Supporting Information for

Quantitative profiling of protein carbonylations in ferroptosis by an aniline-derived probe

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SUPPORTING INFORMATION TABLES

Table S1. List of sites of exogenous HNE modification identified by TOP-ABPP.

Table S2. List of HNE-sensitive cysteines quantitatively profiled by isoTOP-ABPP.

Table S3. Comparison of the HNE-hypersensitive cysteines profiled by our study with that from a previous competitive isoTOP-ABPP study by Wang *et al*.

Table S4. Quantitative profiling of endogenously carbonylated proteins in ferroptosis by RD-ABPP.

Table S5. List of endogenously LDE-modified residue sites in ferroptotic cells.

Table S6. List of the HNE-modified Cysteines identified by the AOyne probe.

SUPPORTING INFORMATION FIGURES

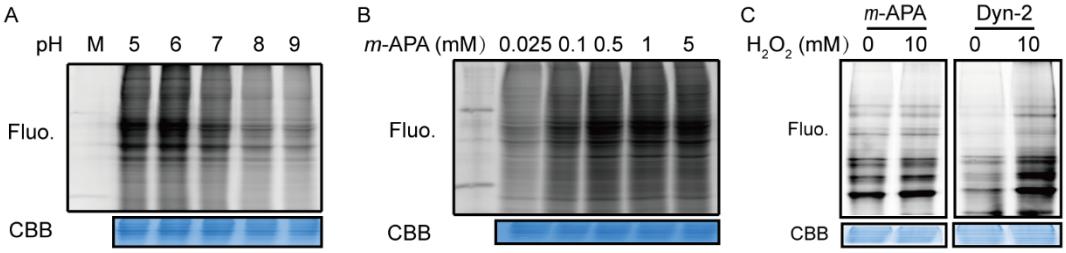


Figure S1. Optimization of *m*-APA labeling. (a) Optimization of pH for *m*-APA labeling. (b) Optimization of probe concentration for *m*-APA labeling. (c) *m*-APA does not react with protein sulfenylations. HT1080 cell lysates were incubated with 10 mM H_2O_2 to induce proteome-wide cysteine oxidation which could be detected by a sulfenylation-reactive dimedone (Dyn-2) probe but not the *m*-APA probe.

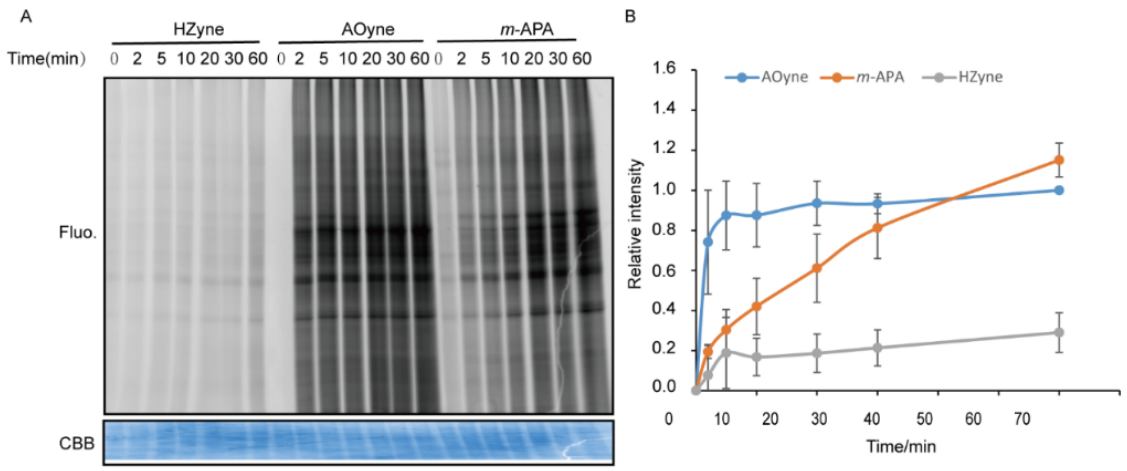


Figure S2. Comparison of the labeling efficiency of *m*-APA with another two aldehyde-directed probes, HZyne and AOyne. (a) Comparison of the labeling efficiency of the three aldehyde-directed probes in proteomes via in-gel fluorescence. (b) Relative quantification of the intensity of in-gel fluorescence at different probe concentrations to reflect the labeling kinetics and strengths of the three probes.

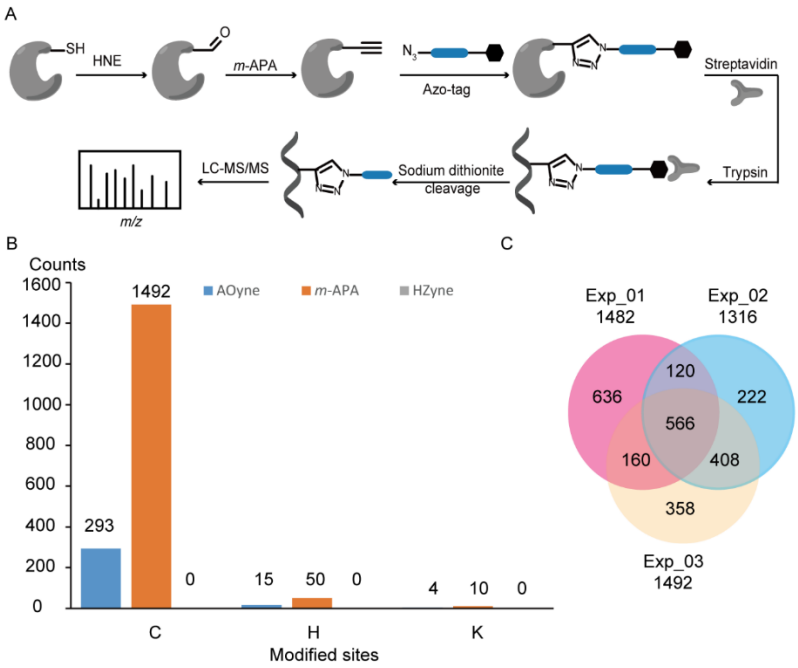


Figure S3. Identification of sites of exogenous HNE modification by TOP-ABPP. (a) Scheme of identifying the HNE modified sites in proteomes using *m*-APA with TOP-ABPP. (b) Number of Cys, His and Lys that were identified by each of the AOyne, *m*-APA and HZyne probes side-by-side. (c) A venn diagram showing the number of HNE modified cysteines identified in three replicates of TOP-ABPP experiments using the *m*-APA probe.

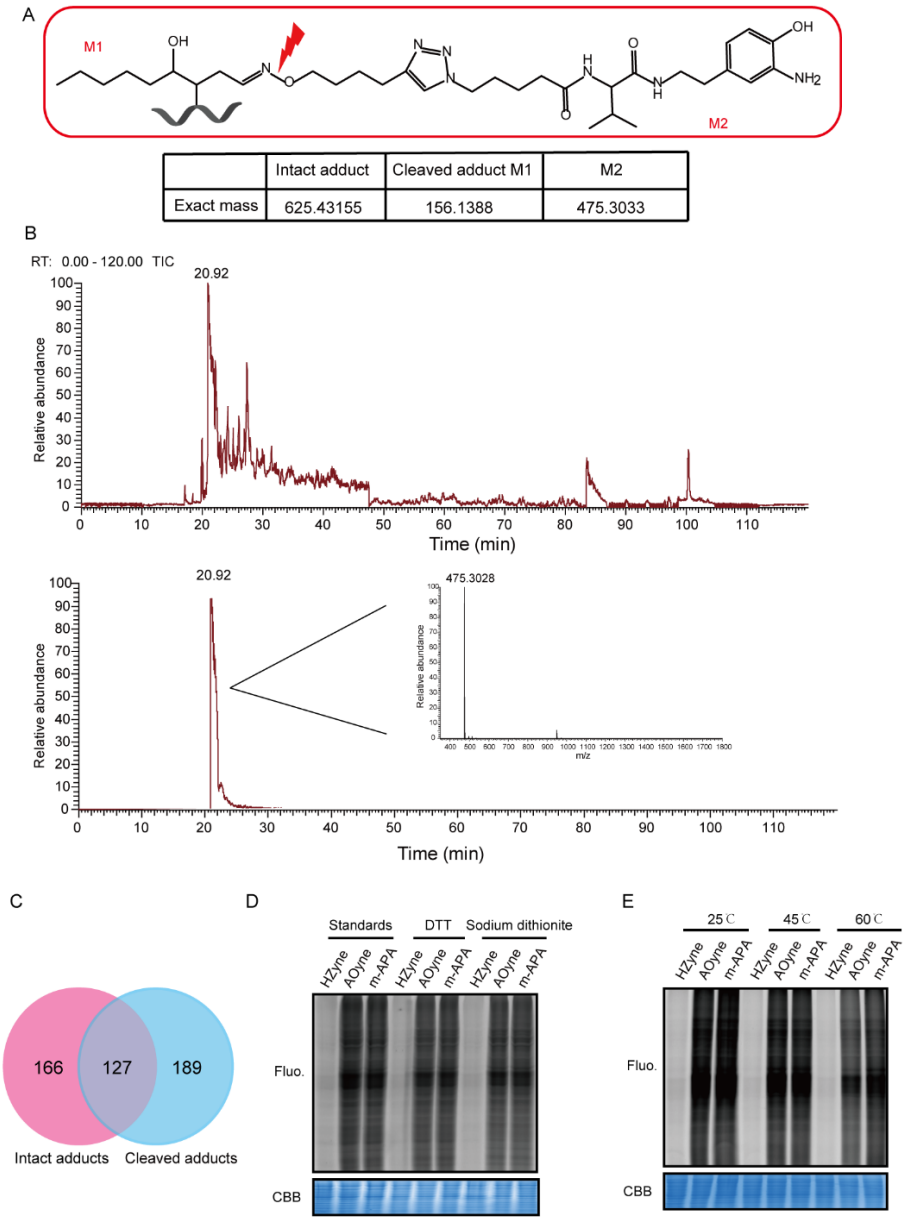


Figure S4. Observation of an unstable product from AOyne-adducted peptide samples during sample preparation. (a) Top: structure of the expected adduct of an HNE modified peptide that was captured by AOyne, conjugated with the azide-azobenzene-biotin tag, enriched by streptavidin and cleaved by sodium dithionite. Bottom: summary of exact masses of intact and cleaved adducts using AOyne probe. (b) Top: total ion chromatograms of a standard TOP-ABPP sample prepared using AOyne probe and analyzed by LC-MS/MS. Bottom: The extracted chromatographic peak with the m/z of 475.3028 and a retention time of 20.92 min indicating the possible unstable product broken at the N-O bond of AOyne adducted peptides. (c) Intact and cleaved adducts were searched, and 293 and 316 sites were identified, respectively (Table S6). There are 127 overlapping sites shared between the intact adducts and cleaved adducts. (d-e) Proteomes labeled by HZyne, AOyne and *m*-APA were treated with DTT and $Na_2S_2O_4$ or incubated with 0.1% formic acid at different temperatures according to the conditions used in LC-MS/MS sample preparation. In-gel fluorescence showed an obvious signal loss for the AOyne-labeled proteome at 60°C in 0.1% formic acid.

