

Electronic Supplementary Material (ESI) for

**Dehydrocurvularin is a potent antineoplastic agent irreversibly
blocking ATP-citrate lyase: Evidence from chemoproteomics**

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1. Supplementary Tables and Figures

Supplementary Table S1. The IC₅₀ values (μM) of DCV and its derivatives in cancerous (HeLa, Jurkat, HCT116, K562) and normal (CD4, CHO) cells.

	DCV	CV	1	2	DCV-yne	CV-yne
HeLa	3.86 ± 0.66	>100	5.52 ± 0.78	17.6 ± 2.26	6.89 ± 0.81	>100
Jurkat	1.55 ± 0.24	>100	1.64 ± 0.13	8.22 ± 1.38	3.50 ± 0.46	>100
HCT116	4.60 ± 0.82	>100	8.28 ± 0.47	25.5 ± 2.64	12.2 ± 1.56	>100
K562	3.66 ± 0.24	>100	2.66 ± 0.25	7.21 ± 0.64	7.48 ± 0.88	>100
CD4	19.6 ± 0.86	>100	25.8 ± 1.20	>100	28.4 ± 1.35	>100
CHO	29.8 ± 4.44	>100	>100	>100	48.6 ± 3.46	>100

Supplementary Table S2. Proteins identified from the pull-down/LC-MS experiments.

Accession	Description	Ratio-1st	Ratio-2nd	Average Ratio	p-Value
P53396	ATP-citrate synthase OS=Homo sapiens GN=ACLY PE=1 SV=3	4.93	4.89	4.91	0.0013
Q3SXM5	Inactive hydroxysteroid dehydrogenase-like protein 1 OS=Homo sapiens GN=HSDL1 PE=1 SV=3	1.76	2.79	2.27	0.1414
P45880	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens GN=VDAC2 PE=1 SV=2	2.28	2.21	2.25	0.0096
P20645	Cation-dependent mannose-6-phosphate receptor OS=Homo sapiens GN=M6PR PE=1 SV=1	2.43	1.81	2.12	0.0920
Q9Y277	Voltage-dependent anion-selective channel protein 3 OS=Homo sapiens GN=VDAC3 PE=1 SV=1	2.31	1.81	2.06	0.0773
P27797	Calreticulin OS=Homo sapiens GN=CALR PE=1 SV=1	1.64	1.62	1.63	0.0056
P62979	Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens GN=RPS27A PE=1 SV=2	1.74	1.41	1.58	0.0676
P05141	ADP/ATP translocase 2 OS=Homo sapiens GN=SLC25A5 PE=1 SV=7	1.41	1.72	1.57	0.0637
P35579	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	1.58	1.28	1.43	0.0646
P10809	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2	1.37	1.48	1.42	0.0226
Q06323	Proteasome activator complex subunit 1 OS=Homo sapiens GN=PSME1 PE=1 SV=1	1.73	1.09	1.41	0.1406
Q7Z6Z7	E3 ubiquitin-protein ligase HUWE1 OS=Homo sapiens GN=HUWE1 PE=1 SV=3	1.50	1.20	1.35	0.0702
Q9NZ08	Endoplasmic reticulum aminopeptidase 1 OS=Homo sapiens GN=ERAP1 PE=1 SV=3	0.96	1.72	1.34	0.1736
O60506	Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2	1.58	0.96	1.27	0.1533
P12268	Inosine-5'-monophosphate dehydrogenase 2 OS=Homo sapiens GN=IMPDH2 PE=1 SV=2	1.30	1.21	1.26	0.0215
P49588	Alanine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=AARS PE=1 SV=2	1.29	1.15	1.22	0.0346
O75746	Calcium-binding mitochondrial carrier protein Aralar1 OS=Homo sapiens GN=SLC25A12 PE=1 SV=2	1.51	0.90	1.20	0.1557
P68363	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1	1.11	1.28	1.20	0.0428
O00410	Importin-5 OS=Homo sapiens GN=IPO5 PE=1 SV=4	0.70	1.55	1.12	0.2291
Q00325	Phosphate carrier protein, mitochondrial OS=Homo sapiens GN=SLC25A3 PE=1 SV=2	1.04	1.11	1.08	0.0199
P61978	Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens GN=HNRNPK PE=1 SV=1	1.17	0.96	1.07	0.0626
P22234	Multifunctional protein ADE2 OS=Homo sapiens GN=PAICS PE=1 SV=3	1.05	1.07	1.06	0.0067
O75533	Splicing factor 3B subunit 1 OS=Homo sapiens GN=SF3B1 PE=1 SV=3	1.15	0.87	1.01	0.0864

Q12906	Interleukin enhancer-binding factor 3 OS=Homo sapiens GN=ILF3 PE=1 SV=3	0.84	1.06	0.95	0.0741
P48643	T-complex protein 1 subunit epsilon OS=Homo sapiens GN=CCT5 PE=1 SV=1	1.23	0.64	0.93	0.1928
P07237	Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3	0.88	0.95	0.92	0.0237
Q15067	Peroxisomal acyl-coenzyme A oxidase 1 OS=Homo sapiens GN=ACOX1 PE=1 SV=3	1.04	0.76	0.90	0.0981
P11142	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1	0.94	0.84	0.89	0.0344
P21796	Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2	0.81	0.93	0.87	0.0445
Q9Y4L1	Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1	1.05	0.69	0.87	0.1271
Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens GN=DDX17 PE=1 SV=2	0.82	0.91	0.87	0.0336
O14980	Exportin-1 OS=Homo sapiens GN=XPO1 PE=1 SV=1	0.80	0.84	0.82	0.0166
P33527	Multidrug resistance-associated protein 1 OS=Homo sapiens GN=ABCC1 PE=1 SV=3	0.91	0.70	0.81	0.0816
P27708	CAD protein OS=Homo sapiens GN=CAD PE=1 SV=3	1.08	0.52	0.80	0.2116
P50990	T-complex protein 1 subunit theta OS=Homo sapiens GN=CCT8 PE=1 SV=4	0.82	0.78	0.80	0.0176
Q16881	Thioredoxin reductase 1, cytoplasmic OS=Homo sapiens GN=TXNRD1 PE=1 SV=3	0.91	0.66	0.78	0.0995
Q01813	ATP-dependent 6-phosphofructokinase, platelet type OS=Homo sapiens GN=PFKP PE=1 SV=2	0.76	0.75	0.75	0.0026
P42704	Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens GN=LRPPRC PE=1 SV=3	0.90	0.58	0.74	0.1354
Q15233	Non-POU domain-containing octamer-binding protein OS=Homo sapiens GN=NONO PE=1 SV=4	0.73	0.72	0.73	0.0010
O43143	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15 OS=Homo sapiens GN=DHX15 PE=1 SV=2	0.23	1.18	0.71	0.3754
P17987	T-complex protein 1 subunit alpha OS=Homo sapiens GN=TCP1 PE=1 SV=1	0.76	0.63	0.69	0.0562
P78527	DNA-dependent protein kinase catalytic subunit OS=Homo sapiens GN=PRKDC PE=1 SV=3	0.71	0.62	0.67	0.0425
Q86VP6	Cullin-associated NEDD8-dissociated protein 1 OS=Homo sapiens GN=CAND1 PE=1 SV=2	0.42	0.89	0.65	0.2198
P14618	Pyruvate kinase PKM OS=Homo sapiens GN=PKM PE=1 SV=4	0.71	0.57	0.64	0.0670
P12236	ADP/ATP translocase 3 OS=Homo sapiens GN=SLC25A6 PE=1 SV=4	0.21	1.07	0.64	0.3768
O43175	D-3-phosphoglycerate dehydrogenase OS=Homo sapiens GN=PHGDH PE=1 SV=4	0.33	0.93	0.63	0.2807
P06576	ATP synthase subunit beta, mitochondrial OS=Homo sapiens GN=ATP5B PE=1 SV=3	0.72	0.53	0.63	0.0932

