Supporting Information

Installation of electrophiles onto to the C-terminus of recombinant ubiquitin and ubiquitin-like proteins

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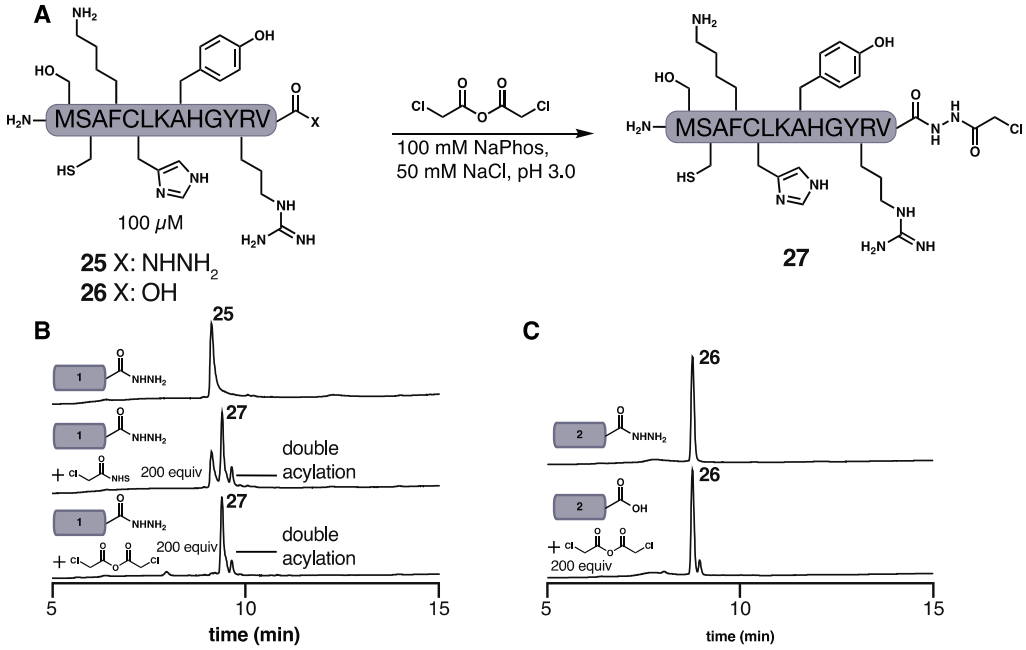


Figure S1 Selective modification of peptide hydrazides by carboxylic acid anhydrides. **A** Reaction scheme showing selective modification of peptide hydrazide **25** with α-chloroacetic acid anhydride. **B** Analytical RP-HPLC chromatogram of unmodified peptide hydrazide **25**, analytical RP-HPLC chromatogram of modification of **25** with α-chloroacetic acid NHS-ester, analytical RP-HPLC chromatogram of modification of **25** with α-chloroacetic acid anhydride. **C** Analytical RP-HPLC chromatogram of unmodified peptide carboxylate **26**, Analytical RP-HPLC chromatogram of modification of **26** with α-chloroacetic acid anhydride.

1.2. pH-dependence screening

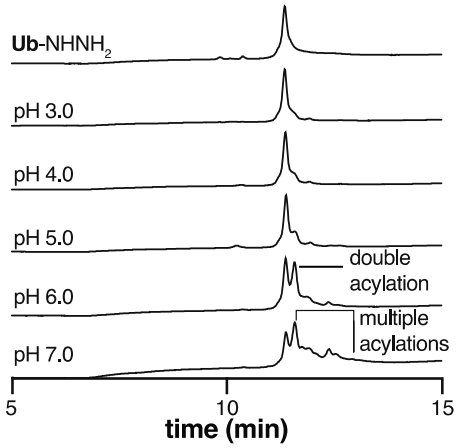


Figure S2 pH dependence of Ub(ΔG76)–$NHNH_2$. Analytical RP-HPLC of acylated ubiquitin hydrazide at various pHs. Ub(ΔG76)–$NHNH_2$ was diluted with 100 mM NaPhos, 50 mM NaCl of varying pHs (3, 4, 5, 6, 7). Acylation was carried out according to the general method.

1.3. CD spectra of Ub(ΔG76) hydrazide and acylated Ub(ΔG76) 2

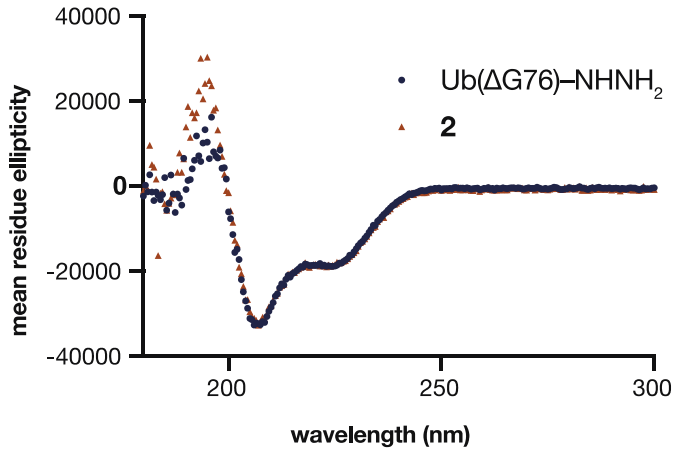


Figure S3 CD spectra of Ub(ΔG76)–$NHNH_2$ and acylated Ub(ΔG76)–NHNH α-chloroacetyl **2**.

1.4. CD spectra of SUMO2(ΔG93) hydrazide and acylated SUMO2(ΔG93) 18

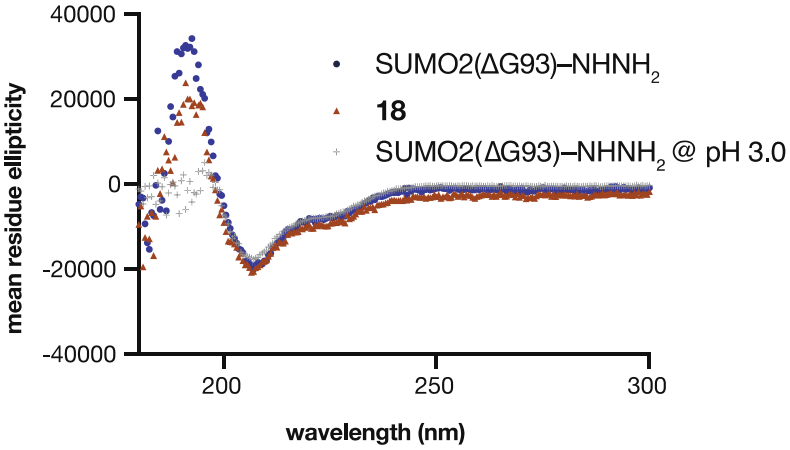


Figure S4 CD spectra of SUMO2(ΔG93)–$NHNH_2$ and acylated SUMO2(ΔG93)–NHNH α-chloroacetyl **18**.

1.5. CD spectra of NEDD8(ΔG76) hydrazide and acylated NEDD8(ΔG76) 12

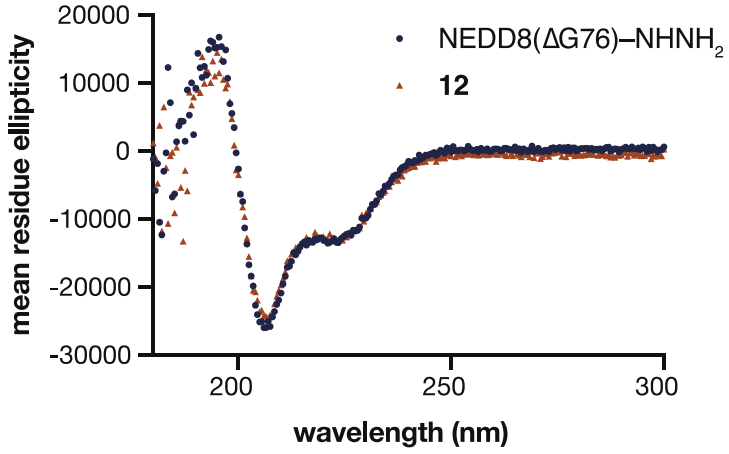


Figure S5 CD spectra of NEDD8(ΔG76) $NHNH_2$ and acylated NEDD8(ΔG76)–NHNH α-chloroacetyl **12**.

1.6. CD spectra of ISG15(ΔG157) hydrazide and acylated ISG15(ΔG157) 25

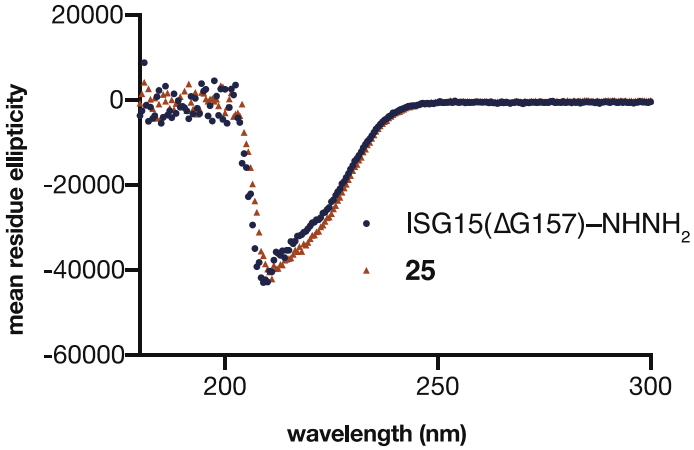


Figure S6 CD spectra of ISG15(ΔG157)–$NHNH_2$ and acylated ISG15(ΔG157)–NHNH methyl fumarate **25**.

1.7. Reaction of SUMO2(ΔG93) probes with SENP1, SENP8 and USP21

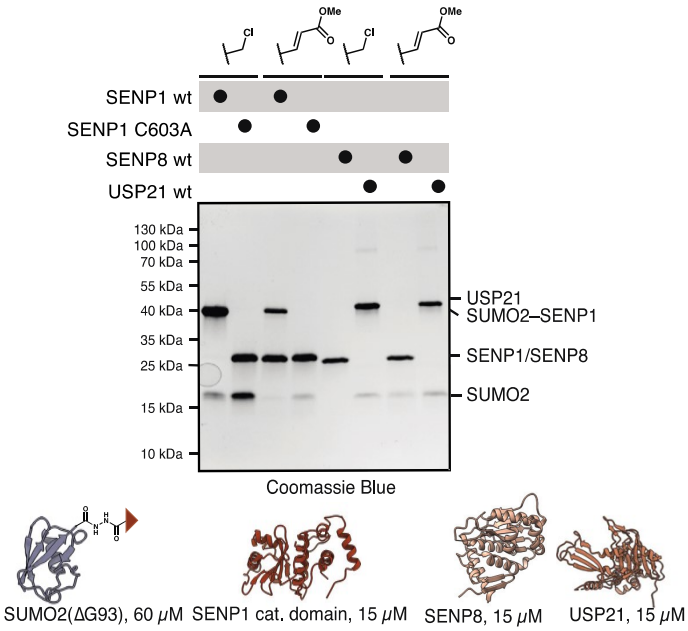


Figure S7 Reaction of SUMO2(ΔG93)–NHNH α-chloroacetyl and methyl fumarate probes with DUBs, SENP1 wt, SENP1 C603A, SENP8 wt and USP21 wt.

1.8. Reaction of Ub(ΔGG) probes with USP21, SENP1 and SENP8

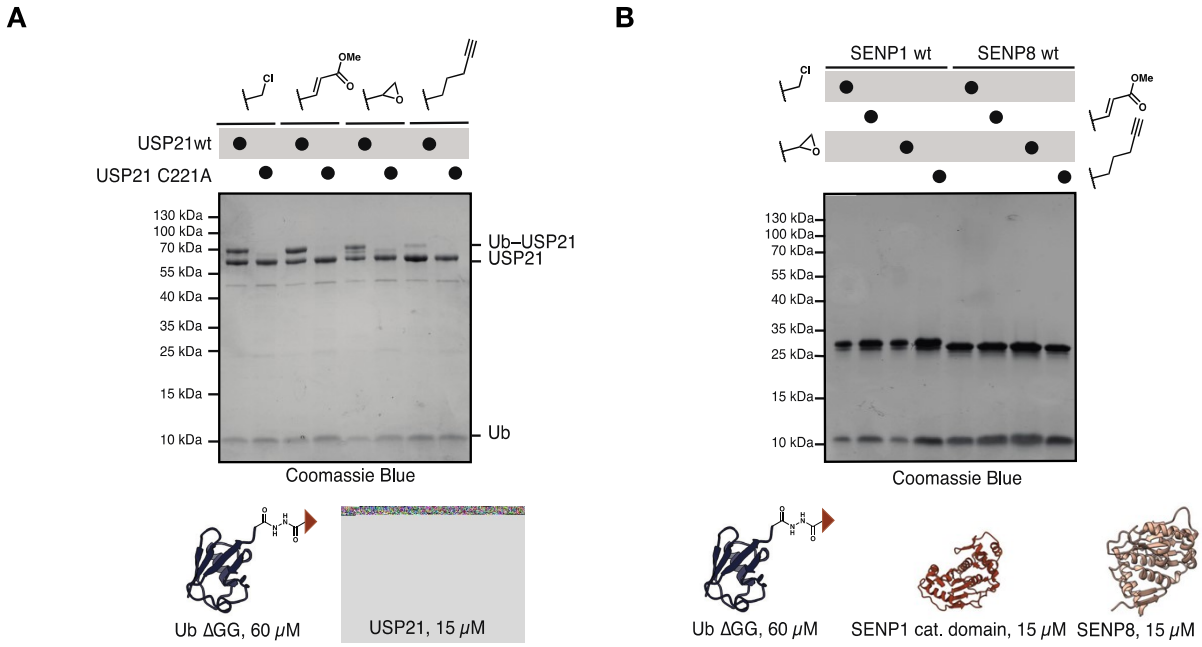


Figure S8 Reaction of Ub(ΔGG)–NHNH α-chloroacetyl, methyl fumarate, glycidate and pentynoate probes with DUBs. **A** Reaction of Ub probes with USP21 wt and its catalytically inactive variant C221A. **B** Reaction of Ub probes with SENP1 and SENP8.

1.9. Reaction of Ub(ΔG76) probes with USP21, SENP1 and SENP8

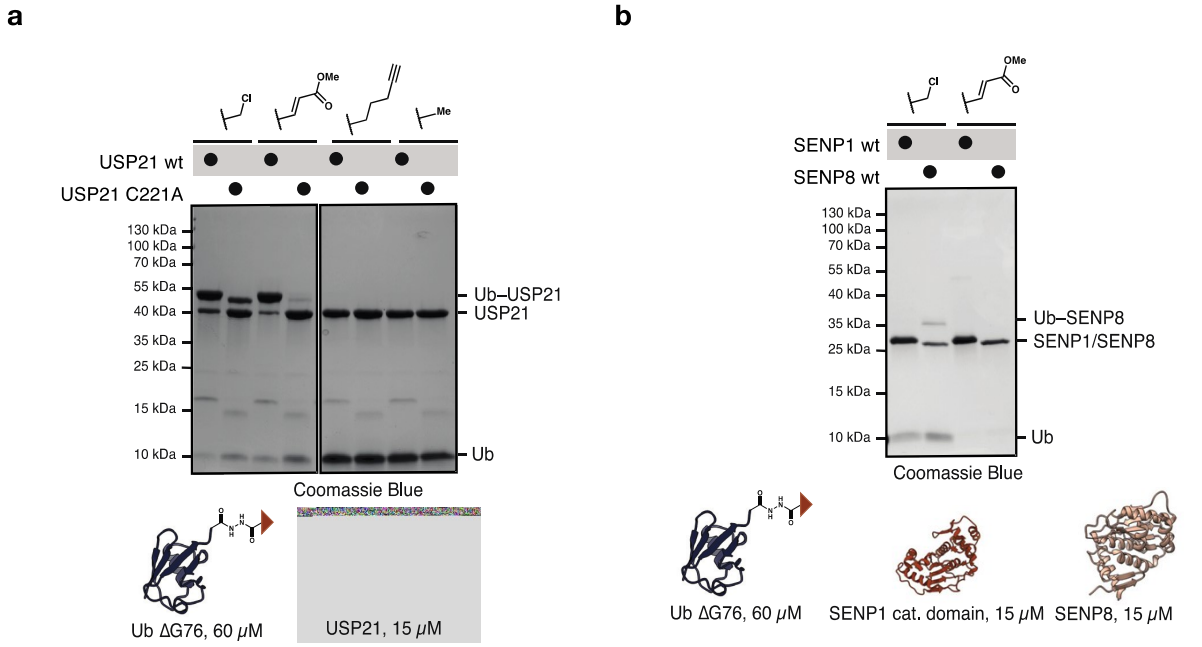


Figure S9 Reaction of Ub(ΔG76)–NHNH α-chloroacetyl, methyl fumarate, pentynoate and acetate probes with DUBs. **a** Reaction of Ub probes with USP21 wt and its catalytically inactive variant C221A. **b** Reaction of Ub probes with SENP1 and SENP8.

1.10. Reaction of NEDD8(ΔGG) probes with SENP8, SENP1 and USP21

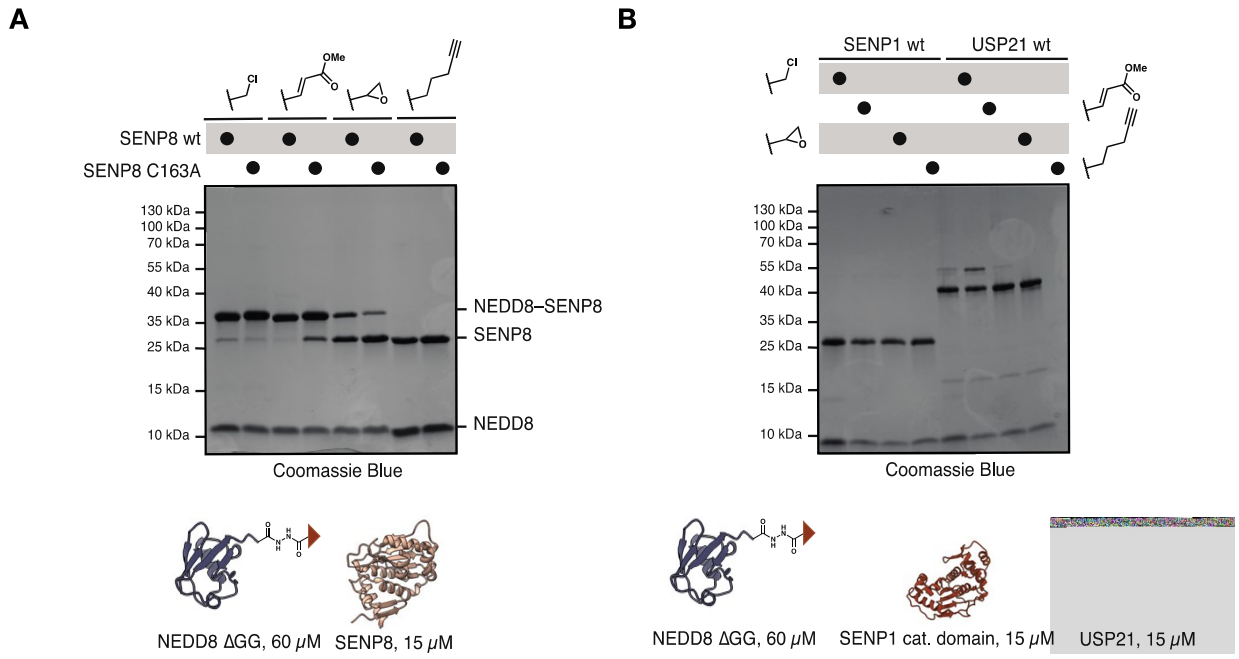


Figure S10 Reaction of NEDD8(ΔGG)–NHNH α-chloroacetyl, methyl fumarate, glycidate and pentynoate probes with DUBs. **A** Reaction of NEDD8 probes with SENP8 wt and its catalytically inactive variant C163A. **B** Reaction of NEDD8 probes with SENP1 and USP21.

1.11. Reaction of NEDD8(ΔG76) probes with SENP8, SENP1 and USP21

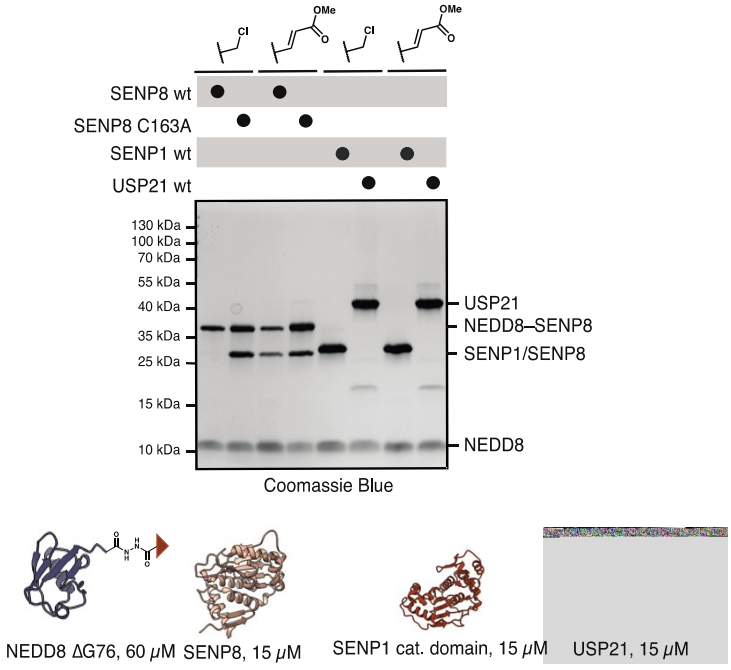


Figure S11 Reaction of NEDD8(ΔGG)–NHNH α-chloroacetyl and methyl fumarate probes with DUBs, SENP8 wt, SENP8 C163A, SENP1 wt and USP21 wt.

1.12. Reaction of Ub(ΔGG) probes with Ube2K, Ube2M and Ubc9

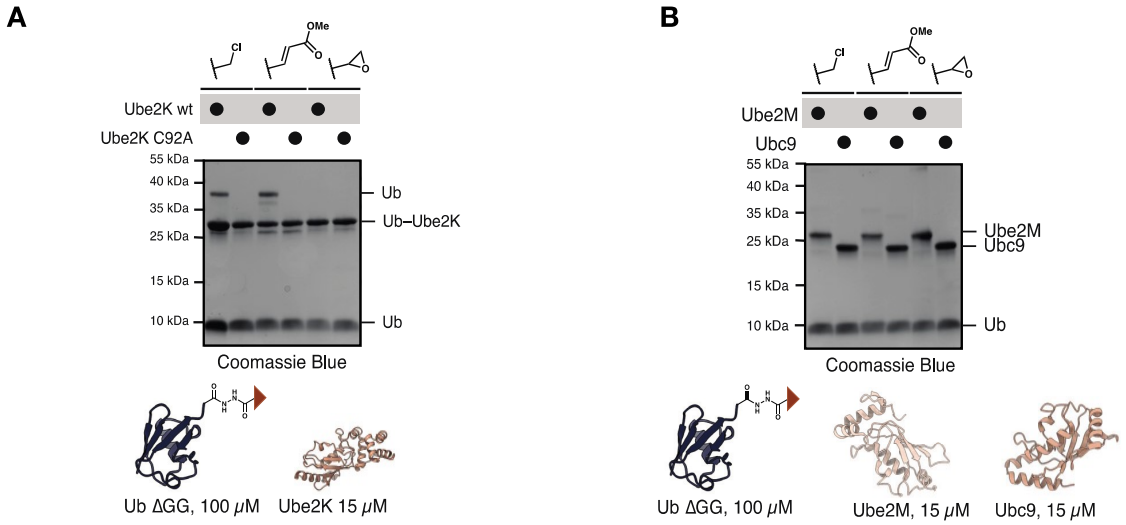


Figure S12 Reaction of Ub(ΔGG)–NHNH α-chloroacetyl, methyl fumarate and glycidate probes with E2s. **A** Reaction of Ub probes with Ube2K wt and its catalytically inactive variant C92A. **B** Reaction of Ub probes with Ube2M and Ubc9.

1.13. Reaction of Ub(ΔGG) and(ΔG76) probes with Ube2K C92A, Ube2G1 C90A and Ube2L3 C86A

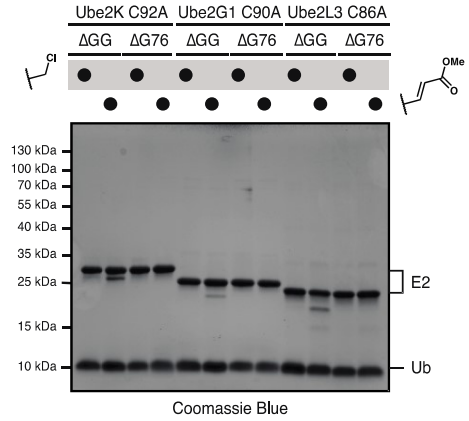


Figure S13 Reaction of Ub(ΔGG)–NHNH α-chloroacetyl, methyl fumarate and Ub(ΔG76)–NHNH α-chloroacetyl, methyl fumarate probes with E2s. Reaction of probes with cysteine-to-alanine mutants of E2s used in Fig 6B.

1.14. Reaction of SUMO2(ΔGG) probes with Ubc9, Ube2M and Ube2K

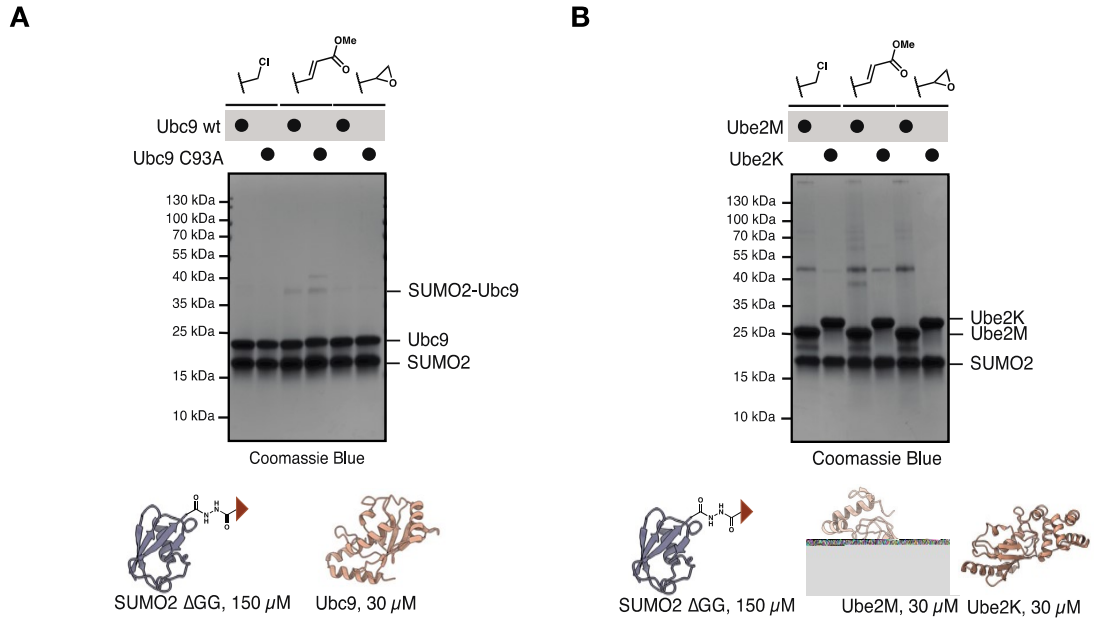


Figure S14 Reaction of SUMO2(ΔGG)–NHNH α-chloroacetyl, methyl fumarate and glycidate probes with E2s. **A** Reaction of SUMO2 probes with Ubc9 wt and its catalytically inactive variant C93A. **B** Reaction of SUMO2 probes with Ube2M and Ube2K.

1.15. Reaction of NEDD8(ΔGG) probes with Ube2M, Ubc9 and Ube2K

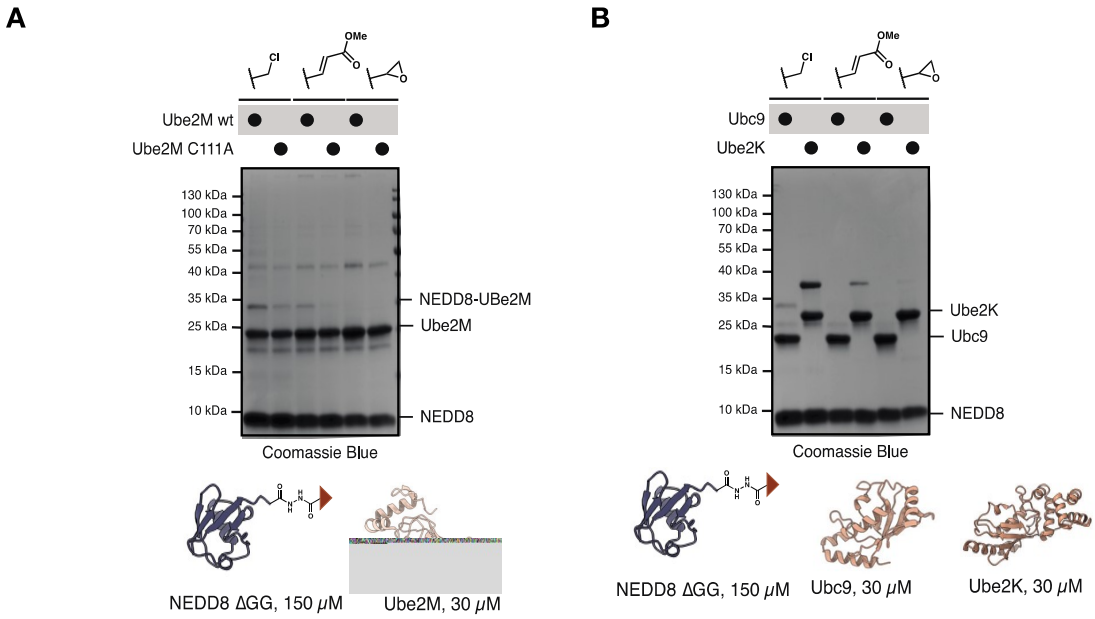


Figure S15 Reaction of NEDD8(ΔGG)–NHNH α-chloroacetyl, methyl fumarate and glycidate probes with E2s. **A** Reaction of NEDD8 probes with Ube2M wt and its catalytically inactive variant C111A. **B** Reaction of NEDD8 probes with Ubc9 and Ube2K.