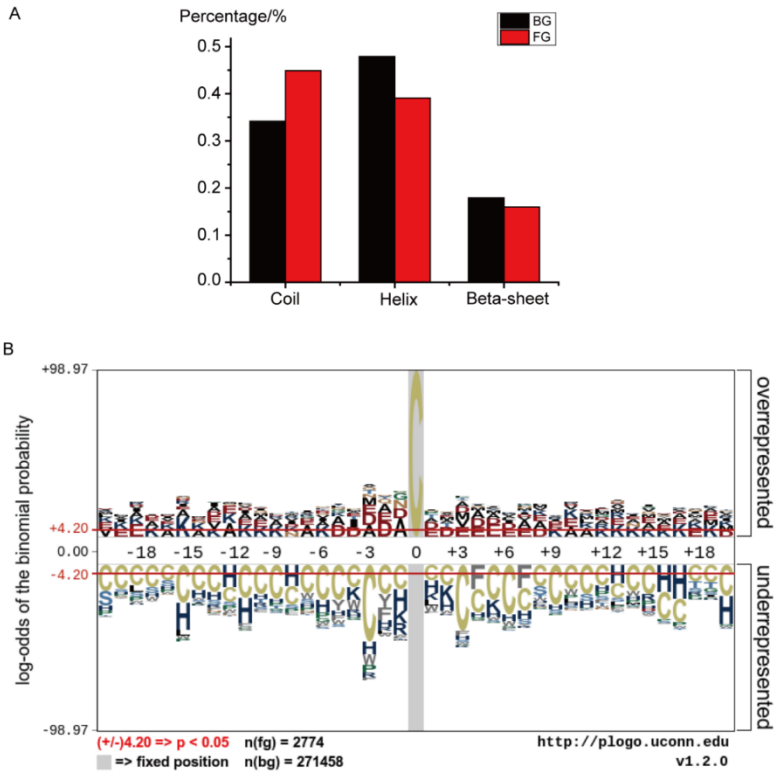


Figure S5. Sequence analysis of cysteines modified by exogenous HNE in proteomes. (a)Analysis of HNE modified cysteines in terms of their secondary structure propensity, showing HNE prefers to target cysteines in the random coil. (b) Local sequence preference for HNE modified cysteines.

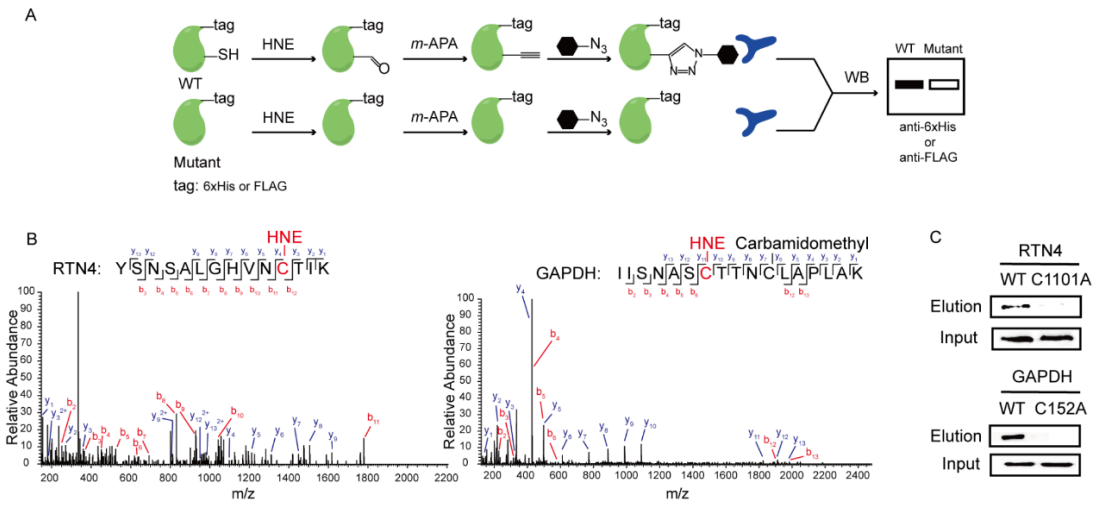


Figure S6. Biochemical validation of HNE-modified cysteines identified by TOP-ABPP. (a) Scheme of verifying HNE modifications on target cysteines by site-directed mutagenesis, *m*-APA labeling and enrichment, and immunoblotting. (b) MS/MS spectra supporting the HNE modification on C1101 of RTN4 and C152 of GAPDH. (c) Confirmation of RTN4^{C1101} and GAPDH^{C152} as sites of HNE modification as the *m*-APA labeling disappeared when the corresponding cysteines are mutated.

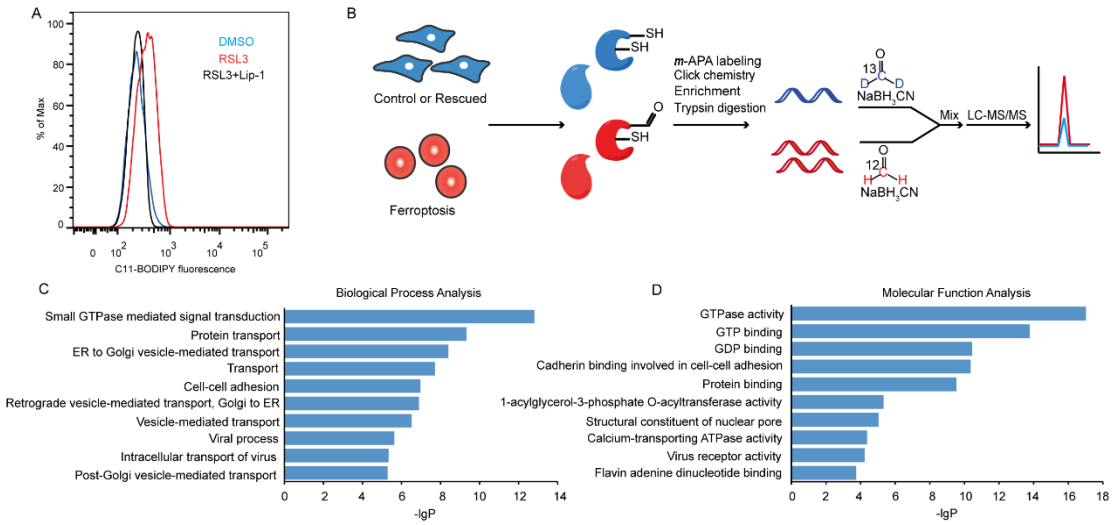


Figure S7. Profiling of endogenous carbonylated proteins in ferroptosis and functional analysis these targets. (a) Monitoring levels of lipid peroxides in ferroptotic cells induced with RSL3 and rescued with Lip-1. RSL3 will increase the level of lipid peroxide, while Lip-1 can decrease the level of lipid peroxide during cell ferroptosis as detected using a C11-BODIPY fluorescent probe and analyzed by FACS. (b) Scheme of RD-ABPP to profile endogenous carbonylated proteins in ferroptosis. (c) GO analysis of carbonylated proteins in ferroptosis in terms of biological processes. (d) GO analysis of carbonylated proteins in ferroptosis in terms of molecular functions.

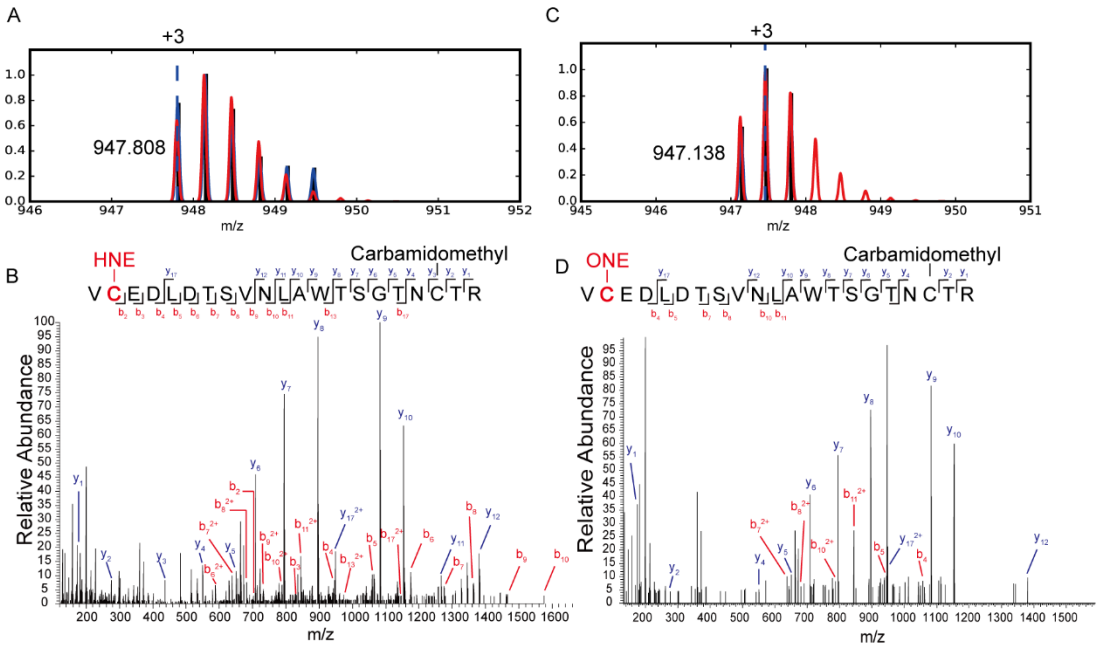


Figure S8. MS spectrum supporting HNE & ONE modification on C210 of VDAC2 in ferroptosis. (a) Validation of full MS spectrum of the adducted peptide supporting HNE modification on C210 of VDAC2 by isotopic envelope check. The observed (blue) and predicted (red) full MS spectra were shown with the dashed line indicating the observed precursor monoisotopic m/z value. (b) MS/MS spectra supporting the HNE modification on C210 of VDAC2. (c) Full MS spectrum of the adducted peptide supporting ONE modification on C210 of VDAC2 which is different from HNE by only 2 Da. (d) MS/MS spectra supporting the ONE modification on C210 of VDAC2.