Q92945	Far upstream element-binding protein 2 OS=Homo sapiens GN=KHSRP PE=1 SV=4	0.65	0.58	0.61	0.0352
Q8WUM4	Programmed cell death 6-interacting protein OS=Homo sapiens GN=PDCD6IP PE=1 SV=1	0.64	0.55	0.60	0.0464
P06239	Tyrosine-protein kinase Lck OS=Homo sapiens GN=LCK PE=1 SV=6	0.65	0.53	0.59	0.0658
P13796	Plastin-2 OS=Homo sapiens GN=LCP1 PE=1 SV=6	0.49	0.61	0.55	0.0656
P49368	T-complex protein 1 subunit gamma OS=Homo sapiens GN=CCT3 PE=1 SV=4	0.58	0.52	0.55	0.0371
P62736	Actin, aortic smooth muscle OS=Homo sapiens GN=ACTA2 PE=1 SV=1	0.59	0.50	0.55	0.0503
P27824	Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2	0.55	0.54	0.54	0.0060
P52272	Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens GN=HNRNPM PE=1 SV=3	0.36	0.72	0.54	0.2001
P34932	Heat shock 70 kDa protein 4 OS=Homo sapiens GN=HSPA4 PE=1 SV=4	0.50	0.58	0.54	0.0448
P21333	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4	0.54	0.46	0.50	0.0475
Q8TCT9	Minor histocompatibility antigen H13 OS=Homo sapiens GN=HM13 PE=1 SV=1	0.45	0.55	0.50	0.0617
P68104	Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1	0.45	0.54	0.50	0.0612
P63104	14-3-3 protein zeta/delta OS=Homo sapiens GN=YWHAZ PE=1 SV=1	0.51	0.48	0.49	0.0217
Q14980	Nuclear mitotic apparatus protein 1 OS=Homo sapiens GN=NUMA1 PE=1 SV=2	0.16	0.77	0.47	0.3643
B011T2	Unconventional myosin-Ig OS=Homo sapiens GN=MYO1G PE=1 SV=2	0.94	-0.02	0.46	0.5109
A0FGR8	Extended synaptotagmin-2 OS=Homo sapiens GN=ESYT2 PE=1 SV=1	0.57	0.35	0.46	0.1504
P26641	Elongation factor 1-gamma OS=Homo sapiens GN=EEF1G PE=1 SV=3	0.77	0.15	0.46	0.3774
P49589	Cysteine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=CARS PE=1 SV=3	0.16	0.75	0.45	0.3654
P68371	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1	0.84	0.05	0.45	0.4567
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2	0.42	0.47	0.44	0.0356
P17858	ATP-dependent 6-phosphofructokinase, liver type OS=Homo sapiens GN=PFKL PE=1 SV=6	0.40	0.48	0.44	0.0621
O95202	LETM1 and EF-hand domain-containing protein 1, mitochondrial OS=Homo sapiens GN=LETM1 PE=1 SV=1	0.35	0.53	0.44	0.1255
P22314	Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3	0.44	0.44	0.44	0.0032
O75369	Filamin-B OS=Homo sapiens GN=FLNB PE=1 SV=2	0.50	0.37	0.44	0.0928
Q9NY33	Dipeptidyl peptidase 3 OS=Homo sapiens GN=DPP3 PE=1 SV=2	0.80	0.07	0.44	0.4409

P14625	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1	0.50	0.36	0.43	0.1048
P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1 PE=1 SV=2	0.41	0.44	0.43	0.0254
P30101	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4	0.53	0.31	0.42	0.1618
P60709	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1	0.37	0.48	0.42	0.0792
P34897	Serine hydroxymethyltransferase, mitochondrial OS=Homo sapiens GN=SHMT2 PE=1 SV=3	0.45	0.38	0.42	0.0575
P53618	Coatomer subunit beta OS=Homo sapiens GN=COPB1 PE=1 SV=3	0.67	0.12	0.40	0.3808
O00483	Cytochrome c oxidase subunit NDUFA4 OS=Homo sapiens GN=NDUFA4 PE=1 SV=1	0.23	0.55	0.39	0.2462
P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	0.51	0.26	0.39	0.1972
P39023	60S ribosomal protein L3 OS=Homo sapiens GN=RPL3 PE=1 SV=2	0.34	0.43	0.39	0.071
P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	0.45	0.22	0.33	0.2055
P04843	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapiens GN=RPN1 PE=1 SV=1	0.45	0.22	0.33	0.2094
O43390	Heterogeneous nuclear ribonucleoprotein R OS=Homo sapiens GN=HNRNPR PE=1 SV=1	0.40	0.26	0.33	0.1355
Q00839	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6	0.58	0.07	0.32	0.4199
P17252	Protein kinase C alpha type OS=Homo sapiens GN=PRKCA PE=1 SV=4	0.56	0.09	0.32	0.3932
P49736	DNA replication licensing factor MCM2 OS=Homo sapiens GN=MCM2 PE=1 SV=4	0.83	-0.20	0.31	0.6472
P00505	Aspartate aminotransferase, mitochondrial OS=Homo sapiens GN=GOT2 PE=1 SV=3	0.20	0.41	0.31	0.2056
O75083	WD repeat-containing protein 1 OS=Homo sapiens GN=WDR1 PE=1 SV=4	0.14	0.46	0.30	0.3122
P62258	14-3-3 protein epsilon OS=Homo sapiens GN=YWHAE PE=1 SV=1	0.17	0.42	0.30	0.2485
P60842	Eukaryotic initiation factor 4A-I OS=Homo sapiens GN=EIF4A1 PE=1 SV=1	0.18	0.39	0.29	0.2206
O94919	Endonuclease domain-containing 1 protein OS=Homo sapiens GN=ENDOD1 PE=1 SV=2	0.13	0.41	0.27	0.3027
P13639	Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4	0.12	0.40	0.26	0.3088
P35580	Myosin-10 OS=Homo sapiens GN=MYH10 PE=1 SV=3	0.20	0.32	0.26	0.1464
Q9NQ29	Putative RNA-binding protein Luc7-like 1 OS=Homo sapiens GN=LUC7L PE=1 SV=1	0.76	-0.25	0.25	0.6998
P17844	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens GN=DDX5 PE=1 SV=1	0.22	0.28	0.25	0.0729
Q12931	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3	0.38	0.09	0.23	0.3456

Q14204	Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens GN=DYNC1H1 PE=1 SV=5	0.19	0.26	0.22	0.1049
Q15366	Poly(rC)-binding protein 2 OS=Homo sapiens GN=PCBP2 PE=1 SV=1	0.18	0.27	0.22	0.1314
P25705	ATP synthase subunit alpha, mitochondrial OS=Homo sapiens GN=ATP5A1 PE=1 SV=1	0.17	0.23	0.20	0.0975
Q06830	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1	0.14	0.26	0.20	0.1776
Q9Y490	Talin-1 OS=Homo sapiens GN=TLN1 PE=1 SV=3	0.37	0.03	0.20	0.4478
Q15084	Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1	0.31	0.03	0.17	0.4277
P69905	Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2	0.08	0.26	0.17	0.3127
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2	-0.16	0.48	0.16	0.6953
P40926	Malate dehydrogenase, mitochondrial OS=Homo sapiens GN=MDH2 PE=1 SV=3	0.24	0.06	0.15	0.3298
P02768	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	-0.03	0.29	0.13	0.5527
P55060	Exportin-2 OS=Homo sapiens GN=CSE1L PE=1 SV=3	-0.35	0.60	0.13	0.8291
Q63HN8	E3 ubiquitin-protein ligase RNF213 OS=Homo sapiens GN=RNF213 PE=1 SV=3	0.71	-0.46	0.12	0.8613
Q8TCJ2	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B OS=Homo sapiens GN=STT3B PE=1 SV=1	0.36	-0.11	0.12	0.6822
Q14697	Neutral alpha-glucosidase AB OS=Homo sapiens GN=GANAB PE=1 SV=3	-0.10	0.35	0.12	0.6678
Q14974	Importin subunit beta-1 OS=Homo sapiens GN=KPNB1 PE=1 SV=2	-0.01	0.21	0.10	0.5107
O60831	PRA1 family protein 2 OS=Homo sapiens GN=PRAF2 PE=1 SV=1	0.42	-0.24	0.09	0.8208
P46782	40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=4	0.07	0.09	0.08	0.0632
P55072	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4	-0.04	0.20	0.08	0.6188
P48735	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2	-0.11	0.26	0.07	0.7385
Q99832	T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2	-0.03	0.17	0.07	0.5788
P50991	T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4	0.26	-0.12	0.07	0.7539
P78417	Glutathione S-transferase omega-1 OS=Homo sapiens GN=GSTO1 PE=1 SV=2	0.18	-0.05	0.07	0.6556
P00338	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2	0.15	-0.02	0.06	0.5822
Q00534	Cyclin-dependent kinase 6 OS=Homo sapiens GN=CDK6 PE=1 SV=1	-0.97	1.08	0.06	0.962
P14324	Farnesyl pyrophosphate synthase OS=Homo sapiens GN=FDPS PE=1 SV=4	-0.18	0.27	0.05	0.8604