1.16. Reaction of probes 2, 4 and Ub–VME and Ub–Dha with Ube2K

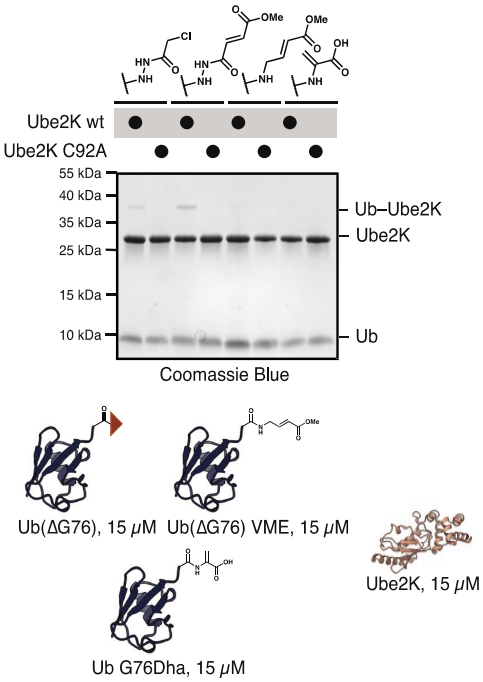


Figure S16 Ub(ΔG76)–NHNH a-chloroacetyl and methyl fumarate probes **2** and **4**, Ub(ΔG76)–VME and Ub G76Dha were reacted with Ube2K wt and Ube2K C92A for 3 h at 37 ºC. Reaction was quenched by addition of SDS-PAGE buffer and analyzed by Coomassie-Blue staining.

1.17. Reaction of FLAG-SUMO2(ΔGG) probes in HEK293 cell lysate

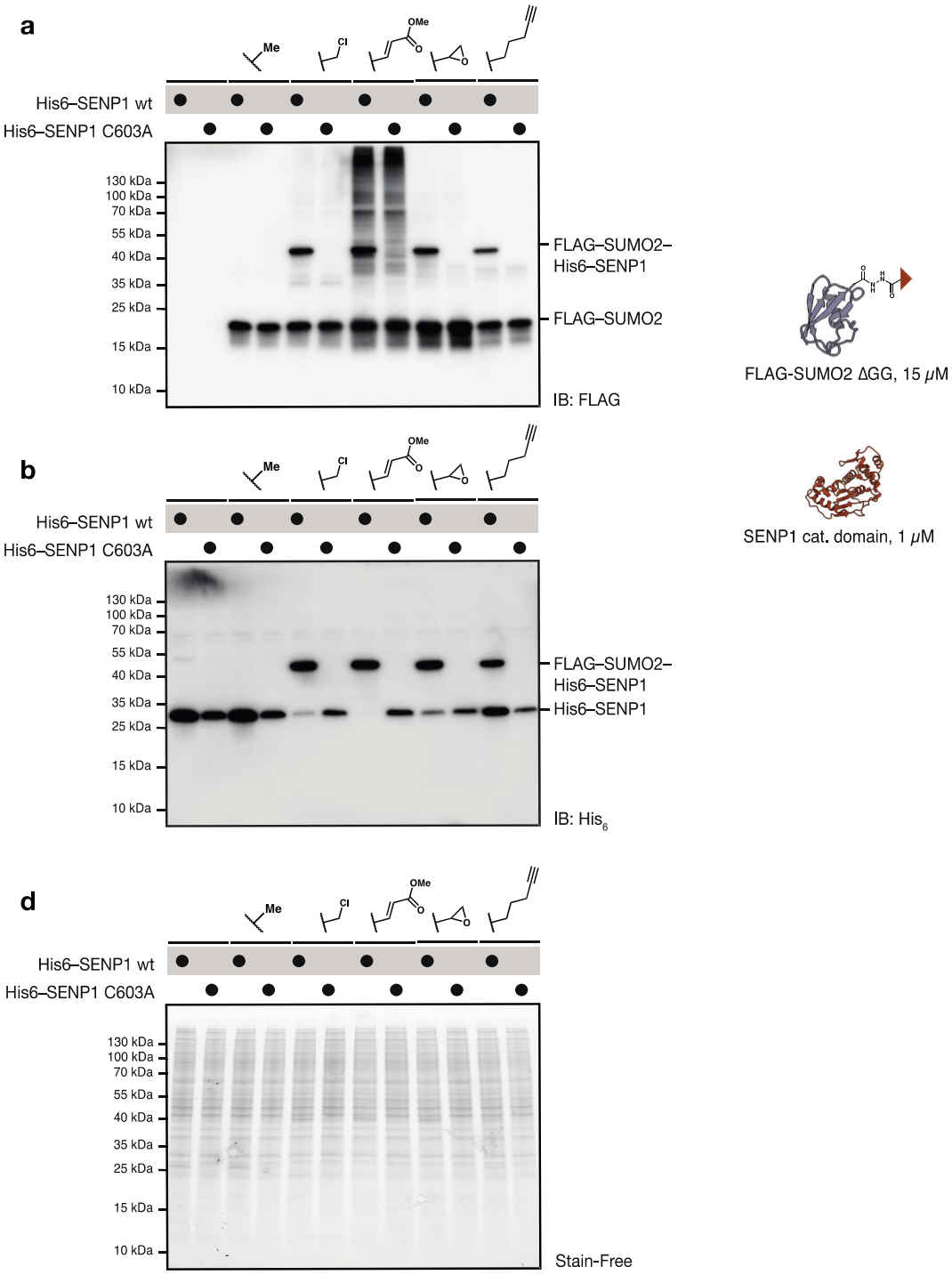


Figure S17 Reaction of FLAG-SUMO2(ΔGG) probes with recombinant SENP1 in HEK293T cell lysate. **A**, **B** His_6-SENP1 (cat. domain) wt, C603A (1 µM) were mixed with HEK293T cell lysate (50 µg), FLAG-SUMO2(ΔGG) probes (15 µM) were added and incubated for 2 h at 37 ºC. **A** Analysis by SDS-PAGE and Western blotting against FLAG-tag. **B** Analysis by SDS-PAGE and Western blotting against His_6-tag. **C** Stain-Free visualization showing total protein content.

1.18. Reaction of FLAG-SUMO2(ΔGG) probes in HeLa cell lysate

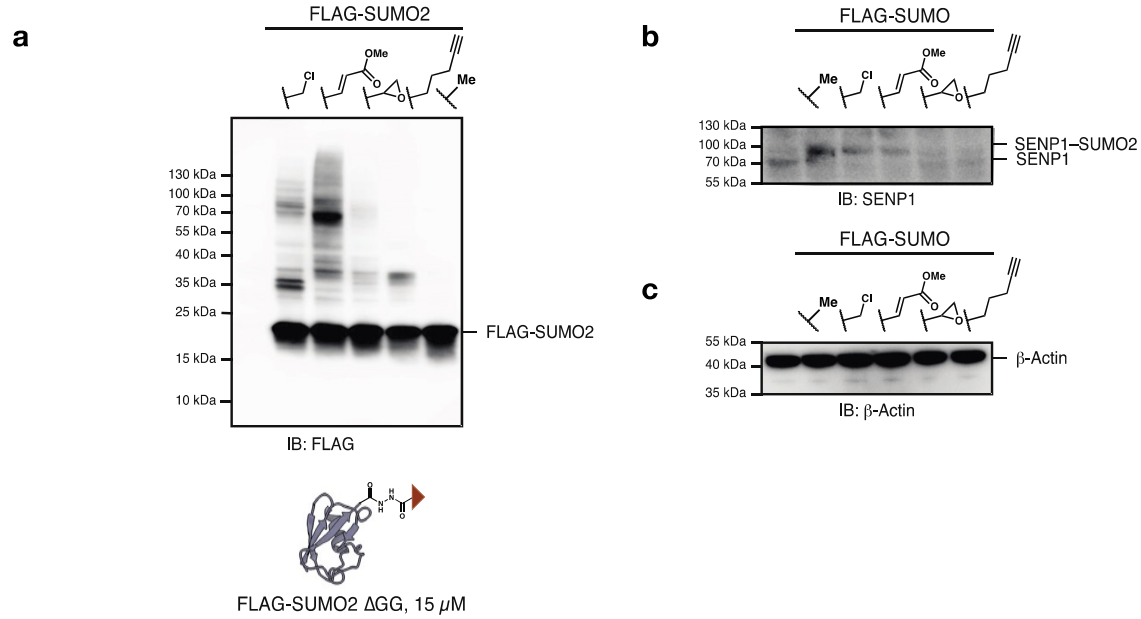


Figure S18 FLAG-SUMO2(ΔGG) probes were incubated with HeLa cell lysate for 2h at 37 ºC. Reaction was quenched by addition of SDS-PAGE buffer and analyzed by Western blotting against (a) FLAG-epitope, (b) SENP1 and (c) β-actin.

1.19. Full-gels

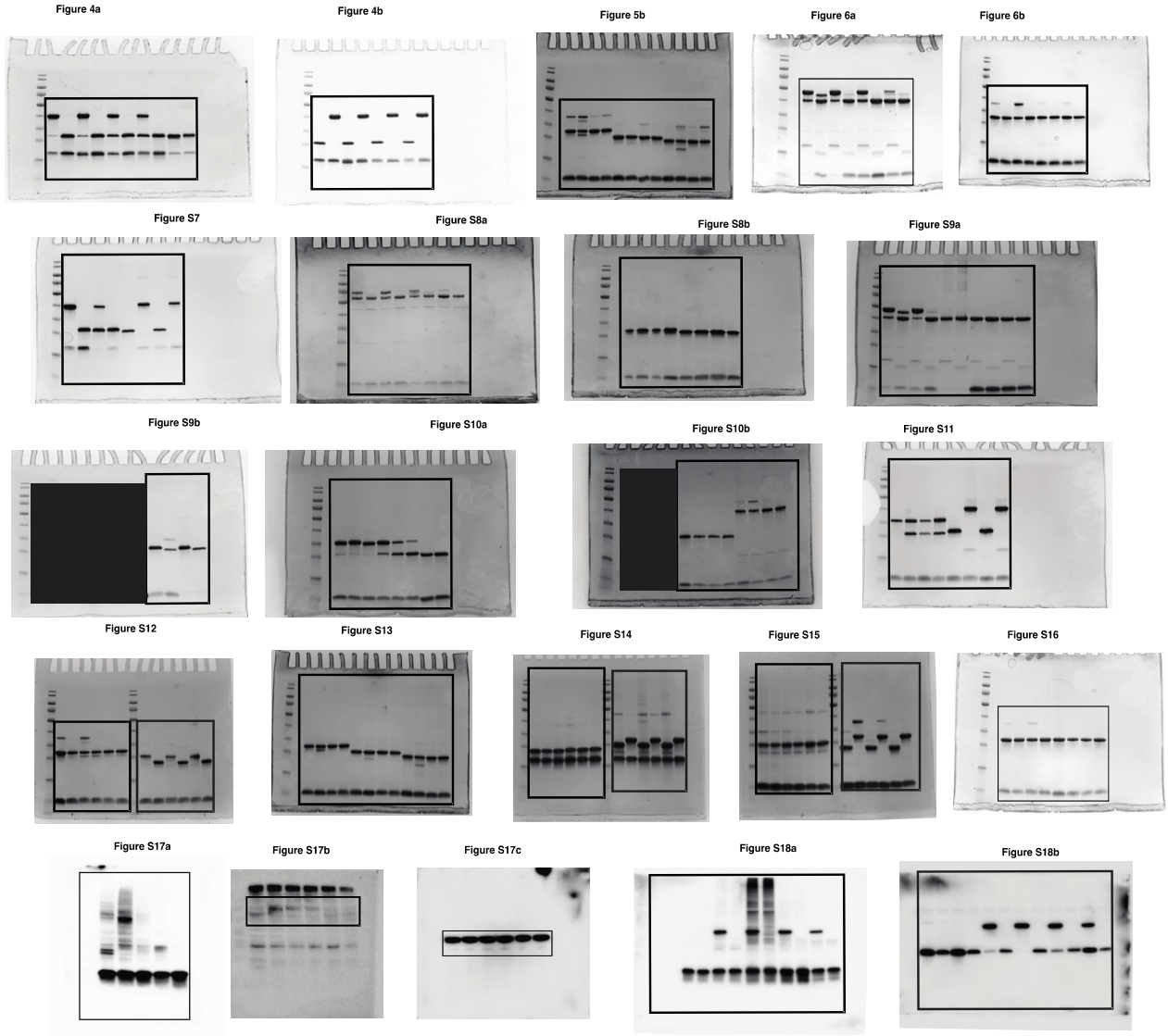


Figure S18 Full SDS-PAGE gels and Western blots. Cropped sections shown in figures are marked.

2. Materials and Methods

2.1. Solvents and Chemicals

Chemical reagents were purchased from Sigma Aldrich (Buchs, Switzerland), Acros Organics (Geel, Belgium), TCI Europe (Zwijndrecht, Belgium) and used without further purification. CH_2Cl_2, DMF, EtOH, THF and MeOH were purchased from Fisher Scientific (Geel, Belgium) and Sigma Aldrich (Buchs, Switzerland) and used without further purification (reagent or HPLC grade). Milli-Q water was obtained from a Millipore purification system. Q5 High-Fidelity DNA Polymerase, PCR reagents, Gibson Assembly Master Mix, were purchased from New England BioLabs (Ipswich, MA, USA). DNase I was obtained from Roche Diagnostics GmbH (Mannheim, Germany). DNA purification kits were purchased from Fisher Scientific (Geel, Belgium) and Zymo Research (Irvine, CA, USA). Lysozyme (22500 U/mg) was obtained from Axon Lab AG (Baden, Switzerland). Kanamycin sulfate and ampicillin were obtained from AppliChem GmbH (Darmstadt, Germany). Ni-NTA agarose resin was obtained from Qiagen GmbH (Hilden, Germany). Bradford protein assays were performed using Protein Asaay Dye Reagent Concentrate from Bio-Rad. Dialysis tubing was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Amicon Ultra centrifugal filters were purchased from Merck (Darmstadt, Germany), VivaSpin 500 centrifugal concentrators were purchased from Satorius Stedim Lab (Stonehoude, UK), PD MiniTrap desalting columns were purchased from Cytiva. All buffers were preperad

using Mili-Q water, sterile filtered (0.2 µm membrane filter), pH was adjusted at the temperature the buffer was used. Oligonucleotide synthesis and sequencing was carried out by Microsynth AG (Balgach, Switzerland). Synthetic genes were ordered from Thermo Fisher Scientific (Waltham, MA, USA), ATG Biosynthetics (Merzhausen, Germany) and GenScript Biotech (New Jersey, USA). 100 µL and 10 µL Pierce™ C18 Tips were purchased from Thermo Fisher Scientific. Pierce Trypsin Protease was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Cell culture reagents including Dulbecco's modified eagle medium (DMEM), phosphate-buffered saline (PBS), trypsin-EDTA. Fetal Bovine Serum (FBS) was purchased from Thermo Fisher Scientific

Fmoc-amino acids with suitable side-chain protecting groups, HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) were purchased from Peptides International (Louisville, KY, USA), ChemImpex (Wood Dale, IL, USA) and Merck Milipore. HPLC grade CH_3CN from Sigma-Aldrich was used for analytical and preparative HPLC purification. Trifluoroacetic acid for HPLC analytical and preparative HPLC purification was purchased from ABCR. DMF (> 99.8%) from Sigma-Aldrich and N-methylpyrrolidine from ABCR were directly used without further purification for solid phase peptide synthesis. Other commercially available reagents and solvents were purchased from Sigma-Aldrich (Buchs, Switzerland), Acros Organics (Geel, Belgium) and TCI Europe (Zwijndrecht, Belgium).

2.2. Mass spectrometric characterization

High-resolution mass spectra were recorded by the Molecular and Biomolecular Analysis Service (MoBiAS) at ETH Zurich with a Bruker maXis instrument (ESI-MS measurements) equipped with an ESI source and a Q-TOF detector. Reaction monitoring was performed on a Bruker microFLEX instrument (MALDI-TOF) using 4-hydroxy-α-cyanocinnamic acid as matrix.

LC-MS analysis was performed on a Waters Xevo G2-XS QTof. Mass analysis was performed using MassLynx. Sample peaks were integrated and deconvoluted using MaxEnt1.

Tandem MS experiments were performed using ESI-TIMS-QTOF-MS system (TimsTOF Pro, Bruker Daltonics, Germany) with collision-induced dissociation (CID) and N_2 as the collision gas. Tryptic peptides were pressure loaded onto a reversed phase 25 cm × 75 µm i.d. C18 1.6 µm column (Ionoptics Ltd., Australia) with a reversed phase 5 mm × 0.3 mm i.d. C18 5 µm column (Thermo Scientific, Lithuania) as guard column at 40 °C. The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The gradient started at 2% of B and was linearly increased to 35% in 120 min at flowrate of 300 µl min^{-1}. A second gradient profile, started at 35% of B and was linearly increased to 95% in 2 min at flowrate of 300 µl min^{-1}. Followed by isocratic conditions of 95% B at flowrate of 300 µl min^{-1} for 8 min. Total run time, including the conditioning of the column to the initial conditions, was 163 min. Further data processing was performed with Data Analysis 5.3 software (Bruker Daltonics, Germany) using a processing script to generate export files and reports. Advanced raw data processing for bioanalytical interpretations were performed using PEAKS Studio software (Bioinformatics Solutions Inc., Canada).

2.3. RP-HPLC analysis and purification

Peptides were analyzed and purified by reverse phase high performance liquid chromatography (RP-HPLC) on JASCO analytical and preparative instruments equipped with dial pump, mixed and in-line degasser, a variable wavelength UV detector (simultaneous detection of the eluent at 220 nm, 254 nm and 301 nm) and a Rheodyne injector with a 200 µL or 10 mL injection loop. Columns were heated to 60 ºC using a Jetstream 2 column heater (analytical) or a H_2O water bath (preparative). The mobile-phases for RP-HPLC were Milipore-H_2O containing 0.1% (v/v) TFA and HPLC grade CH_3CN containing 0.1% (v/v) TFA. Analytical HPLC was performed at a flow rate of 1 mL/min. Analytical columns used were Shiseido Capcell Pak C18 (5 µm, 4.6 mm I.D. x 250 mm) or Agilent Eclipse XDB-C8 (5 µm, 4.6 mm x 150 mm). Preparative HPLC was performed on Shiseido Capcell Pak MGIII (5 µm, 20 mm I.D. x 250 mm) at a flow rate of 40 mL/min.

General *analytical* HPLC methods:
flow 1 mL/min, isocratic 10% CH_3CN for 3 min, then gradient from 5% to 95% CH_3CN in 14 min

General *preparative* HPLC methods:

- flow 40 mL/min, isocratic 5% CH_3CN for 5 min, then gradient from 5% to 65% CH_3CN in 28 min.
- flow 10 mL/min, isocratic 5% CH_3CN for 5 min, then a variable gradient of CH_3CN in 28 min.
- flow 5 mL/min, isocratic 5% CH_3CN for 5 min, then a variable gradient of CH_3CN in 28 min.

2.4. Fast protein liquid chromatography (FPLC)

Chromatographic protein purification was performed on an Äkta Pure system (GE Healthcare) at 4 ºC. Buffers were prepared and pH-adjusted at 4 ºC. Buffers were filtered (2 µm) and degassed prior to use. Ion exchange chromatography was performed using MonoS 5/50 GL, or MonoQ 5/50 GL. Size exclusion chromatography was performed on a Superdex 75 Increase 10/300 G, or HiLoad 16/600 Superdex 200. Protein elution was monitored at 280 nm and acquired using Unicorn (6.3.2.89).

2.5. Solid phase peptide synthesis

Loading of amino acids on solid support was performed as followed:

- C-terminal carboxylic acid: The amino acid (1.20 equiv. of desired loading) was dissolved in CH_2Cl_2 (200 mM). NMM (2 equiv.) was added to the solution. The solution was given to preswollen chloro-trityl resin and shaken for 1h. The resin was washed with CH_2Cl_2 and DMF. Remaining chloro-trityl moieties were capped with CH_2Cl_2/MeOH/NMM (17:2:1, v:v:v) for 1 min. The capping step was repeated once. The resin was washed with CH_2Cl_2 and DMF. The resin was dried using a N_2 stream. The resin was stored at 4 ºC.
- C-terminal hydrazide: A 2 vol% solution of hydrazine monohydrate in DMF was given to preswollen 2-chlorotrityl chloride resin and shaken for 15 min at room temperature. This step was repeated once. The resin was thoroughly washed with DMF and CH_2Cl_2. Remaining chloro-trityl moieties were capped with CH_2Cl_2/MeOH/NMM (17:2:1, v:v:v) for 1 min. Resin was washed with DMF and CH_2Cl_2. The desired amino acid (1.20 equiv. of desired loading) and HATU (0.95 equiv. of amino acid) were dissolved in DMF (200 mM). NMM (2 equiv.) was added to the solution. The preactivated solution was added to the hydrazine resin and shaken overnight. The resin was washed with CH_2Cl_2 and DMF and dried using a N_2 stream. The resin was stored at 4 ºC.

Peptides were synthesized on a Multisyntech Syro I parallel synthesizer using Fmoc-SPPS chemistry. The following Fmoc amino acids with side-chain protection groups were used: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-His(1-Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Boc-Lys(Fmoc)-

OH Fmoc-Nle-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH.

General methods on Multisyntech Syro I parallel synthesizer:

- Amino acids were dissolved in DMF to a concentration of 0.5 M. HATU was dissolved in DMF to a concentration of 0.5 M. DIPEA was dissolved in NMP to a concentration of 2 M. Amino acid, HATU and DIPEA are mixed to a final concentration of 0.2 M, 0.2 M and 0.4 M, respectively, and added to the resin. The resin was agitated for 45 min. Coupling steps were repeated once.
- Capping was performed with acetic anhydride. 20 vol% acetic anhydride in DMF was mixed with 2 M DIPEA at a ratio of 3:2 and added to the resin. The resin was agitated for 5 min. The capping step was repeated once.
- Fmoc deprotection was performed with 20 vol% piperidine in DMF for 10 min. The deprotection step was repeated once.

Amino acids were loaded according to the general methods. Automated peptide elongation was carried out on a Multisyntech Syro I parallel synthesizer according to the general peptide methods. The peptide was cleaved form the resin using TFA/DODT/H_2O (95:2.5:2.5, v/v) for 1 h. The resin was removed by filtration and the filtrate concentrated under reduced pressure. The solution was triturated with Et_2O and centrifuged to obtain crude peptide. The crude peptide was dissolved in H_2O/CH_3N (1:1, v/v) + 0.1% (v/v) TFA and purified using preparative HPLC.

2.6. Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a Mini-PROTEAN Tetra Cell system (Bio-Rad) connected to the PowerPac Basic (Bio-Rad power supply. Reducing samples were treated with sample buffer (Laemmli 2x or 4x Concentrate, Sigma Aldrich), heated at 95 °C for 5 min and used for separation. A 10-180 kDa pre-stained protein ladder (Thermo Fisher) was applied to at least one well of each gel (3 µL). Samples were separated on 8-16% gradient Mini-PROTEAN TGX Precast gels (Bio-Rad) for 30 minutes at 200 V. Gels were stained with Coomassie (0.1% Coomassie Brilliant Blue R, 40% MeOH, 10% acetic acid) with agitation for 1h and subsequent destaining (40% MeOH, 10% acetic acid). Destained gels were imaged on a Bio-Rad Molecular Imager Pharos FX. Gels were cropped in Adobe Illustrator. Full gels are shown in Figure S18.

2.7. Western blotting

Proteins were electrophoretically transferred from resolved gels to low-fluorescence PVDF membranes using TransBlot (Bio-Rad). For blots anti-FLAG, His_6, and β-actin, transfer was performed using Mixed MW method (7 min, 1.3 A). Blots were blocked with 5 wt% dry skim milk powder in TBS-T for 2 h at room temperature. Blots were washed with TBS-T and then incubated with antibodies at indicated dilutions in TBS-T with 1 wt% BSA for 18 h at 4 ºC. Blots were exhaustively washed with TBS-T and incubated with secondary antibody at indicated dilutions in TBS-T with 1 wt% BSA for 1.5 h at room temperature. Blots were exhaustively washed and incubated with Clarity Western ECL (Bio-Rad) and visualized using Bio-Rad Molecular Imager Pharos FX (Chemiluminescence). Blots were cropped in Adobe Illustrator. Full blots are shown in Figure S18.

2.8. Antibodies

Plasmid	Supplier	Identifier	Dilution
Anti-FLAG	Thermo-Fisher	#MA1-91878	1:1,000
Anti-His6	Thermo-Fisher	#MA1-21315	1:1,000
Anti-β-Actin	Thermo-Fisher	#BA3R	1:2,000
Anti-Mouse	Thermo-Fisher	#62-6520	1:3,000
Anti-SENP1	Cell-Signalling	#D16D7	1:1,000
Anti-Rabbit	Cell-Signalling	#7074	1:3,000

Table S1 Antibodies used in this study.

2.9. Gene constructs

Ubiquitin (P0CG48), NEDD8 (Q15843), and ISG15 (C78A) (P05161) were synthesized by Thermo-Fisher Scientific, ATG-Biosynthetics or Genescript. Genes were codon-optimized for *E. coli*. SUMO2 (P61956, Addgene plasmid #53142) was a gift from Frauke Melchior. Genes were subcloned into *Mxe*-GyrA-His_6 pET28a (Merck Novagen) using Gibson assembly. Ube2K C170S (P61086, Addgene plasmid #18892) was a gift from Cecile Pickart, Ube2G1 (P62253, Addgene plasmid #15790) was a gift from Wade Harper, Ube2L3 (P68036, Addgene plasmid #15795) was a gift from Wade Harper, SENP1 cat. domain (Q9P0U3, Addgene plasmid #16356) was a gift from Guy Salvesen, SENP8 (Q96LD8, Addgene plasmid #16361) was a gift from Guy Salvesen. Genes were cloned into His_6-TEV pET28b vector or used as obtained. USP21 196-565 (Q9UK80, addgene plasmid #61585) was a gift from David Komander and used as obtained. Mutations were introduced using inverse PCR. All sequences were confirmed by DNA Sanger sequencing (Microsynth).

2.10. Protein expression

Chemically competent BL21 (DE3) cells (ubiquitin, NEDD8, SUMO2, Ube2K, Ube2G1, Ube2L3) or BL21-CodonPlus (DE3)-RIL (SENP1, SENP8, USP21) were heat-shock transformed with plasmids and single colonies were used to inoculate overnight precultures in selective lysogeny broth (LB) Miller medium. Following 1:1000 dilution with fresh selective LB Miller medium cultures were grown in baffled shake flasks at 37 °C. *Mxe*-GyrA fusion proteins were grown to OD_{600} 0.6. The temperature was reduced to 25 ºC and protein expression induced by addition of isopropyl β-D-1-thiogalactopyranoside at a final concentration (0.5 mM). Expression was allowed to proceed for 18 h. Cells were harvested (4,500 g, 35 min, 4 ºC) and resuspended in intein lysis buffer (20 mM Tris, 200 mM NaCl, pH 7.2). Cells were lysed by addition of lysoszyme, DNase I. Cells were further lysed by sonication and suspension clarified by centrifugation (20,000 g, 35 min, 4 ºC). Intein fusion proteins were purified by gravity Ni-NTA purification. Resin was washed with excess intein lysis buffer and eluted using intein elution buffer (20 mM Tris, 200 mM NaCl, 300 mM imidazole pH 7.2). The isolated fusion protein was dialyzed against intein cleavage buffer (see below). Ube2K, Ube2G1, Ube2L3, SENP1 and SENP8, and USP21 were expressed and purified as previously reported.[1-5] Protein concentrations were estimated by absorption at 280 nm using extinction coefficients calculated using the online tool ProtParam (https://web.expasy.org/protparam). Proteins were aliquoted, flash frozen in liquid N_2 and stored at –80 ºC until use.

2.11. Circular dichroism spectroscopy

CD spectra were recorded on a JASCO J-1500 CD spectrometer in a 0.1 cm cuvette. Samples were measured in potassium phosphate (20 mM, pH 7.2) at 20 ºC. Data was recorded between 180-300 nm with a measuring speed of 20 nm/min. Measurements were repeated (5x) and averaged. Background spectrum was recorded and subtracted from measured spectra. Data were converted to mean residue ellipticity.

2.12. Acylation of hydrazides with anhydrides

Peptides or proteins were dissolved or diluted in acylation buffer (100 mM NaPhos, 50 mM NaCl, pH 3.0) to a concentration of 100 µM. The anhydride was dissolved in THF or DMF to a final concentration of 500 mM. The anhydride was added to the desired concentration (1 mM – 20 mM) and mixed. Reaction was allowed to proceed for ca. 5 min or analyzed directly after addition. The reaction was analyzed by RP-HPLC or LC-MS analysis. The reaction was purified by dialysis at 4 ºC or by buffer exchange using desalting columns (Cytiva). Products could be used without further purification.

2.13. Generation of protein hydrazides from protein-*Mxe* GyrA-His_6 fusions

Protein-*Mxe* GyrA-His_6 fusion after Ni-NTA purification was dialyzed against intein cleavage buffer (25 mM HEPES, 200 mM NaCl, 1 mM EDTA pH 7.2 at 4 ºC). After dialysis hydrazine monohydrate (100 mM) was added and the pH adjusted to 7.8. The solution was allowed to stand at room temperature and monitored using LC-MS analysis. Upon completion the solution was dialyzed against 25 mM HEPES, 200 mM NaCl pH 7.2 at 4 ºC. Cleaved intein was removed using gravity Ni-NTA purification. The flow-through was collected and combined with resin washes. The combined fractions were dialyzed and further purified using ion exchange. ISG15-*Mxe*-GyrA-His_6 was cleaved in the presence of 20 mM hydrazine monohydrate and 50 mM MESNa.

2.14. Synthesis of symmetrical anhydrides

Carboxylic acids were dissolved in CH_2Cl_2 (300 mM) and dicyclohexylcarbodiimide (0.5 equiv.) was added in one portion. The reaction was stirred under N_2 for 18 h. The suspension was filtered and concentrated to 10% of the initial volume. The solution was placed at 4 ºC for 1h and filtered again. The filtrate was concentrated to obtain the symmetrical anhydride which was used without further purification. Anhydrides can be stored at –20 ºC for several weeks.

2.15. Digestion of modified Ub(ΔG76)–$NHNH_2$

Ubiquitin was modified with α-chloroacetic anhydride. The reaction mixture was diluted with 50 mM Tris-HCl, 0.5 mM $Zn(OAc)_2$. (Asp-N (0.5 µg) was added and the mixture was incubated for 18 h at 37 ºC. The digestion was desalted using C_{18} ZipTips according to the manufacturers protocol. The desalted peptides were diluted with 5 vol% MeCN and 0.1 vol% formic acid. Peptides were analyzed using a Bruker timsTOFpro. Analysis was performed using MASCOT searching against SwissProt using a custom modification for the acylated hydrazide.