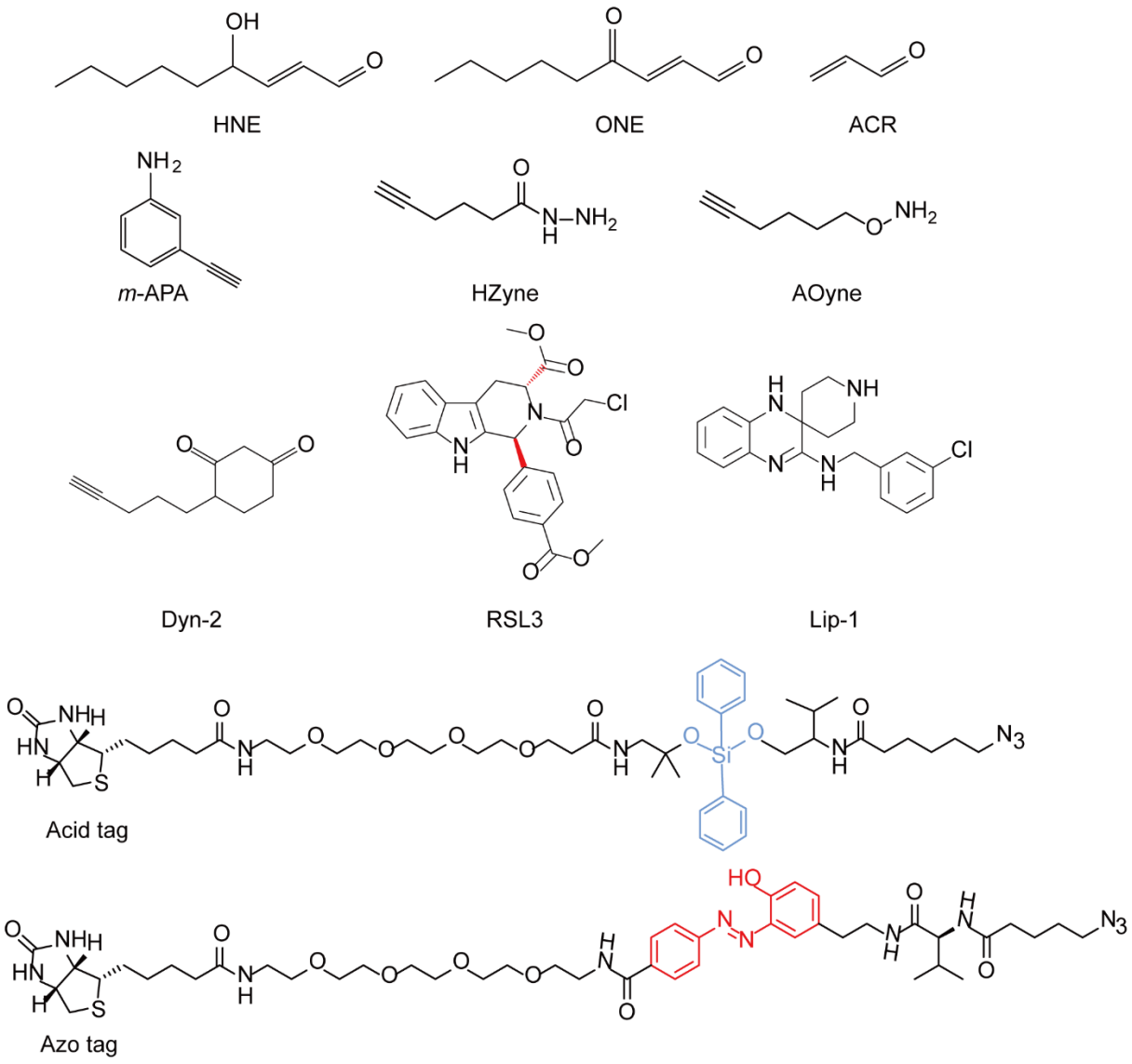


Figure S9. Chemical structures of compounds and probes used in this work.

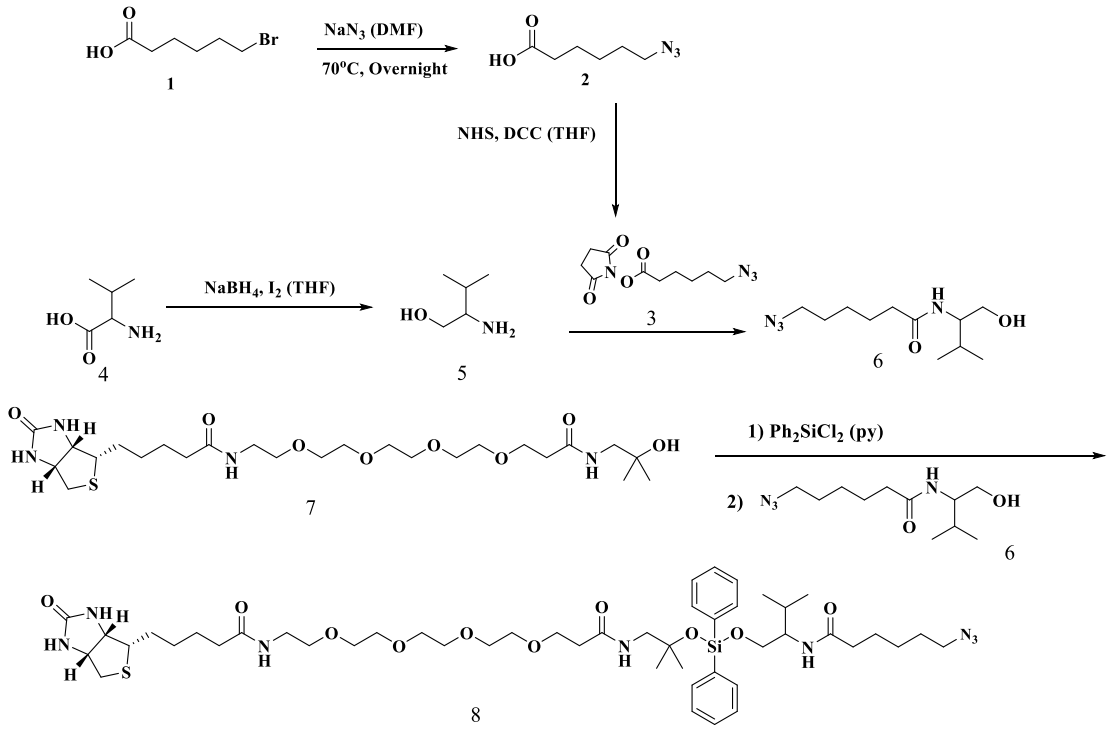


Figure S10. Synthetic roots of acid tag used in this work.

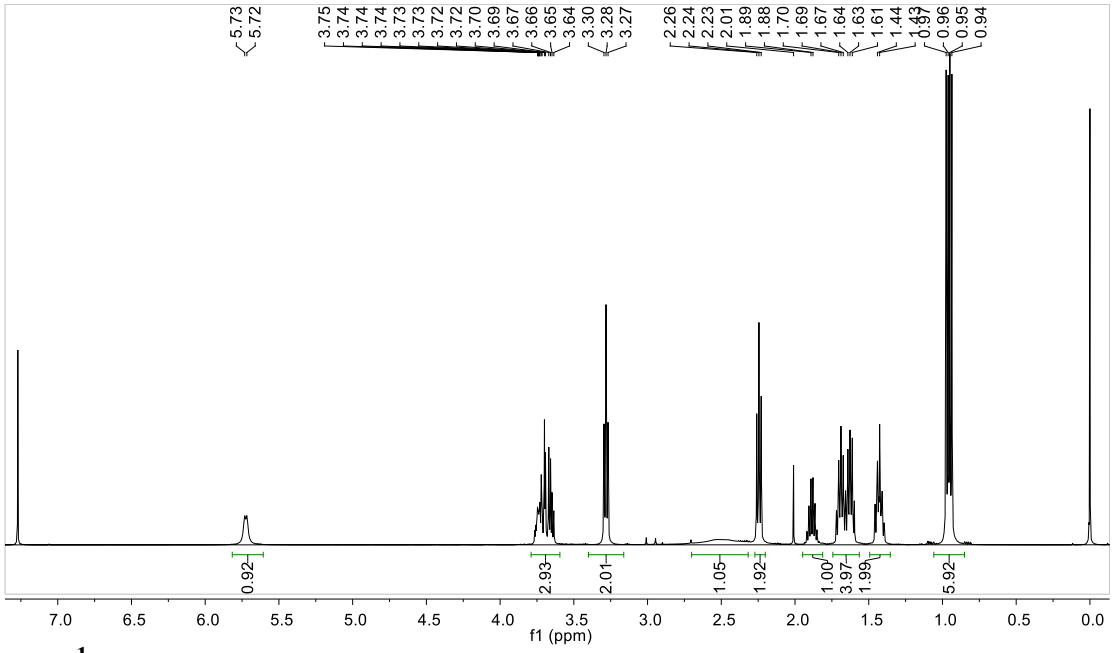


Figure S11. ^{1}H NMR spectrum of AzValOH (500 MHz, CDCl3).

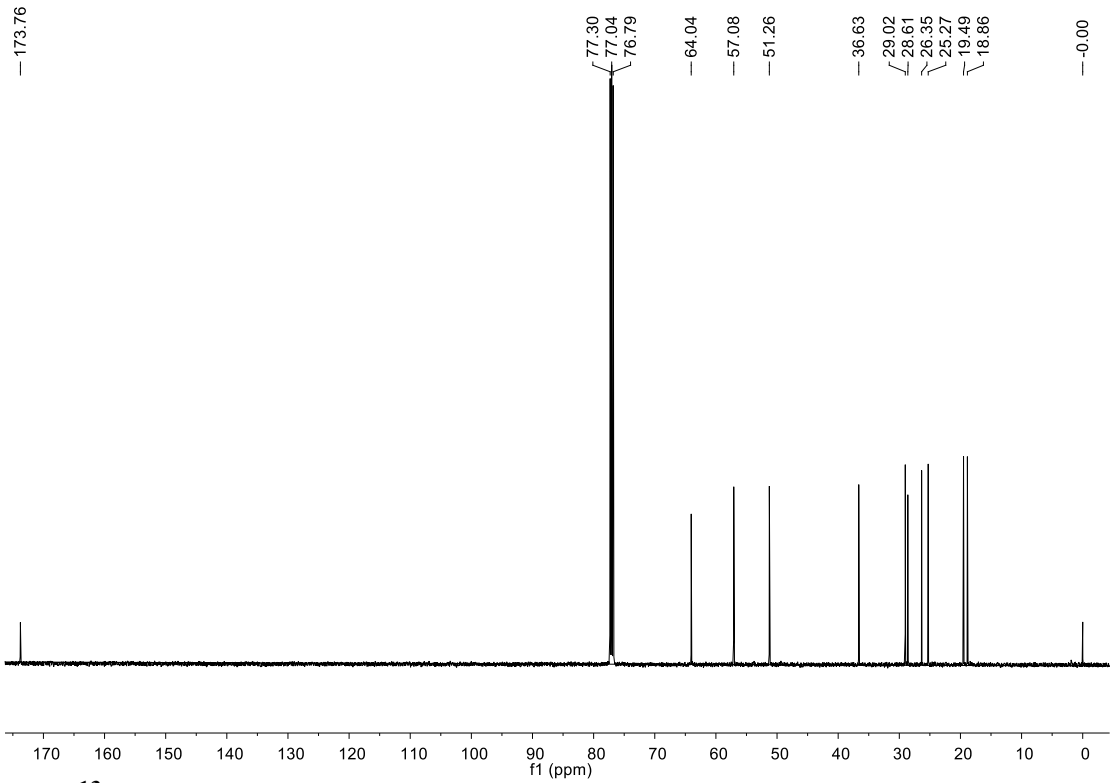
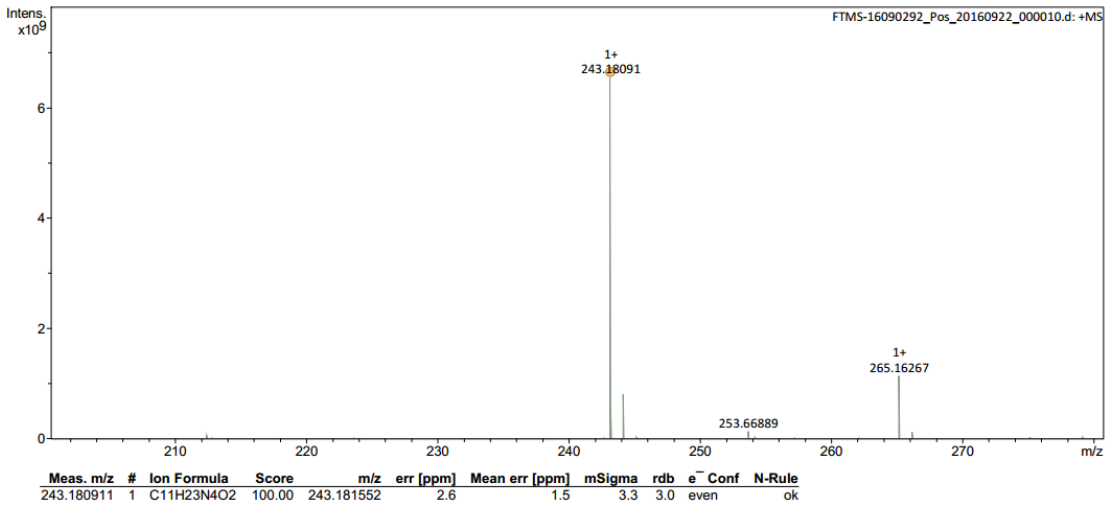


Figure S12. ^{13}C NMR spectrum of AzValOH (126 MHz, CDCl3).