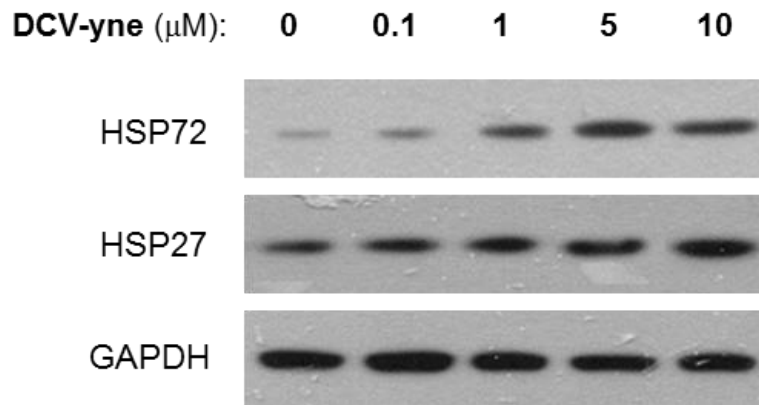
P31948	Stress-induced-phosphoprotein 1 OS=Homo sapiens GN=STIP1 PE=1 SV=1	0.21	-0.16	0.02	0.8986
P23284	Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2	0.12	-0.08	0.02	0.8358
Q00610	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5	0.26	-0.21	0.02	0.9282
Q6P2Q9	Pre-mRNA-processing-splicing factor 8 OS=Homo sapiens GN=PRPF8 PE=1 SV=2	-0.07	0.10	0.02	0.8437
A0AVT1	Ubiquitin-like modifier-activating enzyme 6 OS=Homo sapiens GN=UBA6 PE=1 SV=1	-0.44	0.47	0.01	0.9738
P62701	40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2	-0.28	0.30	0.01	0.9708
P31146	Coronin-1A OS=Homo sapiens GN=CORO1A PE=1 SV=4	-0.07	0.03	-0.02	0.8084
P40227	T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3	0.22	-0.27	-0.02	0.9520
P07195	L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2	-0.02	-0.04	-0.03	0.1705
P30044	Peroxiredoxin-5, mitochondrial OS=Homo sapiens GN=PRDX5 PE=1 SV=4	0	-0.07	-0.03	0.5698
	U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Homo sapiens GN=SNRNP200 PE=1				
O75643	SV=2	0.04	-0.11	-0.04	0.7352
P36578	60S ribosomal protein L4 OS=Homo sapiens GN=RPL4 PE=1 SV=5	-0.04	-0.07	-0.06	0.1847
P16615	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2 OS=Homo sapiens GN=ATP2A2 PE=1 SV=1	0.07	-0.19	-0.06	0.7476
P46940	RasGTPase-activating-like protein IQGAP1 OS=Homo sapiens GN=IQGAP1 PE=1 SV=1	-0.16	0.03	-0.06	0.6565
P52209	6-phosphogluconate dehydrogenase, decarboxylating OS=Homo sapiens GN=PGD PE=1 SV=3	0	-0.13	-0.07	0.4999
P31939	Bifunctional purine biosynthesis protein PURH OS=Homo sapiens GN=ATIC PE=1 SV=3	-0.17	0.03	-0.07	0.6148
P11717	Cation-independent mannose-6-phosphate receptor OS=Homo sapiens GN=IGF2R PE=1 SV=3	-0.07	-0.11	-0.09	0.1612
	Sodium/potassium-transporting ATPase subunit alpha-3 OS=Homo sapiens GN=ATP1A3 PE=1				
P13637	SV=3	-0.67	0.46	-0.11	0.8879
P68871	Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2	-0.02	-0.21	-0.11	0.4534
P08133	Annexin A6 OS=Homo sapiens GN=ANXA6 PE=1 SV=3	-0.12	-0.12	-0.12	0.0076
P49257	Protein ERGIC-53 OS=Homo sapiens GN=LMAN1 PE=1 SV=2	0	-0.28	-0.14	0.5212
P11586	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens GN=MTHFD1 PE=1 SV=3	-0.17	-0.13	-0.15	0.0995
P13010	X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3	-0.21	-0.09	-0.15	0.2522
Q08211	ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4	-0.26	-0.04	-0.15	0.4107

P04075	Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2	-0.01	-0.35	-0.18	0.4828
P25789	Proteasome subunit alpha type-4 OS=Homo sapiens GN=PSMA4 PE=1 SV=1	-0.16	-0.24	-0.20	0.1274
P61981	14-3-3 protein gamma OS=Homo sapiens GN=YWHAG PE=1 SV=2	-0.28	-0.13	-0.21	0.2272
P12956	X-ray repair cross-complementing protein 6 OS=Homo sapiens GN=XRCC6 PE=1 SV=2	-0.21	-0.22	-0.22	0.0203
Q9H9B4	Sideroflexin-1 OS=Homo sapiens GN=SFXN1 PE=1 SV=4	-0.43	-0.03	-0.23	0.4670
P15153	Ras-related C3 botulinum toxin substrate 2 OS=Homo sapiens GN=RAC2 PE=1 SV=1	-0.21	-0.25	-0.23	0.0585
P62937	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2	-0.19	-0.28	-0.23	0.1186
P26038	Moesin OS=Homo sapiens GN=MSN PE=1 SV=3	-0.19	-0.27	-0.23	0.1134
P00558	Phosphoglycerate kinase 1 OS=Homo sapiens GN=PGK1 PE=1 SV=3	-0.22	-0.26	-0.24	0.0551
P60953	Cell division control protein 42 homolog OS=Homo sapiens GN=CDC42 PE=1 SV=2	-0.11	-0.37	-0.24	0.3281
P52566	Rho GDP-dissociation inhibitor 2 OS=Homo sapiens GN=ARHGDI2 PE=1 SV=3	-0.27	-0.22	-0.24	0.0747
P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5	-0.19	-0.3	-0.25	0.1345
P02786	Transferrin receptor protein 1 OS=Homo sapiens GN=TFRC PE=1 SV=2	-0.34	-0.17	-0.25	0.2026
Q9BRX8	Redox-regulatory protein FAM213A OS=Homo sapiens GN=FAM213A PE=1 SV=3	-0.52	0	-0.26	0.4999
Q14152	Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens GN=EIF3A PE=1 SV=1	-0.40	-0.16	-0.28	0.2675
P00813	Adenosine deaminase OS=Homo sapiens GN=ADA PE=1 SV=3	-0.46	-0.12	-0.29	0.3454
P08195	4F2 cell-surface antigen heavy chain OS=Homo sapiens GN=SLC3A2 PE=1 SV=3	-0.17	-0.41	-0.29	0.2449
P62826	GTP-binding nuclear protein Ran OS=Homo sapiens GN=RAN PE=1 SV=3	-0.12	-0.50	-0.31	0.3585
P78371	T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4	-0.38	-0.26	-0.32	0.1229
Q7KZF4	Staphylococcal nuclease domain-containing protein 1 OS=Homo sapiens GN=SND1 PE=1 SV=1	-0.36	-0.29	-0.33	0.0720
Q13813	Spectrin alpha chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTAN1 PE=1 SV=3	-0.24	-0.42	-0.33	0.1685
P23396	40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=2	-0.32	-0.35	-0.34	0.0329
P41252	Isoleucine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=IARS PE=1 SV=2	-0.07	-0.62	-0.34	0.4302
Q99536	Synaptic vesicle membrane protein VAT-1 homolog OS=Homo sapiens GN=VAT1 PE=1 SV=2	-0.23	-0.52	-0.37	0.2407
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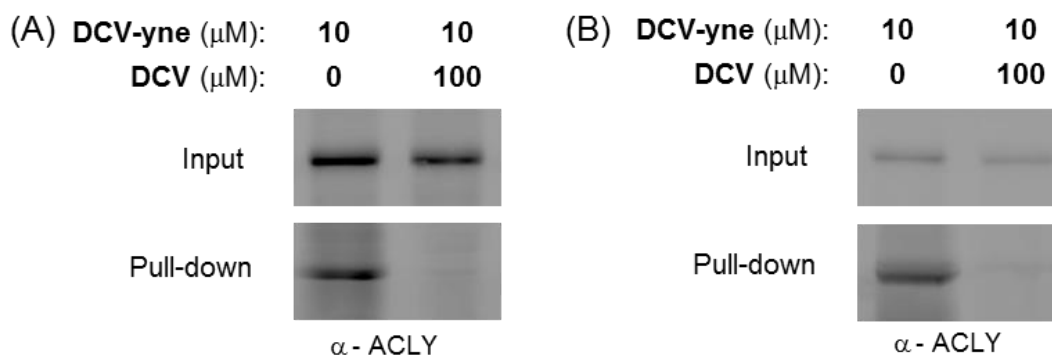
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P12235	ADP/ATP translocase 1 OS=Homo sapiens GN=SLC25A4 PE=1 SV=4	-0.77	-0.02	-0.39	0.4894
Q9NQC3	Reticulon-4 OS=Homo sapiens GN=RTN4 PE=1 SV=2	-0.29	-0.51	-0.4	0.1756
Q01518	Adenylyl cyclase-associated protein 1 OS=Homo sapiens GN=CAP1 PE=1 SV=5	-0.48	-0.34	-0.41	0.1055
P08575	Receptor-type tyrosine-protein phosphatase C OS=Homo sapiens GN=PTPRC PE=1 SV=2	-0.44	-0.42	-0.43	0.0085
P09622	Dihydrolipoyl dehydrogenase, mitochondrial OS=Homo sapiens GN=DLD PE=1 SV=2	-0.21	-0.68	-0.45	0.3138
P29401	Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3	-0.54	-0.41	-0.47	0.0832
P54136	Arginine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=RARS PE=1 SV=2	-0.39	-0.58	-0.48	0.1216
P08621	U1 small nuclear ribonucleoprotein 70 kDa OS=Homo sapiens GN=SNRNP70 PE=1 SV=2	-0.37	-0.60	-0.49	0.1517
P62851	40S ribosomal protein S25 OS=Homo sapiens GN=RPS25 PE=1 SV=1	-0.31	-0.67	-0.49	0.2260
P22392	Nucleoside diphosphate kinase B OS=Homo sapiens GN=NME2 PE=1 SV=1	-0.53	-0.49	-0.51	0.0290
P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3	-0.61	-0.42	-0.52	0.1181
Q9NYU2	UDP-glucose:glycoprotein glucosyltransferase 1 OS=Homo sapiens GN=UGGT1 PE=1 SV=3	-0.47	-0.65	-0.56	0.1023
P19338	Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3	-0.51	-0.67	-0.59	0.0881
P62280	40S ribosomal protein S11 OS=Homo sapiens GN=RPS11 PE=1 SV=3	-0.76	-0.52	-0.64	0.1196
P62906	60S ribosomal protein L10a OS=Homo sapiens GN=RPL10A PE=1 SV=2	-0.51	-0.79	-0.65	0.1363
P07737	Profilin-1 OS=Homo sapiens GN=PFN1 PE=1 SV=2	-0.67	-0.74	-0.71	0.0287
O75390	Citrate synthase, mitochondrial OS=Homo sapiens GN=CS PE=1 SV=2	-0.62	-0.80	-0.71	0.0832
P15311	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4	-1.17	-0.25	-0.71	0.3666
Q96GQ5	RUS1 family protein C16orf58 OS=Homo sapiens GN=C16orf58 PE=1 SV=2	-0.97	-0.48	-0.73	0.2101
Q16629	Serine/arginine-rich splicing factor 7 OS=Homo sapiens GN=SRSF7 PE=1 SV=1	-0.73	-0.79	-0.76	0.0245
Q5T4S7	E3 ubiquitin-protein ligase UBR4 OS=Homo sapiens GN=UBR4 PE=1 SV=1	-0.62	-0.94	-0.78	0.1290
P39019	40S ribosomal protein S19 OS=Homo sapiens GN=RPS19 PE=1 SV=2	-0.79	-0.84	-0.82	0.0200
P61604	10 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPE1 PE=1 SV=2	-0.84	-0.83	-0.83	0.0029
P62424	60S ribosomal protein L7a OS=Homo sapiens GN=RPL7A PE=1 SV=2	-0.73	-0.95	-0.84	0.0844
P06702	Protein S100-A9 OS=Homo sapiens GN=S100A9 PE=1 SV=1	-0.59	-1.12	-0.86	0.1929