2.16. Preparation of Ub(ΔG76)–VME

Ub(ΔG76)–VME was prepared according to previously reported procedures[6]. In brief, Ub(ΔG76) was expressed as an intein fusion with *Mxe*-GyrA. Following Ni-NTA purification the fusion protein was buffer exchanged to 25 mM HEPES, 200 mM NaCl, 1 mM EDTA, pH 7.2 using a HiPrep desalting column (Cytiva). Fractions were pooled and MESNA (100 mM) and TCEP (1 mM) were added and pH adjusted to 7.8. Full intein-cleavage was observed after 3d. Buffer was exchanged to 0.5 vol% acetic acid using HiPrep desalting column (Cytiva). The pooled fractions were purified using preparative reverse phase HPLC, product fractions were combined and lyophilized. Lyophilized Ub(ΔG76)–Mes thioester was dissolved in H_2O (6 mg/mL). N-hydroxysuccinimide (1 mM) and VME-TFA salt (250 mM) in 1M $NaHCO_3$ (pH 8.0) were added and shaken at 37 ºC for 24 h. No reaction progress was observed after 24 h. The reaction was buffer exchanged to PBS using G25 MidiTrap desalting columns (Cytiva). Ub(ΔG76)–VME was concentrated and stored at –80 ºC.

2.17. Preparation of Ub G76C

Ub G76Dha was prepared according to previously reported procedures[7]. In brief, Ub G76C (225 µM) in 50 mM HEPES, 50 mM K_2CO_3 pH 8.0 was treated with 2,5 dibromohexanediamide (10 equivalents) and incubated at 37 ºC for 18 h. The reaxtion mixture was diluted with 50 mM NH_4OAc, pH 4.5 and purified by cation exchange chromatography using a MonoS column (Cytiva) with a gradient of 0-1 M NaCl in 50 mM NH_4OAc, pH 4.5. Fractions were combined buffer exchanged to PBS and stored at –80 ºC.

2.18. Reaction of modified Ubl probes with E2s

Ubl probes (100 µM) were mixed with E2s (15 µM) in PBS. The mixtures were incubated at 37 ºC in a water bath for 1-3 h. The reaction was quenched by addition of 2x Laemmli buffer and boiled at 95 ºC for 5 min. Samples were resolved by SDS-PAGE (8-16%, Bio-Rad) and visualized by Coomassie-Blue staining. If required gel bands were quantified by gel densitometry using ImageLab (Bio-Rad).

2.19. Reaction of Ubl probes with DUBs

Ubl probes (60 µM) were mixed with DUBs (15 µM) in PBS. The mixture was incubated at 37 ºC in a water bath for 1 h. The reaction was quenched by addition of 2x Laemmli buffer and boiled at 95 ºC for 5 min. Samples were resolved by SDS-PAGE (8-16%, Bio-Rad) and visualized by Coomassie-Blue staining.

2.20. Mammalian cell culture and cell lysate generation

HEK293T cells were obtained from ATCC. Cells were cultured in DMEM supplemented with FBS and Penicillin/Streptomycin. Cells were seeded at appropriate densities in 6-well, T25 or T75 flasks. Cells were maintained at 37 ºC with 5 vol% CO_2. Cells were lysed with NP-40 buffer (50 mM Tris, 150 mM NaCl, 1 vol% nonidet-P40, pH 8.0) in the presence of protease inhibitors (Thermo-Fisher). Insoluble cell components were removed by centrifugation and lysate concentration determined by BCA assay (Thermo-Fisher). Lysates were stored at –80 ºC until use.

2.21. Reaction of FLAG-SUMO probes in cell lysate

FLAG-SUMO probes (15 µM) were incubated with HEK293T lysate (25 µg) or HeLa lysate (200 µg) for 2 h at 37 ºC. The reaction was quenched by addition of 4x Laemmli buffer. Reaction was analyzed by Western blotting against FLAG-tag.

2.22. Reaction of FLAG-SUMO probes with recombinant SENP1 in cell lysate

FLAG-SUMO probes (15 µM) were incubated with HEK293T lysate (25 µg, 0.5 mg/mL), recombinant SENP1 (wt, C603A, 1 µM) for 2 h at 37 ºC. The reaction was quenched by addition of 4x Laemmli buffer. Reaction was analyzed by Western blotting against FLAG-tag and His_6-tag.

3. Characterization of peptides and proteins

3.1. Synthesis of peptide 25

Nle-Ser-Ala-Phe-Cys-Leu-Lys-Ala-His-Gly-Tyr-Arg-Val–$NHNH_2$

The peptide was obtained as a white solid.

HRMS (ESI): calculated for $[C_{67}H_{107}N_{21}O_{15}S]^+$: m/z 1477.7976, found: m/z 1477.8118

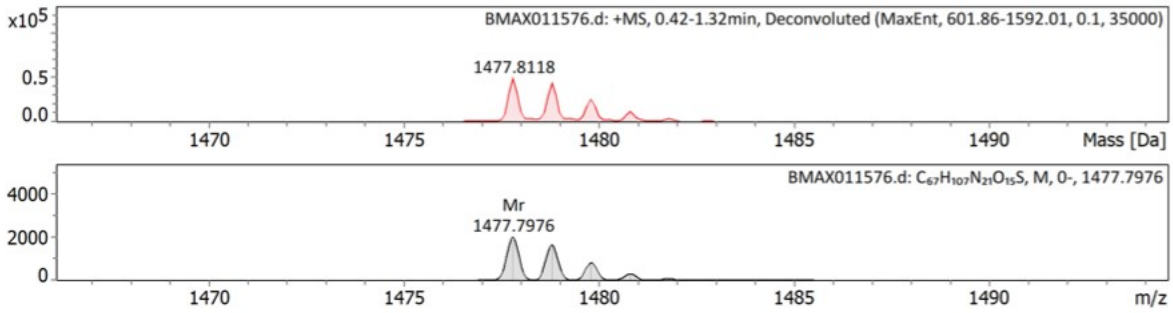


Figure S19 Characterization of peptide 25 HRMS (ESI) spectrum of purified peptide showing recorded mass spectrum (upper panel) and calculated spectrum (lower panel). Corresponding analytical RP-HPLC trace shown in Figure S1.

3.2. Synthesis of peptide 26

Nle-Ser-Ala-Phe-Cys-Leu-Lys-Ala-His-Gly-Tyr-Arg-Val–OH

The peptide was obtained as a white solid.
HRMS (ESI): calculated for $[C_{67}H_{105}N_{19}O_{16}S]^+$: m/z 1463.7707, found: m/z 1463.7798

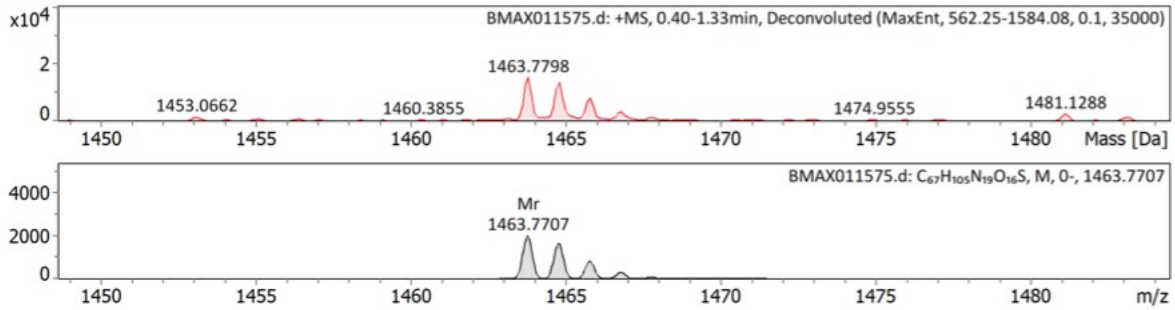
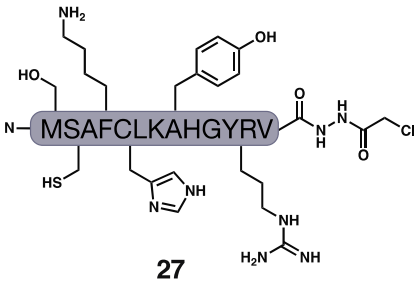


Figure S20 Characterization of peptide 26 HRMS (ESI) spectrum of purified peptide showing recorded mass spectrum (upper panel) and calculated spectrum (lower panel). Corresponding analytical RP-HPLC trace shown in Figure S1.

3.3. Synthesis of peptide 27



27

HRMS (ESI): calculated for $[C_{67}H_{105}N_{19}O_{16}S]^+$: m/z 1553.7692, found: m/z 1553.8089

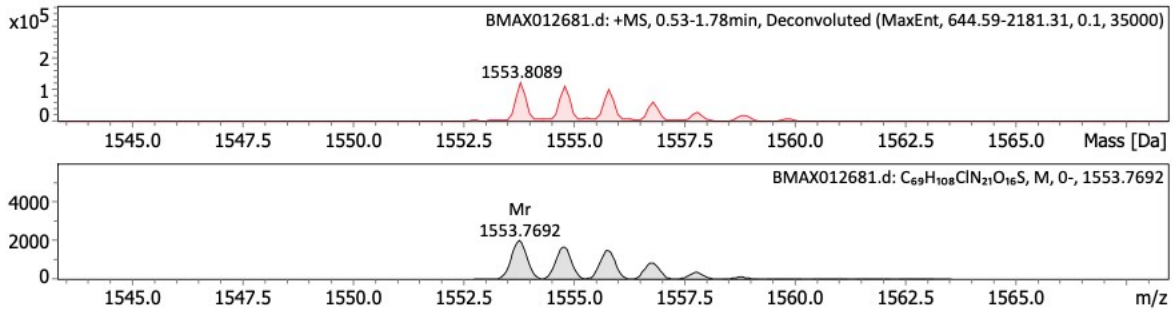
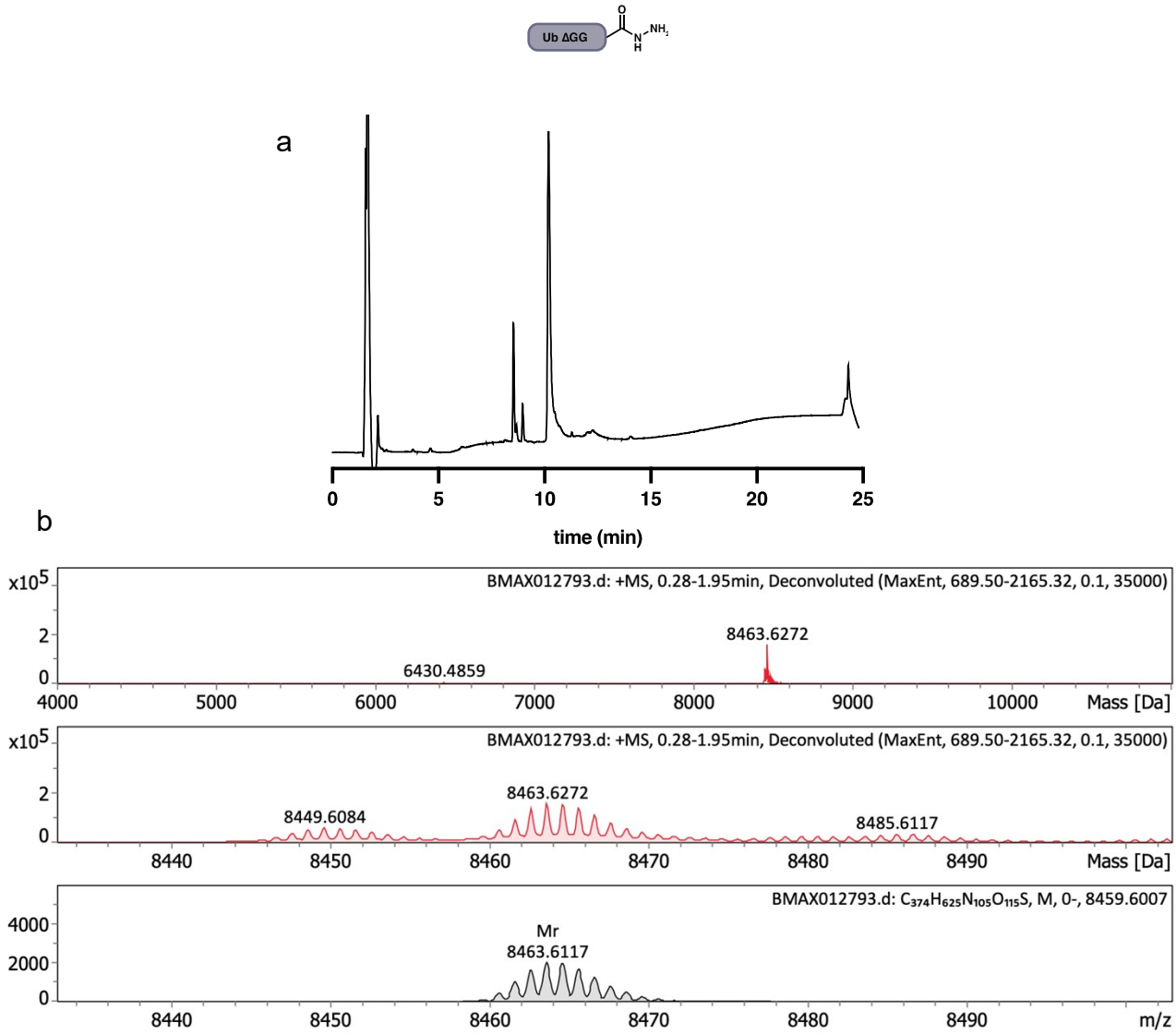


Figure S21 Characterization of peptide 27 HRMS (ESI) spectrum of purified peptide showing recorded mass spectrum (upper panel) and calculated spectrum (lower panel). Corresponding analytical RP-HPLC trace shown in Figure S1.

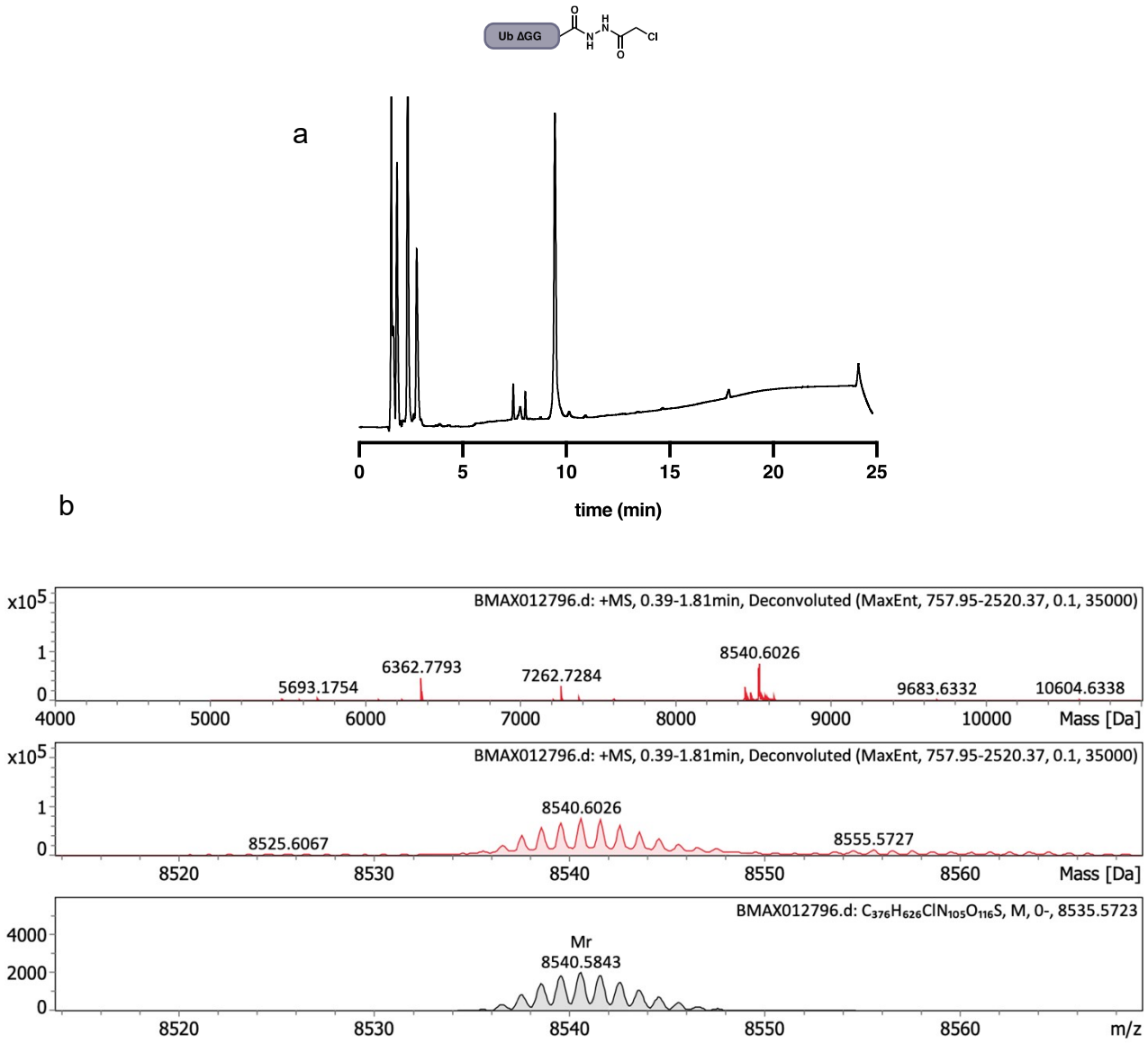
3.4. Ub(ΔGG)–$NHNH_2$



HRMS (ESI): calculated for $[C_{374}H_{625}N_{105}O_{115}S]^+$: m/z 8459.6007, found: m/z 8459.6599

Figure S22 Characterization of Ub(ΔGG)–$NHNH_2$ (a) Analytical RP-HPLC of purified Ub(ΔGG)–$NHNH_2$. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔGG)–$NHNH_2$ showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel). A small amount of hydrolyzed Ub(ΔGG) –OH is observed with mass of 8449.6084 Da.

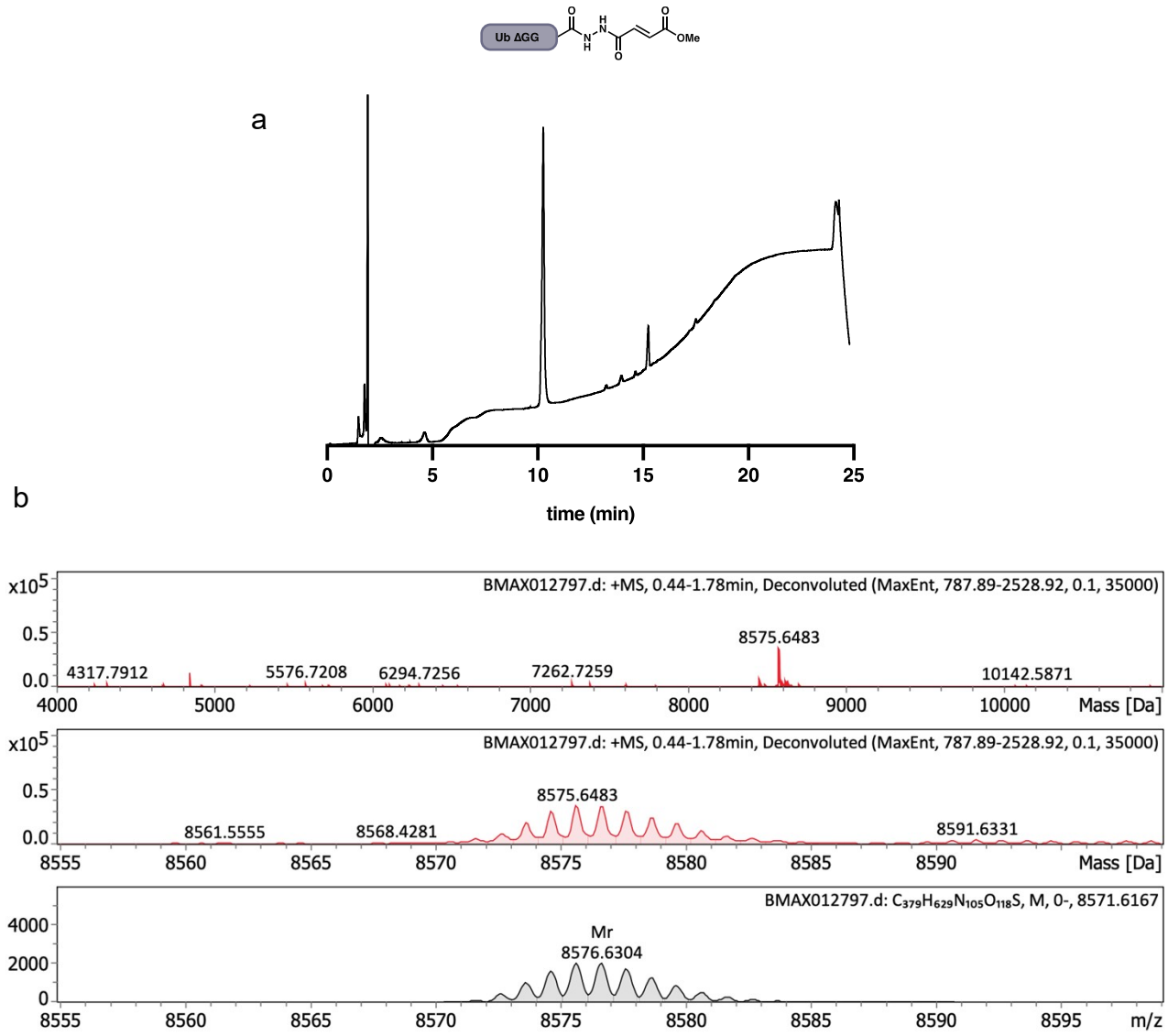
3.5. Ub(ΔGG)–NHNH α-chloroacetyl 1



HRMS (ESI): calculated for $[C_{376}H_{626}ClN_{105}O_{116}S]^{+}$: m/z 8535.5723, found: m/z 8535.6696

Figure S23 Characterization of Ub(ΔGG)–NHNH α-chloroacetyl (a) Analytical RP-HPLC of purified Ub(ΔGG)–NHNH α-chloroacetyl. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔGG)–NHNH α-chloroacetyl showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

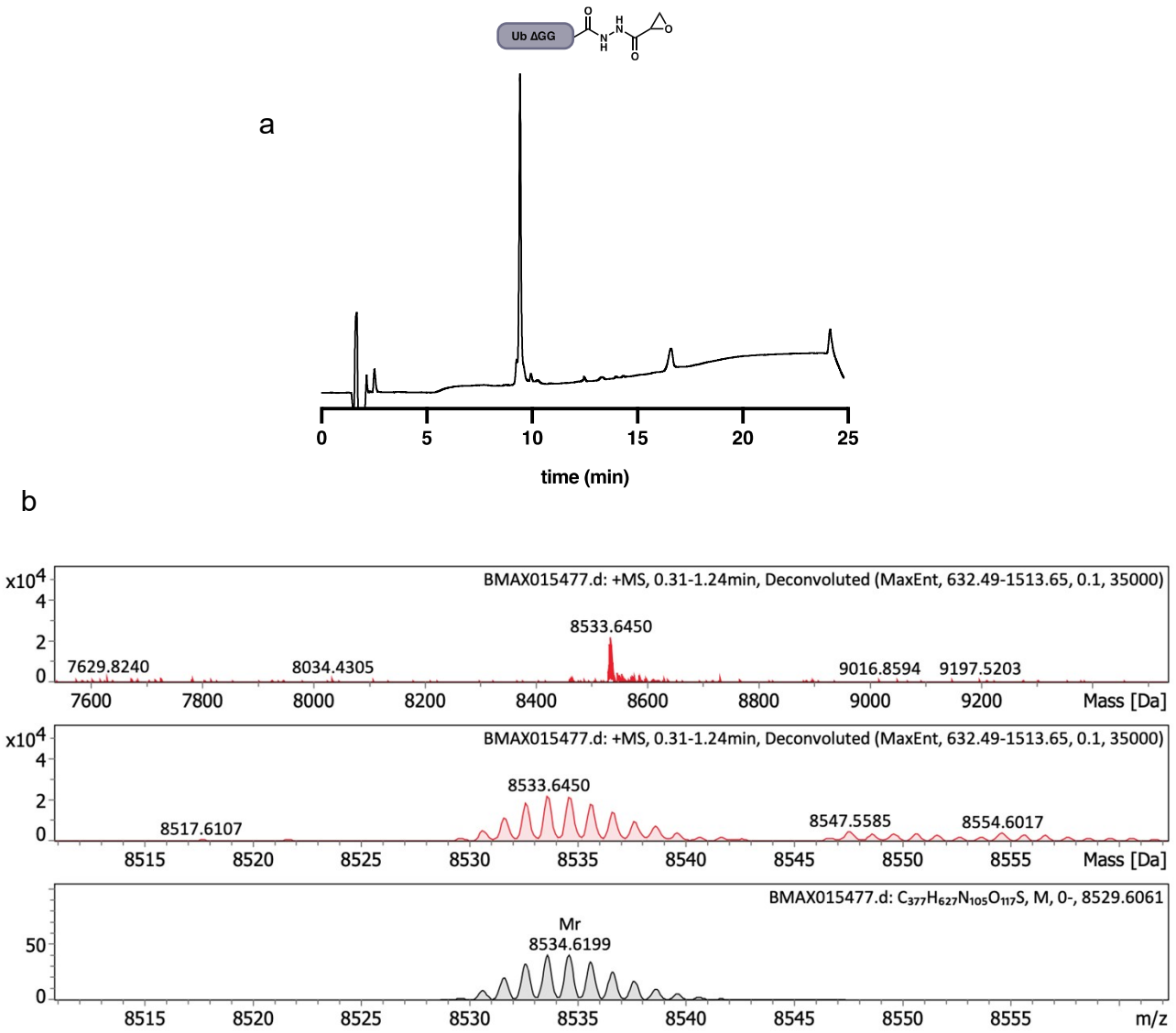
3.6. Ub(ΔGG)–NHNH methyl fumarate 3



HRMS (ESI): calculated for $[C_{379}H_{629}N_{105}O_{118}S]^{+}$: m/z 8571.6167, found: m/z 8571.6662

Figure S24 Characterization of Ub(ΔGG)–NHNH methyl fumarate (a) Analytical RP-HPLC of purified Ub(ΔGG)–NHNH methyl fumarate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔGG)–NHNH methyl fumarate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

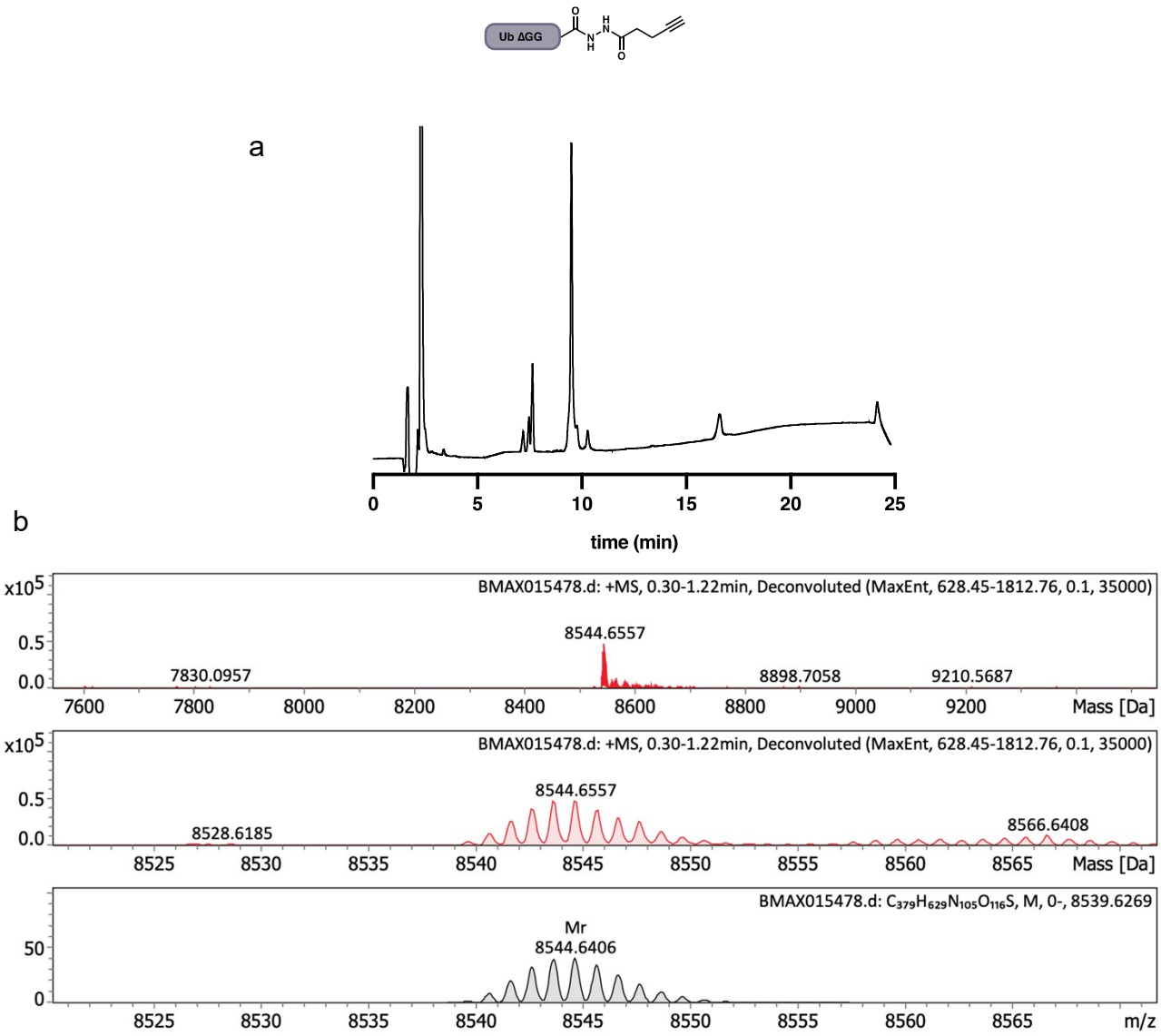
3.7. Ub(ΔGG)–NHNH glycidate 5



HRMS (ESI): calculated for $[C_{377}H_{627}N_{105}O_{117}S]^+$: m/z 8530.6090, found: m/z 8530.6545

Figure S25 Characterization of Ub(ΔGG)–NHNH glycidate (a) Analytical RP-HPLC of purified Ub(ΔGG)–NHNH glycidate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔGG)–NHNH glycidate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