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
243.180911	1	C11H23N4O2	100.00	243.181552	2.6	1.5	3.3	3.0	even	ok

Figure S13. HRMS of AzValOH.

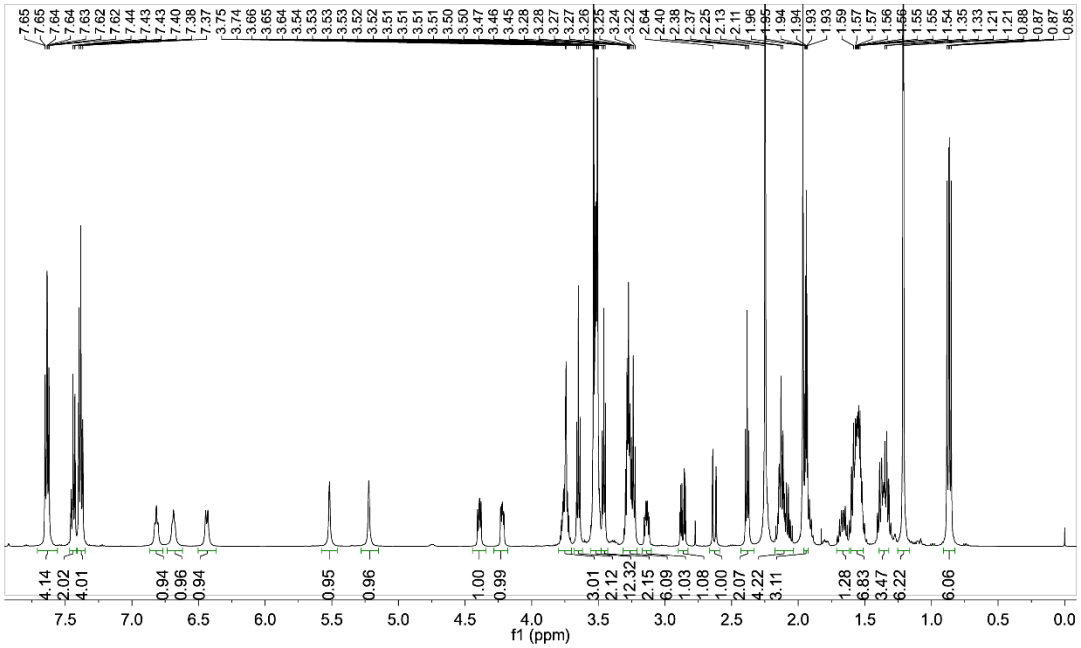


Figure S14. ^{1}H NMR spectrum of acid tag (500 MHz, CD_3CN).

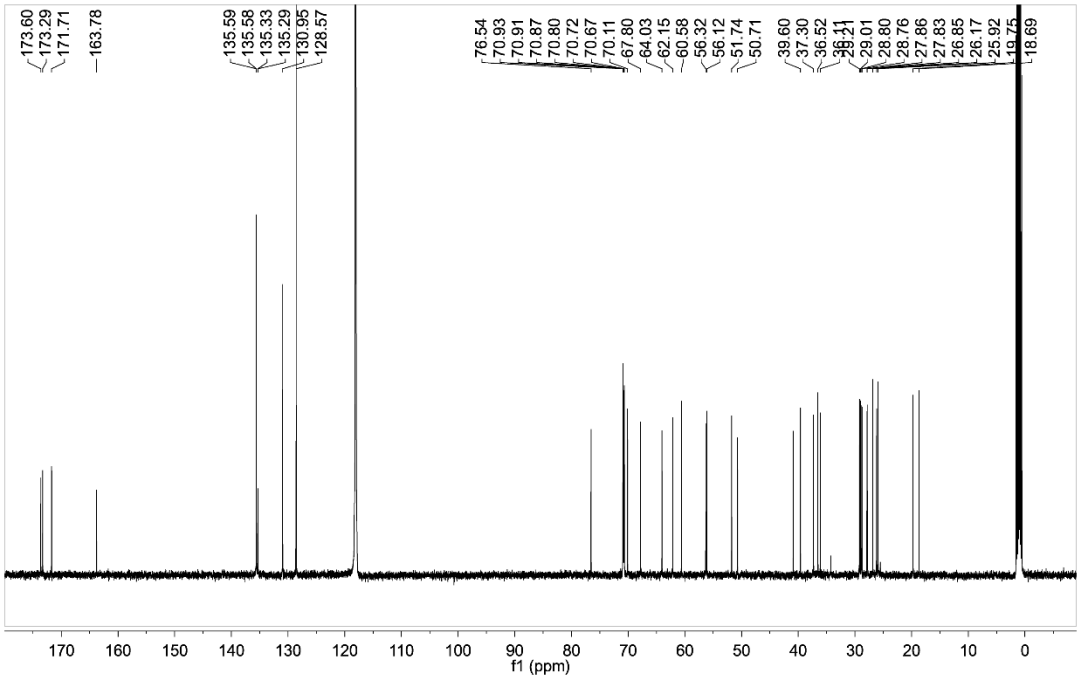
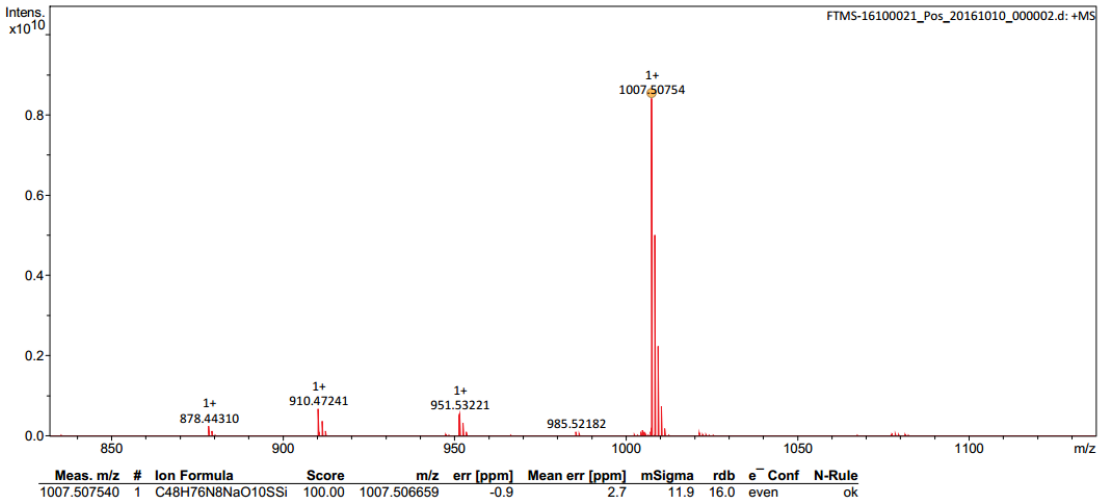


Figure S15. ^{13}C NMR spectrum of acid tag (126 MHz, CD_3CN).



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
1007.507540	1	C48H76N8NaO10SSi	100.00	1007.506659	-0.9	2.7	11.9	16.0	even	ok

Figure S16. HRMS of acid tag.

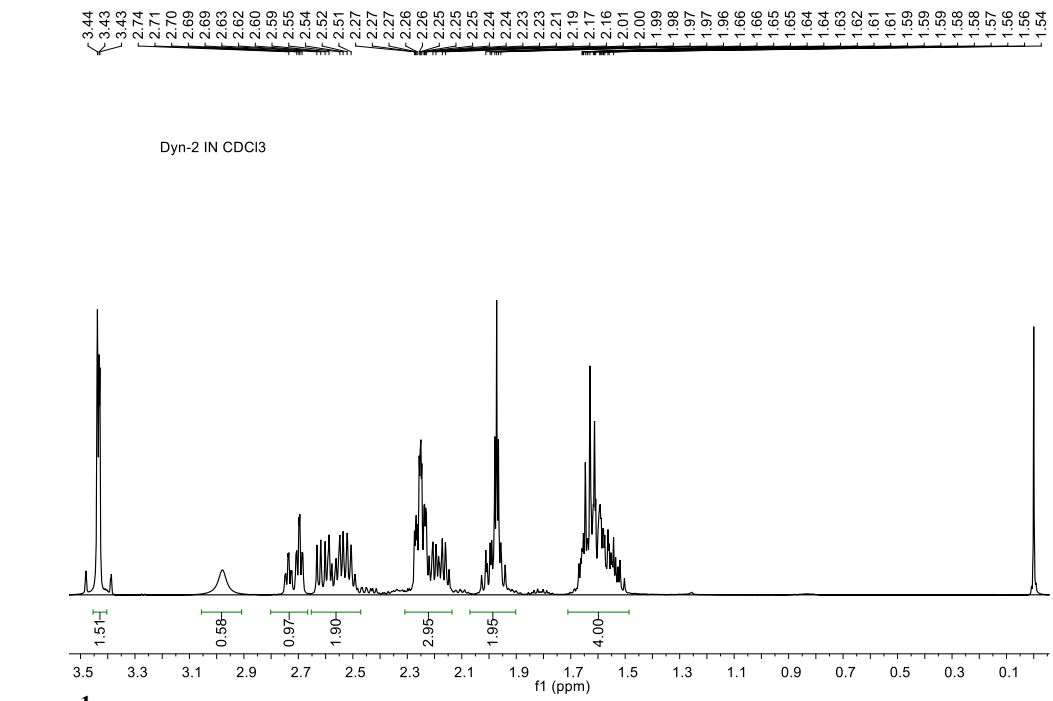


Figure S17. ^{1}H NMR spectrum of Dyn-2 (400 MHz, CDCl3).

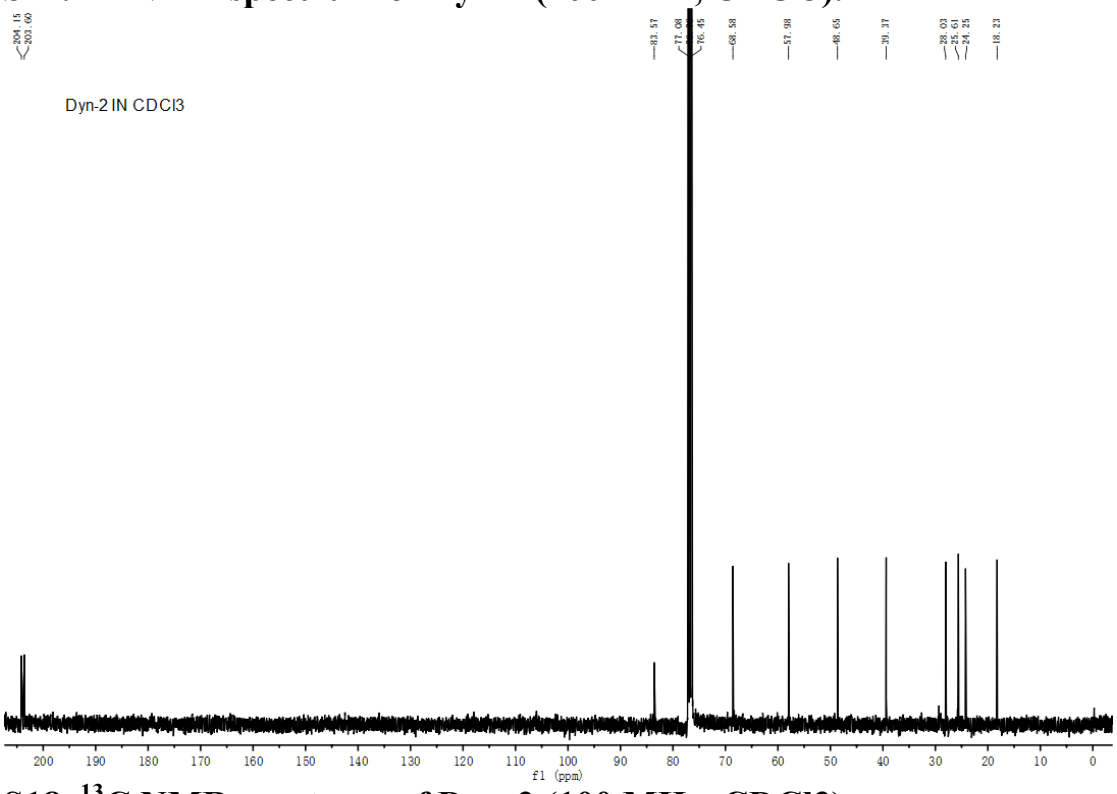


Figure S18. ^{13}C NMR spectrum of Dyn-2 (100 MHz, CDCl3).