P15880	40S ribosomal protein S2 OS=Homo sapiens GN=RPS2 PE=1 SV=2	-0.95	-0.84	-0.90	0.0408
P05165	Propionyl-CoA carboxylase alpha chain, mitochondrial OS=Homo sapiens GN=PCCA PE=1 SV=4	-0.85	-1.00	-0.92	0.0507
P81605	Dermcidin OS=Homo sapiens GN=DCD PE=1 SV=2	-0.83	-1.02	-0.93	0.0665
P18621	60S ribosomal protein L17 OS=Homo sapiens GN=RPL17 PE=1 SV=3	-0.95	-0.91	-0.93	0.0164
Q01130	Serine/arginine-rich splicing factor 2 OS=Homo sapiens GN=SRSF2 PE=1 SV=4	-1.06	-0.91	-0.98	0.0479
P18124	60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1	-1.00	-1.00	-1.00	0.0004
P04908	Histone H2A type 1-B/E OS=Homo sapiens GN=HIST1H2AB PE=1 SV=2	-1.76	-0.28	-1.02	0.4002
P62750	60S ribosomal protein L23a OS=Homo sapiens GN=RPL23A PE=1 SV=1	-0.88	-1.24	-1.06	0.1078
P40429	60S ribosomal protein L13a OS=Homo sapiens GN=RPL13A PE=1 SV=2	-1.13	-1.06	-1.10	0.0202
Q07020	60S ribosomal protein L18 OS=Homo sapiens GN=RPL18 PE=1 SV=2	-1.03	-1.18	-1.10	0.0434
P11498	Pyruvate carboxylase, mitochondrial OS=Homo sapiens GN=PC PE=1 SV=2	-0.97	-1.27	-1.12	0.0869
Q01082	Spectrin beta chain, non-erythrocytic 1 OS=Homo sapiens GN=SPTBN1 PE=1 SV=2	-1.00	-1.32	-1.16	0.0899
P27635	60S ribosomal protein L10 OS=Homo sapiens GN=RPL10 PE=1 SV=4	-1.16	-1.19	-1.17	0.0087
Q96RQ3	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial OS=Homo sapiens GN=MCCC1 PE=1 SV=3	-1.34	-1.06	-1.20	0.0731
Q9Y383	Putative RNA-binding protein Luc7-like 2 OS=Homo sapiens GN=LUC7L2 PE=1 SV=2	-1.16	-1.29	-1.22	0.0344
P62917	60S ribosomal protein L8 OS=Homo sapiens GN=RPL8 PE=1 SV=2	-1.28	-1.23	-1.26	0.0126
Q02878	60S ribosomal protein L6 OS=Homo sapiens GN=RPL6 PE=1 SV=3	-1.16	-1.40	-1.28	0.0606
Q02978	Mitochondrial 2-oxoglutarate/malate carrier protein OS=Homo sapiens GN=SLC25A11 PE=1 SV=3	-1.28	-1.38	-1.33	0.0236
P62753	40S ribosomal protein S6 OS=Homo sapiens GN=RPS6 PE=1 SV=1	-1.41	-1.40	-1.41	0.0024
P84098	60S ribosomal protein L19 OS=Homo sapiens GN=RPL19 PE=1 SV=1	-1.23	-1.62	-1.42	0.0863
P62241	40S ribosomal protein S8 OS=Homo sapiens GN=RPS8 PE=1 SV=2	-1.38	-1.52	-1.45	0.0308
P62910	60S ribosomal protein L32 OS=Homo sapiens GN=RPL32 PE=1 SV=2	-1.49	-1.48	-1.48	0.0027
P50416	Carnitine O-palmitoyltransferase 1, liver isoform OS=Homo sapiens GN=CPT1A PE=1 SV=2	-1.49	-1.60	-1.54	0.0229
P47914	60S ribosomal protein L29 OS=Homo sapiens GN=RPL29 PE=1 SV=2	-1.60	-1.58	-1.59	0.0046
P16401	Histone H1.5 OS=Homo sapiens GN=HIST1H1B PE=1 SV=3	-1.67	-1.58	-1.63	0.0177