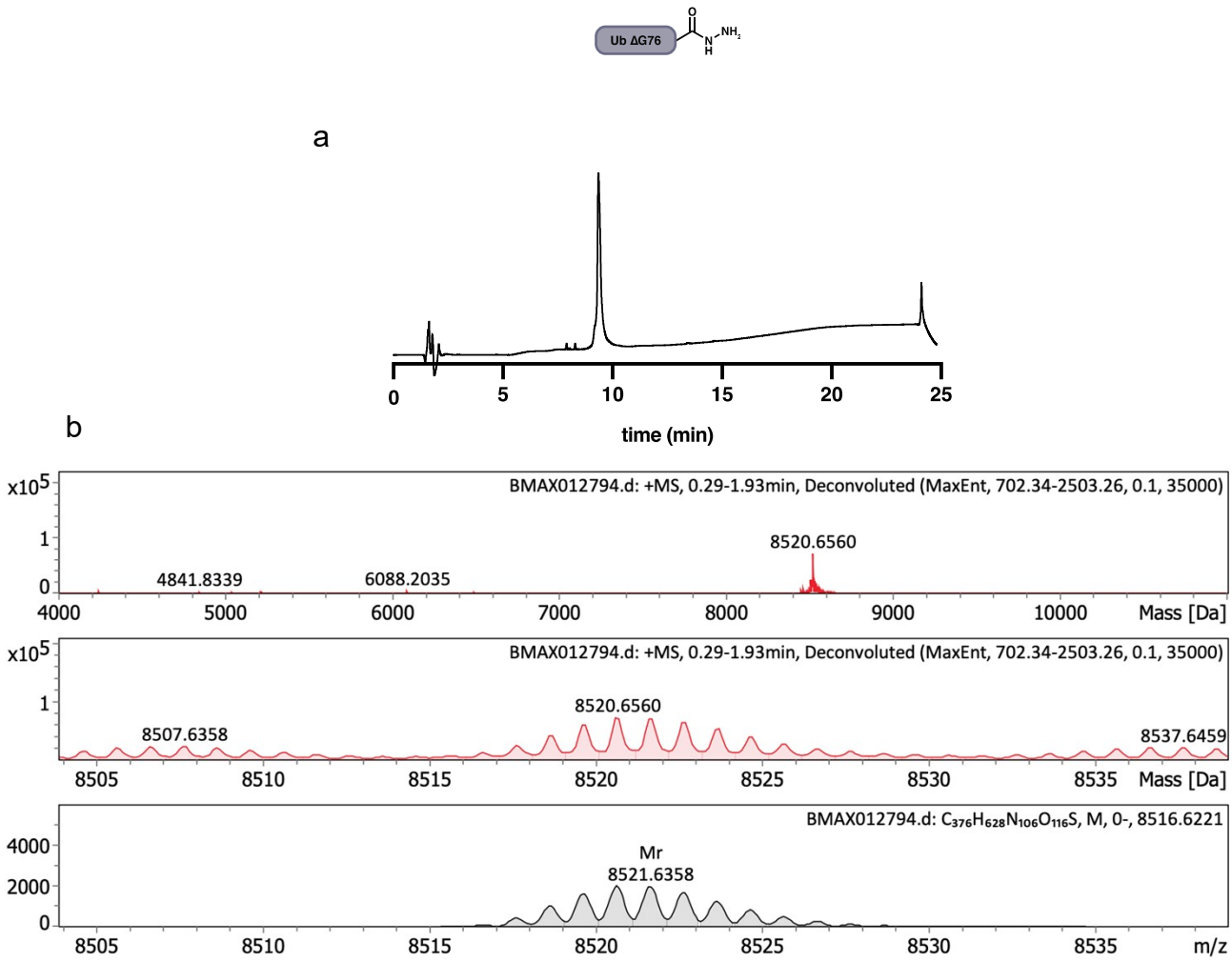
3.8. Ub(ΔGG)–NHNH pentynoate 7



HRMS (ESI): calculated for $[C_{379}H_{629}N_{105}O_{116}S]^+$: m/z 8539.6269, found: m/z 8539.6585

Figure S26 Characterization of Ub(ΔGG)–NHNH pentynoate (a) Analytical RP-HPLC of purified Ub(ΔGG)–NHNH pentynoate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔGG)–NHNH pentynoate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

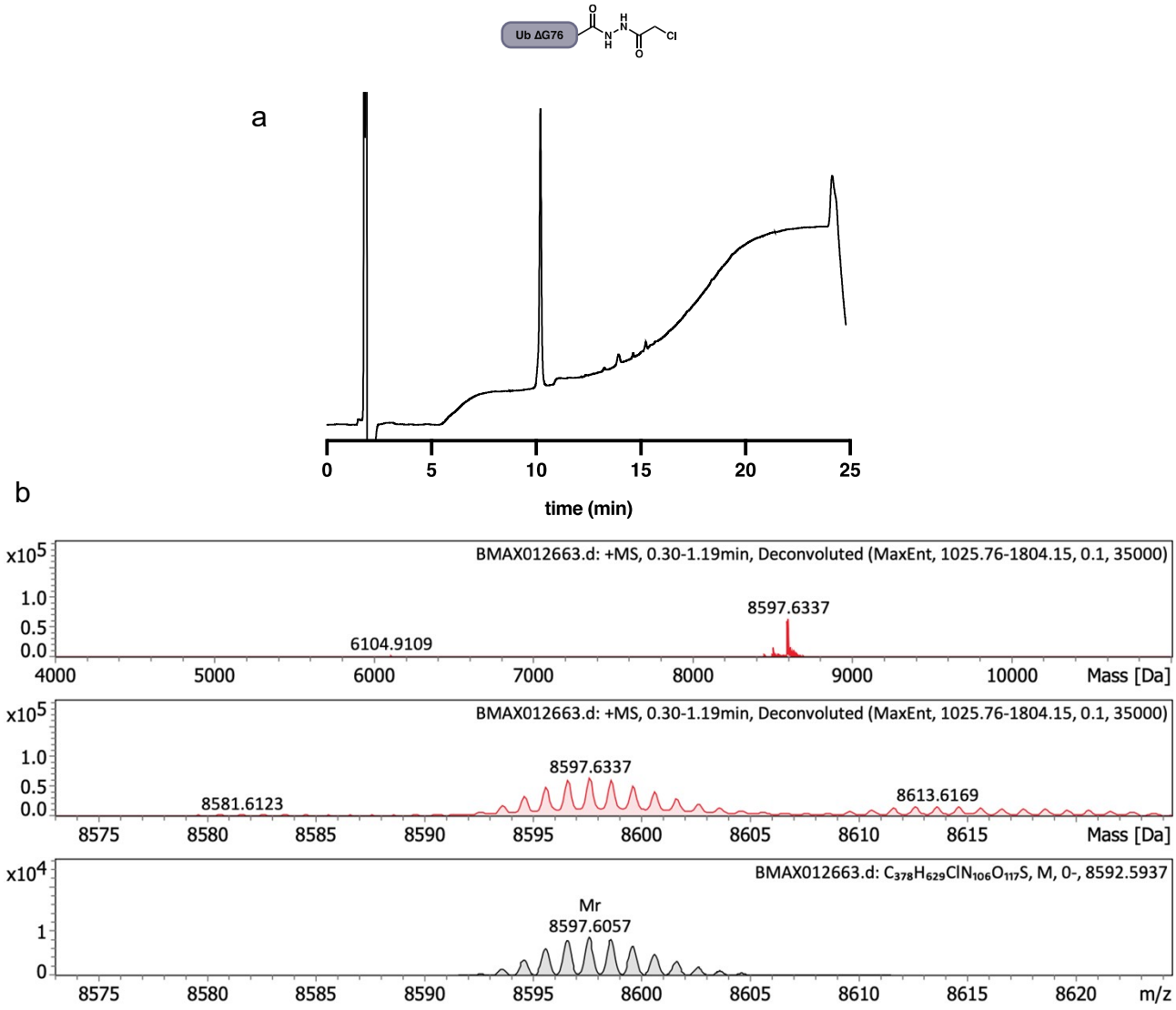
3.9. Ub(ΔG76)–$NHNH_2$



HRMS (ESI): calculated for $[C_{376}H_{628}N_{106}O_{116}S]^+$: m/z 8516.6221, found: m/z 8516.6825

Figure S27 Characterization of Ub(ΔG76)–$NHNH_2$ (a) Analytical RP-HPLC of purified Ub(ΔG76)–$NHNH_2$. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔG76)–$NHNH_2$ showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.10. Ub(ΔG76)–$NHNH_2$ α-chloroacetyl 2



HRMS (ESI): calculated for $[C_{378}H_{629}ClN_{106}O_{117}S]^{+}$: m/z 8592.5937, found: m/z 8592.6766

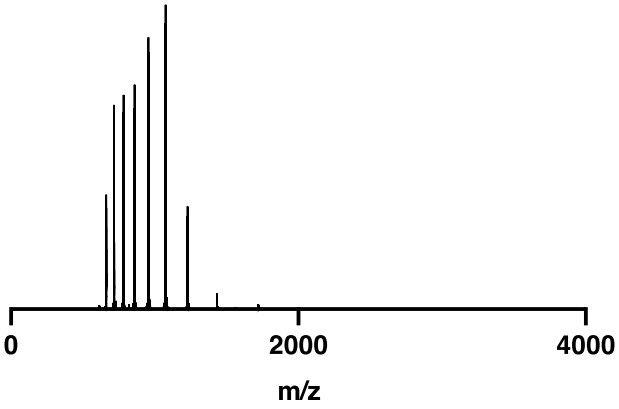
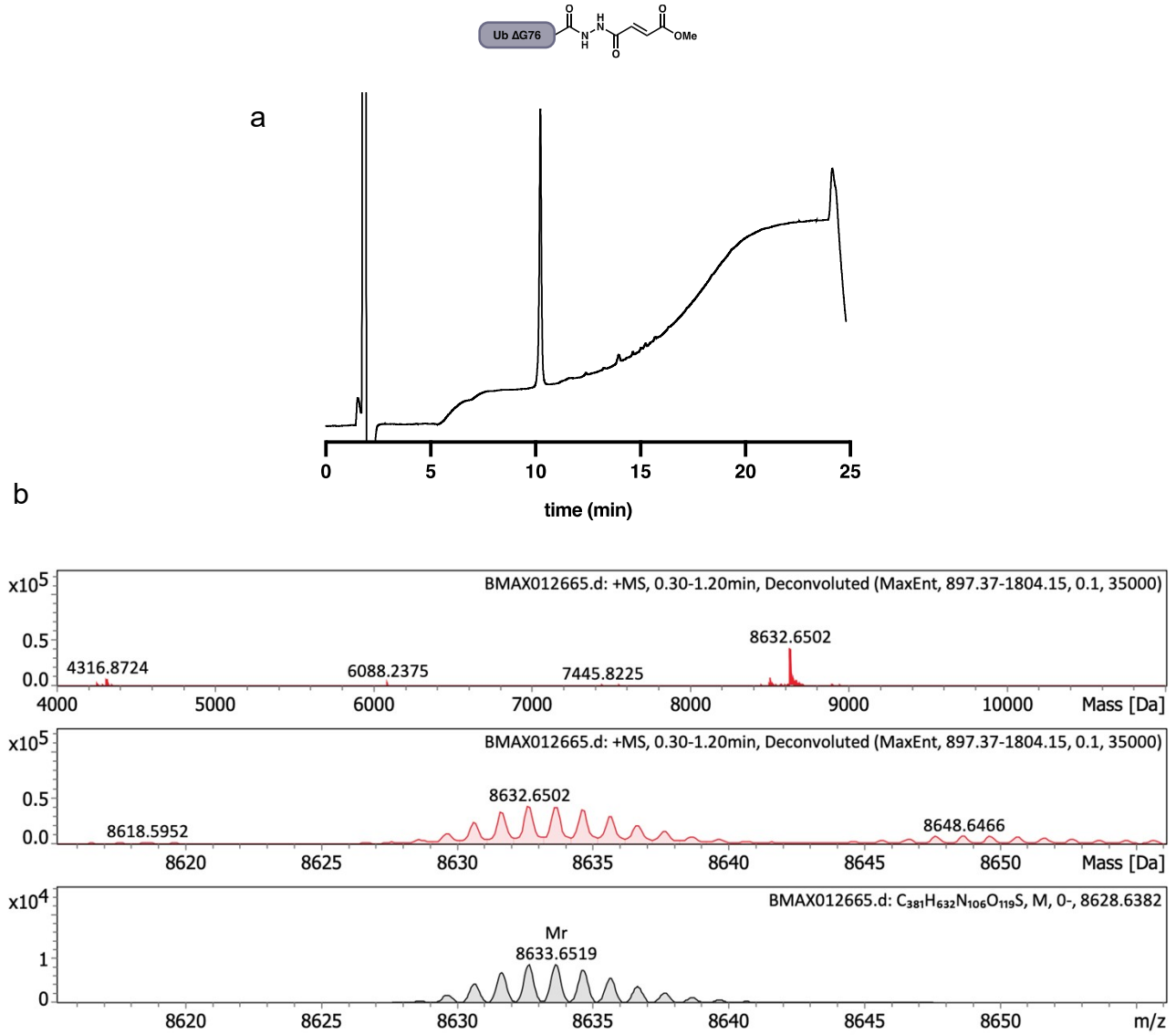


Figure S28 Characterization of Ub(ΔG76)–NHNH α-chloroacetyl (a) Analytical RP-HPLC of purified Ub(ΔG76)–NHNH α-chloroacetyl. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔG76) NHNH α-chloroacetyl showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel). Mass spectrum (ESI) obtained from LC-MS analysis. Deconvoluted mass spectrum shown in Figure 3a.

3.11. Ub(ΔG76)–NHNH methyl fumarate 4



HRMS (ESI): calculated for $[C_{381}H_{632}N_{106}O_{119}S]^+$: m/z 8628.6382, found: m/z 8628.6843

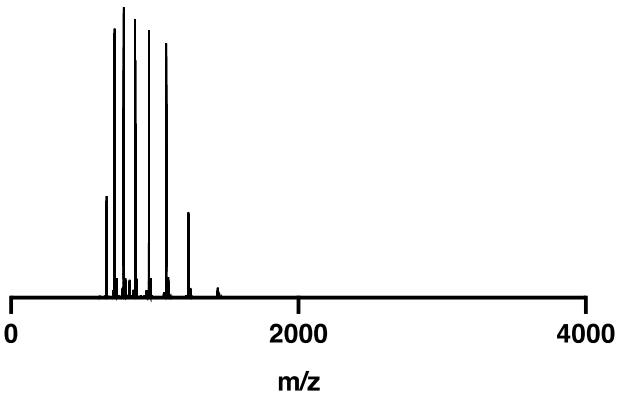


Figure S29 Characterization of Ub(ΔG76)–NHNH methyl fumarate (a) Analytical RP-HPLC of purified Ub(ΔG76)–NHNH methyl fumarate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of Ub(ΔG76)–NHNH methyl fumarate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel). Mass spectrum (ESI) obtained from LC-MS analysis. Deconvoluted mass spectrum shown in Figure 3a.

3.12. Ub(ΔG76)–NHNH glycidate 6

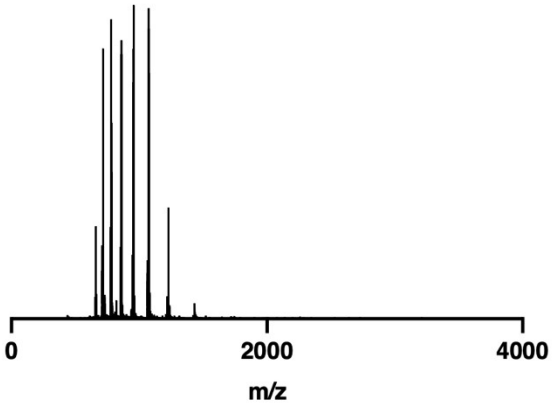


Figure S30 Characterization of Ub(ΔG76)–NHNH glycidate Mass spectrum (ESI) obtained from LC-MS analysis. Deconvoluted mass spectrum shown in Figure 3a.

3.13. Ub(ΔG76)–NHNH pentynoate 8

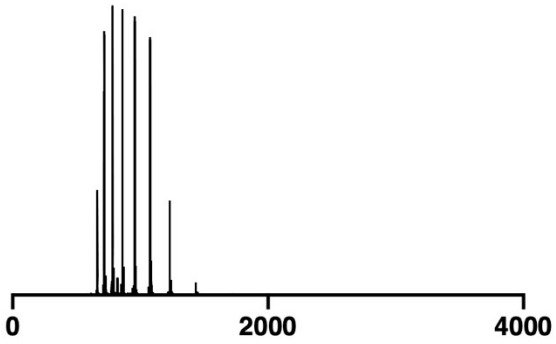


Figure S31 Characterization of Ub(ΔG76)–NHNH pentynoate Mass spectrum (ESI) obtained from LC-MS analysis. Deconvoluted mass spectrum shown in Figure 3a.

3.14. Ub(ΔG76)–NHNH acetyl 10

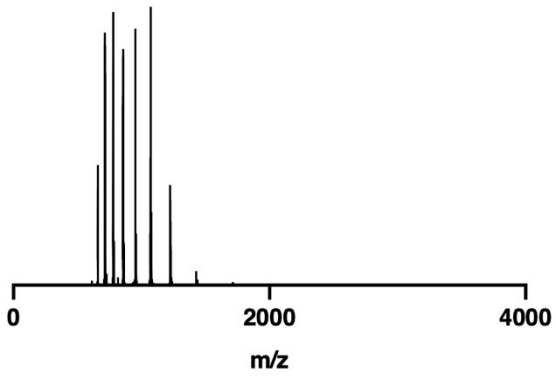
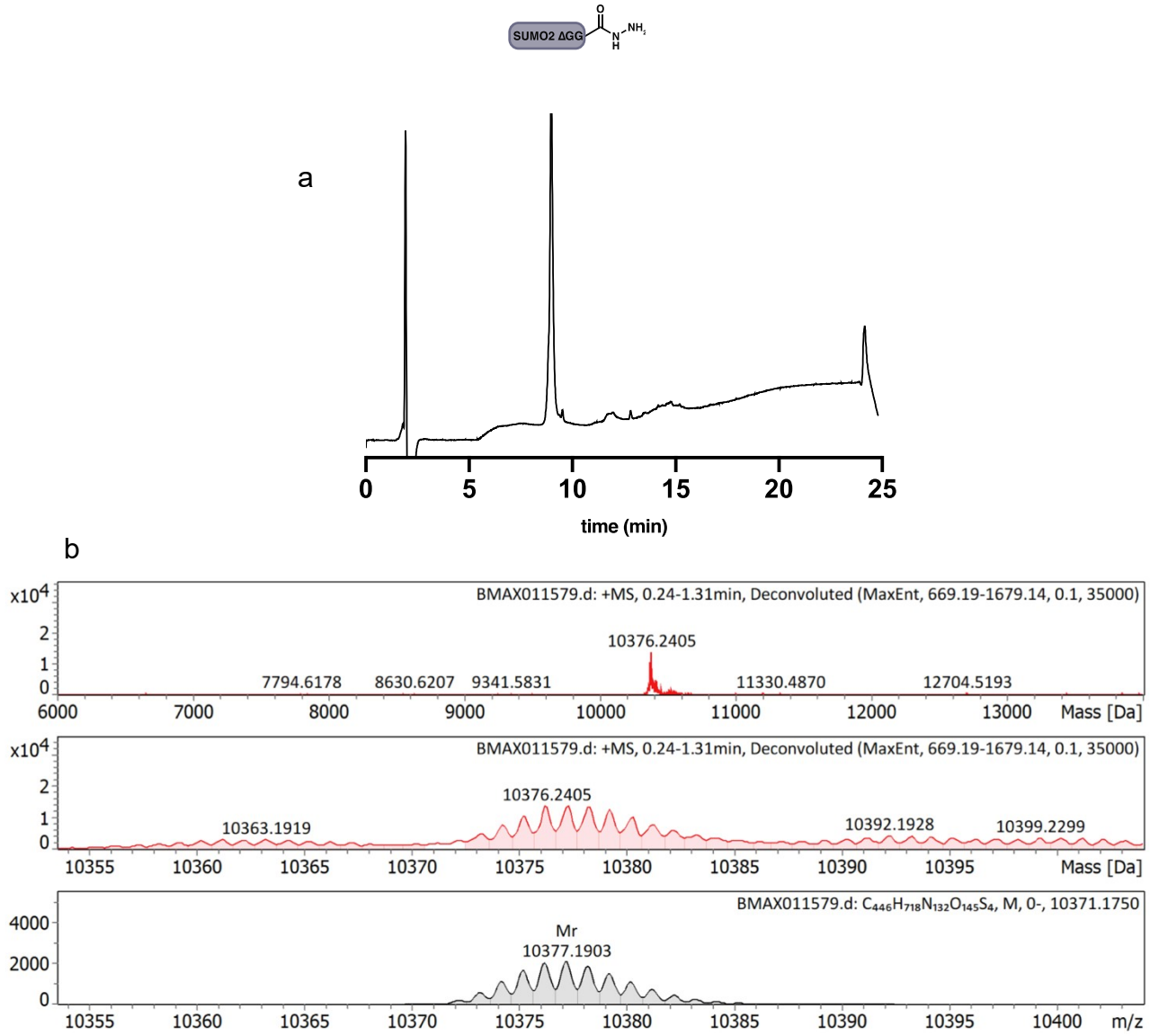


Figure S31 Characterization of Ub(ΔG76)–NHNH acetyl Mass spectrum (ESI) obtained from LC-MS analysis. Deconvoluted mass spectrum shown in Figure 3a.

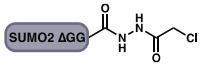
3.15. SUMO2(ΔGG)–$NHNH_2$

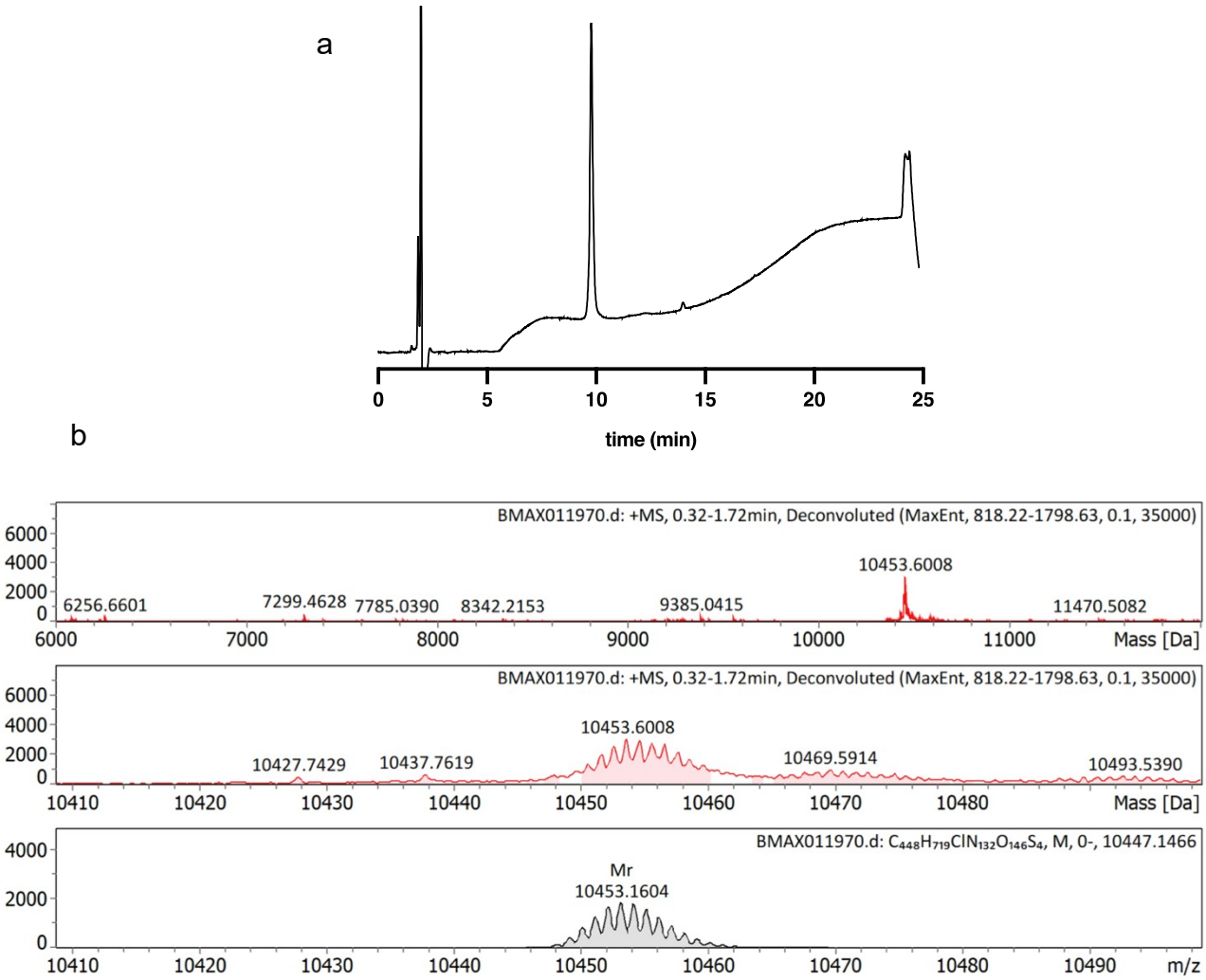


HRMS (ESI): calculated for $[C_{446}H_{718}N_{132}O_{145}S_4]^+$: m/z 10376.1918, found: m/z 10376.2405

Figure S33 Characterization of SUMO2(ΔGG)–NHNH$_2$ (a) Analytical RP-HPLC of purified SUMO2(ΔGG)–NHNH$_2$. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔGG)–NHNH$_2$ showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.16. SUMO2(ΔGG)–NHNH α-chloroacetyl 17

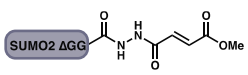




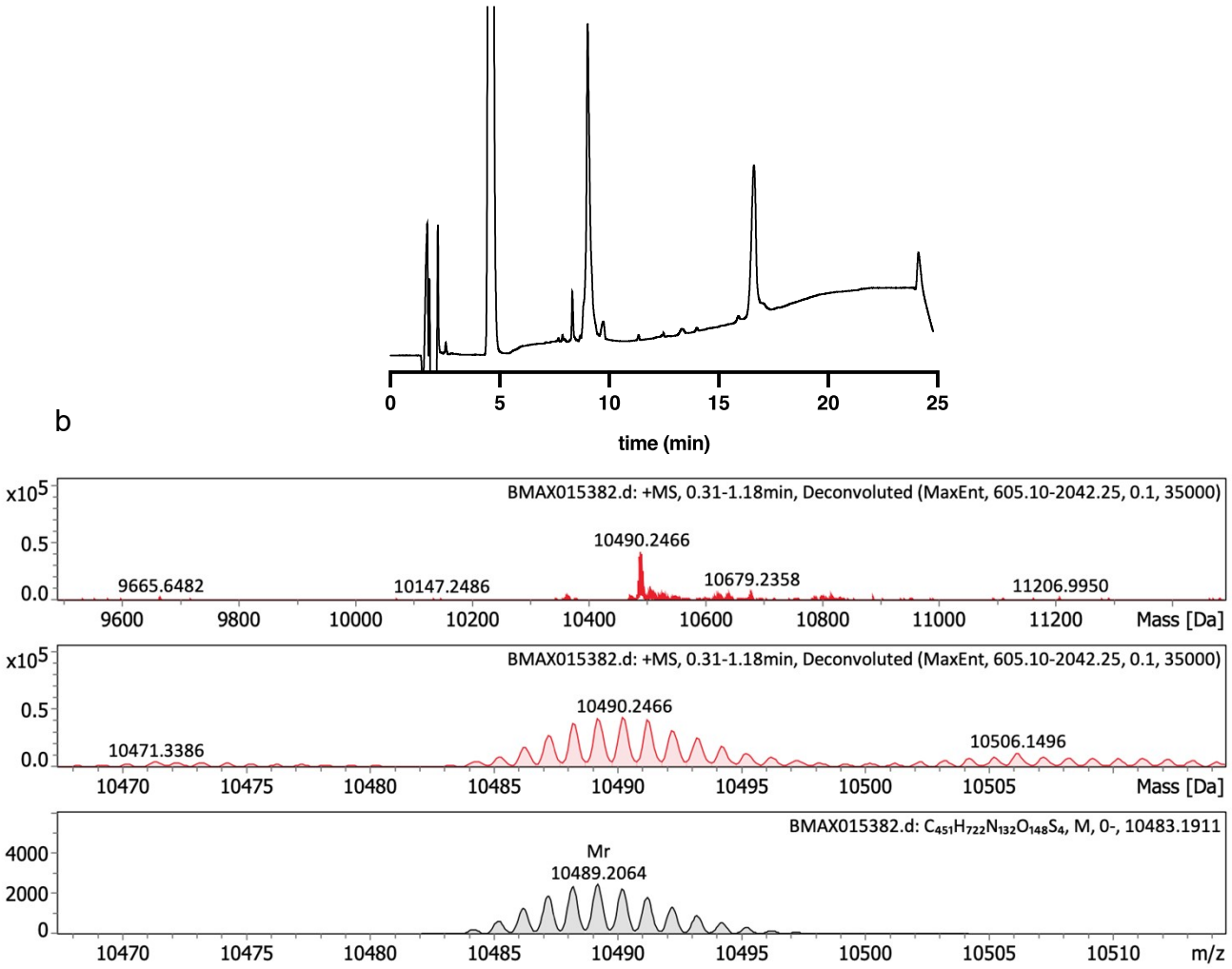
HRMS (ESI): calculated for $[C_{448}H_{719}N_{132}O_{146}S_4]^+$: m/z 10447.1466, found: m/z 10447.9337

Figure S34 Characterization of SUMO2(ΔGG)–NHNH α-chloroacetyl (a) Analytical RP-HPLC of purified SUMO2(ΔGG)–NHNH α-chloroacetyl. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔGG)–NHNH α-chloroacetyl showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.17. SUMO2(ΔGG)–NHNH methyl fumarate 19