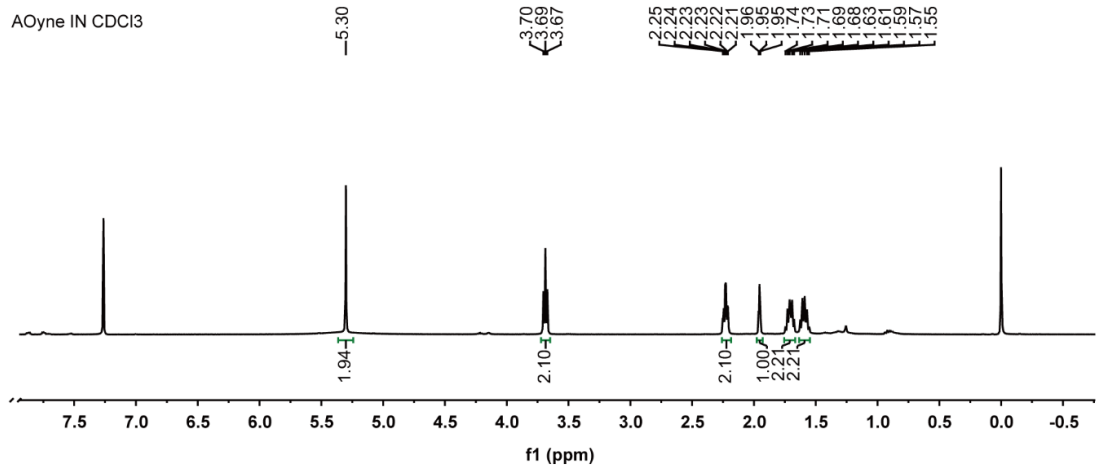


Figure S19. ^{1}H NMR spectrum of AOyne (400 MHz, CDCl3).

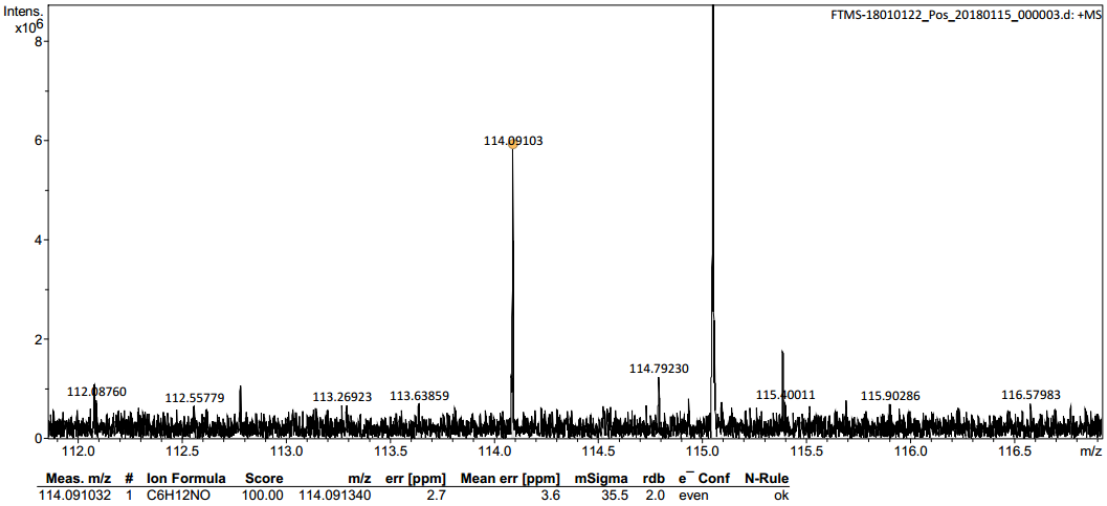


Figure S20. HRMS of AOyne.

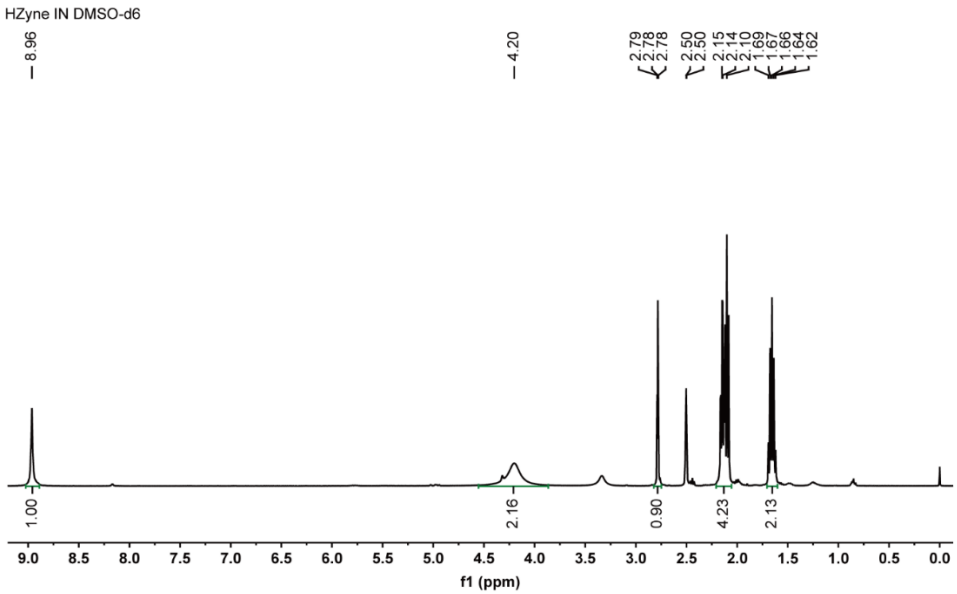


Figure S21. ^{1}H NMR spectrum of HZyne (400 MHz, DMSO-d6).

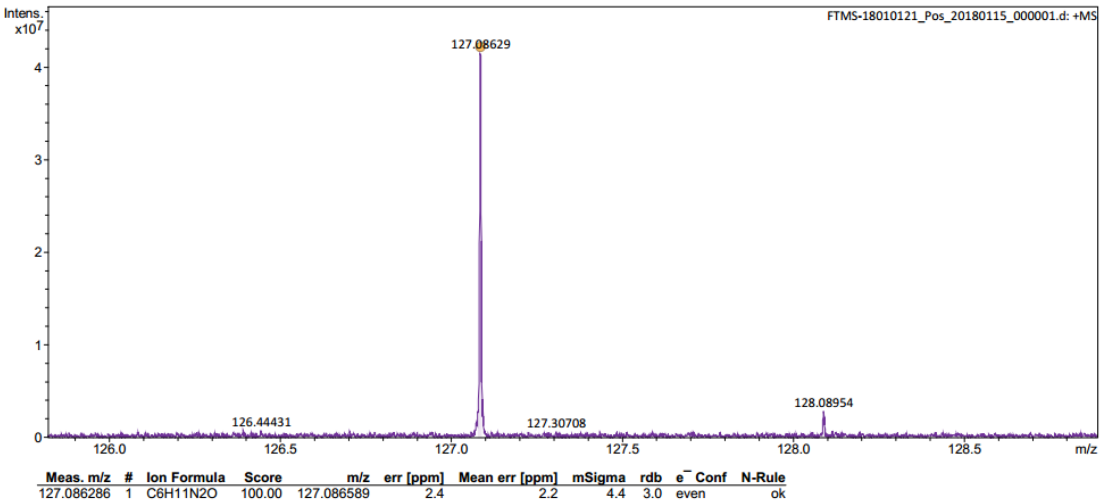


Figure S22. HRMS of HZyne.

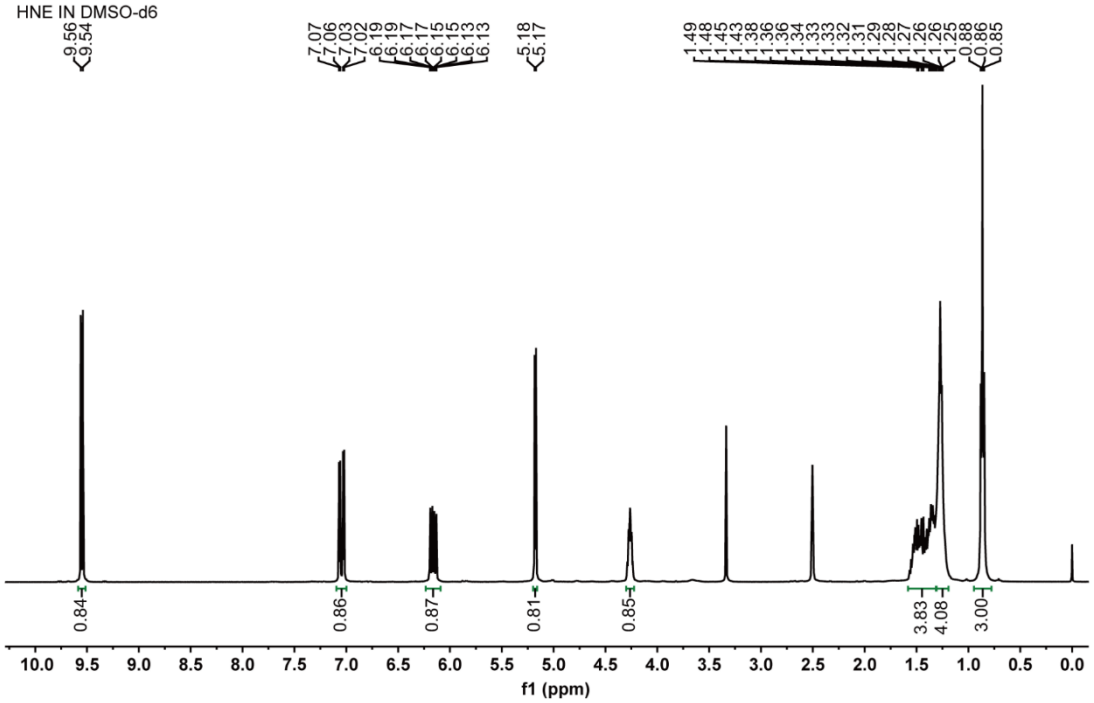


Figure S23. ^{1}H NMR spectrum of HNE (400 MHz, DMSO-d6).