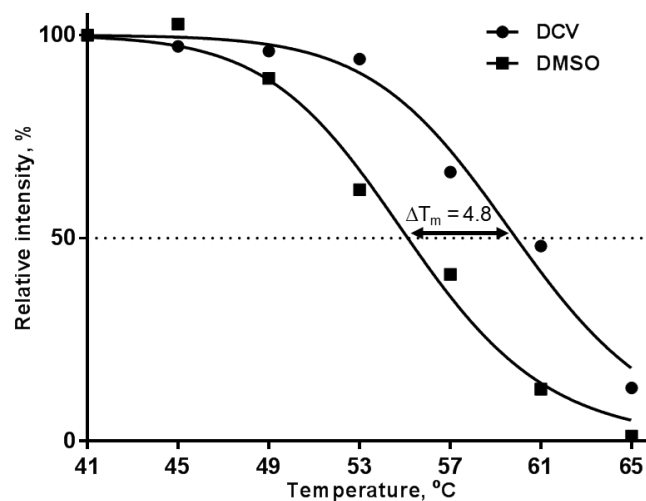
P46778	60S ribosomal protein L21 OS=Homo sapiens GN=RPL21 PE=1 SV=2	-1.52	-1.76	-1.64	0.0462
P46781	40S ribosomal protein S9 OS=Homo sapiens GN=RPS9 PE=1 SV=3	-1.74	-1.56	-1.65	0.0335
P46779	60S ribosomal protein L28 OS=Homo sapiens GN=RPL28 PE=1 SV=3	-1.58	-1.79	-1.68	0.0395
P61313	60S ribosomal protein L15 OS=Homo sapiens GN=RPL15 PE=1 SV=2	-1.74	-1.63	-1.68	0.0210
Q13085	Acetyl-CoA carboxylase 1 OS=Homo sapiens GN=ACACA PE=1 SV=2	-1.66	-1.76	-1.71	0.0182
Q9Y3U8	60S ribosomal protein L36 OS=Homo sapiens GN=RPL36 PE=1 SV=3	-1.65	-1.89	-1.77	0.0429
P68431	Histone H3.1 OS=Homo sapiens GN=HIST1H3A PE=1 SV=2	-1.73	-1.87	-1.80	0.0247
Q02543	60S ribosomal protein L18a OS=Homo sapiens GN=RPL18A PE=1 SV=2	-1.97	-1.68	-1.82	0.0510
P62805	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2	-2.00	-1.80	-1.90	0.0329
P26373	60S ribosomal protein L13 OS=Homo sapiens GN=RPL13 PE=1 SV=4	-1.77	-2.03	-1.90	0.0437
P61927	60S ribosomal protein L37 OS=Homo sapiens GN=RPL37 PE=1 SV=2	-1.84	-1.98	-1.91	0.0238
P16403	Histone H1.2 OS=Homo sapiens GN=HIST1H1C PE=1 SV=2	-2.16	-1.68	-1.92	0.0790
P51572	B-cell receptor-associated protein 31 OS=Homo sapiens GN=BCAP31 PE=1 SV=3	-1.95	-2.03	-1.99	0.0124
P10412	Histone H1.4 OS=Homo sapiens GN=HIST1H1E PE=1 SV=2	-1.95	-2.04	-2.00	0.0150
P62899	60S ribosomal protein L31 OS=Homo sapiens GN=RPL31 PE=1 SV=1	-1.85	-2.17	-2.01	0.0506
P42766	60S ribosomal protein L35 OS=Homo sapiens GN=RPL35 PE=1 SV=2	-1.92	-2.11	-2.01	0.0297
P40939	Trifunctional enzyme subunit alpha, mitochondrial OS=Homo sapiens GN=HADHA PE=1 SV=2	-1.87	-2.45	-2.16	0.0861
P49207	60S ribosomal protein L34 OS=Homo sapiens GN=RPL34 PE=1 SV=3	-2.81	-2.66	-2.73	0.0175



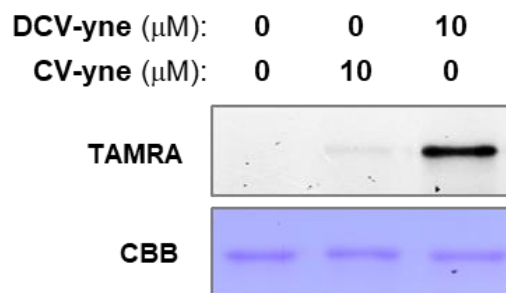
Supplementary Fig. S1 Induction of endogenous heat shock proteins by DCV-yne in Jurkat. Jurkat cells were treated with various concentrations of DCV-yne in culture medium for 24 h.



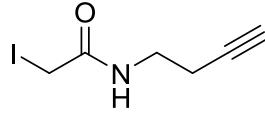
Supplementary Fig. S2 Pull-down/Western blot validation: (A) HeLa and (B) HCT116 cells were treated with DCV-yne (10 μM , 2 h) with or without DCV (100 μM , 1 h) preincubation as indicated. Cells were lysed and subjected to click reaction with biotin- N_3 and pulled-down with streptavidin beads. Bound proteins were eluted by boiling in loading buffer, separated by SDS-PAGE, transferred to membrane, and visualized by ACLY antibody.



Supplementary Fig. S3 Cellular thermal-shift assay (CETSA) revealed the stabilization of ACLY upon DCV treatment: The band intensity (Fig. 2E) of soluble ACLY at 41 °C is set at 100%; the band intensities (Fig. 2E) of the remaining soluble ACLY at different temperatures were measured, and the data were fitted to obtain apparent ΔT_m values using the Boltzmann Sigmoid equation within GraphPad Prism 7.0.

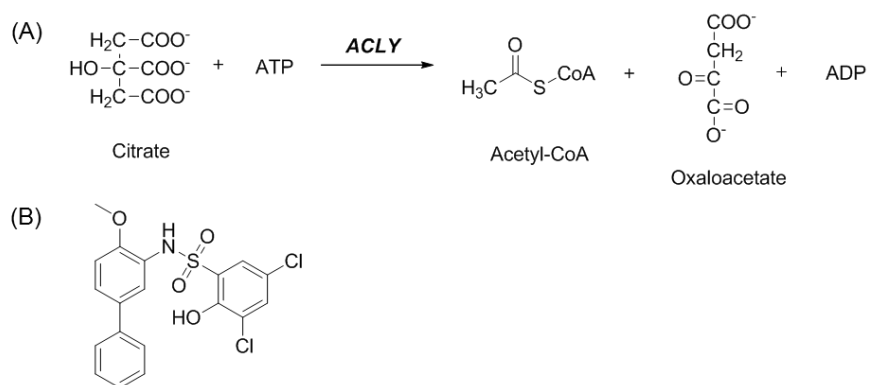


Supplementary Fig. S4 The negative probe CV-yne cannot label recombinant ACLY: Recombinant ACLY protein was incubated with DCV-yne, CV-yne, or DMSO as indicated at room temperature for 1 h, followed by click reaction with TAMRA- N_3 and in gel fluorescence.

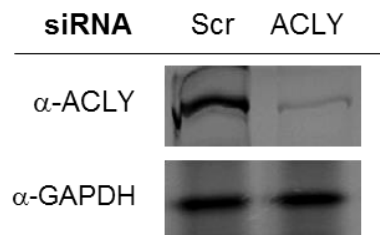


IA-yne

Supplementary Fig. S5 The structure of IA-yne which used in competitive labeling experiment of Fig. 3C.



Supplementary Fig. S6 (A) The reaction catalyzed by ACLY protein. (B) The structure of known ACLY inhibitor BMS-303141.



Supplementary Fig. S7 Validation of siRNA-mediated ACLY knockdown by western blot

2. Biological Experiments

2.1 Cell proliferation assay

All cell lines (Jurkat, K562, HeLa, HCT116, and CHO) were ordered from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Human PBMCs were obtained from the Blood Center of Shanghai, China. CD4 T cells were purified from PBMCs by use of anti-CD4-specific antibody-coated microbeads (Miltenyi Biotec) as described previously.¹

Jurkat, K562, and CD4 cells were grown in RPMI medium (Gibco) supplemented with 1% L-Glutamine, 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum (FBS, Gibco). HeLa, HCT116, and CHO cells were grown in DMEM (Gibco) supplemented with 1% L-Glutamine, 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum (FBS, Gibco). The culture incubator set is 37°C with 5% CO₂. Cells cultured for three passages were diluted in culture medium to 1×10⁶ cells per mL. 100 μL of cell suspension were seeded to each well (10000 cells/well) of 96-well plate and incubated at 37 °C overnight. Cells in 96-well plate were treated with various concentrations of compounds and DMSO (negative control) for 48 h in a 37 °C incubator. Cell viability was assessed by CellTiter-Glo[®] Luminescent Kit (Promega). Data analysis was performed with GraphPad Prism 7.0.

2.2 Western blot

The primary antibodies of Hsp27 and Hsp72 were purchased from Santa Cruz, USA. The primary antibodies of ACLY and GAPDH were purchased from Sangon Biotech, Shanghai. After SDS-PAGE, the proteins were transferred onto nitrocellulose membrane (Millipore) and the membrane was blocked with 3% (w/v) BSA in tris-buffered saline (TBS) for 2 h at room temperature. After blocking, membranes were incubated with primary antibody at room temperature for 1 h. After washing with TBST (TBS containing 0.1% Tween-20) three times, blots were further incubated with the HRP-conjugated anti-rabbit (Sangon Biotech) secondary antibody for 1 h at room temperature. After incubation, the blot was washed again with TBST three times and developed by enhanced ECL chemiluminescent substrate kit (Pierce).