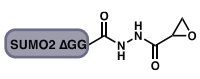
a

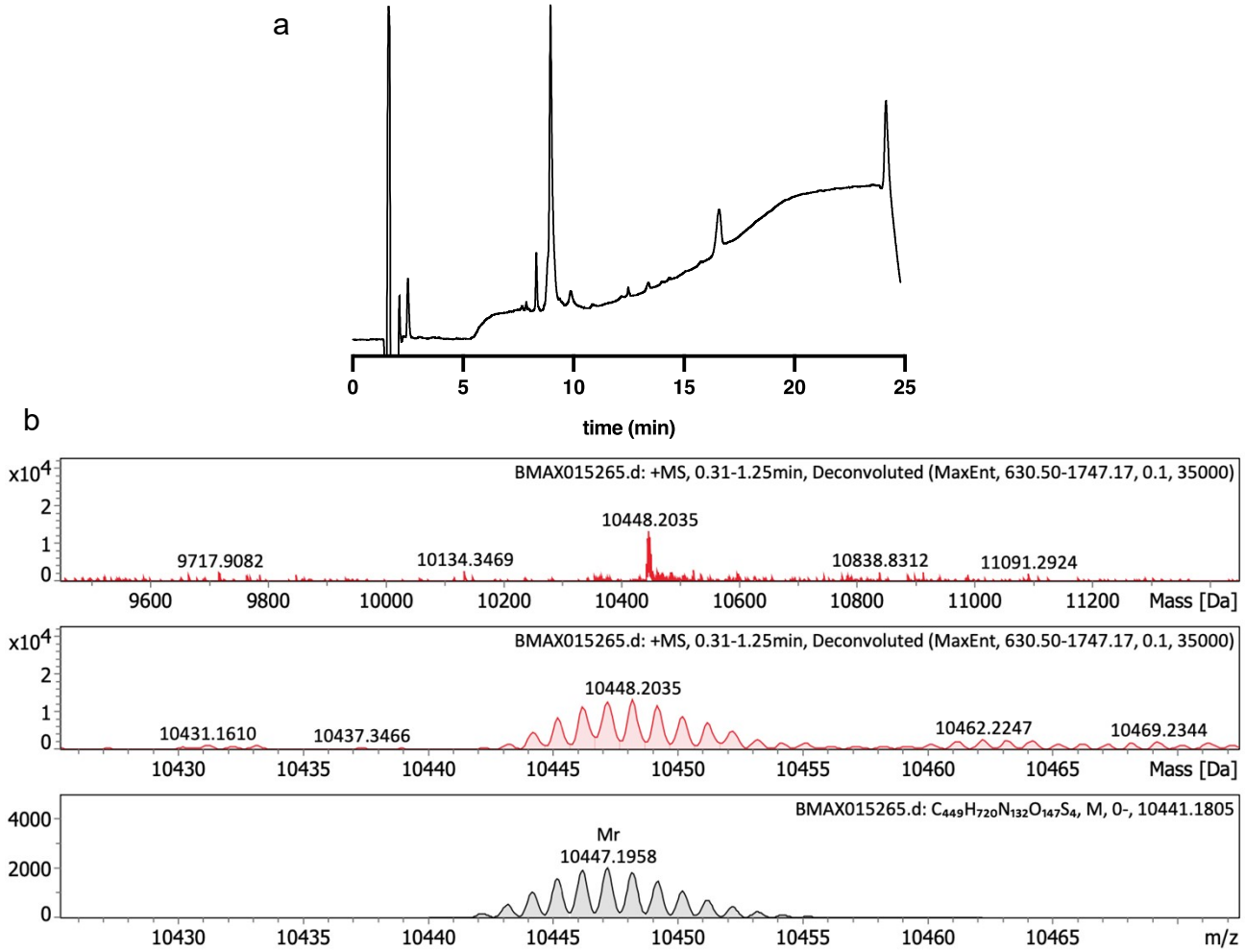


HRMS (ESI): calculated for $[C_{451}H_{722}N_{132}O_{148}S_4]^+$: m/z 10489.2064, found: m/z 10489.2477

Figure S35 Characterization of SUMO2(ΔGG)–NHNH methyl fumarate (a) Analytical RP-HPLC of purified SUMO2(ΔGG)–$NHNH_2$ methyl fumarate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔGG)–NHNH methyl fumarate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.18. SUMO2(ΔGG)–$NHNH_2$ glycidate 21

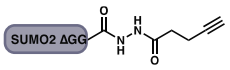


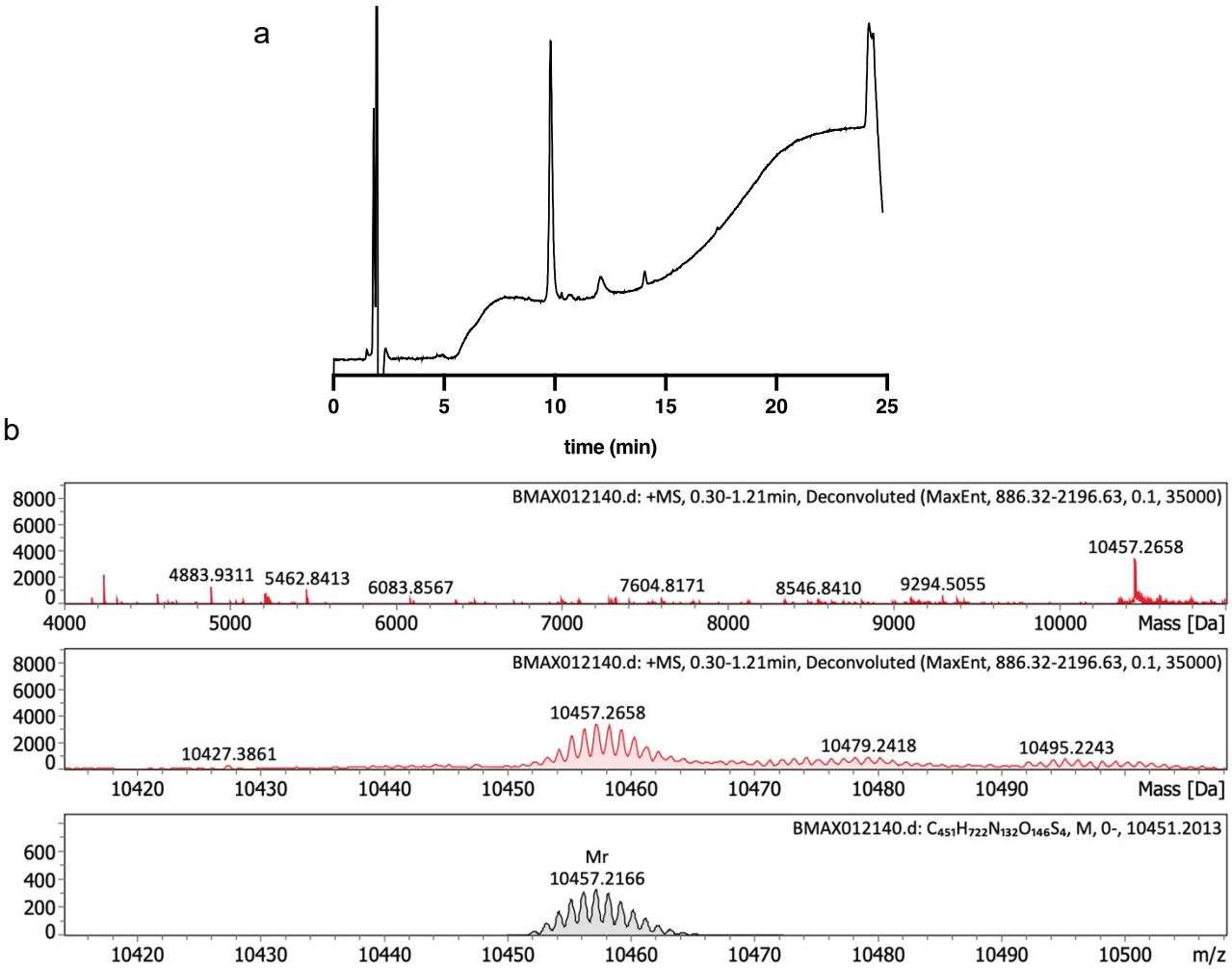


HRMS (ESI): calculated for $[C_{449}H_{720}N_{132}O_{147}S_4]^+$: m/z 10447.1958, found: m/z 10447.1998

Figure S36 Characterization of SUMO2(ΔGG)–NHNH glycidate (a) Analytical RP-HPLC of purified SUMO2(ΔGG)–NHNH glycidate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔGG)–NHNH glycidate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.19. SUMO2(ΔGG)–NHNH pentynoate 22





HRMS (ESI): calculated for $[C_{451}H_{722}N_{132}O_{146}S_4]^+$: m/z 10457.2166, found: m/z 10457.2658

Figure S37 Characterization of SUMO2(ΔGG)–NHNH pentynoate (a) Analytical RP-HPLC of purified SUMO2(ΔGG)–NHNH pentynoate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔGG)–NHNH pentynoate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.20. SUMO2(ΔGG)–NHNH acetyl 23

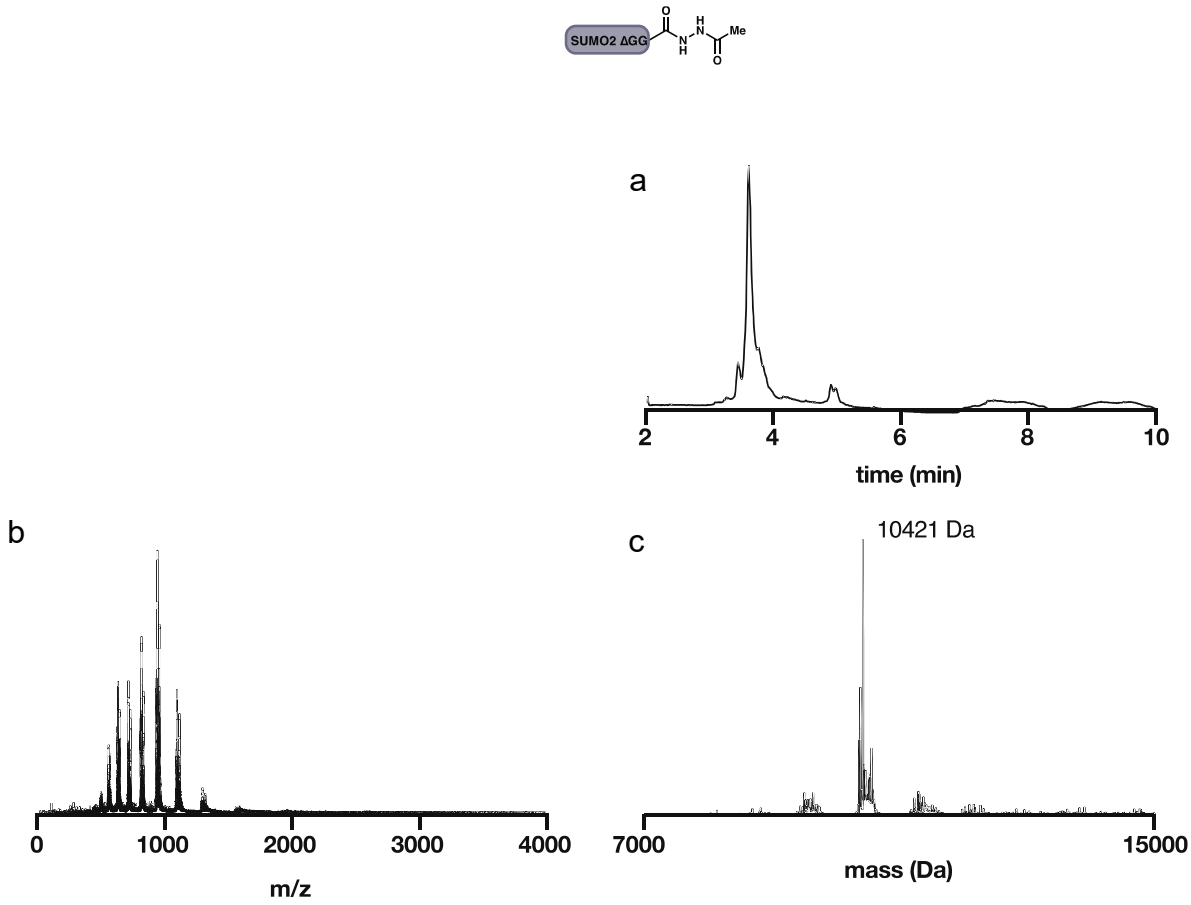
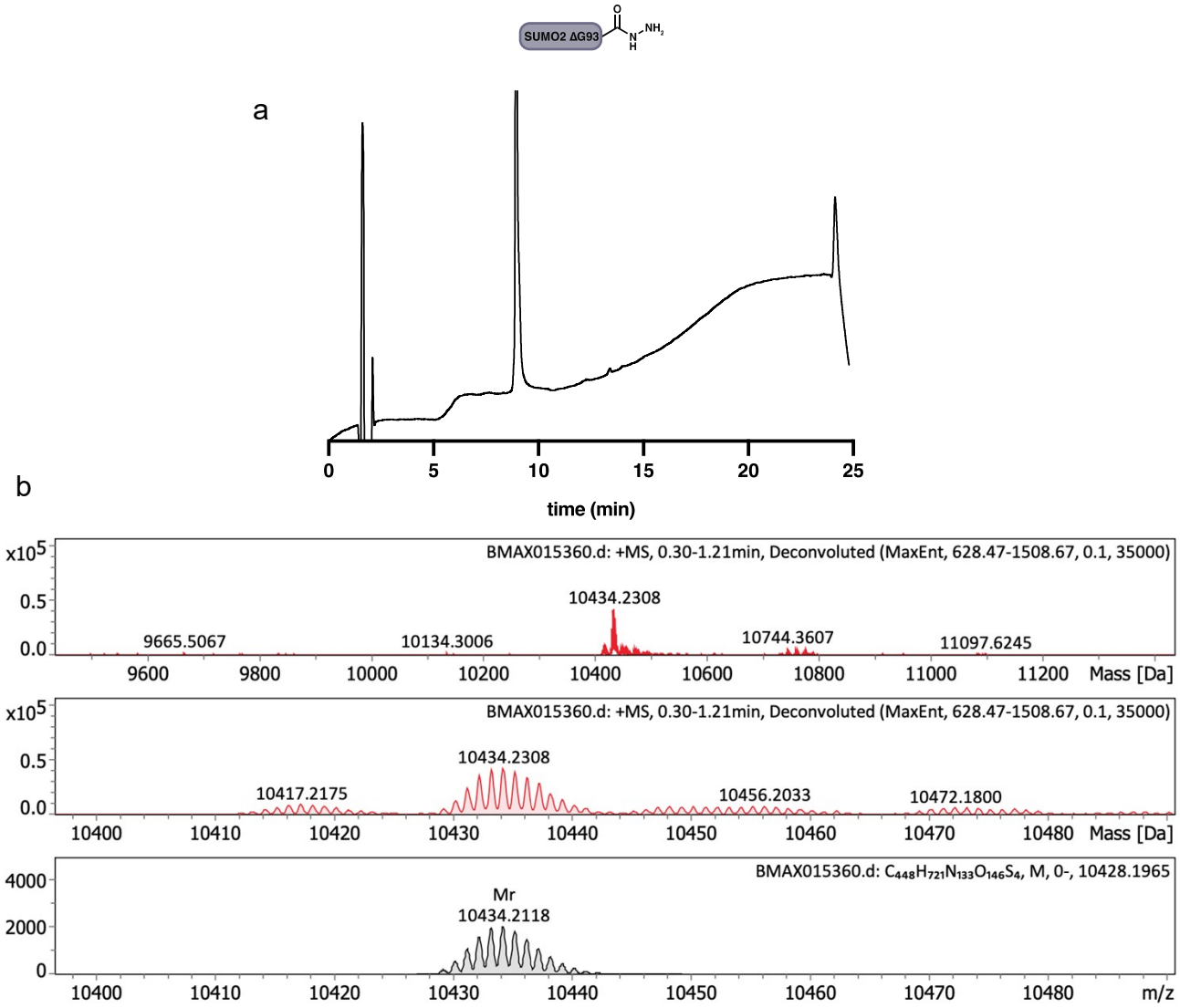


Figure S38 Characterization of SUMO2(ΔGG)–NHNH acetyl (a) Analytical RP-HPLC of purified SUMO2(ΔGG)–NHNH acetyl. TIC shown. (b) MS (ESI) spectrum (left panel), (c) deconvoluted mass spectrum (right panel), calculated mass 10419 Da.

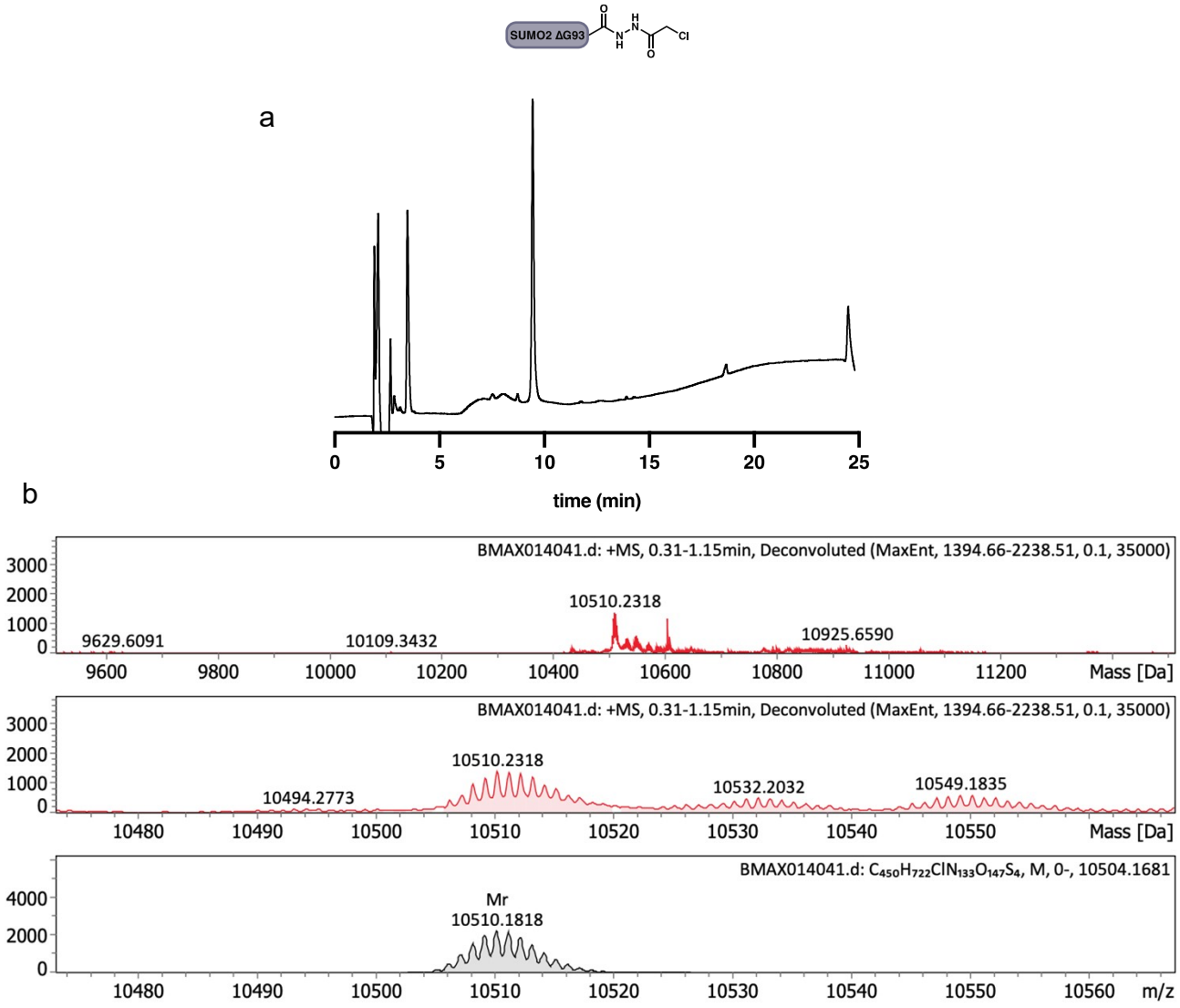
3.21. SUMO2(ΔG93)–$NHNH_2$



HRMS (ESI): calculated for $[C_{448}H_{721}N_{133}O_{146}S_4]^+$: m/z 10434.2118, found: m/z 10434.2308

Figure S39 Characterization of SUMO2(ΔG93)–NHNH$_2$ (a) Analytical RP-HPLC of purified SUMO2(ΔG93)–NHNH$_2$. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔG93)–NHNH$_2$ showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

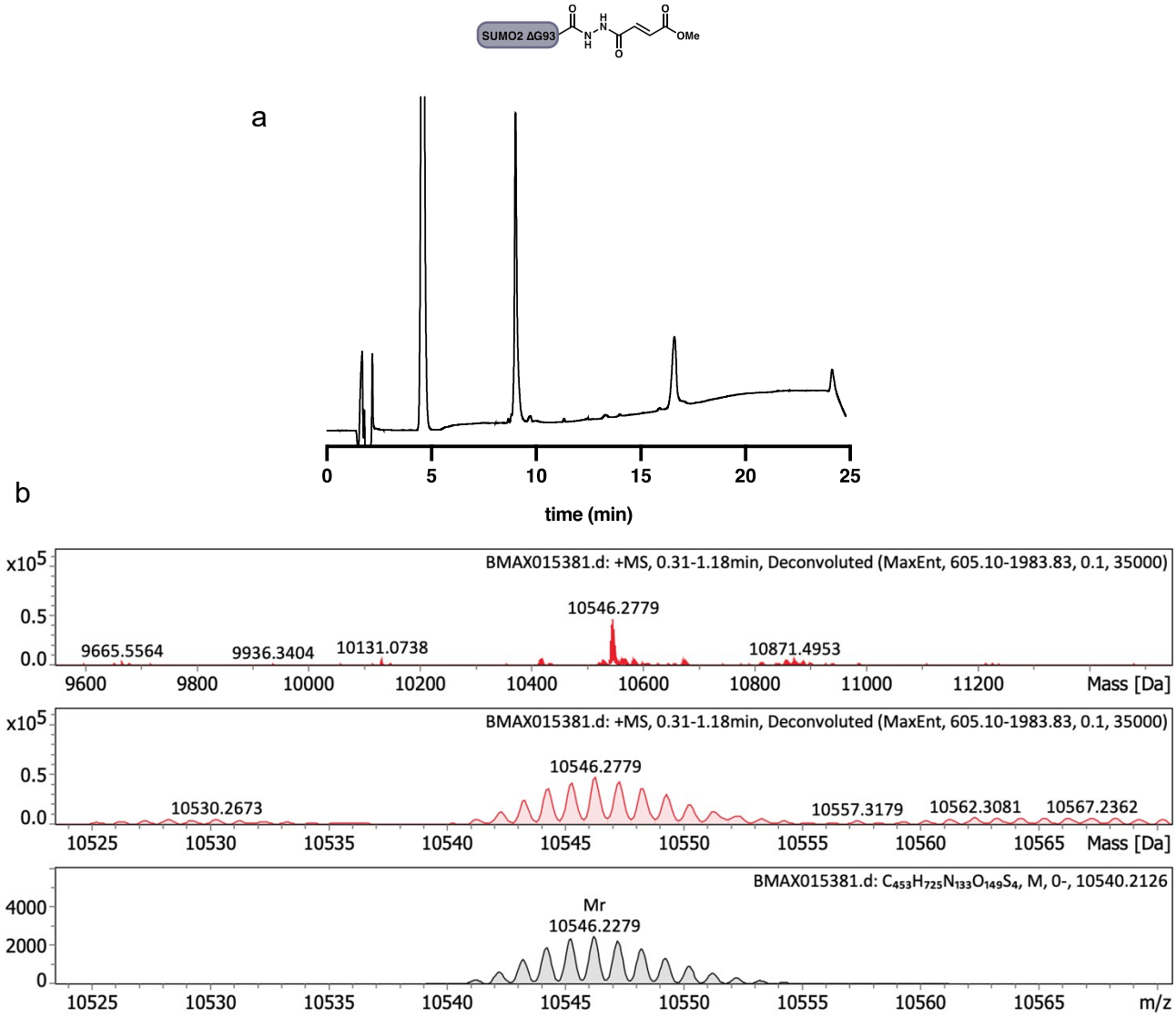
3.22. SUMO2(ΔG93)–NHNH α-chloroacetyl 18



HRMS (ESI): calculated for $[C_{450}H_{722}ClN_{133}O_{147}S_4]^+$: m/z 10510.1818, found: m/z 10510.2318

Figure S40 Characterization of SUMO2(ΔG93)–NHNH α-chloroacetyl (a) Analytical RP-HPLC of purified SUMO2(ΔG93)–NHNH. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔG93)–NHNH α-chloroacetyl showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

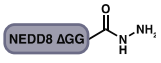
3.23. SUMO2(ΔG93)–NHNH methyl fumarate 20

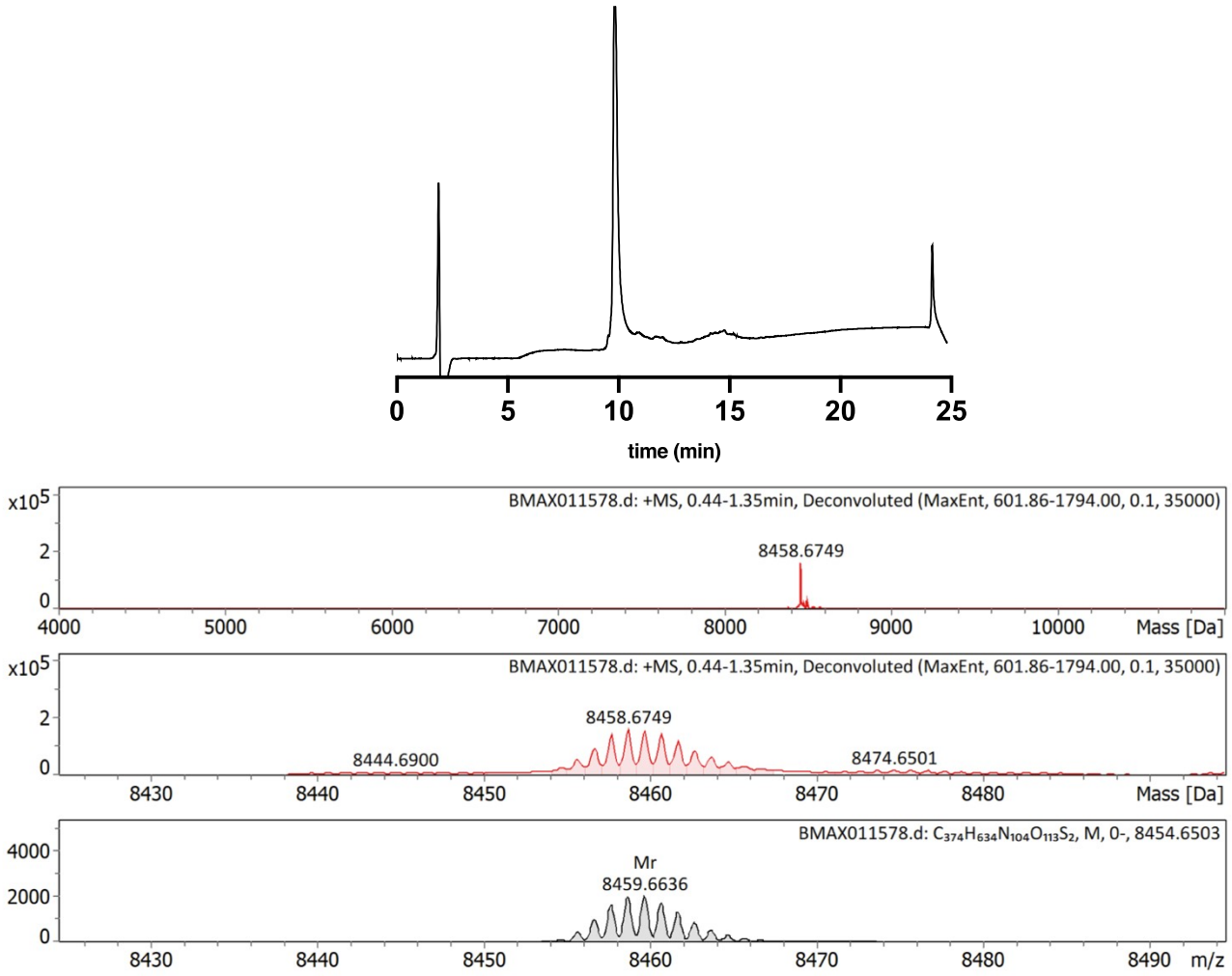


HRMS (ESI): calculated for $[C_{453}H_{725}N_{133}O_{149}S_4]^+$: m/z 10546.2279, found: m/z 10546.2779

Figure S41 Characterization of SUMO2(ΔG93)–NHNH methyl fumarate (a) Analytical RP-HPLC of purified SUMO2(ΔG93)–NHNH methyl fumarate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of SUMO2(ΔG93)–NHNH methyl fumarate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.24. NEDD8(ΔGG)–$NHNH_2$

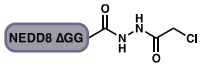


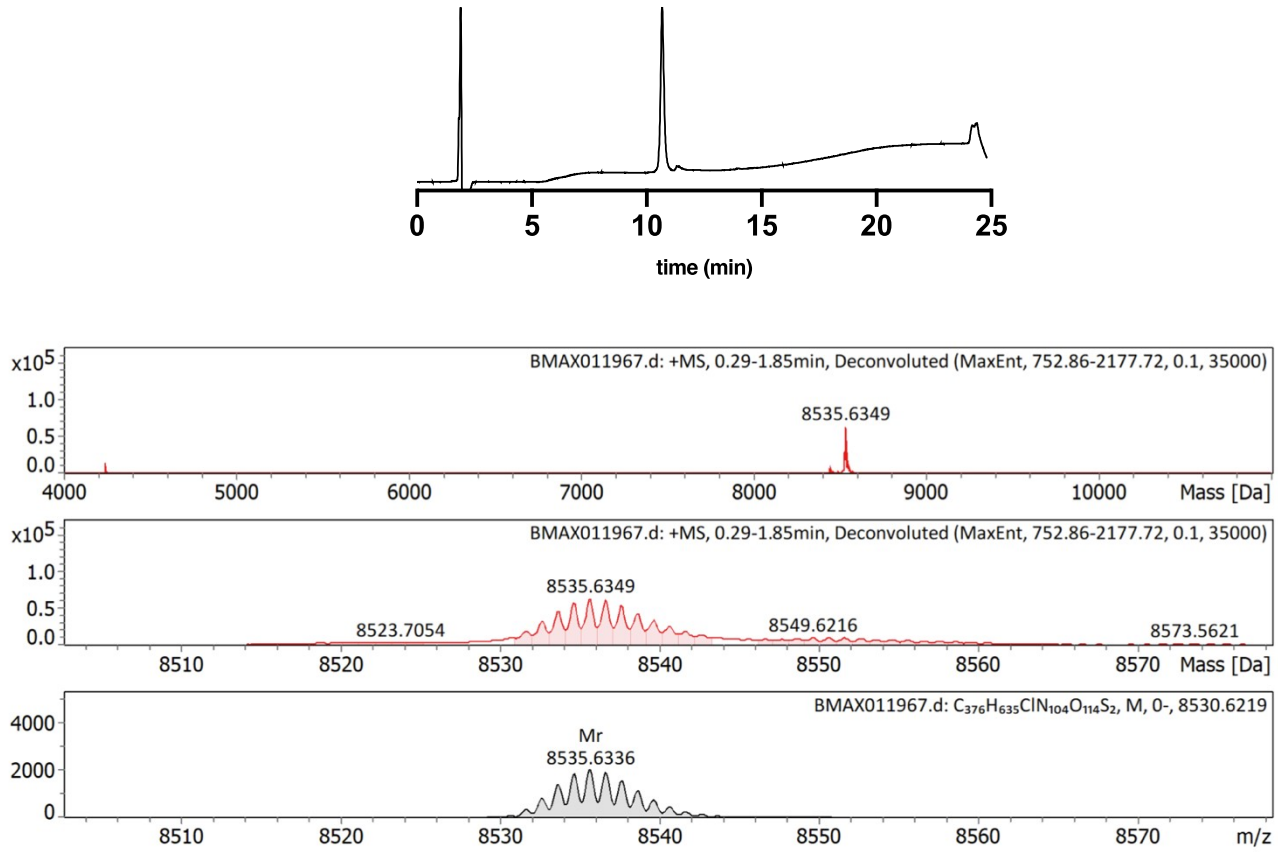


HRMS (ESI): calculated for $[C_{374}H_{634}N_{104}O_{113}S_2]^+$: m/z 8454.6503, found: m/z 8454.7913