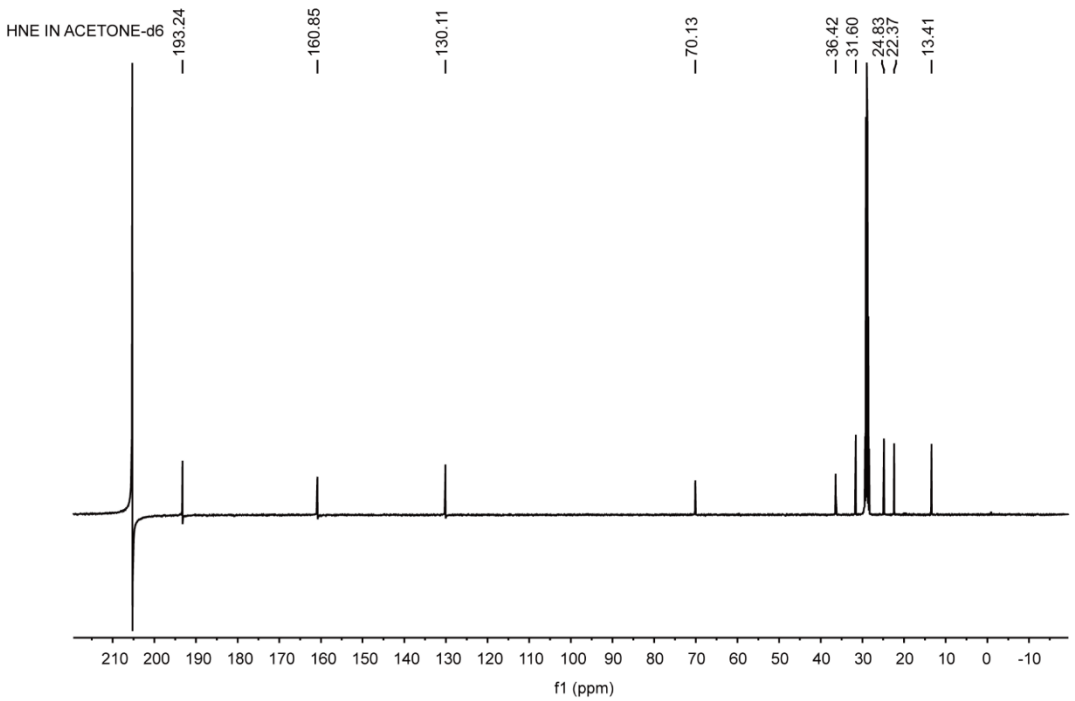


Figure S24. ^{13}C NMR spectrum of HNE (100 MHz, ACETONE-d6).

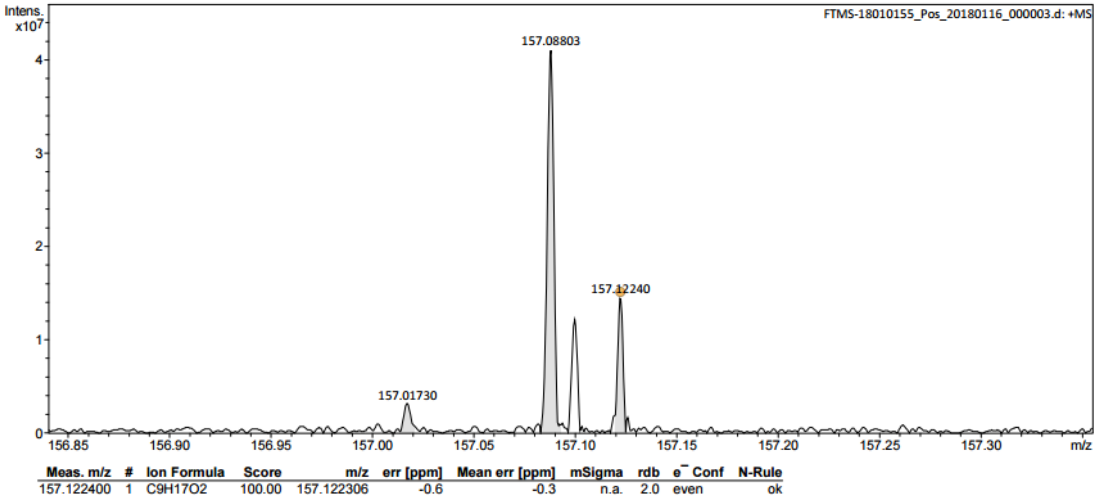


Figure S25. HRMS of HNE.

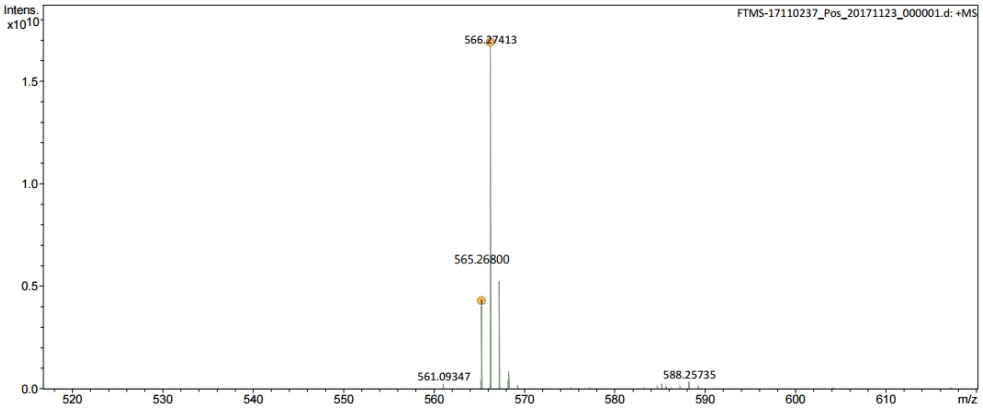


Figure S26. HRMS of the final adduct of GSH_HNE_*m*-APA.

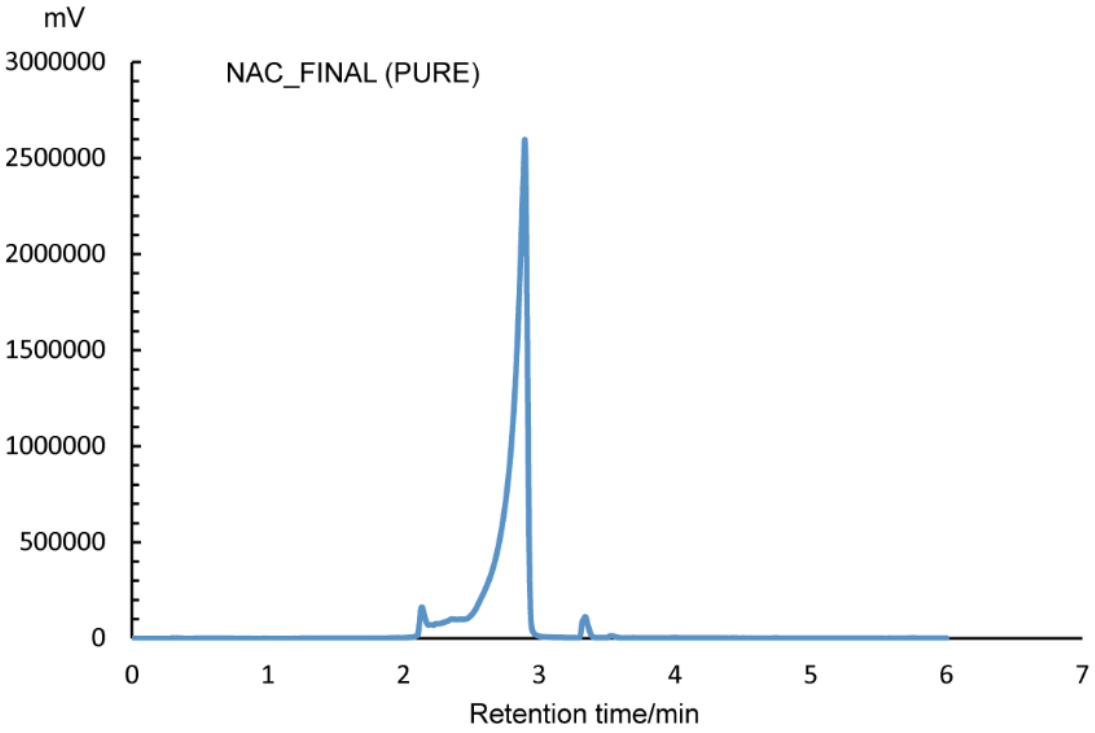


Figure S27. HPLC trace of the final adduct of NAC_HNE_*m*-APA.

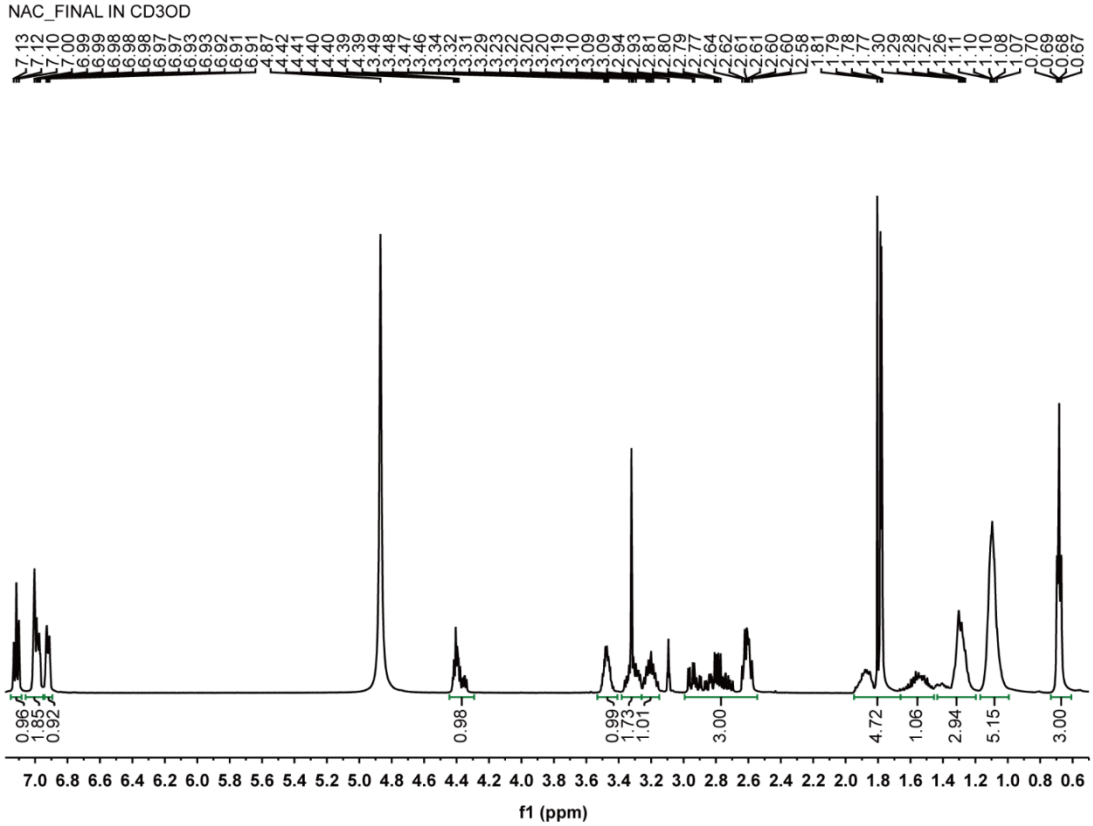


Figure S28. ^{1}H NMR spectrum of NAC_HNE_*m*-APA final adduct (500 MHz, CD3OD).