2.3 Gel-based probes labeling of living Jurkat cells

Jurkat cells grown in culture medium were incubated with DCV-yne or CV-yne as indicated for 3 h. After harvesting, cell pellets were sonicated and homogenized in cold lysis buffer (50 mM HEPES pH 8.0, 150 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100 and complete protease inhibitors) for 30 minutes. The lysed cells were centrifuged at 14000 g for 5 minutes and the soluble fractions were collected as whole cellular proteomes. The samples were diluted to 2 mg/mL with lysis buffer and 1% SDS (w/v) was added to each sample and click reaction was performed as below: for each reaction, 20 μL of protein samples were added to freshly prepared 0.2μL each of TAMRA-N₃ (10 mM in DMSO,

Lumiprobe), CuSO₄ (100 mM in H₂O), THPTA (Tris(3-hydroxypropyltriazolylmethyl)amine, 10 mM in H₂O, Sigma) and sodium ascorbate (NaVc, 100 mM in H₂O). The samples were incubated at room temperature for 1 h, added with SDS-PAGE sample loading buffer (5×, 4 μL), applied to SDS-PAGE and imaged by Pharos-FX imaging system (Bio-rad).

2.4 Chemoproteomic profiling of Jurkat cells with DCV probes

The probe incubation and proteome preparation procedures were same as that of labeling studies mentioned above in section 2.2, ESI. Each of 500 μL of proteome sample was subjected to click reaction with Biotin-N₃ (500 μM, Biomatrix Inc.), CuSO₄ (1 mM), THPTA (100 μM) and NaVc (1 mM). The samples were precipitated with CH₃OH (600 μL) / CHCl₃ (150 μL) / H₂O (300 μL) sequentially and vortexed for a while. After centrifuge at 14,000 g for 3 minutes, the protein disk was washed twice with CH₃OH (500 μL), air-dried and re-dissolved in 200 μL of click buffer (50 mM HEPES pH 8.0, 1% SDS) by sonication. 50 μL of streptavidin-sepharose (GE Healthcare) beads were added to each sample and incubated at room temperature with continuous rotation for 2 h. The beads were washed with PBS with 1% SDS (w/v) three times, PBS with 0.5 M NaCl three times, 4 M urea in 100 mM triethylammonium bicarbonate (TEAB, Thermo Scientific) twice, and 100 mM TEAB five times. Each wash was performed on a rotator for 15 minutes. The bounded proteins were subjected to on-beads reductive alkylation with 200 μL of 10 mM of tris(2-carboxyethyl)phosphine (TCEP, Pierce) at 56 °C for 30 minutes and 200 μL of 55 mM iodoacetamide (Sigma) at 37 °C in dark for another 30 minutes, followed by wash with 100 mM TEAB three times. Bounded proteins on beads were digested with 1 μg of sequencing grade modified trypsin (Promega) reconstituted in 50 μL of 100 mM TEAB overnight at 37 °C. The digests were labeled with respective tandem mass tagging (TMT-²plex) Isobaric Label Reagent (Thermo Scientific) according to the manufacturer's procedures. The digested CV-yne pull-down sample (negative group) was labeled with TMT reagent 126, the digested DCV-yne pull-down sample (positive group) was labeled with reagent 127. Combined peptides were desalted by C18 spin columns (Pierce) and evaporated to dryness on a SpeedVac. Dried peptides were suspended in 15 μL of ddH₂O containing 0.1% formic acid with sonication. The proteomics experiment was carried out in biological duplicates.

2.5 LC-MS/MS

After filtration through 22 μm membrane the clear solution was subjected to nano LC-MS/MS separation. A volume of 1 μL of each sample was desalted by loading on a Thermo C18 PepMap100 precolumn (300 μm × 5 mm) and eluted on a Thermo Acclaim PepMap RSLC analytical column (75 μm × 15 cm). Mobile phase A (0.1% formic acid in H₂O) and mobile phase B (0.1% formic acid in acetonitrile) were used to establish the 120min gradient comprised of 85 min of 4–30% B, 15 min of 30–50% B, and 5 min of 90% B, followed by re-equilibrating at 4% B for 15 min. The flow rate was 0.3 μL/min. Peptides were then analyzed on a Q-Exactive proteomic mass spectrometer (Thermo Scientific) in a data-dependent manner, with automatic switching between MS and MS/MS scans

using a top 10 method. MS spectra were acquired at a resolution of 70000 with a target value of 3×10^6 ions or a maximum integration time of 50 ms. The scan range was limited from 375 to 1400 m/z . Peptide fragmentation was performed via higher-energy collision dissociation (HCD) with the energy set at 32 NCE. The MS/MS spectra were acquired at a resolution of 35000 with a target value of 1×10^5 ions or a maximum integration time of 100 ms. The fixed first m/z was 100, and the isolation window was 1.2 m/z .

2.6 Data process

Protein identification and TMT quantification were performed using Proteome Discoverer 2.1 software (Thermo Scientific). Peptide sequences (and hence protein identity) were determined by matching protein databases (UniProt) with the acquired fragmentation pattern by SEQUEST HT algorithm. The precursor mass tolerance was set to 10 ppm and fragment ion mass tolerance to 0.02 Da. One missed cleavage site of trypsin was allowed. Carbamidomethyl (C) and TMT-²plex (K and N-terminal) were used as a fixed modification. Oxidation (M) was used as variable modifications. All spectra were searched against protein database using a target false discovery rate (FDR) of 1%. Identified proteins were additionally filtered by at least three spectral counts and one unique peptides in each experimental replicate. Protein ratios were calculated as the median of all peptide hits belonging to a protein. TMT ratios obtained from Proteome Discoverer 2.1 were transformed with $\log_2(x)$ and then normalized using Z-score, and $-\log_{10}(p\text{-value})$ of all proteins were obtained by a two-sided one sample t-test over two biological replicates. Only proteins identified have $p\text{-values} < 0.05$ ($-\log_{10}(p\text{-value}) > 1.3$) were considered as statistical significant.

2.7 Target validation by pull-down and western blot

Bound proteins on streptavidin beads from pull-down experiments as described in section 2.4 were eluted by boiling in SDS-PAGE sample loading buffer for 15 minutes and separated by SDS-PAGE. The following western blot analysis was performed as described above utilizing anti-ACLY and anti-GAPDH antibodies in section 2.2.

2.8 *In vitro* probe labeling of recombinant ACLY

Recombinant ACLY protein was ordered from Abcam. ACLY-C20S mutant was prepared according to previously reported procedures.² The protein was diluted to 0.05 mg/mL with PBS, pH 7.5. For each sample, 20 μL of ACLY solution (1 μg) was incubated with different probes (DCV-yne, CV-yne, or IA-yne) as indicated at room temperature for 1 h. In the competitive labeling experiment of Fig. 3A, DCV was incubated with protein samples either after or before probe incubation for 2 h at room temperature; in that of Fig. 3B, DCV was incubated with protein samples before probe incubation for 2 h at room temperature; while in that of Fig. 3C, ATP was incubated with protein samples before probe incubation for 2 h at room temperature. Then, the samples were added with 1% SDS and the following click reaction and in-gel fluorescence scanning were performed as described

above in section 2.3.

2.9 Cellular thermal shift assay (CETSA)

Jurkat cells were incubated with DCV (5 μ M) in PBS for 2 h. The medium was removed and cells were washed with PBS three times. Then the cells were suspended in PBS (1 mL) and aliquot into PCR tubes (each 250 μ L). The tubes were heated individually at various temperatures as indicated for 3 min followed by immediate cooling on ice for another 3 min. The cells were then lysed by freeze (dry ice)-thaw (25 °C water bath) for three cycles. The cell lysates were centrifuged at 18,000 g for 30 min at 4 °C to pellet the denatured and precipitated proteins. Soluble supernatants were analyzed by gel electrophoresis followed by immuno-blotting using ACLY and GAPDH antibodies as described in section 2.2 above.

2.10 ACLY enzymatic assay

The known ACLY inhibitor BMS-303141 was ordered from MedChemExpress. Various concentrations of DCV or BMS-303141 dissolved in DMSO were incubated with 12.5 μ L of recombinant ACLY protein (20 ng) dissolved in 40 mM Tris-HCl, pH 8.0 at room temperature for 3 h. Proteins were added to 12.5 μ L of reaction mixture containing 40 mM Tris-HCl pH 8.0, 20 mM MgCl₂, 5 mM DTT, 5 mM ATP, 0.2 mM CoA and 5 mM sodium citrate. The total volume of reaction is 25 μ L and the final concentration of DMSO is 1% in all reactions. The enzymatic reactions were conducted at 30 °C for 60 min. After the reaction, the amount of ADP was quantified using ADP-Glo luminescence assay kit (Promega) according to the manufacturer's instruction. The luminescence signal was measured using an EnVision Perkin Elmer microplate reader. Data analysis was performed with GraphPad Prism 7.0.