Figure S42 Characterization of NEDD8(ΔGG)–NHNH$_2$ (a) Analytical RP-HPLC of purified NEDD8(ΔGG)–NHNH$_2$. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔGG)–NHNH$_2$ showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.25. NEDD8(ΔGG)–NHNH α-chloroacetyl 11

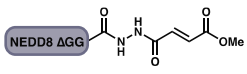




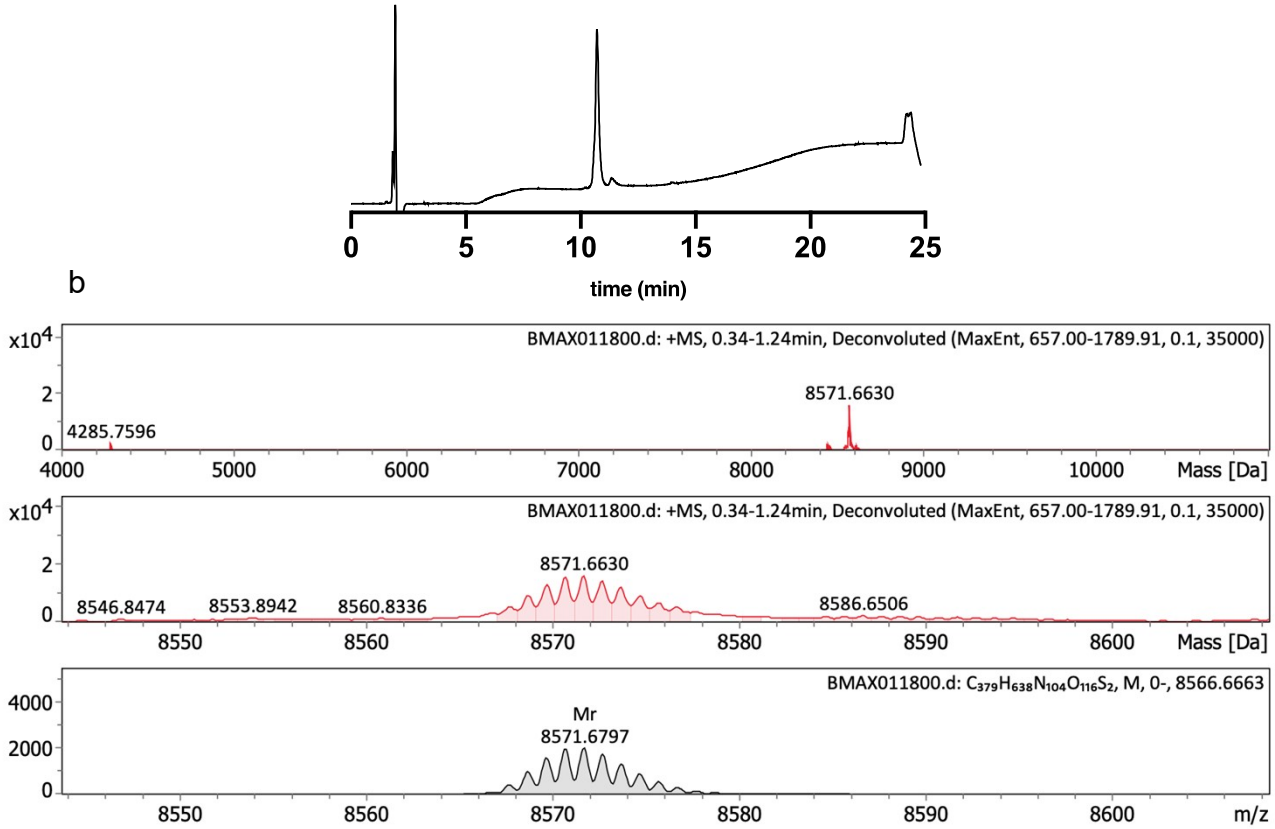
HRMS (ESI): calculated for $[C_{376}H_{635}ClN_{104}O_{114}S_2]^+$: m/z 8535.6336, found: m/z 8535.6349

Figure S43 Characterization of NEDD8(ΔGG)–NHNH α-chloroacetyl (a) Analytical RP-HPLC of purified NEDD8(ΔGG)–NHNH α-chloroacetyl. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔGG)–NHNH α-chloroacetyl showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.26. NEDD8(ΔGG)–NHNH methyl fumarate 13



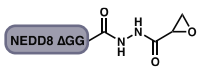
a



HRMS (ESI): calculated for $[C_{379}H_{638}N_{104}O_{116}S_2]^+$: m/z 8571.6797, found: m/z 8571.6630

Figure S44 Characterization of NEDD8(ΔGG)–NHNH methyl fumarate (a) Analytical RP-HPLC of purified NEDD8(ΔGG)–NHNH methyl fumarate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔGG)–NHNH methyl fumarate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.27. NEDD8(ΔGG)–NHNH glycidate 15



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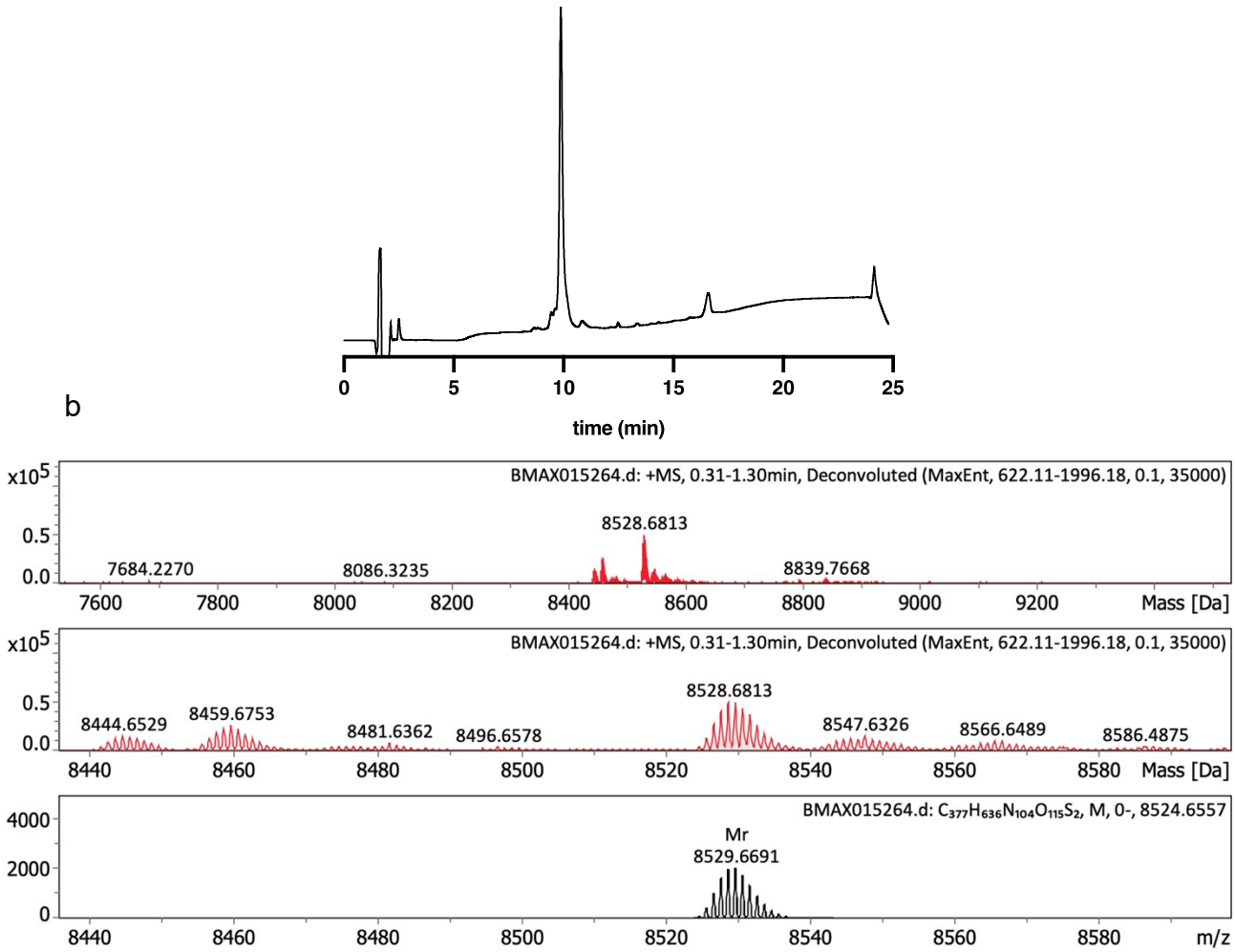
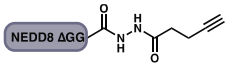


HRMS (ESI): calculated for $[C_{377}H_{636}N_{104}O_{115}S_2]^+$: m/z 8529.6691, found: m/z 8529.6860

Figure S45 Characterization of NEDD8(ΔGG)–NHNH glycidate (a) Analytical RP-HPLC of purified NEDD8(ΔGG)–NHNH glycidate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔGG)–NHNH glycidate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel). Ub(ΔGG)–$NHNH_2$ is seen with mass 8459.6753 Da. Ub(ΔGG) OH is seen with mass 8444.6529 Da.

3.28. NEDD8(ΔGG)–NHNH pentynoate 16



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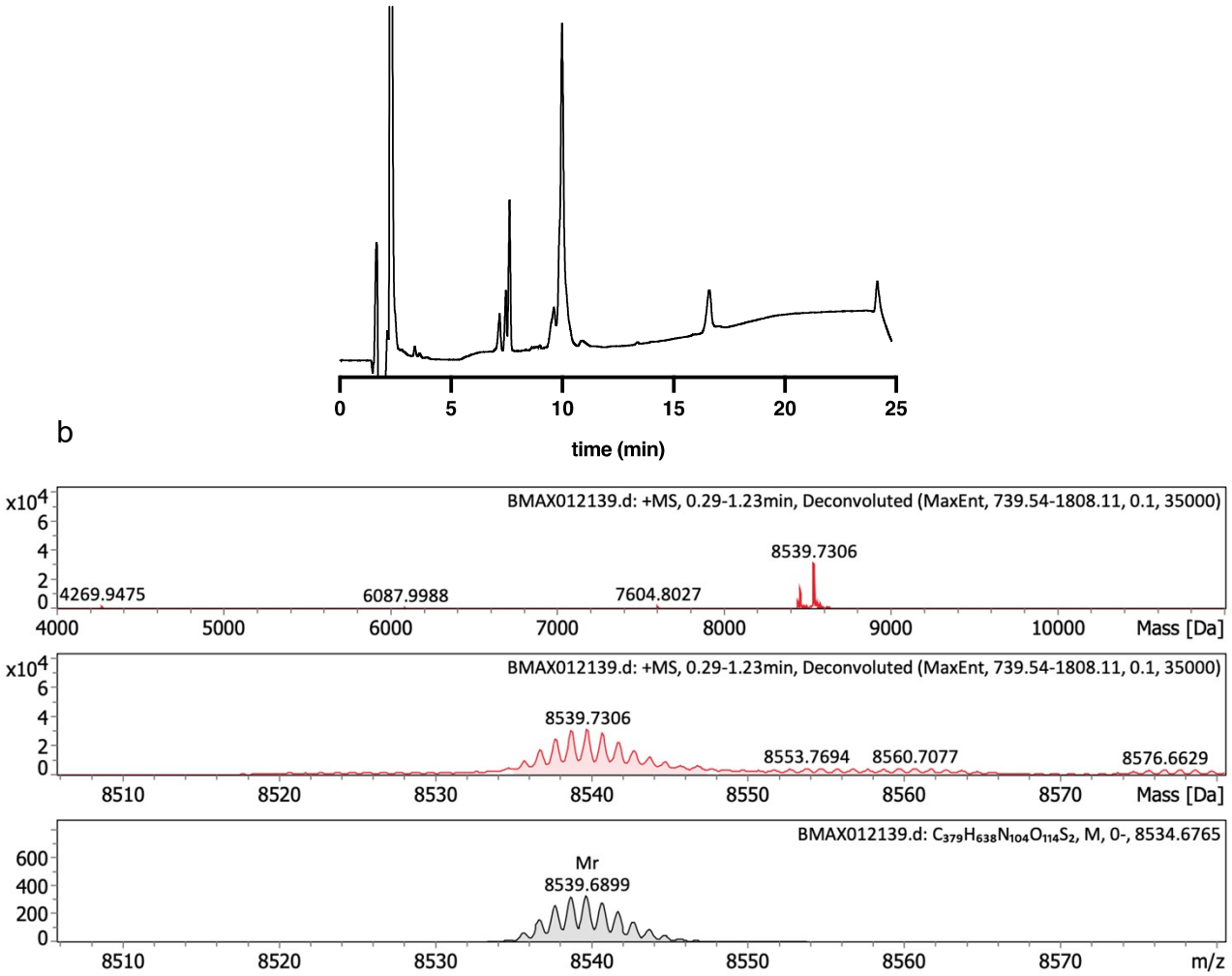
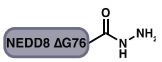
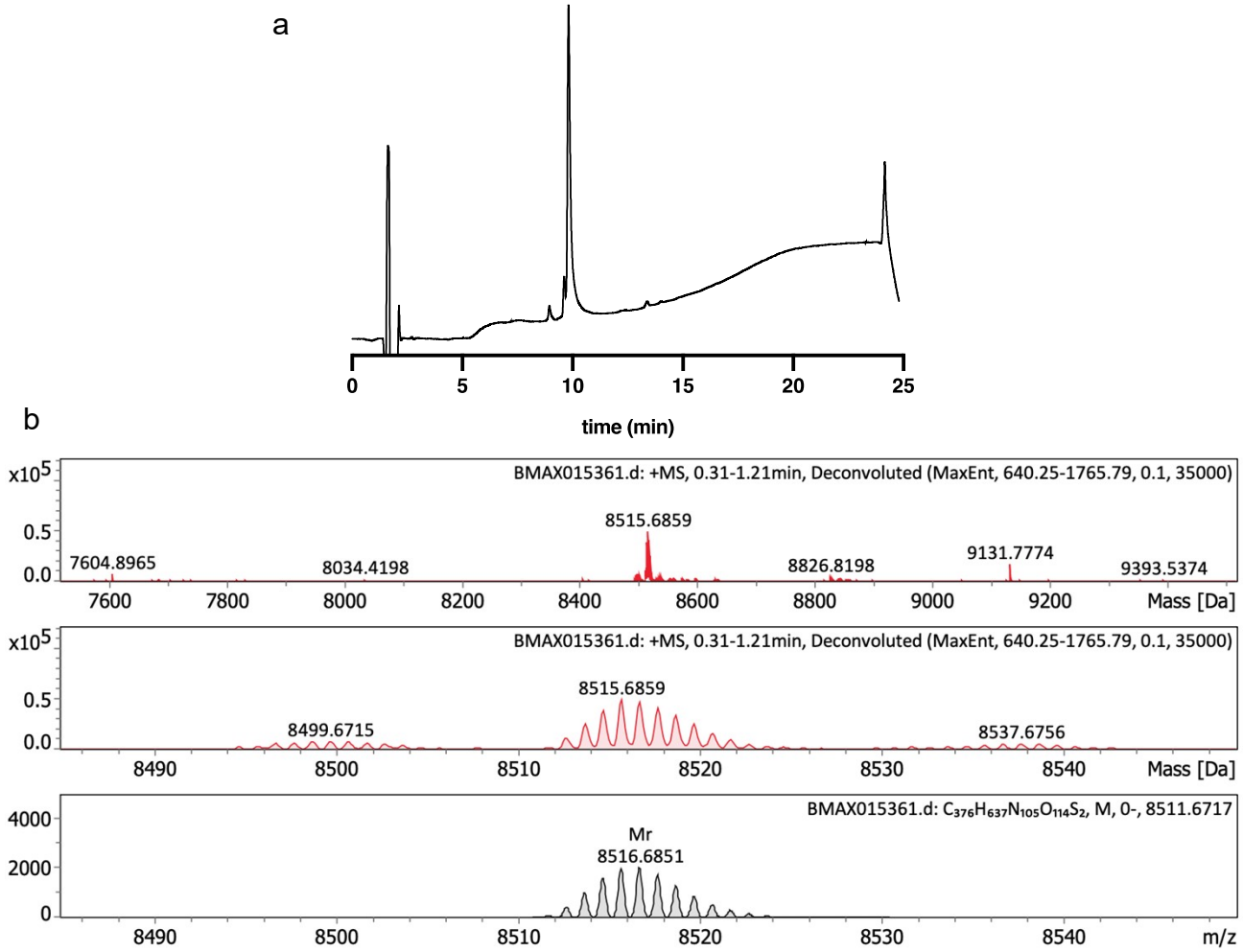


HRMS (ESI): calculated for $[C_{379}H_{638}N_{104}O_{114}S_2]^+$: m/z 8539.6899, found: m/z 8539.7306

Figure S46 Characterization of NEDD8(ΔGG)–NHNH pentynoate (a)Analytical RP-HPLC of purified NEDD8(ΔGG)–NHNH pentynoate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔGG)–NHNH pentynoate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.29. NEDD8(ΔG76)–$NHNH_2$

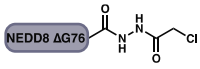


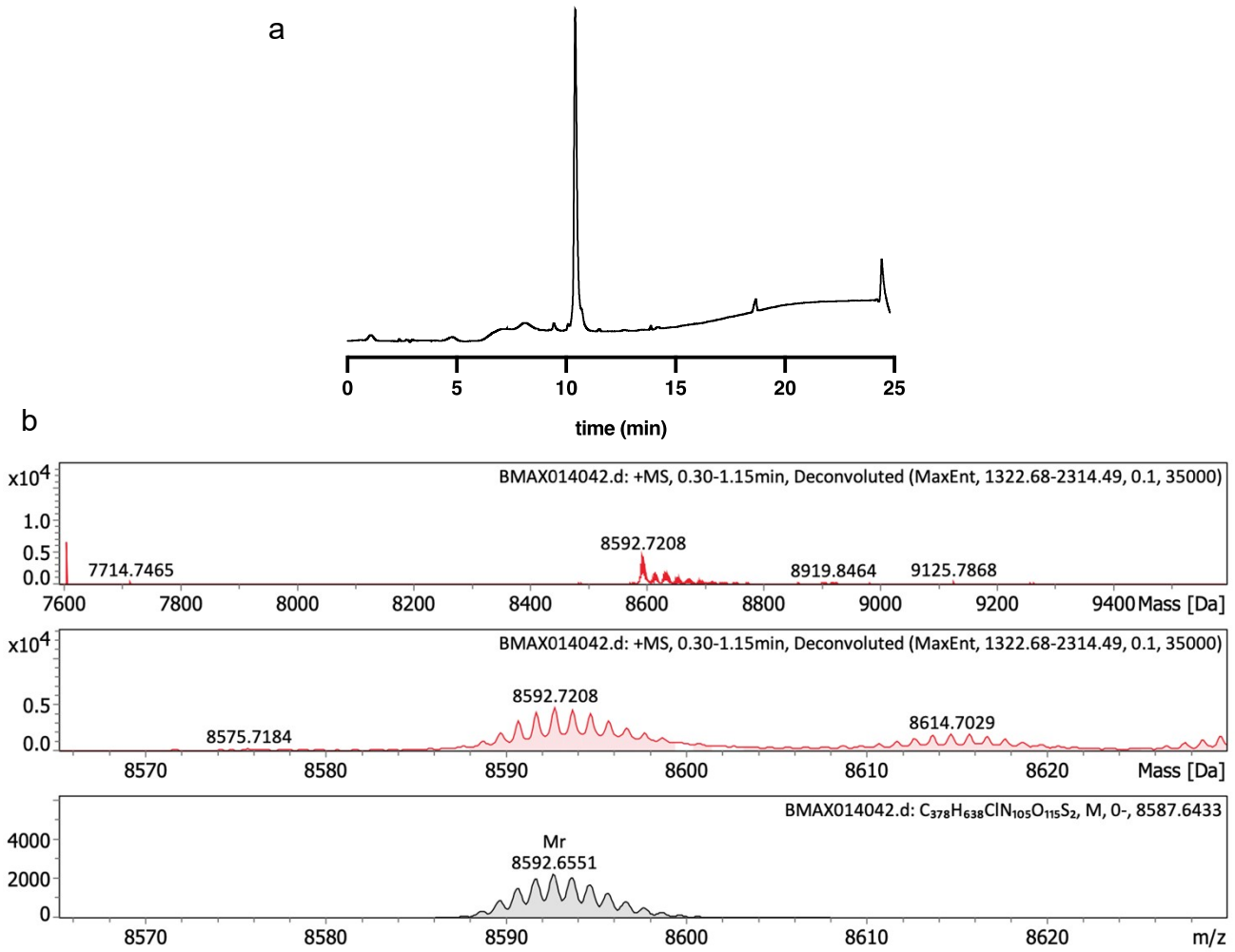


HRMS (ESI): calculated for $[C_{376}H_{637}N_{105}O_{114}S_2]^+$: m/z 8516.6851, found: m/z 8516.6939

Figure S47 Characterization of NEDD8(ΔG76)–NHNH$_2$ (a) Analytical RP-HPLC of purified NEDD8(ΔG76)–NHNH2. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔG76)–NHNH2 showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.30. NEDD8(ΔG76)–NHNH α-chloroacetyl 12

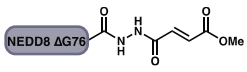




HRMS (ESI): calculated for $[C_{378}H_{638}ClN_{105}O_{115}S_2]^+$: m/z 8592.6551, found: m/z 8592.7208

Figure S48 Characterization of NEDD8(ΔG76)–NHNH α-chloroacetyl (a) Analytical RP-HPLC of purified NEDD8(ΔG76)–NHNH α-chloroacetyl. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔG76)–NHNH α-chloroacetyl showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.31. NEDD8(ΔG76)–NHNH methyl fumarate 14



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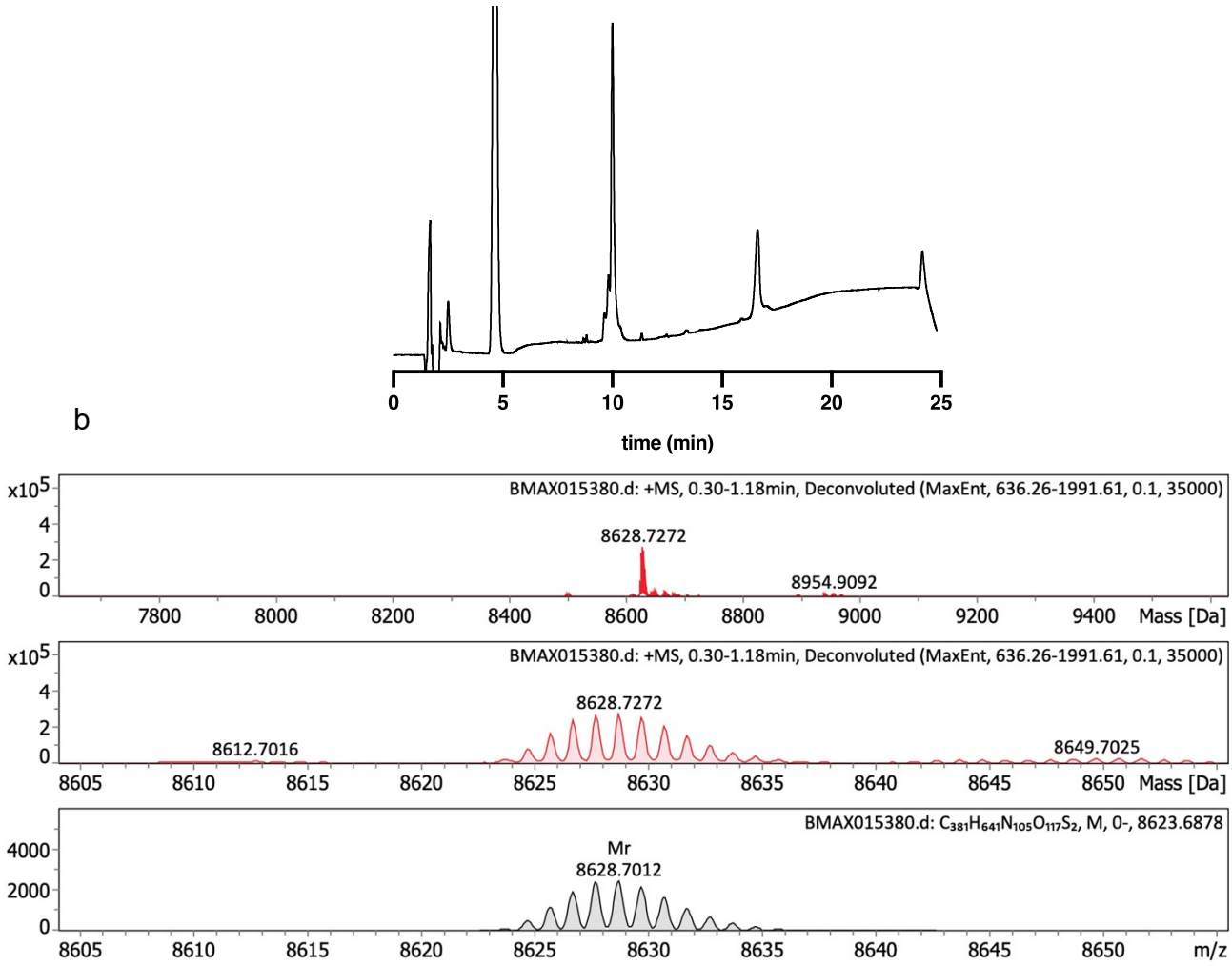
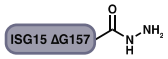


HRMS (ESI): calculated for $[C_{381}H_{641}N_{105}O_{117}S_2]^+$: m/z 8623.6878, found: m/z 8623.7455

Figure S49 Characterization of NEDD8(ΔG76)–NHNH methyl fumarate (a) Analytical RP-HPLC of purified NEDD8(ΔG76)–NHNH methyl fumarate. UV-absorption at 220 nm shown. (b) HRMS (ESI) spectrum of NEDD8(ΔG76)–NHNH methyl fumarate showing recorded mass spectrum (upper panel), zoom in of recorded spectrum (middle panel) and calculated spectrum (lower panel).

3.32. ISG15(ΔG157)–$NHNH_2$



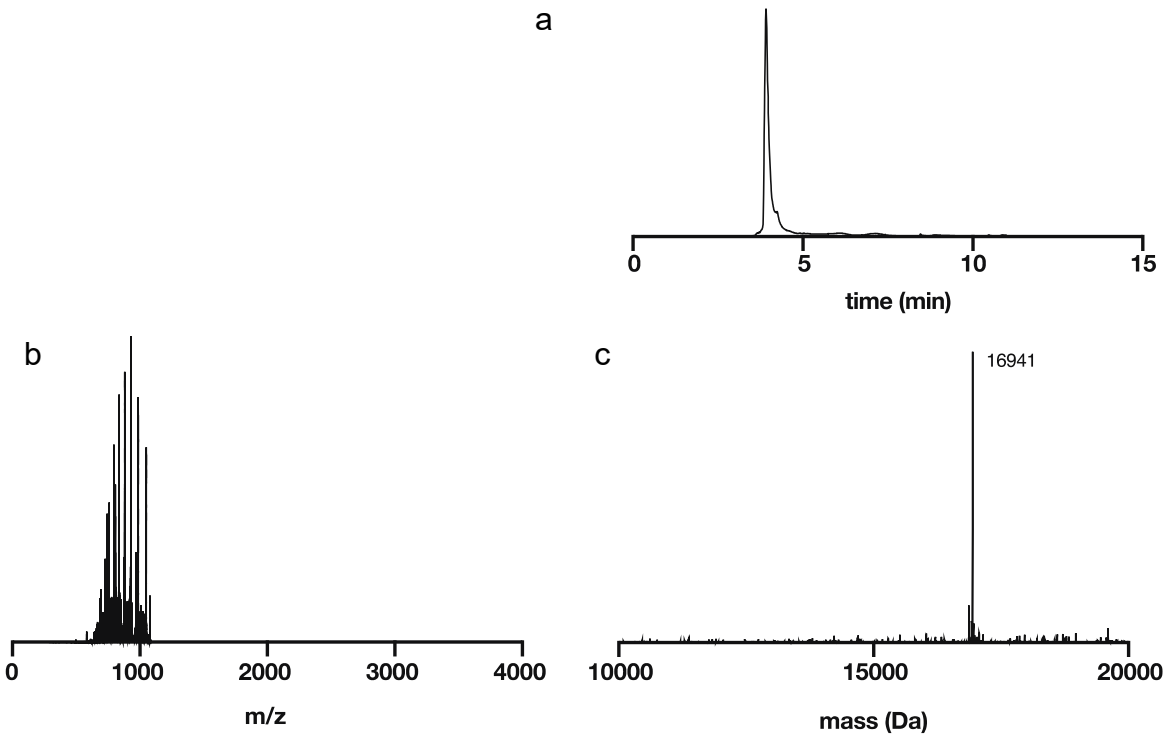
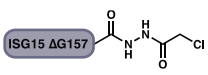


Figure S50 Characterization of ISG15(ΔG157)–NHNH$_2$ (a) Analytical RP-HPLC of purified ISG15(ΔG157)–NHNH$_2$. TIC shown. (b) MS (ESI) spectrum of ISG15(ΔG157)–NHNH$_2$ (left panel), (c) deconvoluted mass spectrum (right panel), calculated mass 16939 Da.