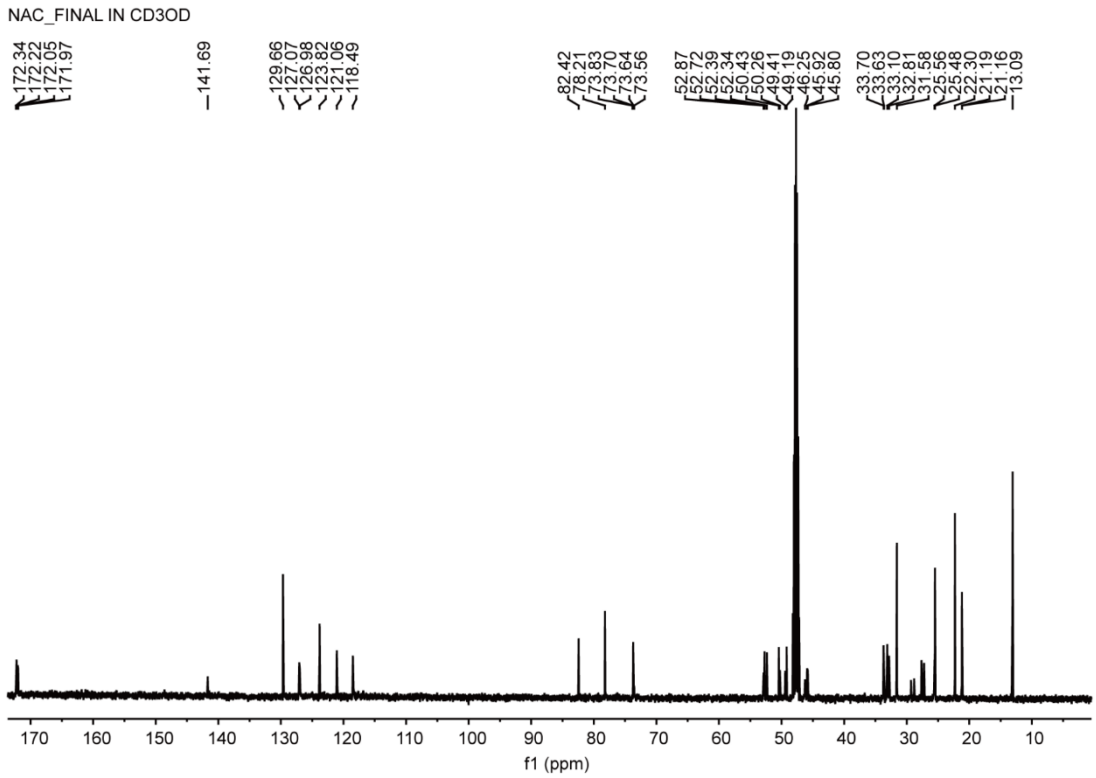


Figure S29. ^{13}C NMR spectrum of NAC_HNE_*m*-APA final adduct (126 MHz, CD3OD).

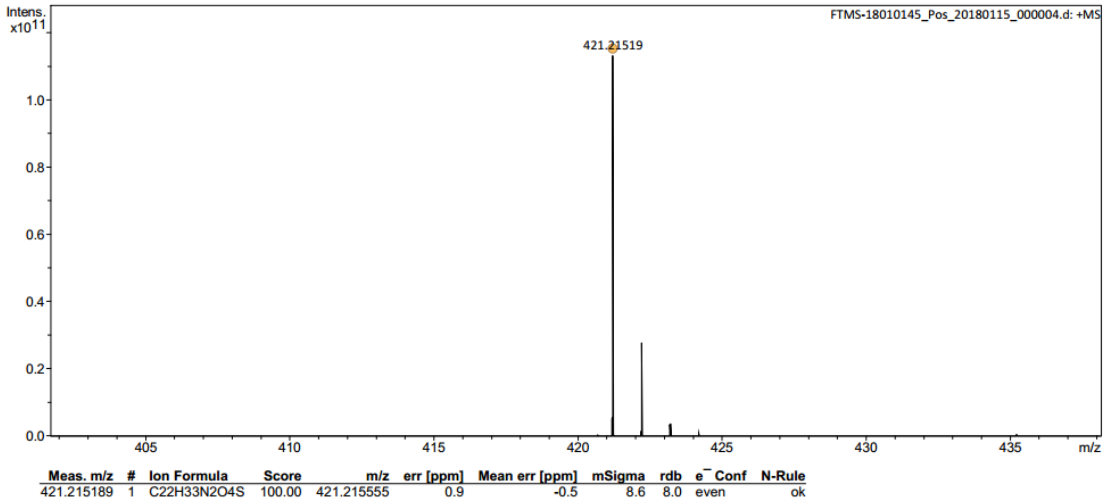


Figure S30. HRMS of NAC_HNE_*m*-APA final adduct.

SUPPORTING INFORMATION METHODS

Reagents

o-APA, *m*-APA, *p*-APA probes were purchased from Sigma-Aldrich (product number: 597651, 498289 and 481122). Other reagents were purchased from Sigma-Aldrich unless individually specified.

Cell culture

H1299, HT1080 and HEK293T cells were cultured at 37℃ under 5% CO_2 atmosphere in DMEM culture medium supplemented with 10% FBS and 1% PS. Cells were subcultured by trypsinization when 80% confluence was reached.

Plasmids

Full-length cDNAs of VDAC2, HMOX2 were obtained from hORFeome Database and subcloned into a modified pCLNCX retroviral vector. The plasmids of $VDAC2^{C210A}$ and $HOMX2^{C282A}$ were generated by using the PCR-based site-directed mutagenesis with TransStart® FastPFU DNA polymerase (Beijing TransGen Biotech Co., Ltd.) and the following primers:

RTN4 (B^{C282A}) (sense, 5'- GGTCATGTGAACGCTACGATAAAGGAACTCAG-3' and antisense, 5'-AGCGTTCACATGACCAAGAGCAGAATTACTG -3').

$VDAC2^{C210A}$ (sense, 5'-GCTGAAGATCTTGACACTTCAGTAAACCTTGCTTG-3' and antisense, 5'-GTCAAGATCTTCAGCAACTTTCTGATAAATTG-3'). $HOMX2^{C282A}$ (sense, 5'-GCTCCCTTCCGAACAGCTATGGCTGTGCTGAGG-3' and antisense, 5'-GTTCGGAAGGGAGCGCTGCTGCCCTCCAGG-3').

$GAPDH^{C152A}$ (sense, 5'- CAATGCCTCCGCTACCACCAACTGCTTAGC -3' and antisense, 5'-AGCGGAGGCATTGCTGATGATCTTGAGGCTG -3').

Transient transfection

$RTN4^{WT}$, $RTN4^{C1101A}$, $HOMX2^{WT}$ and $HOMX2^{C282A}$ were verified with correct sequences before transfection. HEK-293T cells were cultured to ~70% confluency in 6-well plates and were transfected with 3 μg plasmids using Lipo2000 (Thermo Fisher). The cells were changed into fresh DMEM medium 12 hours later. Cells overexpressing $RTN4^{WT}$ and $RTN4^{C1101A}$ were incubated with 100 μM HNE for 1 h after 48 hours, while cells overexpressing $HOMX2^{WT}$ and $HOMX2^{C282A}$ were incubated with 0, 2, 5, 10 and 20 μM HNE as indicated.

Stable overexpression

HEK-293T or HT1080 cell lines with stable overexpression of VDAC2, GAPDH and their corresponding cysteine mutants were constructed by retrovirus transfection. The proteins were fused with a C-terminal FLAG tag and 6xHis tag.

The retrovirus was generated by transfecting 3 μg AMPHOR plasmids and 3 μg targeted modified

pCLNCX plasmids into target cells using Lipo2000 system. The medium was freshly changed after 24 hours and collected after 48 hours. The medium containing virus was collected and filtered by 0.22 μm membrane. The virus was diluted with 3-fold DMEM medium and mixed with 8 μg/ml polybrene. The mixture was added into the plates of targeted cells (~70% confluency) and changed with fresh medium after 24 hours. The cells were passaged and cultured under the treatment of 200 μg/ml hygromycin until all non-transfected cells were killed. The transfected efficiency of pCLHCY plasmids was verified by western blot with anti-FLAG or anti-6x His antibody.

Validation of individual HNE targeted proteins

Cells overexpressing $RTN4^{WT}$, $RTN4^{C1101A}$, $GAPDH^{WT}$, and $GAPDH^{C152A}$ were lysed in 0.1% TritonX-100/PBS, centrifuged at 100000g for 30 minutes to remove cell debris and protein concentrations were determined by BCA protein assay. Proteomes were normalized to 2 mg/mL, incubated with 100 μM HNE for 1h at room temperature and labeled by 0.5 mM *m*-APA at pH 5.0 for 1h. Proteomes were precipitated by methanol/chloroform and resuspended in 0.4% SDS/PBS. The proteomes were reacted with 1 mM $CuSO_4$, 100 μM TBTA ligand, 100 μM biotin-$(PEG)_2$-N_3 and 1 mM TCEP for 1 h at room temperature. And after a second precipitation, the proteomes were enriched with streptavidin beads for 3 hours. The beads were washed with PBS for 3 times, and eluted with loading buffer at 95℃ for 20 min. Samples were separated on a SDS-PAGE gel and immunoblotted with an anti-6xHis or anti-FLAG antibody.

To examine the concentration-dependent labeling of HMOX2 and VDAC2 by HNE, cells were treated with 0, 2, 5, 10 and 20 μM HNE in serum-free media and lysed by sonication in ice-cold PBS containing 0.5% TritonX-100.

To verify the carbonylation on C210 of VDAC2 in ferroptosis, HT1080 cells with stable overexpression of $VDAC2^{WT}$ or $VDAC2^{C210A}$ were incubated with DMSO or 300 nM RSL3 to induce ferroptosis *in situ* for 3h in serum-free medium. The cells were collected and lysed by sonication in ice-cold PBS containing 0.5% TritonX-100.

Analysis of the level of lipid peroxide in ferroptotic cells by FACS

$8x10^5$ HT1080 cells/well were seeded in 6-well dishes to grow overnight. On the day of the experiment, cells were treated with DMSO, 150 nM RSL3 with/without 300 nM Lip-1 in serum-free medium for 3 h. Then the cells were washed 3 times with pre-warmed PBS and incubated with serum-free medium containing 2 μM C11-BODIPY for 15 mins. Cells were washed 3 times with PBS, collected and analyzed using a flow cytometer equipped with 488 nm laser for excitation. A minimum of 10,000 cells were analyzed per condition.

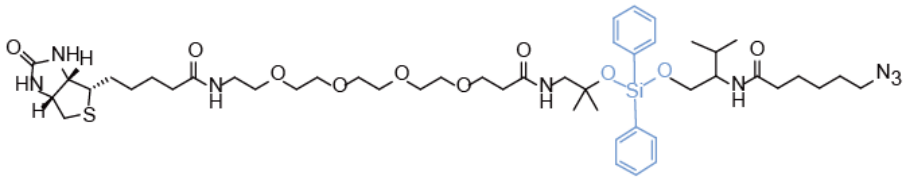
Monitoring Cell viability by MTT

10,000 cells/well were seeded in 96-well dishes to grow overnight. Then the cells were treated with DMSO or 500 nM RSL3 in serum medium for 24 h. The cells were washed with pre-warmed PBS and incubated with serum-free medium containing MTS reagent for 2h. The absorbance of 490 nm was measured and cell viability under test conditions was reported as a percentage relative to the negative control treatment.

In-gel fluorescence scanning of protein sulfenylation

H1080 cells were grown to 80% confluence, washed for 3 times with PBS and centrifuged at 1000 rpm for 3 mins. PBS supernatant were removed and the cell pallets were stored at -80 ℃. The cell pallets were lysed by sonication in ice-cold PBS containing 0.1% TritonX-100, centrifuged at 100,000g for 30 mins to remove cell debris, and protein concentrations were determined by BCA protein assay. Proteomes were incubated with 10 mM H_2O_2 for 1h at room temperature and labeled by 100 μM Dyn-2 for another 1h. The proteomes were reacted with 1 mM $CuSO_4$, 100 μM TBTA ligand, 100 μM fluorophore-N_3 and 1 mM TCEP for 1 h at room temperature. The proteomes were boiled using sample buffer at 90 ℃ for 5 mins, resolved on 10% SDS-PAGE gels and imaged by ChemiDoc XRS+ (Bio-Rad). The gels were then stained by Coomassie brilliant blue (CBB) to demonstrate equal loading.