2.11 Knockdown by siRNA

HeLa cells were transfected in OPTI-MEM with lipofectamin RNAiMAX (Invitrogen) with 20 nM of siRNA of ACLY or scrambled RNA (Dharmacon) for 24 h according to the manufacturer's protocols. The medium was removed and replaced for DMEM containing 10% FBS. Cells were treated with compounds as described in figure legends for 24 h in a 37 °C incubator. Cell viability was assessed by CellTiter-Glo[®] Luminescent Kit (Promega).

2.12 The effect of citrate/acetate on cell proliferation inhibitory effect of DCV

Jurkat cells were cultured in full RPMI medium containing sodium citrate (1 mM) or sodium acetate (5 mM) for 24 h prior to drug treatment. The medium was aspirated and various concentrations of DCV dissolved in fresh sodium citrate- (1 mM) or sodium acetate- (5 mM) containing RPMI medium were added to cells and incubated for another 24 h. Cell viability was assessed by CellTiter-Glo[®] Luminescent Kit (Promega).

3. Chemistry

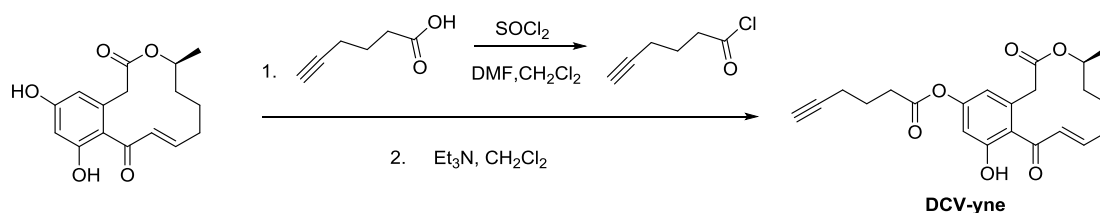
Materials

All the reagents were purchased commercially and used without further purification. Dehydrocurvularin and Curvularin were obtained according to *Ref 3*. Compounds **1** and **2** were obtained according to *Ref 4*.

Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Huanghai silica gel plates (HSGF254) using UV light as visualizing agent. Flash column chromatography was carried out using Yantai Xinnuo silica.

¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker Ultrashield™ 400 PLUS (¹H: 400 MHz, ¹³C: 100 MHz) with chemical shift values in ppm relative to TMS (δ_{H} 0.00 and δ_{C} 0.00) and residual D-chloroform (δ_{H} 7.26 and δ_{C} 77.16) as standard. HR-MS were obtained using an Agilent 6520 Accurate-Mass Q-TOF instrument.

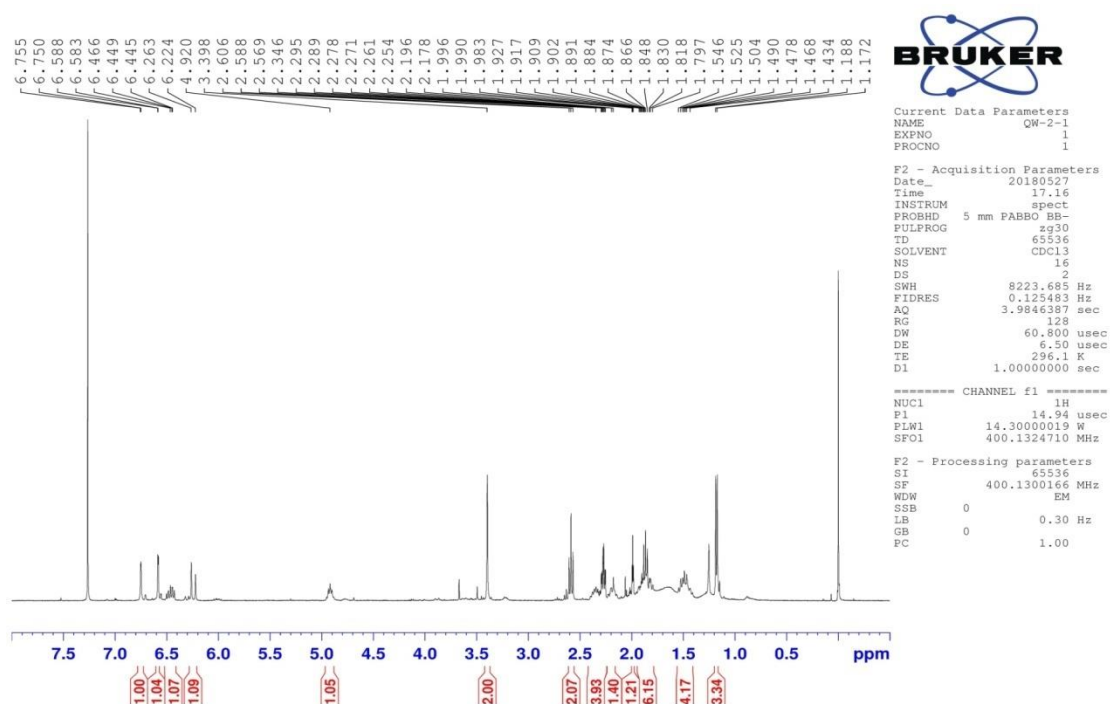
3.1 Synthesis of DCV-yne



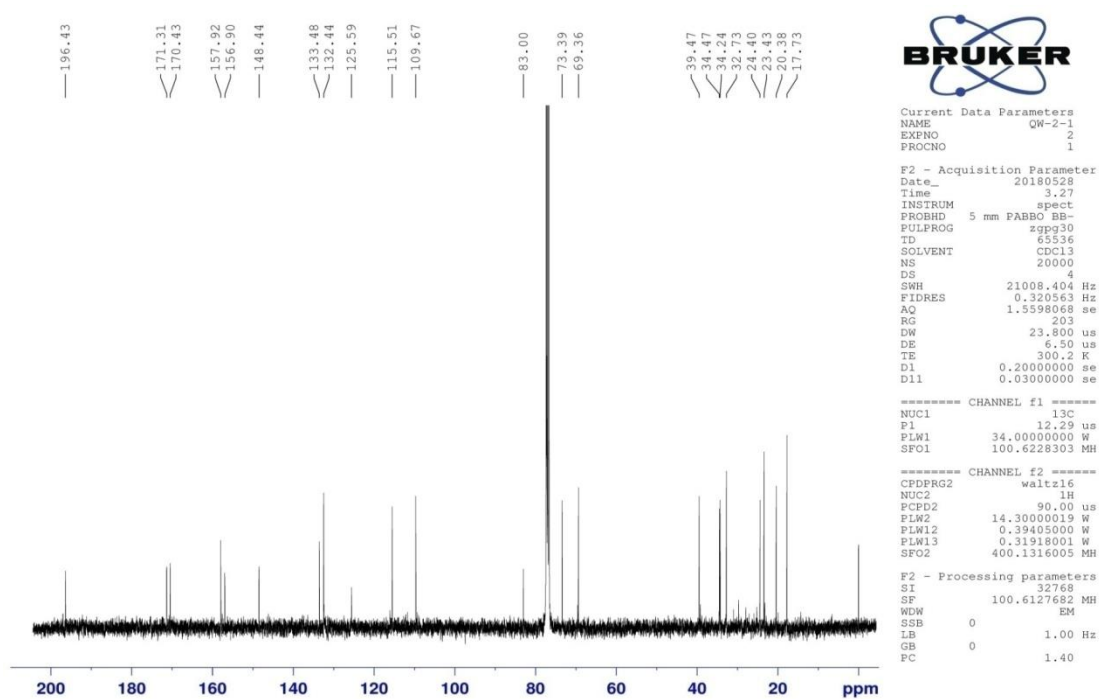
To the solution of 5-hexynoic acid (224 mg, 2 mmol) in dry CH_2Cl_2 (10 mL) were added dropwise thionyl chloride (560 μL , 8 mmol) and catalytic amounts of DMF with stirring. The mixture was stirred for 3h with reflux, and excess thionyl chloride and CH_2Cl_2 was removed under vacuum. The residue was dissolved in CH_2Cl_2 (30 mL). Dehydrocurvularin (10 mg, 0.0345 mmol) and triethylamine (140 μL , 1 mmol) were added to the solution of acyl chloride (2 mL) with stirring. The reaction mixture was stirred for 4h at reflux, concentrated under *vacuum*. The residue was purified with flash column chromatography using trichloromethane/methanol (60:1) as an eluent to give crude products (4 mg), followed by reversed-phase semipreparative HPLC using a C18 chromatographic column (YMC-Pack-ODS-A 5 μm , 4.6x250 mm) to afford DCV-yne (2.3 mg).