3.33. ISG15(ΔG157)–NHNH α-chloroacetyl 24



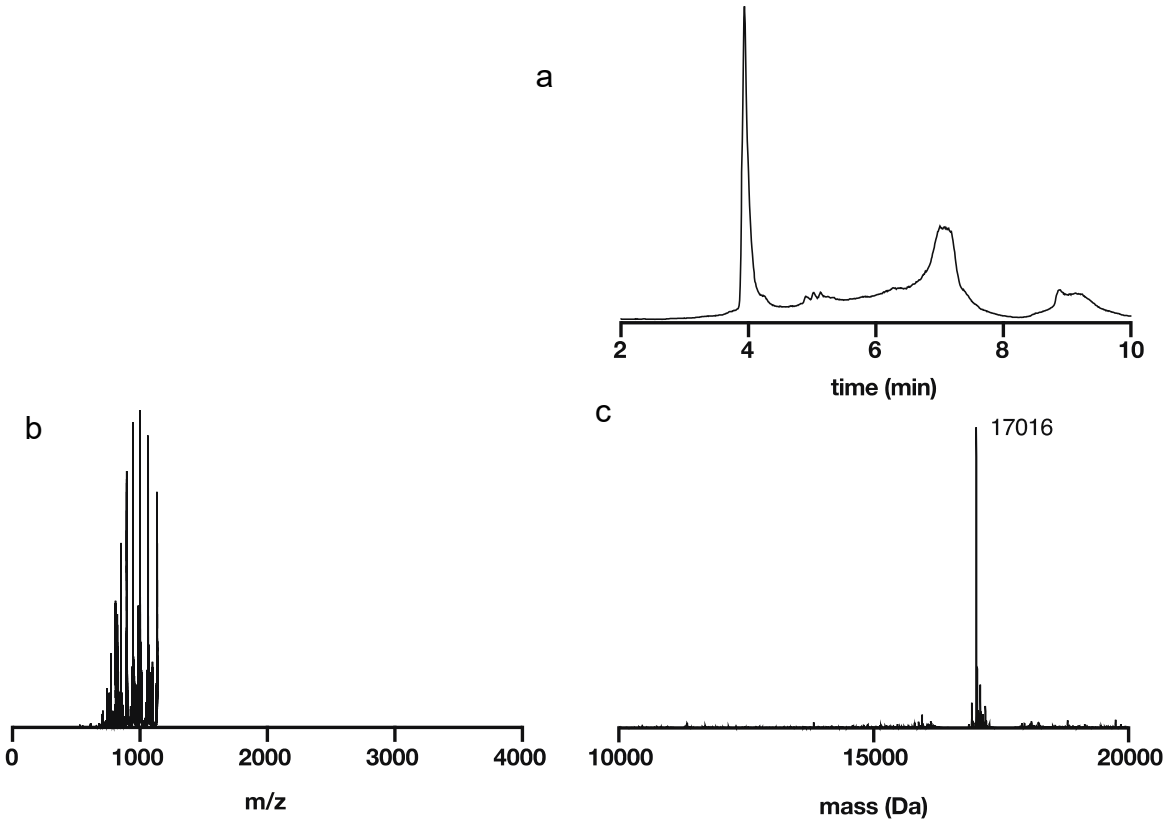
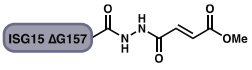


Figure S51 Characterization of ISG15(ΔG157)–NHNH α-chloroacetyl (a) Analytical RP-HPLC of purified ISG15(ΔG157)–NHNH α-chloroacetyl. TIC shown. (b) MS (ESI) spectrum (left panel), (c) deconvoluted mass spectrum (right panel), calculated mass 17016 Da.

3.34. ISG15(ΔG157) NHNH methyl fumarate 25



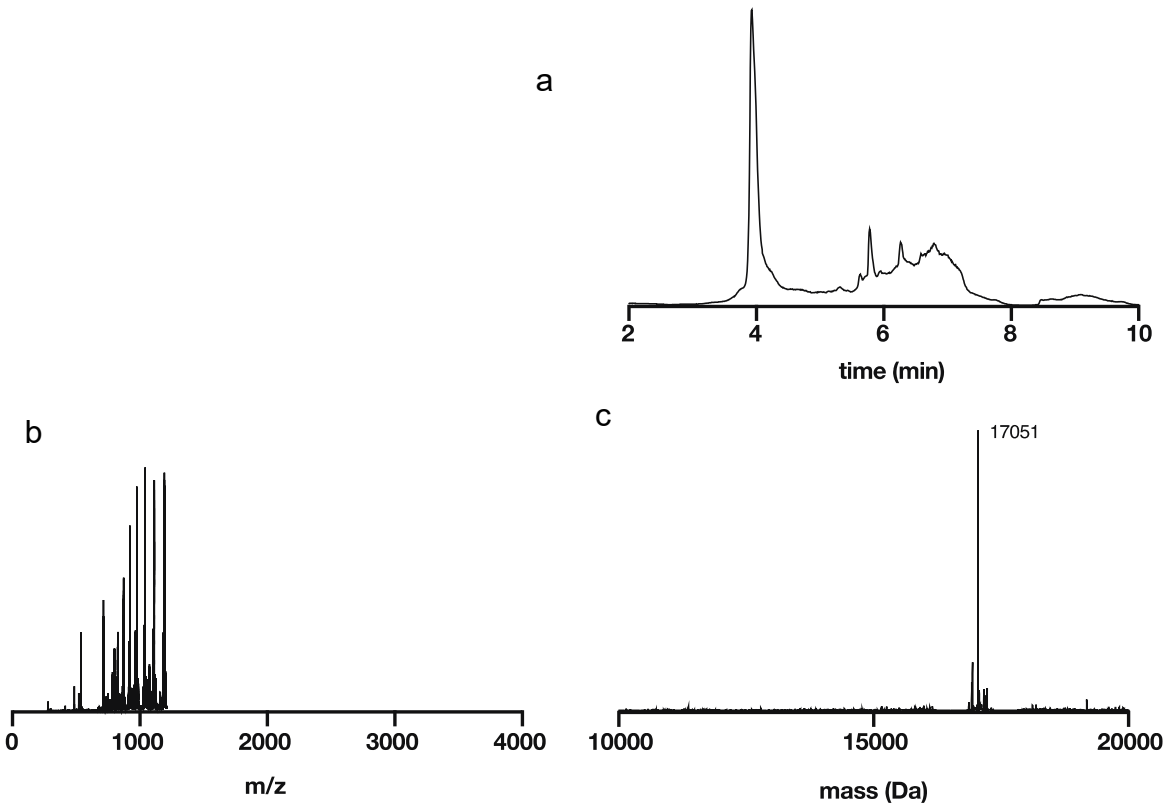


Figure S52 Characterization of ISG15(ΔG157)–NHNH methyl fumarate (a) Analytical RP-HPLC of purified ISG15(ΔG157)–NHNH methyl fumarate. TIC shown. (b) MS (ESI) spectrum (left panel), (c) deconvoluted mass spectrum (right panel), calculated mass 17051 Da.

3.35. FLAG-SUMO2(ΔGG)–$NHNH_2$

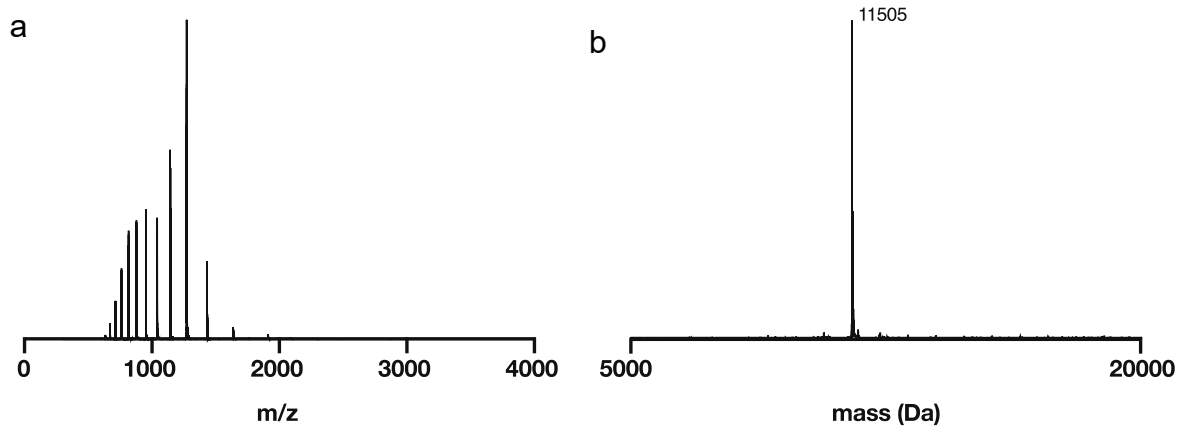
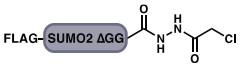


Figure S53 Characterization of FLAG-SUMO2 $NHNH_2$ (a) MS (ESI) spectrum (left panel), (b) deconvoluted mass spectrum (right panel), calculated mass 11504 Da.

3.36. FLAG-SUMO2(ΔGG)–NHNH α-chloroacetyl



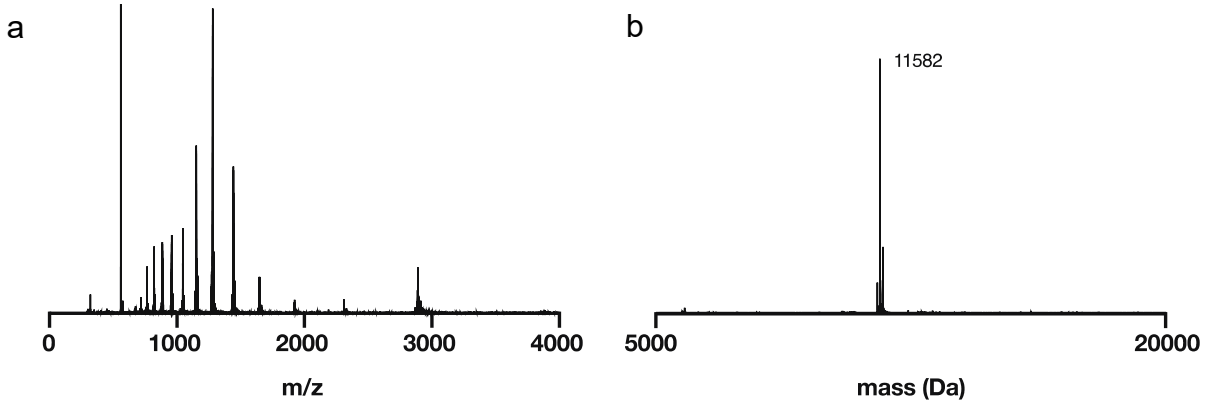
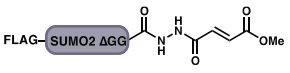


Figure S54 Characterization of FLAG-SUMO2 NHNH α-chloroacetyl (a) MS (ESI) spectrum (left panel), (b) deconvoluted mass spectrum (right panel), calculated mass 11580 Da.

3.37. FLAG-SUMO2(ΔGG)–NHNH methyl fumarate



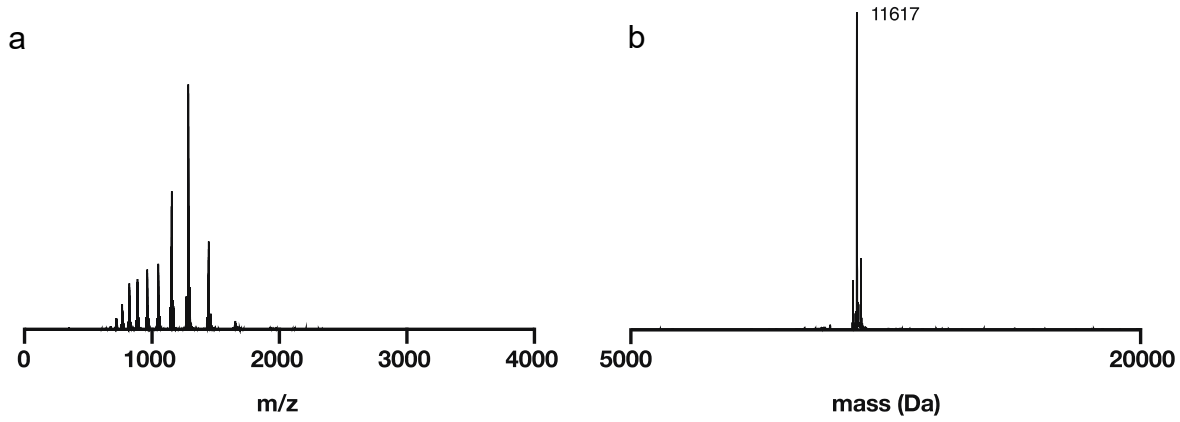
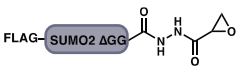


Figure S55 Characterization of FLAG-SUMO2 NHNH methyl fumarate (a) MS (ESI) spectrum (left panel), (b) deconvoluted mass spectrum (right panel), calculated mass 11616.

3.38. FLAG-SUMO2(ΔGG)–$NHNH_2$ glycidate



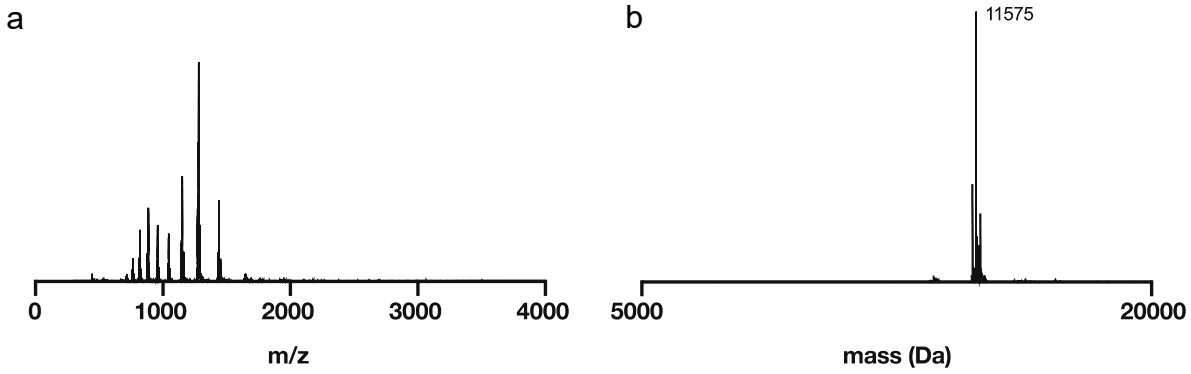
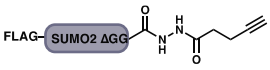


Figure S56 Characterization of FLAG-SUMO2 NHNH glycidate (a) MS (ESI) spectrum (left panel), (b) deconvoluted mass spectrum (right panel), calculated mass 11574 Da.

3.39. FLAG-SUMO2(ΔGG)–NHNH pentynoate



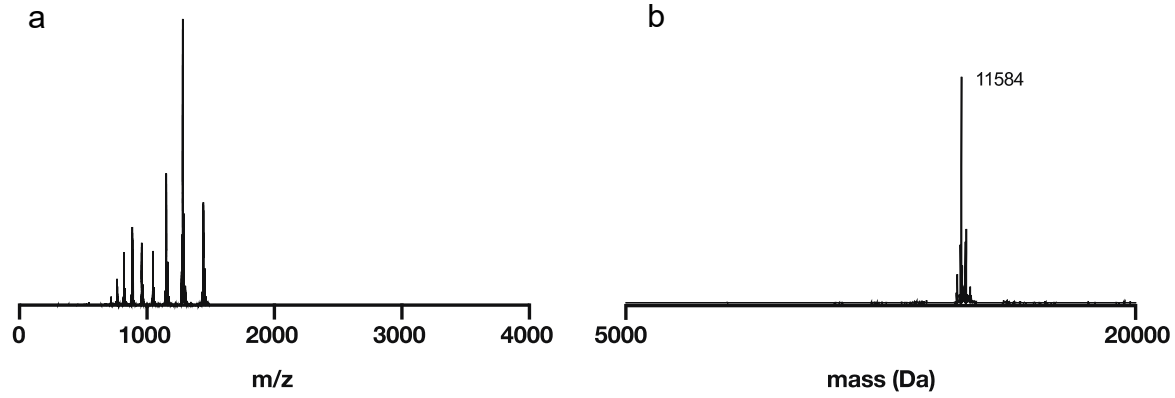


Figure S57 Characterization of FLAG-SUMO2 NHNH pentynoate (a) MS (ESI) spectrum (left panel), (b) deconvoluted mass spectrum (right panel), calculated mass 11584.

3.40. FLAG-SUMO2(ΔGG)–NHNH acetyl

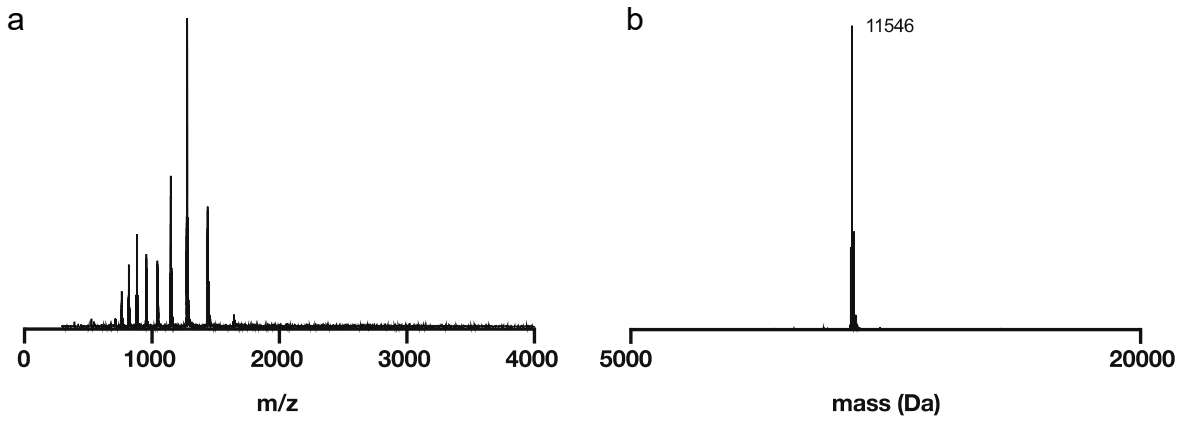


Figure S58 Characterization of FLAG-SUMO2 NHNH acetyl (a) MS (ESI) spectrum (left panel), (b) deconvoluted mass spectrum (right panel), calculated mass 11546.

3.41. Ub(ΔG76)–VME

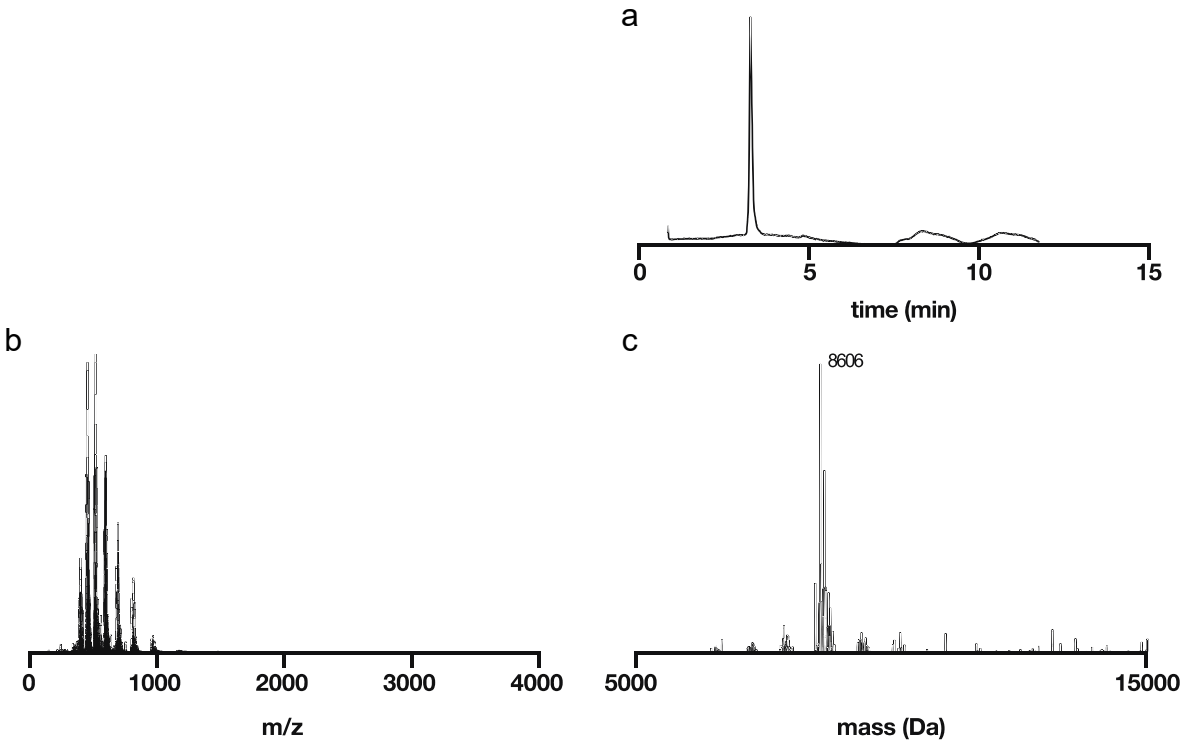


Figure S59 Characterization of Ub(ΔG76)–VME (a) Analytical RP-HPLC of purified Ub(ΔG76)–VME. TIC shown. (b) MS (ESI) spectrum (left panel), (c) deconvoluted mass spectrum (right panel), calculated mass 8605 Da.

3.42. Ub G76Dha

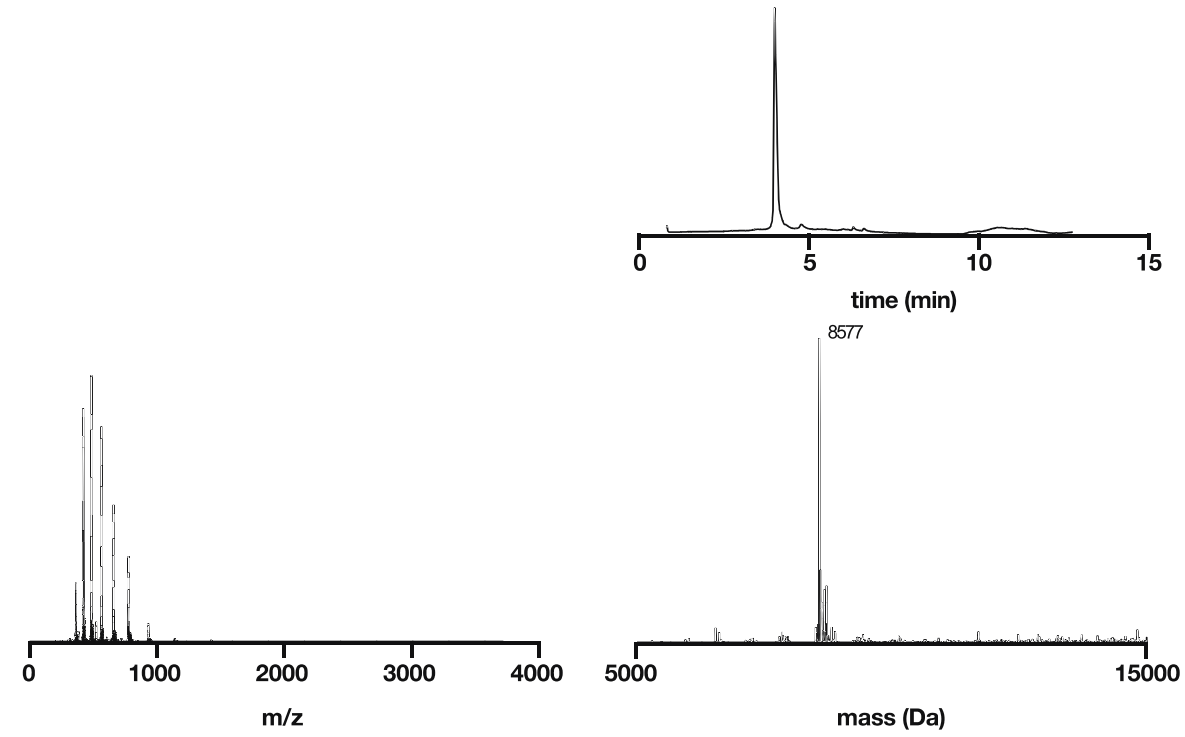


Figure S59 Characterization of Ub G76Dha (a) Analytical RP-HPLC of purified UbG76Dha. TIC shown. (b) MS (ESI) spectrum (left panel), (c) deconvoluted mass spectrum (right panel), calculated mass 8576 Da.

4. Small molecule synthesis

4.1. Synthesis of α-chloroacetyl NHS-ester

N-hydroxysuccinimide (640 mg) was dissolved in $CHCl_3$ (8.5 mL) and cooled to 0 ºC under inert atmosphere. Triethylamine (850 µL, 1.1 eqiv.) was added and the mixture was stirred for 10 min. α-chloroacetyl (880 µL, 2 equiv.) was added over 10 min. After a further 20 min cold H_2O was added. The organic phase was separated and washed with cold brine. The organic phase was further dried using NaS_2O_4. The solution was concentrated to ca. 2 mL and EtOAc (170 µL) and hexane (1.2 mL) were added and stirred for 1 h on ice. The suspension was filtered and the solid was successively washed with cold hexane/EtOAc (4:1, 9:1) and cold hexane. The solid was further dried under vacuum to obtain the product as a white powder (690 mg, 65%).
^{1}H NMR (400 MHz, $CDCl_3$): δ 4.37 (2H, s), 2.86 (4H, s)
^{13}C NMR (101 MHz, $CDCl_3$): δ 168.55, 163.34, 37.97, 25.67.

4.2. Synthesis of glycidic acid

Glycidic acid was synthesized from (±)-glycidol by adapting a previously published procedure.[8] Glycidol (6.75 mmol) was given to MeCN (5 mL), H_2O (300 µL). $NaIO_4$ (23.6 mmol, 3.5 equiv.) was added followed by $RuCl_3 \bullet 3H_2O$ (0.34 mmol, 0.05 equiv.). The reaction was stirred for 3 h. Diethyl ether was added and the reaction mixture filtered through Celite and concentrated. The crude mixture was purified using a short SiO_2-column using EtOAc as eluant. The product was obtained as a light brown oil (259 mg, 44%)
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.48 (1H, dd), 3.01-3.02 (2H, d)
^{13}C NMR (101 MHz, $CDCl_3$): δ 175.13, 47.04, 46.80

4.3. Synthesis of VME-TFA salt

Aminovinylmethyl ester-TFA salt was prepared according to previously reported procedures[9].
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (3H, s), 6.85 (1H, dt), 6.15 (1H, dt), 3.70 (5H, m)
^{13}C NMR (101 MHz, $CDCl_3$): δ 165.84, 158.78, 141.09, 132.77, 119.17, 116.19, 52.17, 36.67

5. Supplementary Tables

5.1. Reaction conditions for Ubl-$NHNH_2$ modification with anhydrides

Ubl-$NHNH_2$	α-chloroacetyl	methyl fumarate	glycidate	pentynoate	acetyl
Ub(ΔGG)	200 equiv.	200 equiv.	300 equiv.	200 equiv.	20 equiv.
Ub(ΔG76)	200 equiv.	200 equiv.	300 equiv.	200 equiv.	10 equiv.
NEDD8(ΔGG)	200 equiv.	200 equiv.	300 equiv.	200 equiv.	Not prepared
NEDD8(ΔG76)	200 equiv.	200 equiv.	Not prepared	Not prepared	Not prepared
SUMO2(ΔGG)	200 equiv.	200 equiv.	300 equiv.	200 equiv.	20 equiv
SUMO2(ΔG76)	200 equiv.	200 equiv.	Not prepared	Not prepared	Not prepared
ISG15(ΔG157)	200 equiv.	8 equiv.	Not prepared	Not prepared	Not prepared
FLAG-SUMO2(ΔGG)	200 equiv.	100 equiv.	300 equiv.	200 equiv.	10 equiv.

Table S2 Reaction conditions for Ubl-$NHNH_2$ modifications. Anhydride equivalents used for each modification shown.