Chemical synthesis



6-bromohexanoic acid (**1**) (20 g, 0.10 mol) was dissolved in 100 ml N, N-Dimethylformamide (DMF), then NaN_3 (20g, 0.31mol) was added. The reaction mixture was stirred at 65 °C for 16h. After cooled to room temperature, the mixture was diluted with 300 mL water. The product (**2**) was extracted with 200 mL ethyl acetate for three times without purification.

2 (1.3 g, 10 mmol) was dissolved in 30 mL tetrahydrofuran (THF), *N*-hydroxysuccinimide (NHS) (1.4 g, 12 mmol) was added. When the solution was cooled to 0 °C, dicyclohexylcarbodiimide (DCC) (2.7 g, 13 mmol) was added. After 12 h, the solid was removed, and the supernatant of NHS ester (**3**) was used directly in the next step without purification.

L-Valine (**4**) (1.00 g, 8.5 mmol) was dissolved in 10 mL THF, $NaBH_4$ (0.8 g, 21.2 mmol) was added under an ice-bath. After 30 min, I_2 (2.16 g, 8.5 mmol) was added. The solution was refluxed overnight. After cooled to room temperature, the reaction mixture was quenched with 10 mL methanol, evaporated to dryness and redissolved in 20 mL 10% KOH (aq). The solution was reflux for 3 h. After cooled to room temperature, the reaction mixture was diluted with 20 mL water and extracted with 30 mL CH_2Cl_2 for

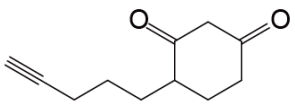
three times. The organic layers were combined and dried with Na_2SO_4, then evaporated to dryness under reduced pressure to give **5** as a clear oil (0.78 g, 88%).

5 (1.25 g, 12mmol) was dissolved in 20 mL MeOH, triethylamine (TEA) (3.66 g, 36 mmol) was added, and then THF solution of **3** was added. After 12 h, the solution was concentrated under vacuum and the product was purified by flash chromatography, eluted with EtOAc : PE gradually from 0:1 to 3:1 to give the product (**6**) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 5.72 (d, J = 7.3 Hz, 1H), 3.96 – 3.53 (m, 3H), 3.28 (t, J = 6.8 Hz, 2H), 2.51 (br, 1H), 2.24 (t, J = 7.5 Hz, 2H), 1.89 (dq, J = 13.6, 6.8 Hz, 1H), 1.66 (ddt, J = 19.3, 14.8, 7.2 Hz, 4H), 1.43 (tt, J = 9.7, 6.1 Hz, 2H), 0.96 (dd, J = 12.6, 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 173.76, 64.04, 57.08, 51.26, 36.63, 29.02, 28.61, 26.35, 25.27, 19.49, 18.86. ESI MS: calcd. for $C_{11}H_{23}N_4O_2^+$[M+H]$^+$ m/z, 243.18211; found, 243.18091.

Dichlorodiphenylsilane (1.70 g, 6.7 mmol) was added in 60 mL anhydrous pyridine under an ice-bath with N_2 protection. Compound **7** [1](510 mg, 0.9 mmol) in 1.5 mL CH_2Cl_2 was added. After 12 h, **6** (1.3 g, 16.9 mmol) was added, and stirred for 24 h. The product was purified by flash chromatography, eluted with MeOH : CH_2Cl_2 gradually from 0:1 to 1:9 to give the product (**8**) as an amorphous solid (170 mg, 19 %)[2].

^{1}H NMR (500 MHz, CD_3CN) δ ppm 7.66 – 7.60 (m, 4H), 7.47 – 7.41 (m, 2H), 7.38 (dd, J = 11.2, 4.3 Hz, 4H), 6.82 (t, J = 5.9 Hz, 1H), 6.69 (s, 1H), 6.44 (d, J = 8.4 Hz, 1H), 5.52 (s, 1H), 5.22 (s, 1H), 4.44 – 4.34 (m, 1H), 4.28 – 4.18 (m, 1H), 3.80 – 3.70 (m, 3H), 3.65 (t, J = 6.2 Hz, 2H), 3.58 – 3.48 (m, 12H), 3.46 (t, J = 5.6 Hz, 2H), 3.31 – 3.21 (m, 6H), 3.14 (td, J = 7.4, 4.5 Hz, 1H), 2.91 – 2.83 (m, 1H), 2.63 (d, J = 12.7 Hz, 1H), 2.38 (t, J = 6.2 Hz, 2H), 2.18 – 2.03 (m, 4H), 1.95 – 1.87 (m, 3H), 1.72 – 1.62 (m, 1H), 1.62 – 1.49 (m, 6H), 1.43 – 1.28 (m, 3H), 1.22 (t, J = 8.3 Hz, 6H), 0.87 (dd, J = 8.9, 6.8 Hz, 6H). ^{13}C NMR (126 MHz, CD_3CN) δ ppm 173.60, 173.29, 171.71, 163.78, 135.59, 135.58, 135.33, 135.29, 130.95, 128.57, 76.54, 70.93, 70.91, 70.87, 70.80, 70.72, 70.67, 70.11, 67.80, 64.03, 62.15, 60.58, 56.32, 56.12, 51.74, 50.50, 40.90, 39.60, 37.30, 36.52, 36.11, 29.21, 29.01, 28.80, 28.76, 27.86, 27.83, 26.85, 26.17, 25.92, 19.75, 18.69. ESI MS: calcd. for $C_{48}H_{76}N_8NaO_{10}SSi^+$[M+Na]$^+$ m/z, 1007.50721; found, 1007.50754.

Dyn-2

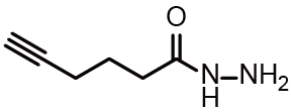


Dyn-2 was synthesized based on previous reports[3]. ^{1}H-NMR: (400 MHz, $CDCl_3$) δ ppm 3.49-3.37 (m, 2H), 2.72 (dtd, J = 16.2, 4.5, 1.2Hz, 1H), 2.65–2.47 (m, 2H), 2.31–2.14 (m, 3H), 2.07–1.90 (m, 2H), 1.71–1.49 (m, 4H). ^{13}C NMR: (100 MHz, $CDCl_3$): δ ppm 204.15, 203.60, 83.57, 68.58, 57.98, 48.65, 39.37, 28.03, 25.61, 24.25, 18.23. ESI-MS, *m/z* calcd. for $C_{11}H_{14}O_2$ [M+H]$^+$ 179.10, found: 179.10.

AOyne

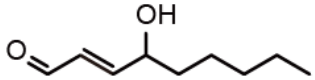
AOyne was synthesized based on previous reports[4]. ^{1}H NMR: (400 MHz, CDCl3) δ ppm 5.30 (s, 2H), 3.69(t, *J*=6.3 Hz 2H), 2.23(td, *J*=7.0, 2.6 Hz 2H), 1.95(t, *J*=2.6 Hz 1H), 1.74-1.68(m, 2H), 1.63-1.55(m, 2H). ESI MS: calcd. for $C_6H_{12}NO^+[M+H]^+$ m/z, 114.09189; found, 114.09103.

HZyne



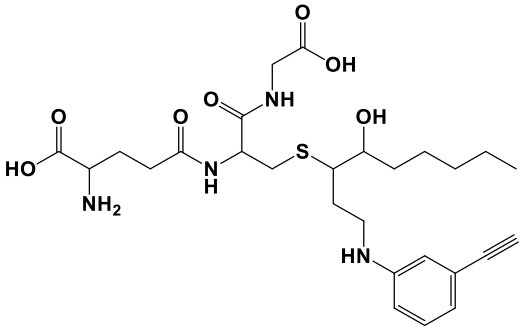
HZyne was synthesized based on previous reports[5]. ^{1}H NMR: (400 MHz, DMSO-d6) δ ppm 8.96 (s, 1H), 4.20(s, 2H), 2.78(t, *J*=2.6 Hz 1H), 2.15-2.07(m, 4H), 1.66 (p, *J*=7.2 Hz 2H). ESI MS: calcd. for $C_6H_{11}N_2O^+[M+H]^+$ m/z, 127.08714; found, 127.08629.

HNE



HNE was synthesized based on previous reports[6]. ^{1}H NMR: (400 MHz, DMSO-d_6): δ ppm 9.55 (d, *J* = 8.1 Hz 1H), 7.04 (dd, *J* = 15.5, 4.3 Hz 1H), 6.16 (ddd, *J* = 1.6, 8.1 and 15.5 Hz 1H), 5.18 (d, *J* = 5.0 Hz 1H), 4.30–4.22 (m, 1H), 1.58–1.31 (m, 4H), 1.31–1.19 (m, 4H), 0.86 (t, *J* = 6.8 Hz 3H). ^{13}C NMR : (100 MHz , acetone-d_6): δ ppm 193.24, 160.85, 130.11, 70.13, 36.42, 31.60, 24.83, 22.37, 13.41. ESI MS: calcd. for $C_9H_{17}O_2^+[M+H]^+$ m/z, 157.12286; found, 157.12240.

The final adduct of GSH_HNE_*m*-APA



50 mg GSH was reacted with equal molar HNE in 50 mL Tris-HCl buffer (pH=7.4) for 1 hour at room temperature. The system was then adjusted to pH 5.0, added with equal molar *m*-APA and $NaBH_3CN$ and

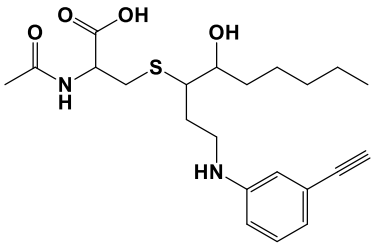
lasted for 60 mins. The crude product was purified by HPLC to give pure product. ESI MS: calcd. for $C_{27}H_{41}N_4O_7S^+[M+H]^+$ m/z, 565.26960; found, 565.26800.

HPLC conditions:

A: water containing 0.1% TFA; B: acetonitrile containing 0.1% TFA; 5mL/min.

Time/min	A/%	B/%
0.00	95	5
8.00	40	60
8.10	0	100
10.00	0	100
10.10	95	5
12.00	95	5

The final adduct of NAC_HNE_*m*-APA



50 mg NAC was reacted with equal molar HNE in 50 mL PBS buffer (pH=7.4) for 1 hour at room temperature. The system was then adjusted to pH 5.0, added with equal molar *m*-APA and $NaBH_3CN$ and lasted for 60 mins. The crude product was purified by HPLC to give the final product containing diastereomer. ^{1}H NMR (500 MHz, CD3OD): δ ppm 7.12 (t, J = 7.9 Hz 1H), 6.98 (ddd, J = 6.4, 5.9, 4.1 Hz 2H), 6.95-6.89 (m, 1H), 4.47-4.29 (m, 1H), 3.53-3.42 (m, 1H), 3.31-3.25 (m, 1H), 3.25-3.14 (m, 1H), 2.99-2.54 (m, 3H),1.97-1.66 (m, 5H), 1.65-1.44 (m, 1H), 1.44-1.18 (m, 3H), 1.17-0.99 (m, 5H), 0.68 (t, J = 6.8 Hz 3H). ^{13}C NMR (126 MHz, CD3OD): δ ppm 172.28, 172.01, 141.69, 129.66, 127.03, 123.82, 121.06, 118.49, 82.42, 78.21, 73.68, 52.28, 49.83, 45.99, 33.24, 31.60, 29.07, 27.43, 25.48, 22.30, 21.17, 13.09. ESI MS: calcd. for $C_{22}H_{33}N_2O_4S^+[M+H]^+$ m/z, 421.21611; found, 421.21519.

SUPPORTING INFORMATION REFERENCES

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