DCV-yne: Colorless amorphous powder, HR-ESI-MS m/z 385.1645 [$\text{M}+\text{H}$]⁺ (calcd for $\text{C}_{22}\text{H}_{25}\text{O}_6$ 385.1651); ¹H NMR (400 MHz, CDCl_3): δ 6.75 (1H, d, $J=2.0$ Hz), 6.59 (1H, d, $J=2.0$ Hz), 6.53-6.40 (1H, m), 6.24 (1H, d, $J=16.0$ Hz), 4.98-4.86 (1H, m), 3.40 (2H, s), 2.59 (2H, t, $J=7.4$ Hz), 2.41-2.30 (1H, m), 2.27 (2H, td, $J=2.6, 6.9$ Hz), 2.24-2.14 (1H, m), 1.99 (1H, t, $J=2.6$ Hz), 1.95-1.78 (4H, m), 1.56-1.39 (2H, m), 1.18 (3H, d, $J=6.3$ Hz); ¹³C NMR (100 MHz, CDCl_3): δ 196.4, 171.3, 170.4, 157.9, 156.9, 148.4, 133.5, 132.4, 125.6, 115.5, 109.7, 83.0, 73.4, 69.4, 39.5, 34.5, 34.2, 32.7, 24.4, 23.4, 20.4, 17.7.

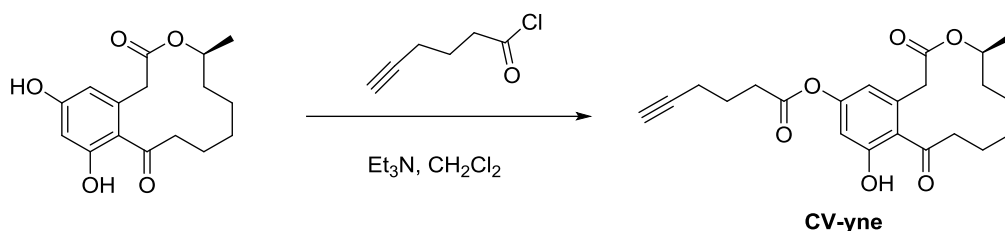
¹H NMR spectrum of DCV-yne:



¹³C NMR spectrum of DCV-yne:



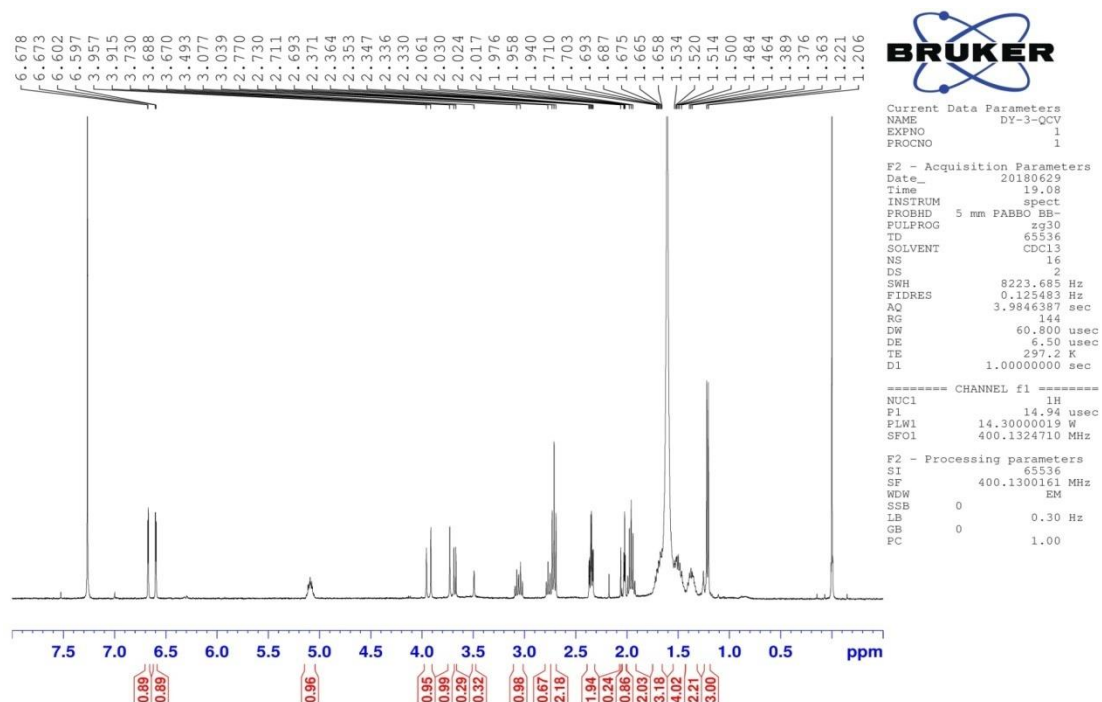
3.2 Synthesis of CV-yne.



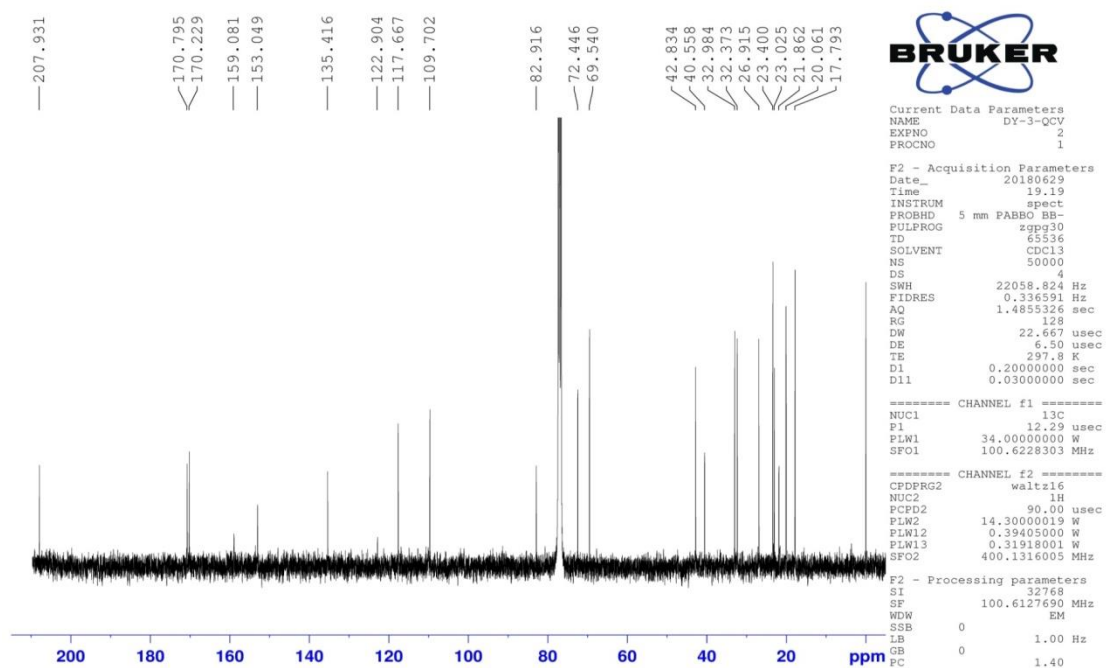
Curvularin (3.4 mg, 0.0116 mmol) and triethylamine (50 μ L, 0.357 mmol) were added to the solution of acyl chloride (1 mL) with stirring. The reaction mixture was stirred for 4h with reflux and concentrated under vacuum. The residue was purified with reversed-phase semi-preparative HPLC using a C18 chromatographic column (YMC-Pack-ODS-A 5 μ m, 4.6x 250 mm) to afford CV-yne (1.1 mg).

CV-yne: Colorless amorphous powder, HR-ESI-MS m/z 387.1801 $[M+H]^+$ (calcd for $C_{22}H_{27}O_6$ 387.1808); 1H NMR(400 MHz, $CDCl_3$): δ 6.68 (1H, d, $J=2.2$ Hz), 6.60 (1H, d, $J=2.2$ Hz), 5.14-5.04 (1H, m), 3.94 (1H, d, $J=16.8$ Hz), 3.71 (1H, d, $J=16.8$ Hz), 3.06 (1H, dt, $J=7.1, 15.2$ Hz), 2.77 (1H, t, $J=7.1$ Hz), 2.71 (2H, t, $J=7.4$ Hz), 2.35 (2H, td, $J=2.6, 6.9$ Hz), 2.02 (1H, t, $J=2.6$ Hz), 1.96 (2H, qui, $J=7.1$ Hz), 1.75-1.43 (6H, m), 1.42-1.31 (2H, m), 1.21 (3H, d, $J=6.4$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.9, 170.8, 170.2, 159.1, 153.0, 135.4, 122.9, 117.7, 109.7, 82.9, 72.4, 69.5, 42.8, 40.6, 33.0, 32.4, 26.9, 23.4, 23.0, 21.9, 20.1, 17.8.

1H NMR spectrum of CV-yne:



¹³C NMR spectrum of CV-yne:



4. References

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