5.2. Amino acid sequences of recombinantly expressed proteins

Protein	Amino acid sequence	Comments

Ubiquitin (ΔGG) -**GyrA-His$_6$**	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQL EDGRTLSDYNIQKESTLHLVLRLR**CITGDALVALPEGESVRIADIVPGAR PNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVYTVRTVEGLRVTGTANHP LLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCAGFARGKPEFAP TTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQP VYSLRVDTADHAFITNGFVSHALEHHHHHH**	*Mxe*-GyrA intein with C-terminal His$_6$
Ubiquitin (ΔG76)-**GyrA-His$_6$**	MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQL EDGRTLSDYNIQKESTLHLVLRLRG**CITGDALVALPEGESVRIADIVPGA RPNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVYTVRTVEGLRVTGTANH PLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCAGFARGKPEFA PTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQ PVYSLRVDTADHAFITNGFVSHALEHHHHHH**	*Mxe*-GyrA intein with C-terminal His$_6$
SUMO2 (ΔGG) -**GyrA-His$_6$**	MADEKPKEGVKTENNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCER QGLSMRQIRFRFDGQPINETDTPAQLEMEDEDTIDVFQQQT**CITGDALVA LPEGESVRIADIVPGARPNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVY TVRTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSA FSVDCAGFARGKPEFAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDG RFYYAKVASVTDAGVQPVYSLRVDTADHAFITNGFVSHALEHHHHHH**	*Mxe*-GyrA intein with C-terminal His$_6$
SUMO2 (ΔG93)-**GyrA-His$_6$**	MADEKPKEGVKTENNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYCER QGLSMRQIRFRFDGQPINETDTPAQLEMEDEDTIDVFQQQTG**CITGDALV ALPEGESVRIADIVPGARPNSDNAIDLKVLDRHGNPVLADRLFHSGEHPV YTVRTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRS AFSVDCAGFARGKPEFAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTD GRFYYAKVASVTDAGVQPVYSLRVDTADHAFITNGFVSHALEHHHHHH**	*Mxe*-GyrA intein with C-terminal His$_6$
NEDD8 (ΔGG) -**GyrA-His$_6$**	MLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQQRLIYSGKQM NDEKTAADYKILGGSVLHLVLALR**CITGDALVALPEGESVRIADIVPGAR PNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVYTVRTVEGLRVTGTANHP LLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCAGFARGKPEFAP TTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQP VYSLRVDTADHAFITNGFVSHALEHHHHHH**	*Mxe*-GyrA intein with C-terminal His$_6$
NEDD8 (ΔG76)-**GyrA-His$_6$**	MLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQQRLIYSGKQM NDEKTAADYKILGGSVLHLVLALRG**CITGDALVALPEGESVRIADIVPGA RPNSDNAIDLKVLDRHGNPVLADRLFHSGEHPVYTVRTVEGLRVTGTANH PLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCAGFARGKPEFA PTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQ PVYSLRVDTADHAFITNGFVSHALEHHHHHH**	*Mxe*-GyrA intein with C-terminal His$_6$
ISG15 (C78A, ΔG157)-**GyrA-His$_6$**	MGWDLTVKMLAGNEFQVSLSSSMSVSELKAQITQKIGVHAFQQRLAVHPS GVALQDRVPLASQGLGPGSTVLLVVDKADEPLSILVRNNKGRSSTYEVRL TQTVAHLKQQVSGLEGVQDDLFWLTFEGKPLEDQLPLGEYGLKPLSTVFM NLRLRG**CITGDALVALPEGESVRIADIVPGARPNSDNAIDLKVLDRHGNP VLADRLFHSGEHPVYTVRTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLI DEIKPGDYAVIQRSAFSVDCAGFARGKPEFAPTTYTVGVPGLVRFLEAHH RDPDAQAIADELTDGRFYYAKVASVTDAGVQPVYSLRVDTADHAFITNGF VSHALEHHHHHH**	*Mxe*-GyrA intein with C-terminal His$_6$
FLAG-SUMO2 (ΔGG) -**GyrA-His$_6$**	MDYKDDDDKADEKPKEGVKTENNDHINLKVAGQDGSVVQFKIKRHTPLSK LMKAYCERQGLSMRQIRFRFDGQPINETDTPAQLEMEDEDTIDVFQQQT**C ITGDALVALPEGESVRIADIVPGARPNSDNAIDLKVLDRHGNPVLADRLF HSGEHPVYTVRTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDEIKPGD YAVIQRSAFSVDCAGFARGKPEFAPTTYTVGVPGLVRFLEAHHRDPDAQA IADELTDGRFYYAKVASVTDAGVQPVYSLRVDTADHAFITNGFVSHALEH HHHHH**	N-terminal FLAG tag, *Mxe*-GyrA intein with C-terminal His$_6$
His-TEV-Ube2K (C170S)	MGSSHHHHHHSSGAENLYFQGMANIAVQRIKREFKEVLKSEETSKNQIKV DLVDENFTELRGEIAGPPDTPYEGGRYQLEIKIPETYPFNPPKVRFITKI WHPNISSVTGAICLDILKDQWAAAMTLRTVLLSLQALLAAAEPDDPQDAV VANQYKQNPEMFKQTARLWAHVYAGAPVSSPEYTKKIENLSAMGFDRNAV IVALSSKSWDVETATELLLSN	N-terminal His$_6$ with TEV cleavage site
His-TEV-Ube2G1	MGSSHHHHHHSSGAENLYFQGMTELQSALLLRRQLAELNKNPVEGFSAGL IDDNDLYRWEVLIIGPPDTLYEGGVFKAHLTFPKDYPLRPPKMKFITEIW	N-terminal His$_6$ with TEV

	HPNVDKNGDVAISILHEPGEDKYGYEKPEERWLPIHTVETIMISVISMLA DPNGDSPANVDAAKEWREDRNGEFKRKVARCVRKSQETAFE	cleavage site
His-TEV-Ube2L3	MGSSHHHHHHSSGAENLYFQGMAASRRLMKELEEIRKCGMKNFRNIQVDE ANLLTWQGLIVPDNPPYDKGAFRIEINFPAEYPFKPPKITFKTKIYHPNI DEKGQVCLPVISAENWKPATKTDQVIQSLIALVNDPQPEHPLRADLAEEY SKDRKKFCKNAEEFTKKYGEKRPVD	N-terminal His_6 with TEV cleavage site
His-thrombin-SENP1 (419-644)	MGSSHHHHHHSSGLVPRGSHMEFPEITEEMEKEIKNVFRNGNQDEVLSEA FRLTITRKDIQTLNHLNWLNDEIINFYMNMLMERSKEKGLPSVHAFNTFF FTKLKTAGYQAVKRWTKKVDVFSVDILLVPIHLGVHWCLAVVDFRKKNIT YYDSMGGINNEACRILLQYLKQESIDKKRKEFDTNGWQLFSKKSQEIPQQ MNGSDAGMFACKYADCITKDRPINFTQQHMPYFRKRMVWEILHRKLL	N-terminal His_6 with thrombin cleavage site
His-thrombin SENP8	MGSSHHHHHHSSGLVPRGSHMDPVVLSYMDSLLRQSDVSLLDPPSWLNDH IIGFAFEYFANSQFHDCSDHVSFISPEVTQFIKCTSNPAEIAMFLEPLDL PNKRVVFLAINDNSNQAAGGTHWSLLVYLQDKNSFFHYDSHSRSNSVHAK QVAEKLEAFLGRKGDKLAFVEEKAPAQQNSYDAGMYVICNTEALCQNFFR QQTESLLQLLTPAYITKKRGEWKDLIATLATK	N-terminal His_6 with thrombin cleavage site
His-SUMO-USP21 (196-565)	**MGSSHHHHHHGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIK KTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIE AHREQIGG**SDDKMAHHTLLLGSGHVGLRNLGNTCFLNAVLQCLSSTRPLR DFCLRRDFRQEVPGGGRAQELTEAFADVIGALWHPDSCEAVNPTRFRAVF QKYVPSFSGYSQQDAQEFLKLLMERLHLEINRRGRRAPPILANGPVPSPP RRGGALLEEPELSDDDRANLMWKRYLEREDSKIVDLFVGQLKSCLKCQAC GYRSTTFEVFCDLSLPIPKKGFAGGKVSLRDCFNLFTKEEELESENAPVC DRCRQKTRSTKKLTVQRFPRILVLHLNRFSASRGSIKKSSVGVDFPLQRL SLGDFASDKAGSPVYQLYALCNHSGSVHYGHYTALCRCQTGWHVYNDSRV SPVSENQVASSEGYVLFYQLMQEPPRCL	N-terminal His_6 SUMO

Table S3 Amino acid sequences of recombinantly expressed proteins used in this study. Purification handles cleaved after expression are shown in bold.

5.3. Amino acid sequences of recombinantly expressed proteins

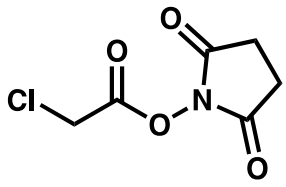
Protein	Mutation	Primer	Sequence
Ubiquitin **GyrA-His_6**	GG deletion	Forward primer (5'–3')	TGCATTACGGGTGATGCGCT
		Reverse primer (5'–3')	GCGCAGACGCAGAACCAG
Ubiquitin **GyrA-His_6**	G76 deletion	Forward primer (5'–3')	TGCATTACGGGTGATGCGC
		Reverse primer (5'–3')	ACCGCGCAGACGCAGAAC
SUMO2 **GyrA-His_6**	GG deletion	Forward primer (5'–3')	TGCATTACGGGTGATGCGCTG
		Reverse primer (5'–3')	CGTCTGCTGTTGGAACACATCAATTG
SUMO2 **GyrA-His_6**	G93 deletion	Forward primer (5'–3')	GGTTGCATTACGGGTGATGCG
		Reverse primer (5'–3')	CGTCTGCTGTTGGAACACATCAAT
NEDD8 **GyrA-His_6**	GG deletion	Forward primer (5'–3')	TGCATTACGGGTGATGCGCTG
		Reverse primer (5'–3')	ACGCAGTGCCAGAACCAGAT
NEDD8 **GyrA-His_6**	G76 deletion	Forward primer (5'–3')	TGCATTACGGGTGATGCGCTG
		Reverse primer (5'–3')	ACCACGCAGTGCCAGAAC
ISG15 **GyrA-His_6**	G157 deletion	Forward primer (5'–3')	TGCATTACGGGTGATGCGC

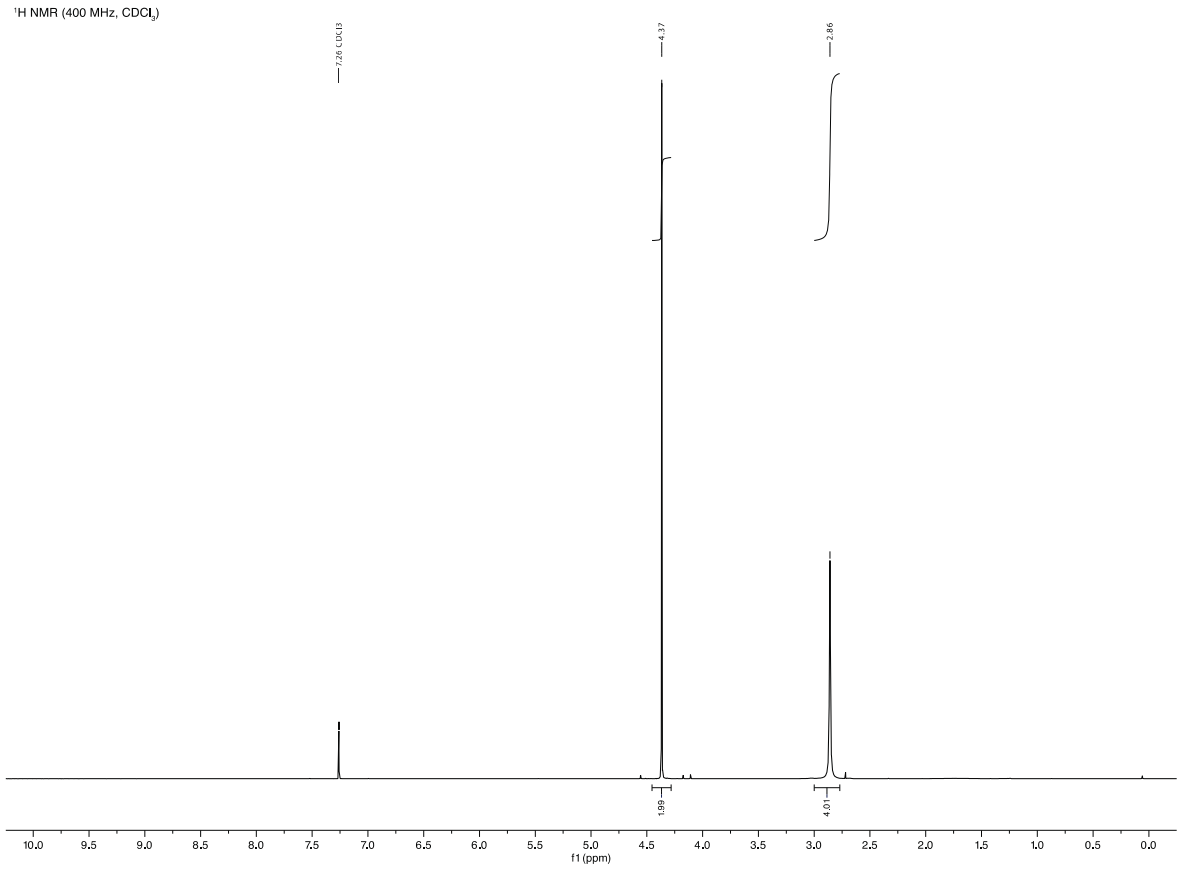
		Reverse primer (5'–3')	GCCACGCAAGCGAAGGTT
His_6-TEV-Ube2K	C92A	Forward primer (5'–3')	GCGTTGGATATCCTGAAAGATCAATGGGCAGC
		Reverse primer (5'–3')	AATAGCCCCTGTGACGGAACTAATATTAGG
His_6-TEV-UbeG1	C90A	Forward primer (5'–3')	GCGATTTCTATTCTTCATGAGCCTGGGGAAGA
		Reverse primer (5'–3')	CACATCACCATTTTTATCAACATTTGGGTGC
His_6-TEV-Ube2L3	C86A	Forward primer (5'–3')	CGGACAGATTGCGCTGCCCATCATCAGCAG
		Reverse primer (5'–3')	TTCTCGTCCACGTTGGGG
His_6-thrombin-SENP1	C603A	Forward primer (5'–3')	TGGAAGTGACGCGGGGATGTTTGCC
		Reverse primer (5'–3')	TTCATCTGCTGAGGAATC
His_6-thrombin-SENP8	C163A	Forward primer (5'–3')	GCGGGGATGTACGTGATATGTAACACTGAGGC
		Reverse primer (5'–3')	GTCATAGCTGTTTTGTTGGGCAGGG
His_6-SUMO-USP21	C221A	Forward primer (5'–3')	GGGAAACACGGCGTTCCTGAATGCTGTG
		Reverse primer (5'–3')	AGGTTTCGAAGGCCAACA

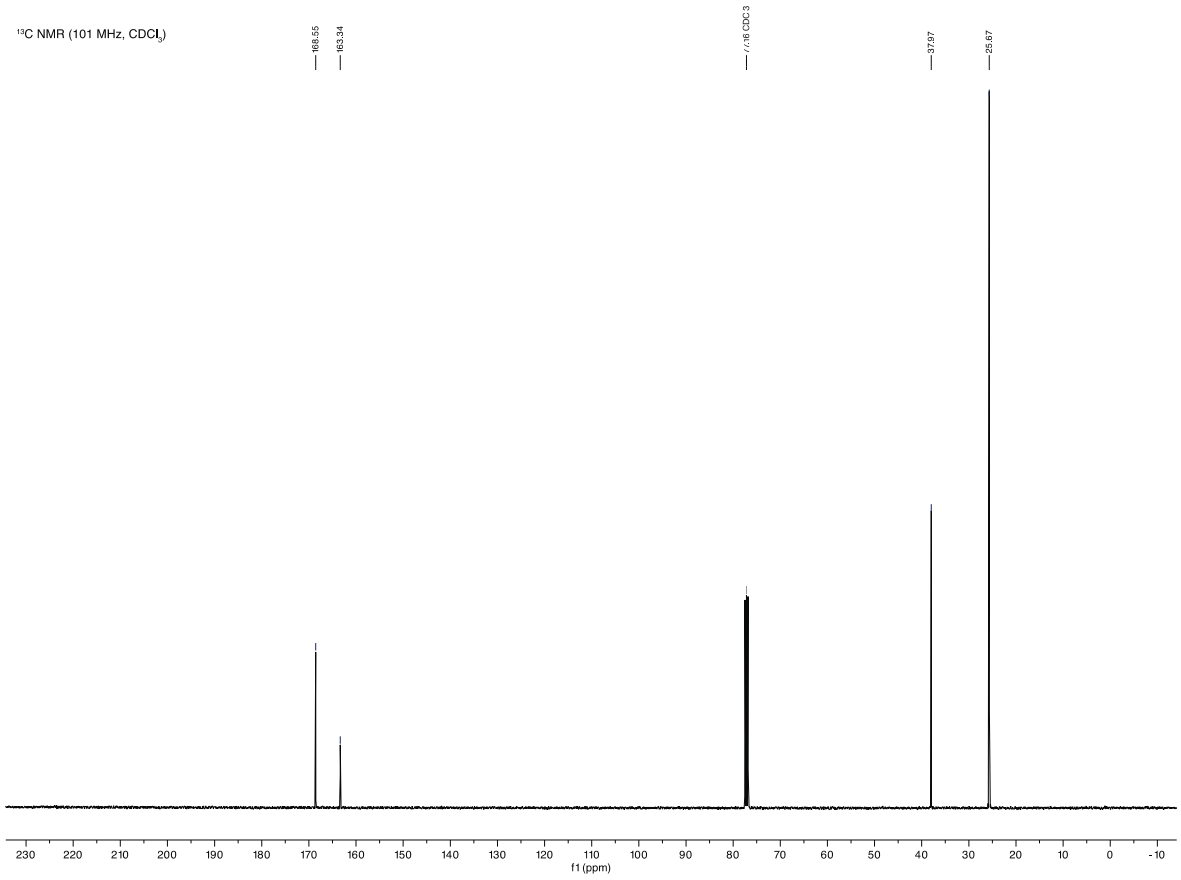
Table S4 Primer sequences used to introduce deletions and point mutations for proteins used in this study.

6. Small molecule characterization

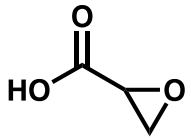
6.1. α-chloroacetyl NHS-ester

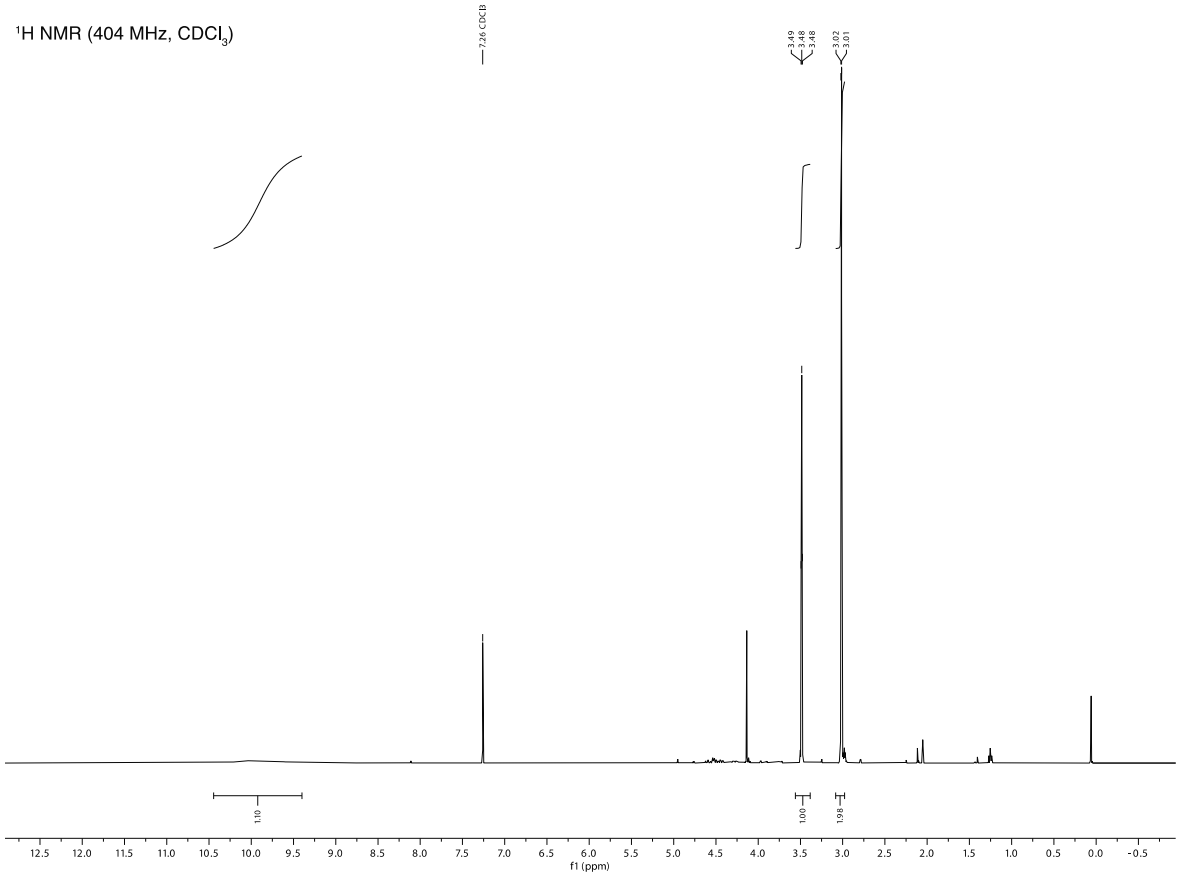


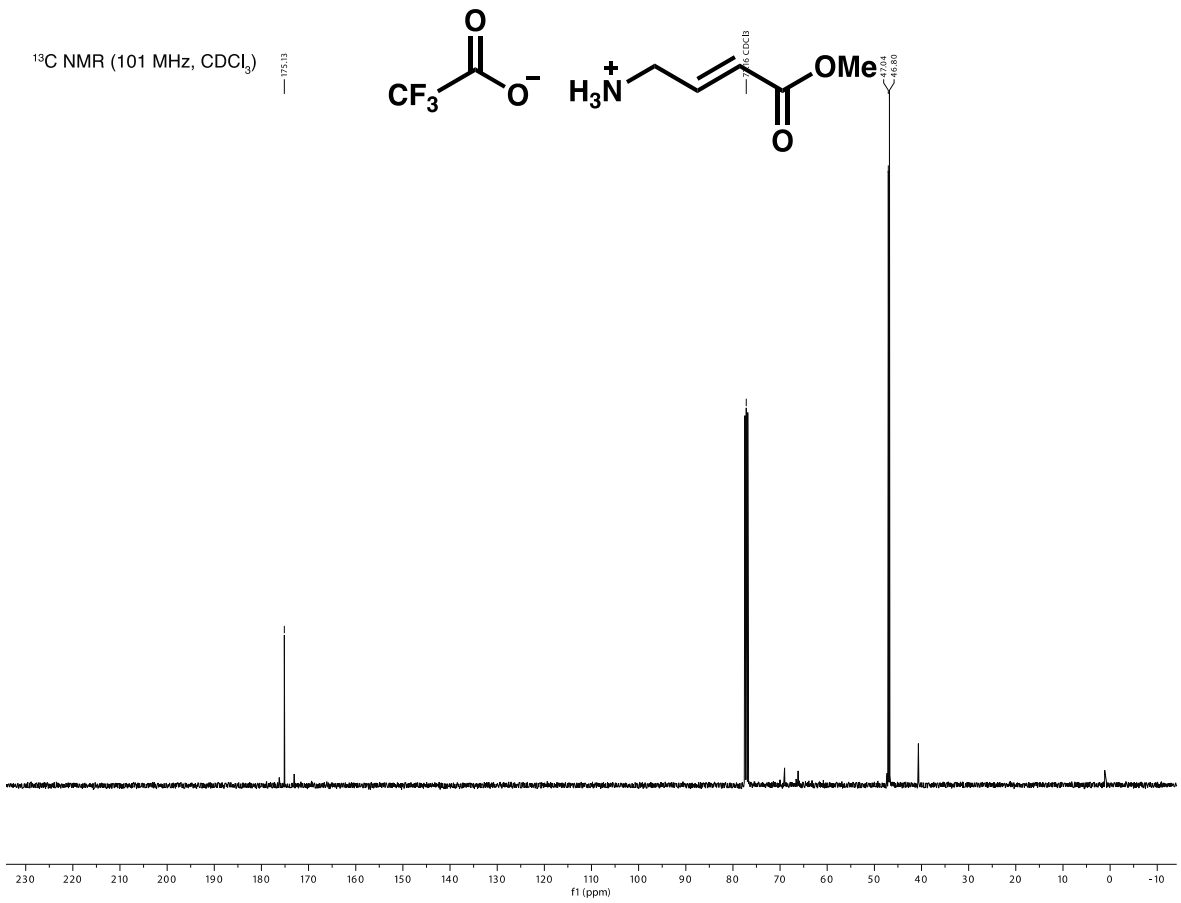

^{1}H NMR (400 MHz, $CDCl_3$)
7.26 CDCl3
4.37
2.86
1.99
4.01
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


^{13}C NMR (101 MHz, $CDCl_3$)
168.55
163.34
77.16 CDCl3
37.97
25.67
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
f1 (ppm)

6.2. Glycidic acid

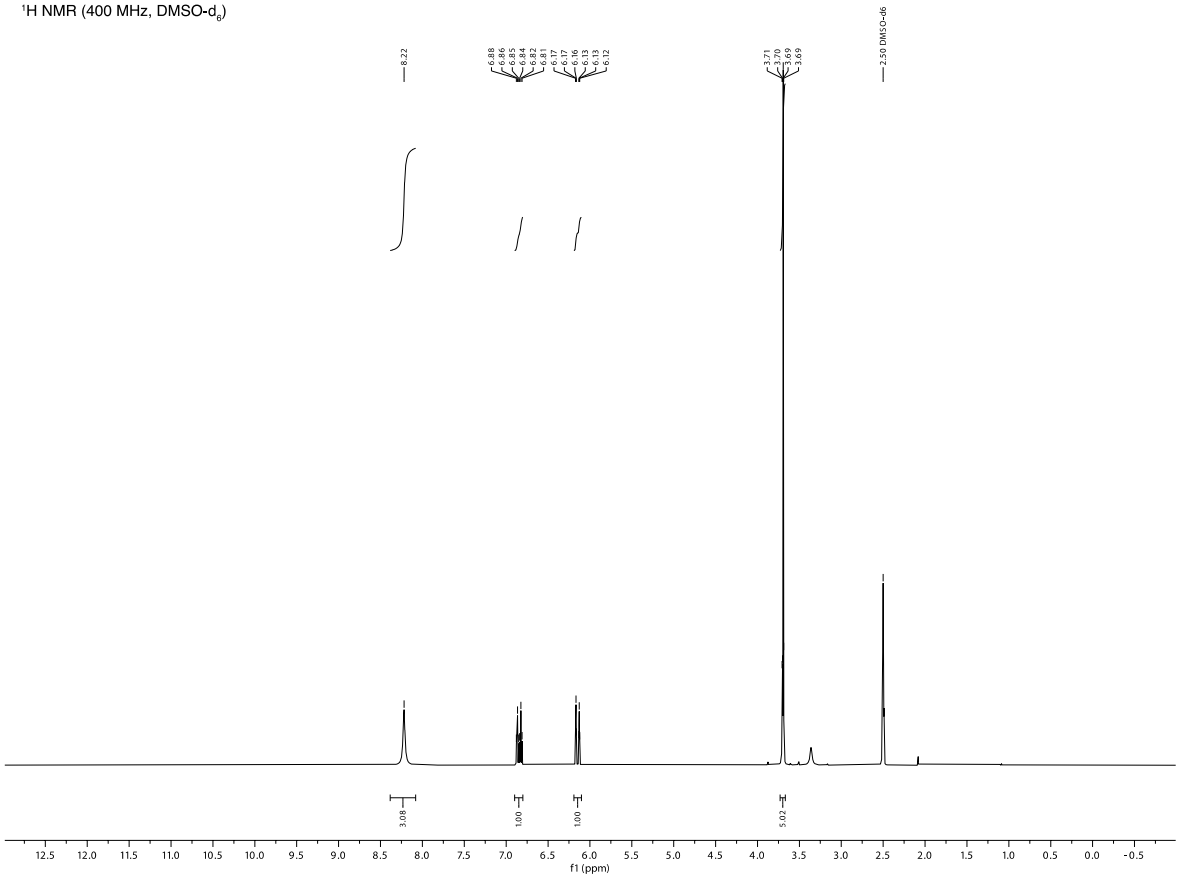




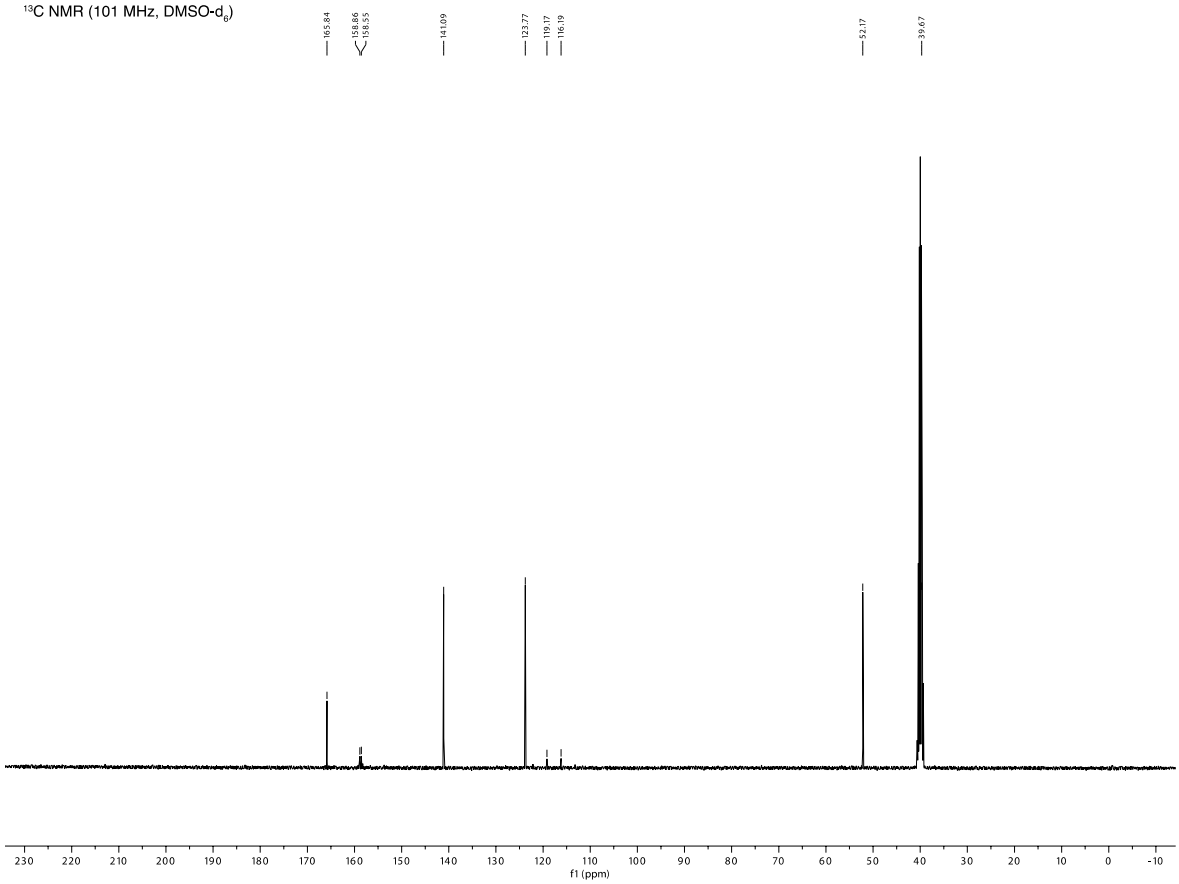


6.3. VME-TFA salt

^{1}H NMR (400 MHz, DMSO-d_6)



^{13}C NMR (101 MHz, DMSO-d_6)



7. References

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