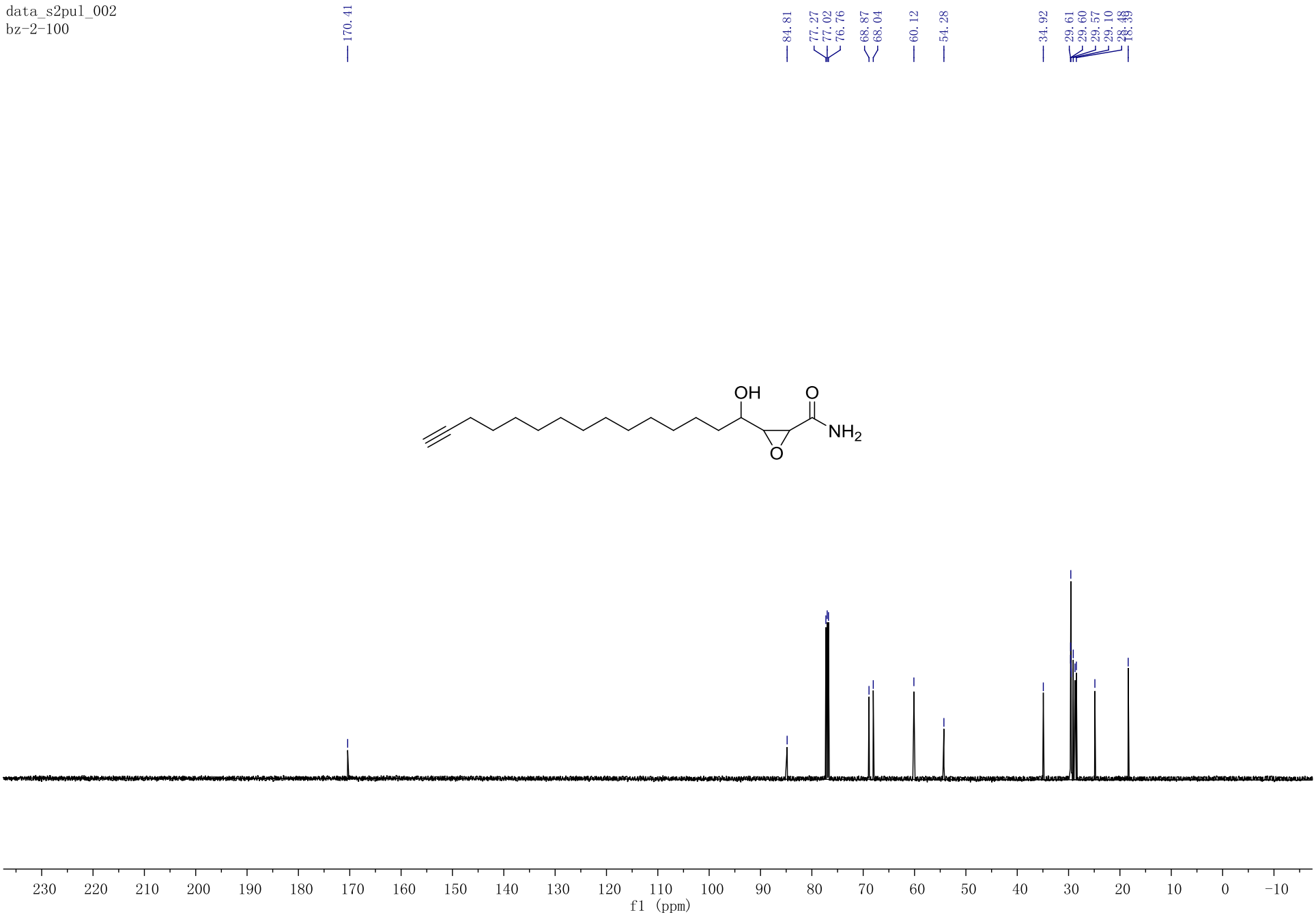


data_s2pul_002
bz-2-109
170.33
84.79
79.81
77.26
77.01
76.76
68.02
58.01
49.79
32.09
29.52
29.46
29.43
29.31
29.22
29.08
28.74
28.48
24.20
18.39
f1 (ppm)
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

data_s2pul_001
bz-2-100



data_s2pul_002
bz-2-100



bz-2-104
bz-2-104

data_s2pul_002
bz-2-104

202.65
167.45
84.81
77.26
77.01
76.75
68.03
58.33
55.32
41.13
29.52
29.51
29.45
29.08
28.74
28.08
18.39

f1 (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10